001 [Oral 036]

Calpain activity is essential for cell recruitment, myofibroblast differentiation and angiogenesis in early stages of skin wounding

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Calpains are calcium-dependant intracellular cysteine proteases that play major role in cytoskeleton and adhesion molecule rearrangements. Since these phenomena alter cell motility, inflammation, angiogenesis and fibrosis, we asked whether such inhibition may affect wound healing. We used a transgenic mouse overexpressing ubiquitously and constitutively the natural calpain inhibitor, calpastatin. Surgical skin wounds were performed on wild-type (WT) and Calpastatin transgenic mice (CPST-TG). Wound closure was delayed in CPST-TG mice on days 3, 7 and 10. The bigger delay was at day 3 (Residual wound: 114% in CPST-TG vs 41 % in WT, $p<0,001$), suggesting impairment of wound contraction. Immunolabeling revealed a reduction in αSMA+ cells in CPST-TG wounds on day 3 (7% v/s 15%, $p<0,05$), with a 71% decrease in α-SMA RNA. We also found less CD45+ cells in CPST-TG wounds (19% v/s 34%, $p<0,05$). On day 7, cell proliferation of the epidermis and the granulation tissue (106 v/s 152 vessel/mm2, $p<0,05$) were decreased in CPST-TG animals. Vessels density was reduced, with a 28% decrease in VEGFa RNA. Epidermization -measured on K14 labelled sections-was also impaired. Finally, WT skin transplanted on CPST-TG recipient showed healing impaired on days 3 and 5, with a reduction in blood vessel density and CD45+ cells counts. In vitro, CPST-TG fibroblasts expressed lower αSMA levels upon TGFβ stimulation. All these results show a prominent role for calpain in the formation of the granular tissue and the differentiation of myofibroblasts resulting in delayed angiogenesis, proliferation and epidermization.

002 [Oral 037]

Topical Simvastatin Accelerates Wound Healing in Diabetes by Enhancing Lymphangiogenesis

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Impaired wound healing is one of the common complications of diabetes. Recent studies have shown reduced lymphangiogenesis and angiogenesis during diabetic wound healing, and this may represent a new therapeutic target. We previously reported that infiltrating macrophages in granulation tissues can be differentiated into new lymphatics during wound healing, and that this function is also attenuated in diabetes. In the present study, we tested the hypothesis that topical application of simvastatin, a HMG-CoA reductase inhibitor, would accerelate wound healing via promotion of lymphangiogenesis in genetically diabetic mice. We also evaluated the effects of simvastatin on lymphatic endothelial cells (LECs) and macrophages, both of which contribute to lymphangiogenesis. Full thickness skin wounds were created on the backs of diabetic mice. Animals were treated with simvastatin (50 μg per wound) or vehicle topically. Application of simvastatin resulted in significant acceleration of wound recovery, and this acceleration was notable for increased lymphangiogenesis in the wound. Simvastatin also promoted infiltration of LYVE-1 positive macrophages, which seemed to be incorporated into newly formed lymphatics. In vitro, simvastatin did not promote LEC proliferation, but directly promoted capillary morphogenesis on both LECs and macrophages. These results suggest that both pre-existing LECs and infiltrating macrophages contribute to newly organized lymphatic vessels induced by simvastatin. In conclusion, a simple strategy of topical application of simvastatin may have significant therapeutic potential for enhanced wound healing in patients with impaired microcirculation, such as that found in diabetes.

003 [Oral 038]

How do Peroxisome-Proliferator-Activated-Receptors regulate the expression of Cathepsin in endothelial cells?

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Malignant tumor cells recruit vasculature and stromal cells through production and secretion of stimulatory growth factors and cytokines to activate their local host tumour microenvironment. In this context, the reorganization of the extracelluar matrix is an important tumour initiated process. In the last years a group of lysosomal proteases, the cathepsins, as well as ligand-activated transcription factors Peroxisome-Proliferator-Activated-Receptors (PPARs), were described to be crucial in the process of metastasis and angiogenesis. We therefore explored the effect of PPARα and PPARδ agonists on cathepsin expression by endothelial cells. Both inhibit the endothelial cathepsin B protein expression in a time and dose concentration dependent manner. We further investigated whether the inhibition in cathepsin B protein synthesis by PPARα and PPARδ ligands is mediated by changes in the mRNA expression level. Treatment with PPARα agonist suppressed cathepsin B mRNA accumulation, whereas PPARδ ligands failed to change the mRNA expression levels. Analysis of 5´-deletional cathepsin B promoter-based constructs revealed, that PPARα ligands mediate their inhibitory effects on cathepsin B expression through an E-box-binding site in close proximity to the transcriptional start site. EMSA analysis demonstrated that PPARα activator suppresses the binding of USF1/USF2 at the E-box located between the base pairs -16 and +17 at the cathepsin B promoter sequence. In conclusion, our data identify for the first time endothelial cathepsin B expression as a novel target for PPARα and PPARδ agonist. That gives a new aspect in their modes of action and might enhance there potential as a class of therapeutic compounds.

004

Egln3 as a key element of differential regulation of HIF1 activity by acute and chronic hypoxia in epidermal keratinocytes

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Hypoxia-induced factor 1 (HIF1 - a heterodimer of HIF1α and HIF1β or ARNT) is essential for cellular adaptation to low oxygen. As such, it plays an important role in skin development, wound healing, tumorigenesis and barrier function. However, mechanisms controlling epidermal HIF1 activity remain unknown. Using primary mouse and N-TERT human epidermal keratinocytes and ARNT-deficient and -overexpressing cells exposed to ambient (21%) or low oxygen conditions (1%) we studied mechanisms controlling HIF1α and ARNT levels in the epidermis. In normoxia HIF1α protein is both nuclear and cytoplasmic whilst ARNT is nuclear. Under hypoxia, HIF1α mRNA falls down in two steps (at 1h and after 24h), while protein increases during acute hypoxia (<5h), then declines way below its normoxic level. ARNT mRNA remains constant all the time, while protein is steady under acute hypoxia only, being significantly downregulated during subsequent chronic stage. This suggests that HIF1α is controlled post-translationally during acute hypoxia and ARNT during chronic hypoxia. As a result, HIF1 transcriptional activity is detected in normoxia, increases in acute hypoxia and drops in chronic phase (TransAm assay). All these effects are not affected by Ca^{++} level. We also found that EGLN3 is a key factor of HIF1α protein stability in the epidermis and is tightly regulated by ARNT through HDAC-dependent mechanisms. EGLN3 mRNA level steadily increases under hypoxia, but protein appears to be post-translationally modified showing decrease during acute phase of hypoxia. Our results expose the complexity of epidermal hypoxia pathway regulation and outlines future steps for its exploration.

005

Blood and lymph vessel density in Caucasian skin

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The aim of the present study was to provide data on blood- and lymph vessel density in human healthy skin in a Caucasian population. Special regard was put on a potential difference in sun-exposed and sun-protected body regions as well as changes possibly occurring during aging. Skin of 10 different body regions was analysed by immunohistochemical staining of paraffin sections for von Willebrand Factor and LYVE-1. The age range of the donors (n=87) was in between 20 - 94 years. The overall amount of blood vessels detected per skin section (=0,6mm²) was 20,62 ± 2,78 (mean ± standard deviation). The highest number was found in breast skin (24,30 ± 8,23) and the lowest in skin from the thigh (15,93 ± 8,32). Lymph vessel density was remarkably lower with 3,89 ± 1,17 lymph vessels per skin section, the highest amount could be demonstrated in neck skin (5,8 ± 3,11) and the lowest in skin from the forehead (2,19 ± 1,02). Blood vessel density was 5,75 ± 1,85 fold higher than lymph vessel density. Neither body region nor donor age exerted a significant influence on blood or lymph vessel density in our study population. In summary, we found a high inter-individual variability of vessel density in every body region but no significant effect of age or sun-exposure in our Caucasian study population. This might reflect ethnic as well as individual differences in skin aging and predisposition to develop certain skin diseases like for example rosacea.

006

The role of thymidine phosphorylase in the induction of early growth response protein-1 and thrombospondin-1 by 5-fluorouracil in human cancer carcinoma cells

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5-Fluorouracil (5-FU) is widely used for treatment of various cancers including cutaneous squamous cell carcinoma, extramammary Paget carcinoma, and so forth. Thymidine phosphorylase (TP), an enzyme involved in reversible conversion of thymidine to thymine, is identical to angiogenic factor and platelet-derived endothelial cell growth factor (PD-ECGF). TP is expressed higher in a variety of malignant tumors than adjacent non-neoplastic tissues and thought to affect the metabolic process and the anticancer effect of 5-FU. To investigate the molecular basis of this effect,human gastric carcinoma AZ521 cells and epidermoid carcinoma KB cells were transfected with TP cDNA, and AZ521/TP and KB/TP were cloned. AZ521/TP and KB/TP cells overexpressed TP, were more sensitive to 5-FU than the counterpart parental cells. 5-Formyltetrahydrofolate (leucovorin; LV) enhanced the sensitivity of AZ521/TP cells to 5-FU, but not of the parental cells by stabilizing the complex of thymidylate synthase (TS) and 5-fluoro-deoxyuridine-monophosphate (FdUMP). The complex of TS and FdUMP was increased in AZ521/TP cells treated with LV but not in the parental cells treated with LV. Furthermore, 5-FU increased the expression of early growth response protein-1 (Egr-1) and an angiogenesis inhibitor, thrombospondin-1 (TSP-1), in KB/TP cells but not in the parental cells. TPI, a inhibitor for TP ($Ki=2.36x10^{-9}M$), attenuated the induction of Egr-1 and TSP-1 by 5-FU, while LV increased the expression of these proteins. These findings demonstrate that the TP activity has a principal role in the production of FdUMP in TP-expressing cells, and FdUMP is implicated in the induction of Egr-1 and TSP-1 in KB cells.

007

Hypothyroidism improves random-pattern skin flap survival in rats

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The protective effect of hypothyroidism against ischemic or toxic conditions is shown in various tissues. We investigated the effect of hypothyroidism and acute local effect of propylthiouracil (PTU) and methimazole (MMI) on the outcome of lethal ischemia in this flap model. Forty-two Sprauge-Dawley rats were randomly divided into 7 groups. In all groups, dorsal flaps with caudal pedicles were elevated at midline and flap survival was measured at the seventh day after surgery. The first group served as control and received 1 ml of 0.9% saline solution into their flap before flap elevation. In groups 2 and 3, hypothyroidism was induced by administration of either PTU 0.05% or MMI 0.04% in their drinking water for four weeks. Next four groups received local injections of MMI (10, 20, 50 or 100 µg/flap) before flap elevation. Local PTU injection was ignored due to insolubility of the agent. Hypothyroidism was induced in chronic PTU and MMI treated groups, and animals in these groups showed significant increase in their flap survival compared to control euthyroid rats (79.47 ± 10.49% and 75.48 ± 12.93% vs. 52.26 ± 5.75%, respectively, $P< 0.01$). Acute local treatment of skin flaps with MMI failed to significantly affect the flap survival. This study demonstrates for the first time that hypothyroidism survives random-pattern skin flaps in rats.

008

VEGF plays a key role enhancing epidermal and blood vessel protection against stress

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Vascular endothelial growth factor (VEGF) is a crucial element of endothelial cells and angiogenesis and plays diverse roles in skin photoaging, hypoxia, and wound healing. We investigated the expression of VEGF-A and VEGFR-2 (Flk-1), its major receptor, in different cell lines by RT-PCR. VEGF-A and Flk-1 immunofluorescence studies showed that IV09.006, a compound designed to target VEGF pathway, increased the expression of these two proteins in normal human keratinocytes (NHK) and endothelial cells. *Ex vivo* studies showed that VEGF-A expression in the epidermis is mainly located in the suprabasal layers. UVB irradiation and H2O2 stresses increased VEGF-A expression in the epidermis. In parallel, pre-treatment with IV09.006 was shown to protect skin from stress damage. For *in vivo* evaluation, we used chicken chorio-allantoïc membrane (CAM). IV09.006 active was applied directly on the CAM for 24h, and then a stress induced by UVB irradiation (60 mJ/cm2) or H2O2 (10 mM) was applied. A time course observation of the blood vessel network was performed after each stress condition. Our study showed that pre-induction of VEGF-A and Flk-1 enabled a better maintenance of the blood vessel network, preventing vasodilatation and coagulation induced by stress. Taken together, these results indicate that the positive modulation of VEGF-A and Flk-1 expression could be linked to a better preservation of the epidermis from UVB and oxidative stress-induced damage, as well as a protection of the blood vessel network from these stresses.

009 [Oral 109]

A unique population of inflammatory macrophages induced by iron impairs cutaneous wound healing

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Uncontrolled macrophage activation is now considered to be a critical event in the pathogenesis of chronic inflammatory disorders like arteriosclerosis, multiple sclerosis and chronic venous leg ulcers (CVU). However, it is still unclear which environmental cues induce persistent activation of macrophages *in vivo* and how macrophage-derived effector molecules maintain chronic inflammation and affect resident fibroblasts essential for tissue homeostasis and repair. We used a complementary approach studying human subjects with CVU, a model disease for macrophage-driven chronic inflammation, while establishing a murine model closely reflecting its pathogenesis. Here we show that iron overloading of macrophages in CVU and in iron-dextran-treated murine full-thickness excisional wounds induce a novel macrophage population in situ which up-regulate CD163, the hemoglobin-haptoglobin scavenger receptor for iron. $CD163^{hi}$ wound macrophages mount a persistent pro-inflammatory activation state with high expression of M1 markers ($TNFa^{high}IL\text{-}12^{high}CD11b^{high}CCR2^{high}$) and the concomitant intermediate expression of anti-inflammatory M2 markers ($IL\text{-}4Ra^{med}Dectin\text{-}1^{med}CD36^{med}CD206^{med}$), suggestive for an incomplete switch towards the tissue repair-promoting M2 activation phenotype. We show that 'hybrid' M1/M2 activated macrophages - via enhanced TNFa and hydroxyl radicals release – perpetuate inflammation and install a $p16^{INK4a}$-dependent senescence program in resident fibroblasts eventually leading to tissue breakdown and impaired wound healing. Understanding the role of macrophage activation for persistency or resolution of inflammation in chronic venous ulcers and other chronic inflammatory diseases holds substantial promise for the development of novel therapies for these difficult-to-treat conditions.

010 [Oral 110]

Toll-like receptor signaling in dendritic cells is sufficient to mediate Imiquimod-induced psoriasis-like skin inflammation in mice

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Psoriasis is a common inflammatory skin disease characterized by sharply demarcated chronic red plaques covered by white scales. Based on the observation that Imiquimod (IMQ) treatment leads to de novo development or relapse of psoriasis in patients, we established a new mouse model for human psoriasis. Daily application of IMQ onto mouse back skin induces inflamed scaly lesions resembling plaque type psoriasis. These lesions show increased epidermal proliferation, abnormal cell differentiation, neoangiogenesis and infiltrates consisting of neutrophils, $CD4^+$ T cells, conventional and plasmacytoid dendritic cells (DC). We previously demonstrated that lesion development is critically dependent on IL-23 and IL-17. However, the cell types triggering the inflammatory process remain elusive. IMQ activates diverse cells of the immune system after binding to toll-like receptors (TLR)-7/8. To investigate the role of different (skin) DC in initiating IMQ-induced psoriasis, we generated MyD88-deficient mice in which TLR-signaling can be conditionally switched on by Cre-mediated excision of a stop cassette ($MyD88^{stp/stp}$ mice). As expected, $MyD88^{stp/stp}$ animals are resistant to IMQ-induced psoriasis. In contrast, mice with selective reconstitution of TLR signaling in all $CD11c^+$ DC acquire full-blown psoriasiform skin disease following IMQ painting. Intriguingly, mice with TLR signaling restricted to $Langerin^+$ DC subsets, including epidermal Langerhans cells and $Langerin^+$ dermal DC, develop attenuated disease. These data imply that DC are sufficient to mediate IMQ-induced psoriasis. In ongoing experiments we are further dissecting the requirement of different DC subsets for the induction of disease.

011 [Oral 063]

Human IL-10 producing regulatory B cells control $CD4^+$ T cell proliferation

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The existence of interleukin 10 (IL-10) producing regulatory B cells that down-modulate inflammation has already been validated in mice. Especially, a potent subset of regulatory B cells with a $CD1d^{high}CD5^+CD19^{high}$ phenotype was found to decrease contact hypersensitivity in an IL-10-dependent manner. In humans, a transitional B cell subset has recently been shown to exhibit regulatory capacities in vitro.We investigated the existence of IL-10 producing B cells in human blood and spleen and evaluated their phenotype. We also tested the optimal in vitro conditions to trigger IL-10 production by B cells and tested their capacities to regulate $CD4^+CD25^-$ T cell proliferation.We were able to detect after *ex vivo* short time stimulation, IL-10 producing B cells in human blood and spleen (1.8% and 1.1% of blood and spleen human B cells produced IL-10 as detected by intracellular cytokine staining). Blood IL-10 producing B cells were relatively enriched within the memory (CD27+) and transitional (CD38highCD24high) B cell subsets but IL-10 producing B cells were detected within the whole B cell lineage. Combined CpG-B and anti-immunoglobulin stimulation, rather than CD40L-CD40 pathway, was the most potent stimulus for inducing IL-10 secretion (ELISA assay). Under these conditions of stimulation, human blood B cells inhibited $CD4^+$ $CD25^-$ T cell proliferation *in vitro* in an IL-10-dependent mechanism.These findings imply that promoting or inhibiting IL-10 production by human B cells may represent potential targets for modulating immune responses in human pathology, including dermatological inflammatory diseases.

012 [Oral 064]

The genetics of murine epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is an autoimmune blistering disease, characterized by auto-antibodies to type VII collagen (COL7). EBA can be induced in mice by immunization with a fragment of the non-collagenous domain 1 of murine COL7 or by transfer of anti-COL7 antibodies. Unlike other autoimmune diseases, e.g. rheumatoid arthritis or Lupus, little is known about the genetic susceptibility for EBA and/or related autoimmune blistering diseases. We therefore used the EBA mouse model to address the hypothesis that disease induction depends on the MHC haplotype and additional genes outside the MHC. Mice from different inbred and MHC congenic strains were immunized with recombinant murine COL7. Three distinct responses were observed: (i) severe disease in SJL/J (H2s) and MRL/MpJ (H2k), (ii) mild and transient disease in C57Bl/10.s (H2s), (iii) resistance in C57Bl/6J (H2b), NZM2410/J (H2z), DBA/1J (H2q), NOD/ShiLtJ (H2g7) and C57Bl/10.q (H2q) mice. Overall, susceptibility to EBA was strongly associated with H2s and H2K. We then used a 4-way intercross we recently generated to map quantitative trait loci, QTL, linked to EBA. Active or passive EBA was induced in 530 intercross mice genotyped with 1400 SNP markers, Illumina MD array, and disease phenotypes were evaluated. HAPPY mapping software was used to identify QTL associated with EBA. In total, we identified six loci controlling disease onset and/or severity, located on Chr. 1, 6, 12, 15, 19. Our findings suggest that susceptibility to experimental EBA is linked to the MHC haplotype. Furthermore, preliminary QTL mapping data suggest that numerous non-MHC genes are involved.

013 [Oral 065]

Novel cleavage sites and specific neoepitopes on shed ectodomain of collagen XVII/BP180: role in autoimmune blistering diseases

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Collagen XVII/BP180 is a type II transmembrane protein in basal keratinocytes which provides stable adhesion between epidermis and dermis. Its ectodomain can be shed from the cell surface, and autoantibodies from patients with bullous pemphigoid and linear IgA bullous dermatosis are known to preferentially react with the cleaved fragment. Previous studies have suggested the development of neoepitopes on the shed ectodomain, however the pathomechanisms are still unclear. In this study, we investigated how certain antibodies can react specifically with the shed ectodomain of collagen XVII, but not with the full-length form, and their role in blister formation. First, a new polyclonal antibody recognizing the stretch from Leu^{524} to Gly^{532} preferentially reacted with the shed ectodomain, but not with the full-length form, indicating that a neoepitope was actually localized at this site. Second, the antibody could induce granulocyte-dependent dermal-epidermal separation in cryosections of normal human skin, suggesting its pathogenicity. Third, a novel method using mass spectrometry revealed physiological cleavage sites, and different amino-termini were found at Asp^{514}, Leu^{524}, Glu^{525} and Gly^{526}, among which Asp^{514} and Glu^{525} were blocked by acetylation and pyroglutaminate, respectively. Finally, in silico prediction of B-cell epitopes demonstrated that the antigenicity of the Leu^{524} to Gly^{532} region increased substantially after shedding, regardless of the different cleavage sites. These data demonstrate that physiological shedding of collagen XVII generates neoepitopes in the released ectodomain and suggest a pathogenic role for neoepitope-specific antibodies in autoimmune blistering diseases.

014 [Oral 066]

Mast cell committed progenitors travel through peripheral tissues and lymph - implication for the accumulation of mature mast cells due to inflammatory signals

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Hematopoietic stem and progenitor cells (HSPCs) have been shown to re-circulate through blood, lymph, and extramedullary tissues and to give rise to tissue-resident myeloid cells, preferentially dendritic cells, upon inflammatory stimuli. Recently, mast cell-committed progenitors (MCPs) have been described among HSPCs in the bone marrow of adult mice. Here, we demonstrate that Lin-Kit+Sca-1-Ly6c-CD27-FcεRIα-β7-integrin+T1/ST2+ MCPs similarly travel though blood, peripheral tissue, and lymph. We show that MCPs are detectible in blood and peripheral tissue, i.e. skin, and that mouse thoracic duct lymph contains MCPs that possess mast cell (MC) reconstitution capacity. FTY720 treatment, a potent sphingosine-1-phosphate (S1P) receptor antagonist, results in the depletion of MCPs from lymph indicating that the egress of MCPs from peripheral tissue into the lymph is a S1P receptor dependent mechanism. Prolonged topical application of the phorbolester phorbol 12-myristate 13-acetate (PMA) results in pronounced dermal MC accumulation. Pertussis toxin (PTx) treatment markedly impaired the recruitment of MCPs to PMA treatment sites and reduced the number of accumulating MCs. Thus, we demonstrate that the migration of MCPs into peripheral tissue, i.e. the skin, requires the expression of functional Gαi-coupled receptors. In summary, our findings indicate that MCPs traffic through bone marrow, blood, periphery tissue and lymph under physiological conditions, and that MC accumulation due to inflammatory signals depends on the recruitment of MCPs and/or the inhibition of their egress from tissue sites. The characterization of the regulation of MCP trafficking in detail could lead to a better understanding of MC-mediated diseases and the development of novel therapeutic strategies.

015 [Oral 067]

Epidermal EGFR signaling controls leukocyte infiltration in the skin and prevents systemic disease

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The skin rash that occurs during the anti EGFR therapy of cancer patients shows the importance of EGFR control on skin immune-homeostasis. To better understand the pathogenetic role of EGFR inhibition in the skin, we developed an epidermally targeted mouse model of EGFR ablation. By crossing Keratin 5 driven Cre recombinase transgenics with EGFR floxed mice, EGFR was ablated in the epidermis. The double transgenic mice did not display a clear phenotype at birth. After the first week and for their entire life, double transgenics showed a strong phenotype with skin inflammation and aberrant hair growth and hair loss cycles. The appearance of the skin phenotype (7/8 days after birth) was concomitant with tissue infiltration of total lymphocytes (CD45), mast cells (toluidine blue) and macrophages (F4/80). In the second week of life, the neutrophilic infiltrate was also abundant as detected by myeloperoxidase assay. Tissue samples isolated from skin of adult double transgenics contained high mRNA levels of a subset of inflammatory mediators namely CCL17, CCL20, CCL2, TNF-alpha, IL-6, IL-17, IL-18, and G-CSF. Targeted knockout mice also displayed hematological abnormalities with a higher number of circulating neutrophils, platelets and lower numbers of lymphocytes. Extramedullary hematopoiesis was detected in the spleen and plasma levels of G-CSF, IL-1ra, IL-17, IL-6 and CCL2 were elevated. These data indicate that the chronic absence of EGFR expression in the epidermis triggers not only local but also systemic inflammatory responses.

016 [Oral 068]

Desmoglein 3-specific T cells induce Experimental Autoimmune Dermatitis (EAD), a novel model for interface dermatitis

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Desmoglein 3 (Dsg3), a transmembrane component of desmosomes in the skin, is the autoantigen in pemphigus vulgaris (PV). We recently established Dsg3-specific $CD4^+$ T cell (Dsg3 T cell) clones that induced PV phenotype. Herein, we cloned T cell receptors (TCR) from Dsg3 T cells and developed transgenic (tg) mice expressing these TCRs. In these tg mice, tgDsg3 T cells escape from central deletion and are found in periphery. Surprisingly, instead of inducing PV phenotype, tgDsg3 T cells induced clinical phenotype characterized by scaly erythematous lesions, which histology displayed interface dermatitis, as determined by $CD4^+$ T cell infiltration in the dermo-epidermal junction with vacuolar degeneration and apoptosis. When these TCRs were retrovirally transduced into wild-type (WT) $CD4^+$ T cells (rvDsg3), the cells acquired antigen-specificity identical to tgDsg3 T cells. RvDsg3 T cells evoked similar clinical phenotype, and histological changes in skin, mucous membranes and esophagus, where Dsg3 was expressed. Cytokine profiles revealed that both rvDsg3 and tgDsg3 T cells had lost the Th2-phenotype that parental T cell clone displayed, and produced IFN-γ and IL-17. To evaluate the roles of these cytokines, we compared the phenotypes induced by WT, IFN-γ KO, or IL-17 KO rvDsg3 T cells. Strikingly, deficiency for IFN-γ, but not IL-17, lead to dramatic loss of acute phenotype. Our model, designated experimental autoimmune dermatitis (EAD), provides a valuable tool to understand the pathophysiology of T cell-mediated inflammatory skin diseases including lichen planus and lupus erythematosus, that display interface dermatitis.

017 [Oral 069]

Platelet-activating factor blockade inhibits Th17 pathway and suppresses psoriatic-like skin disease in K5.hTGF-beta1 transgenic mice

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Platelet activating factor (PAF), a potent biolipid mediator, is involved in a variety of cellular transduction pathways and plays a prominent role in inducing inflammation in different organs, including the skin. We utilized K5.hTGF-beta1 transgenic mice, which exhibit an inflammatory skin disorder and molecular and cytokine abnormalities with strong similarities to human psoriasis, in order to evaluate the role of PAF and its receptor in the pathogenesis of the skin phenotype. We found that injecting PAF into the skin led to inflammation and accelerated the manifestation of the psoriatic phenotype in transgenic mice by a local effect, as assessed by macroscopic and microscopic findings. In contrast, injecting mice with PAF receptor antagonist PCA-4248 lowered the PAF level (most likely by depressing an autocrine loop) and neutrophil accumulation in the skin and blocked disease progression. This effect of PAF blockade was similar to that of PUVA and paralleled by a decrease of abnormally elevated mRNA and/or protein levels of Th17-related cytokines IL-17A, IL-17F, IL-23, IL-12A, and IL-6, and its transcription factor STAT3. In contrast, PCA-4248 treatment upregulated the mRNA levels of COX2 and IL-10 in the dorsal skin and release of IL-10 in the serum and skin. This implies that specifically interfering with PAF may be an option to induce cytokine pathways with disease suppressive activity and develop novel anti-psoriatic therapeutic strategies in particular for inflammatory disease variants.

018 [Oral 070]

MiR-155 is overexpressed in atopic dermatitis and modulates T cell proliferative responses by targeting CTLA-4

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MicroRNAs are short, noncoding RNAs that suppress gene expression at the post-transcriptional level. Atopic dermatitis is a common chronic inflammatory skin disease characterized by the presence of activated T cells within the skin In this study, we aimed to explore the role of miRNAs in the pathogenesis of atopic dermatitis. Using TaqMan MicroRNA Low Density Arrays, we showed that miRNAs are deregulated in atopic dermatitis skin lesions as compared with healthy skin. MiR-155 was one of the highest-ranked up-regulated microRNA in atopic dermatitis. In situ hybridization as well as quantitative PCR analysis of miR-155 in FACS-sorted dermal cells showed that miR-155 was predominantly expressed in infiltrating immune cells in atopic dermatitis lesions. MiR-155 was up-regulated during T cell differentiation/activation and was markedly induced by T cell activators in PBMCs in vitro and by superantigens and allergens in the skin *in vivo*. We identified CTLA-4, an important negative regulator of T cell activation, as a direct target of miR-155, using in silico prediction and validation by luciferase reporter assays. Overexpression of miR-155 in T helper cells resulted in decreased CTLA-4 levels accompanied by an increased proliferative response. Our results show that miR-155 is significantly overexpressed in atopic dermatitis. Overexpression of miR-155 may contribute to chronic skin inflammation by increasing the proliferative response of T helper cells via the down-regulation of CTLA-4.

019 [Oral 071]

A peptide within HSP70i driving vitiligo development

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Published studies from our lab support the ability of HSP70i, secreted in increased amounts by vitiligo melanocytes, to activate dendritic cell (DC) mediated cytotoxicity. HSP70i also accelerated TRP-2 DNA vaccine induced, T cell mediated depigmentation in C57BL/6 mice, but not in HSP70i knockout animals. To challenge the concept that HSP70 may be targeted to interfere with vitiligo development, we set out to identify an intramolecular peptide crucial to the depigmentation process. DNA encoding an exposed 13-mer peptide was subjected to site-directed mutagenesis. The choice was based on homology to a stretch of amino acids within microbial Hsp70 previously held responsible for binding to and activating human DC. Four different mutant sequences and wildtype HSP70 were used to vaccinate C57BL/6 mice in combination with TRP-2 encoding DNA, measuring depigmentation by scanning and image analysis over time. The ability to augment depigmentation was significantly impaired for 3 mutant sequences, implicating an 11-mer peptide crucial to inducing vitiligo. Western blot analysis using polyclonal and monoclonal antibodies to HSP70i probing homogenates of transiently transfected COS cells revealed a monoclonal antibody reactive with the molecular region of interest. The results demonstrate a crucial role for peptide QPGVLIQVYEG in inducing vitiligo and reveal opportunities for blocking the roaming peptide from activating dendritic cells and recruiting melanocyte-reactive T cells to the skin. This work was recently filed as a continuation of parent patent application PCT/US2008/076266

020 [Oral 017]

The Toll-like receptor 4 ligands Mrp8 and Mrp14 are crucial in the development of autoreactive CD8$^+$ T cells

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Autoimmunity results from a conflict of regulatory mechanisms controlling self-tolerance and the interaction of CD40 on antigen-presenting cells with its ligand CD40L plays an important role during (auto)immune responses. Within the skin, transgenic overexpression of CD40L in keratinocytes leads to systemic autoimmunity as evidenced by autoantibodies, nephritis, and autoimmune dermatitis, which can be adoptively transferred by injecting CD8$^+$ T cells into naive recipients. However, the mechanisms linking the local micromilieu and the development of autoreactive CD8$^+$ T cells in CD40L-induced autoimmunity are poorly understood. Myeloid-related proteins (Mrp) such as Mrp8 and Mrp14 are damage associated molecular pattern molecules (DAMPs) highly up-regulated in various autoimmune disorders. Hence, we investigated the relevance of Mrp8 and Mrp14 for the development of functional autoreactive T cells and could show that local Mrp8/14 production is essential for the induction of autoreactivity in CD8$^+$ T cells and the development of systemic autoimmunity. This effect is mediated via TLR4 signaling and up-regulation of the transcription factors Runx-1 and RORc in CD8$^+$ T cells leading to increased IL-17 expression. Notably, Mrp8 and Mrp14 expression was increased in cutaneous lesional lupus erythematosus (LE) and enhanced concentrations of Mrp8/14 were detectable in patient's serum. Strikingly, stimulation of CD8$^+$ T cells from LE patients with Mrp8 and Mrp14 proteins resulted in a significant up-regulation of IL-17 expression indicating that Mrp8/14 play also an important role during human MHC class I-mediated cellular autoimmunity. Together, these data present the first link between local expression of a DAMP-molecule and the development of systemic autoimmunity.

021 [Oral 023]

Identification of slan (6-sulfo LacNAc$^+$) dendritic cells as a novel proinflammatory DC population in atopic dermatitis driving strong Th17 and Th1 responses

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Atopic dermatitis (AD) is a chronic inflammatory skin disease with a key role for dendritic cells (DC) and T cells. Recently several studies provided evidence for the presence of IL-17 and IL-22 producing T cells in AD. IL-17 was shown to augment the production of proinflammatory cytokines by keratinocytes, while IL-22 induced inhibits genes involved in terminal differentiation of keratinocytes. A DC subset that preferentially induces IL-17 and IL-22 producing T cells has not yet been described. Within the perivascular dermal inflammatory infiltrate of lesional AD we identified high numbers of proinflammatory DC selectively expressing 6-sulfo LacNAc (slan). We previously described slanDC as a distinct and separate population of human blood DC serving as the major source of IL-12 as well as of TNF-α. In addition to blood, slanDC were found within the local inflammatory infiltrate in psoriasis and rheumatoid arthritis. When stimulating slanDC and for comparison CD1c$^+$ DC purified from blood with the TLR4-ligand LPS, slanDC produce by far higher levels of the cytokines (IL-1ß, IL-6, IL-23) that are involved in the programming of IL-17 and IL-22 producing T cells. In line with this, slanDC by far excelled CD1c$^+$ DC in programming of IL-17, IL-22 and IFN-γ producing T cells as determined by ELISA and by intracellular cytokine staining. Instead, CD1c$^+$ DC efficiently programmed T cells to produce IL-10. Characterizing slanDC as a DC population highly effective in programming Th17/Th22 cells together with their identification in AD provides strong evidence for an important proinflammatory role for slanDC in AD.

022 [Oral 001]

Halting angiogenesis by non-viral somatic gene therapy alleviates psoriasis and murine psoriasiform skin lesions

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Dysregulated angiogenesis is a hallmark feature of psoriasis, a common skin disorder that affects 2% of the population. Induced expression of adhesion molecules, such as the integrins alpha5beta1 and alphaVbeta3, on sprouting blood vessels is crucial for angiogenesis and is a key to using these receptors as therapeutic targets. Besides targeting components of the VEGF signaling pathway, adamalysin protein family members, also referred to as ADAMs (a dis¬integrin and metalloproteinase), are potential players interfering with angiogenesis. ADAM-15 (metargidin, a metalloprotease-RGD-disintegrin) is the only adamalysin known to bind integrins alpha5beta1 and alphaVbeta3. Using xenotransplantation of human psoriasis in two distinct models as well as a murine psoriasis-like skin disorder in K5.TGFb1 transgenic mice as independent angiogenesis-related skin disorders, we demonstrate that anti-angiogenic non-viral somatic gene therapy reduces the cutaneous microvasculature and alleviates the inflammatory skin disorders. Transient muscular expression of the disintegrin domain (RDD) of metargidin (ADAM-15) by *in vivo* electroporation significantly reduced cutaneous angiogenesis and vascularization in all three models. As demonstrated by red fluorescent protein-coupled RDD, the treatment resulted in muscular expression and cutaneous deposition of the gene product. High-resolution ultrasound demonstrated reduced cutaneous blood flow *in vivo* following electroporation with RDD, but not control plasmids. In addition, angiogenesis- and inflammation-related molecular markers, keratinocyte proliferation, epidermal thickness as well as the clinical disease scores were significantly downregulated in all models. Thus, non-viral antiangiogenic gene therapy can indeed alleviate psoriasis and other angiogenesis-related inflammatory skin disorders.

023 [Oral 008]

The role of human microRNA-125b in psoriasis

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MicroRNAs (miRNA) are recently discovered regulators of gene expression, which play important roles in both physiological and pathological processes. In a genome-wide screen for miRNAs, we identified a specific miRNA expression profile in psoriasis, distinct from that of healthy skin. One of the miRNAs deregulated in psoriasis skin was microRNA-125b (miR-125b). Our studies presently focus on identifying the potential role(s) of miR-125b in psoriasis. MiR-125b is significantly down-regulated in psoriasis compared to healthy skin, as shown by quantitative real-time PCR. In situ hybridization data showed that the major cell type responsible for suppressed miR-125b levels in psoriasis lesions is keratinocytes. To explore the role of miR-125b on cellular functions we transfected miR-125b precursor miRNAs (to overexpress miR-125b) and miR-125b inhibitor oligonucleotides (to reduce its activity) into primary human keratinocytes and performed functional assays. Overexpression of miR-125b suppressed keratinocyte proliferation and induced the expression of several known differentiation markers such as involucrin, cytokeratin 10 and filaggrin. We identified keratinocyte growth factor receptor (KGFR/FGFR2) as a direct target for suppression by miR-125b, which was verified by luciferase reporter assays. Overexpression of miR-125b in keratinocytes suppressed the expression of KGFR at the protein, but not the mRNA level. Immunohistochemistry data showed that the expression of KGFR was up-regulated in psoriasis epidermis. Taken together, our results demonstrate a role for miR-125b in the regulation of keratinocyte proliferation and differentiation, partially through the regulation of KGFR. Loss of miR-125b in psoriasis skin lesions may contribute to the hyperproliferation and aberrant differentiation of keratinocytes.

024

Generalized alopecia patients have an abnormal retro-nuchal erythema which responds to freezing with cessation of hair loss and regrowth

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In a long term survey, about 90% of 270 alopecia universalis patients demonstrated a geographic, pink and symptom free erythema in the back of the neck which resolved during treatment for alopecia with medications such as systemic corticosteroids, Hydroxychloroquine and Diphencyprone and even spontaneous remission and recurred after recurrence of the alopecia. Less than 10% of normal individuals showed this newly defined sign. This difference was statistical highly significance ($p<0.003$). We concluded that this might be the etiology of alopecia in terms of site of antigen shedding and not an epiphenomenon.Twelve patients underwent skin biopsy at the site which showed mild partial atrophy of the epidermis, fine fibrosis of the dermis with a mild increase and ectasia of superficial vascular plexus associated with mild melanin incontinence in pigmented macrophages, orthokeratotic hyperkeratosis, irregular acanthosis, perivascular accumulation of lymphoid cells, individual apoptotic cells resembling civette bodies in addition to classic histology of alopecia. Thirty three patients at different stages of alopecia received single session of local freezing with Nitrous oxide at -79 degrees centigrade. Six patients did not responded completely and received another session 3-4 weeks later. All patients responded with a 60-100% reduction of surface area. Thirteen patients and 9 additional patients showed reduction of hair loss and regrowth of hair, respectively. Provided the very large number of our alopecia patients at different stages of disease and under different treatments this local physical modality shows much promise. A large immune histological study is underway.

025

Cerebrolysine improves angiogenesis in a local skin irradiated chronic wounds via IL-1β - and IL-8-mediated pathway

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Chronic wounds (CW) are having failed to progress through the normal healing stages and therefore enter a state of pathologic inflammation. The CW are mostly caused by ischemia. The aim of the present study was to discover the effect of cerebrolysine (aminoacid medicine with neurotrophic action) on the interleikin-8 (IL-8) -involved angiogenesis pathway, and on the interleikin-1β (IL-1β) level in a local skin irradiated CW model produced on the thigh of guinea pigs (GP). Animals were randomized to the following groups: intact, GP with radiated CW without treatment and animals with CW treated by paravulnar cerebrolysine administration (1 ml/kg 1 time a 2 days). The animals were sacrificed on 21st day after treatment beginning and wounded skin tissues were used for histological evaluation and for ELISA cytokines analysis and the blood for ELISA. Non-treated animals showed impaired wound healing with delayed angiogenesis: increased IL-1β and IL-8 levels in blood and tissue comparing with control without any morphological improving features. Cerebrolysine significantly decreased IL-1β and IL-8 levels: in blood to 70% and 38%, in tissue to 23% and 45%, respectively. Moreover, histologicaly epithelium had basal membrane, dermis – new collagen fibers, and microcirculatory system demonstrated high CD34 expression (new endothelial cells). The current study provides evidence that cerebrolysine improves angiogenesis in CW and promotes switching pathological inflammatory stage to proliferative. Thus, cerebrolysine action doesn't limited by neurotrophic function but extends to the dermatrophic, which is can be explained by the common origin of both nerve tissue and skin from embryonic ectoderm.

026

Inhibitory effect of vitamin C and sodium L-ascorbyl-2-phosphate on the production of inflammatory cytokines

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Anti-inflammatory effect of vitamin C and its derivatives against oxidative stress-induced inflammation has been reported. However, there are few reports on the effect of vitamin C and its derivatives for their inhibitory action against bacterial-stimulated inflammation. We investigated the anti-inflammatory effect of vitamin C and L-ascorbyl-2-phosphate (APS; a stable derivative of vitamin C) on bacterial peptidoglycan-induced production of inflammatory cytokines. Neonatal human keratinocytes (NHEK(F)) were cultured for a period of 48 hours. 80-90 percent-confluet cells were treated for 2 hours with vitamin C and APS. Peptidoglycan from Staphylococcus aureus was then added at a final concentration of 0.1 mg/mL. Twenty-four hours after the stimulation with peptidoglycan, the amount of each cytokine such as TNF-α, IL-1α, IL-8 in the culture supernatant fluid was measured by ELISA and compared. While treated with vitamin C and APS, and twenty-four hours of a peptidoglycan stimulation, the production of TNF-α, IL-1α, and IL-8 were significantly inhibited in the culture medium. The inhibitory effect was concentration-dependent of the sample in the range from 0.02mM to 2mM. This showed that vitamin C and APS have an in vitro anti-inflammatory effect. According to some papers, the antioxidant effect of vitamin C inhibits the activation of transcription factor, NF-κB. The anti-inflammatory effect discussed in this study may also arise from antioxidant effect.

027

Topical therapeutic effect of Korean red ginseng and its genuine constituents in an atopic dermatitis mouse model

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Ginseng (the root of Panax ginseng C.A. Meyer, family Araliaceae) possesses various biological activities, including anti-inflammatory and anti-tumor actions. Korean red ginseng saponin fraction (KRGS) and its constituents, ginsenosides Rg3, Rf and Rg2 have been shown to potently inhibit mouse passive cutaneous anaphylaxis reaction induced by IgE. They also have anti-allergic properties, proved by the potent inhibition of β-hexosaminidase release from RBL-2HE cells induced by IgE with antigen. However, their effects on atopic dermatitis (AD) have not been studied yet. In this study, we induced atopic dermatitis (AD)-like skin lesions in NC/Nga mice by repeatedly applying 2-choloro-1,3,5-trinitrobenzene (TNCB). We then examined whether topical application of KRGS and its ginsenosides Rh2 and Rg3 have active immune modulating effect in this AD animal model. The therapeutic effects of these agents were evaluated using clinical skin severity scores, histological changes in skin (epidermal thickness and mast cell infiltration), cytokine (IL-4, TNF-a, IFN-r) expressions in skin and total serum IgE. 0.1% KRGS, 0.1% Rh2 and 0.1% Rh2 +0.1% Rg3 all potently suppressed mouse ear swelling and also significantly reduced the mRNA expression of IL-4 and TNF-a compared with the TNCB control group ($p < 0.05$). Our results indicate that topical KRGS and its ginsenosides Rh2 and Rg3 have therapeutic potential as an effective and safe topical supplement for AD.

028

Differential Regulation of Antimicrobial Peptides in Contact Dermatitis

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Antimicrobial Peptides (AMP) are important effector molecules of the skin to protect the host from surrounding microorganisms. Their expression can be induced by various stimuli. Herein, we analysed the expression of AMP in contact dermatitis. Patients undergoing epicutaneous testing were included in this study (n=32). Contact dermatitis was induced by sensitization with ≥ one allergen in approximately 50% of these patients, all other patients served as controls. Skin washing fluids were obtained during contact eczema. Secreted AMP were measured by established ELISA in the washing fluids. In addition, colony-forming units (CFU) of bacteria were quantified by plating a standardized amount of washing fluid onto blood agar plates. The expression of AMP was further assessed by immunohistochemical staining in skin biopsies 72 hours after the contact eczema was induced. Psoriasin secretion was significantly increased 72 hours after the contact eczema was induced; however, 24 hour occlusion per se induced psoriasin expression at control sites. RNase 7 secretion did not increase after contact eczema was induced. Yet, RNase 7 secretion was decreased 24 hours under occlusive conditions. Immunohistochemical analyses revealed an up-regulation of psoriasin and hBD-3 expression at sites of contact eczema, whereas RNase 7 and hBD-2 were not induced. The number of bacteria increased significantly 24 hours under occlusive conditions and decreased to starting levels in contact eczema. In conclusion, psoriasin and hBD-3 in contrast to RNase 7 and hBD-2 are induced during contact dermatitis. Increased psoriasin expression might be caused by occlusion and not by allergic reaction.

029

Interferon alpha enhances IL-22 receptor expression on epidermal keratinocytes

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It has been noted that interferon alpha (IFN-α) often exacerbates psoriasis. Moreover, recent reports demonstrated that plasmacytoid dendritic cells, principal IFN-α-producing cells, infiltrate into the psoriatic lesions in the early phase. These findings indicate that IFN-α plays a pathogenetic role in the development of psoriasis. However, the role of IFN-α in psoriasis has not been fully elucidated. We recently reported that IL-22 receptor (IL-22R) expression is enhanced in the epidermis of psoriatic lesion. The increase of IL-22R enhances the function of IL-22, which is one of the most important cytokines in the pathogenesis of psoriasis. Therefore, we examined whether IFN-αmediates the expression of IL-22R. The IL-22R expression in epidermal keratinocytes was examined using living skin equivalent model (LSE). An LSE is constructed by stratified and air-lifted keratinocytes on collagen gel containing fibroblasts. Interestingly, IFN-α significantly enhanced IL-22R mRNA expression in the epidermal keratinocytes of LSE four hours after stimulation in a dose-dependent manner. The increase of IL-22R expression on cell membrane was confirmed by immunostaining and by flow cytometry analysis. Finally, STAT3 phosphorylation of epidermal keratinocytes was enhanced, when LSE pre-treated with IFN-α was stimulated by IL-22. These findings suggest that IFN-α participates in the development of psoriasis by enhancing epidermal keratinocyte responsiveness to IL-22 via increase of IL-22R expression.

030

Towards a better understanding of paraneoplastic pemphigus pathogenesis : identification of the p170 antigen recognized by PNP autoantibodies.

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Paraneoplastic pemphigus (PNP) is a devastating autoimmune blistering disease, involving mucocutaneous and internal organs, and associated with underlying neoplasms. PNP is characterized by the production of autoantibodies targeting proteins of the plakin and cadherin families involved in maintenance of cell architecture and tissue cohesion. Nevertheless, the identity of an antigen of Mr 170,000 (p170), thought to be critical in PNP pathogenesis, has remained unknown. Using an immunoprecipitation and mass spectrometry based approach, we identified a candidate protein (CP) as p170. We assess that p170 is CP, based on several lines of evidence : 1) the tryptic mass profile of the immunoprecipitated p170 has a significant match with that expected for CP; 2) p170 is recognized by anti-CP antibodies and can be immunoprecipitated from culture media of human keratinocytes, in the same manner as CP; 3) PNP sera immunoprecipitate recombinant CP from cell extracts, whereas binding to CP was never observed with sera obtained from normal volunteers as well as patients with autoimmune bullous diseases of the skin; 4) pre-incubation of p170-reactive PNP sera with recombinant CP selectively abrogated reactivity of the PNP sera with p170 by immunoblot or immunoprecipitation, as well as the labelling of the epidermal granular cell layers, where CP is predominantly expressed. Our study unravels CP, a non structural protein expressed in stratified epithelia and other tissues as a new class of target antigens in this paraneoplastic autoimmune multiorgan syndrome and opens a new challenging investigation avenue for a better understanding of PNP pathogenesis.

031

Time-dependent increase in growth associated protein (GAP)-43 immunoreactive nerve fibres in allergic contact dermatitis

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Our aim was to investigate the expression of the axonal growth marker, growth associated protein (GAP)-43, in skin biopsies from nickel-allergic patients (n= 10) obtained at different time points (0, 6, 24, 48, and 72 h) after challenge with nickel sulphate.The skin biopsies were fixed in a mixture of 4% formalin with 0.2% picric acid and rinsed in 0.1 M phosphate buffer containing 10% sucrose for at least 24 h, before being cut into 14 µm thick sections and stained using immunohistochemistry and a streptavidin-biotin technique. The total (epidermis+dermis) numbers of GAP-43 immunoreactive nerve fibres were found to be increased ($p<0.05$) in a time-dependent manner from 28 (median) fibres/section recorded at 0 h, to 43, 57, 64 and 89 fibres/section recorded at 6, 24, 48 and 72 h, respectively. Also when counting the immunoreactive nerves in individual layers of the skin, epidermal GAP-43 positive nerve fibres were noted to be higher ($p<0.01$) at 48 h (45 fibres/section) and 72 h (72 fibres/section), compared to 0 and 6 h (medians 14 and 23, respectively). At 24 h the median for epidermal GAP-43 labeled nerves was 34. Individual counting of the dermal GAP-43 immunoreactive nerve fibres also revealed a time dependent increase ($p<0.01$) in the number of these fibres particularly when comparing 0 h with 48 and 72 h. Our findings suggest the contribution of re-generating nerves to allergic contact dermatitis during the kinetics of inflammation.

032

Fli1 expression is epigenetically suppressed in dermal fibroblast and endothelium in systemic sclerosis

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Fli1 is a physiological negative regulator of collagen gene expression in dermal fibroblast. The reduced expression of Fli1 was reported in systemic sclerosis (SS) fibroblast. In the study of cultured fibroblast, gene promoter methylation of Fli1 was related to reduced expression of Fli1 and to overexpression of type 1 collagen. Herein we demonstrate that Fli1 promoter was specifically methylated in dermal fibroblast and endothelium in SS with in situ study. Fli1 protein expression was reduced in dermal fibroblast and endothelium in SS disclosed with anti-Fli1 antibody stain. The reduction was more pronounced in sclerotic SS than in atrophic SS. mRNA in situ hybridization revealed reduced transcription of Fli1 in those cells, but not in other cell types, e.g. adipocyte, arector pili myocyte. When RT-PCR for Fli1 was performed on whole tissue extract, no difference was observed among sclerotic and atrophic stage SS, and control. Methylation specific PCR on bisulfite-treated DNA extracted from whole tissue revealed that Fli1 promoter was partly methylated. In order to disclose which cell's Fli1 promoter was specifically methylated, we performed methylation detection with in situ hybridization (MDISH). MDISH revealed that Fli1 promoter was specifically methylated in dermal fibroblast and endothelium, but not in other cell types, e.g. adipocyte, arector pili myocyte. The mechanism of the cell-type specific DNA methylation remains to be elucidated.

033

Mouse model with bi-phasic skin-reaction induced by trimellitic anhydride closely reflects atopic dermatitis and the profile of some compounds which attenuated the late phase reaction

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Atopic dermatitis is a common, chronic, relapsing inflammatory skin disease frequently affecting infants and children. Due to the steadily increasing incidence, especially in children, there is a high medical need for new therapies as well as improved animal models. In this study, we developed a new mouse model by repeated treatment with Trimellitic anhydride, and investigated the suitability as an atopic dermatitis model for the prediction of the treatment efficacy. We studied the profile of the model and demonstrated that it showed bi-phasic skin reaction with marked infiltration of inflammatory cells and histological changes of the inflamed skin. The late-phase reaction observed 8-hours after the evocation was mediated by T cells as well as mast cells. We also tested the effect of various anti-allergic agents and found that only prednisolone suppressed the late-phase reaction in contrast the immediate-phase was suppressed by anti-histamines. These findings gathering with the results of cytokine profile and increased serum IgE levels indicated that our model closely reflects disease characteristics. Besides the characterization of the models, we also tested some newly synthesized compounds on this model and found one that showed strong inhibition of late-phase reaction even treated after evocation. In summary, we present an improved mouse model for atopic dermatitis suggesting possession of the predictive power for therapeutic treatment.

034

Tape-stripping -induced psoriasis is associated with mast cell IL-6 and appearance of dermal cells showing IL-33 and IL-6 receptor

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Degranulation of mast cells is among the earliest changes during the development of psoriatic lesion, and activated mast cells can release a wide range of proinflammatory cytokines, e.g. IL-6 and TNF-α. To study expression of IL-6 in mast cells and changes in cells positive for IL-6 receptor (IL-6R, CD126) and IL-33 (a known inducer of IL-6 in mast cells), the nonlesional skin of 18 psoriatic patients was tape-stripped, the Köbner reaction was induced in 8 patients as judged at the 2-3 week follow-up, and skin biopsies were collected before the tape-stripping and at time points 2h and 3d or at 1d and 7d for immunohistochemical and double-staining analyses. The PASI score and total mast cell numbers did not differ between Köbner-positive and -negative groups. In contrast, the percentage of mast cells showing IL-6 was about 1.5-fold higher in all time-point biopsies in the Köbner-positive than in the Köbner-negative subjects, even in the untreated skin. The total number of IL-6R$^+$ cells increased in the upper dermis in the Köbner-positive group at 3-7 days. However, the cells increased even more in the Köbner-negative group, a change possibly related to Foxp3$^+$ cells. Similarly, dermal IL-33$^+$ cells increased in number in the Köbner-positive group at 3-7 days, but did not so in the Köbner-negative group. In summary, mast cell IL-6 is associated with the lesion induction already in the untreated nonlesional skin and thereafter both IL-6R$^+$ and IL-33$^+$ cells increase at 3-7 days. This suggests a new "vicious circle" in the early psoriatic pathogenesis.

035

Familial Japanese fever (Nakajo-Nishimura syndrome): a novel autoinflammatory syndrome with periodic fever, skin eruptions and partial lipodystrophy

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There is a distinct disease reported first by Nakajo in 1939 and second by Nishimura et al in 1950, both written in Japanese, and further by Kitano et al in 1975 as "a syndrome with nodular erythema, elongated and thickened fingers, and emaciation" in the Archives of Dermatology. Only about 20 cases have been reported exclusively in Japan, however, the disease is registered in databases for rare inheritable diseases as Nakajo syndrome (MIM256040) or Nakajo-Nishimura syndrome (ORPHA2615). This disease seems to be autosomal-recessively inherited, because of frequent parental consanguinity and appearance in sibships. It begins with pernio-like eruptions in early-infancy getting worse in winter. Some patients periodically develop high fever, nodular erythemas and myositis. Lipomusculoatrophy, without significant power loss, and joint contracture gradually progress mainly in upper part of the body forming the characteristic thin facial appearance and elongated clubbed fingers. Inflammatory changes, such as high serum CRP and hyper-g-globulinemia are observed without appearance of distinct autoantibodies. Skin biopsy shows infiltration of inflammatory cells in whole dermis and subcutaneous tissue with vasculopathy. Calcification of basal ganglia is revealed by head CT scanning. Notably, most cases were concentrated in Kansai and Tohoku areas of Japan, including Wakayama prefecture. Here we report the summary of 12 cases experienced in our Department. Among them, 9 cases were accompanied with periodic fever. Based on our observations, we propose to designate the disease as "familial Japanese fever", in contrast with familial Mediterranean fever, a distinct entity of hereditary autoinflammatory syndrome discovered in Japan.

036

Autoantibodies from patients with bullous pemphigoid cross-react with murine collagen XVII/BP180, but do not activate murine complement and granulocytes

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Bullous pemphigoid (BP) is an autoimmune disease characterized by the production of autoantibodies against two hemidesmosomal proteins BP230 and BP180. A major limitation for reproducing the disease by transferring patients' autoantibodies into mice has been attributed to a lack of pathogenic autoantibody cross-reactivity with murine BP180. Thus, several animal models were developed using the passive transfer of antibodies generated against the murine autoantigens or by the use of patients' autoantibodies in mice expressing human BP180. However, these models do not reproduce all main features of the human disease. Interestingly, in silico mapping of B cell epitopes shows partly overlapping antigenic determinants in the murine compared with the human antigen. Therefore, in this study we analyzed the cross-reactivity of BP patients' autoantibodies with the murine skin. We show, that IgG autoantibodies from 17 of 25 BP sera (68%) and, importantly, BP autoantibodies purified against the immunodominat domain of BP180 stained the murine skin by indirect immunofluorescence microscopy. When injected into neonatal mice (n=6), BP autoantibodies bound to the epidermal basement membrane, but failed to activate murine complement and to induce subepidermal blisters. These findings were confirmed *ex vivo* by a lower ability of BP autoantibodies to fix murine compared with human complement and a reduced ability to activate murine granulocytes when compared with human cells. In addition to providing essential mechanistic insights into the experimental models of pemphigoids, these findings have broad implications for pathogenetic studies and for the development of more specific therapies of autoimmune diseases.

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Different expression patterns of podoplanin in psoriasis, atopic dermatitis and lichen ruber

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The 38 kDa glycoprotein podoplanin is expressed in lymphatic endothelial cells and keratinocytes. Our previous studies showed that podoplanin deficiency results in congenital lymphedema. However, its biological function has remained mainly unknown. In the present study we investigated the podoplanin expression pattern in inflammatory skin diseases such as psoriasis, atopic dermatitis and lichen ruber for deduction of possible regulatory mechanisms podoplanin is involved in. Thirty tissue samples of patients suffering from psoriasis vulgaris, atopic dermatitis and lichen ruber were collected. Non affected skin of safety margins after excision of malignant tumors was used as a control. The mouse monoclonal anti-human D2-40 and the rat monoclonal Ki67 antibodies were used for immunohistochemical stainings of paraffin embedded tissue sections. A positive immunoreaction product for podoplanin was found in 53% (16/30) of the psoriasis patients in the basal keratinocytes along the basal membrane. 77% (23/30) tissue samples showed podoplanin expression in the suprapapillary plates, whereas this staining pattern was not found in the control skin. Podoplanin positive lymphatic endothelial cells highlighted elongated and dilated lymphatic capillaries in the dermal papillae of all tissue samples from psoriasis patients and all patients suffering from atopic dermatitis. However, elongated lymphatics in sections showing lichen ruber were restricted to areas occupied by an inflammatory infiltrate of lymphocytes. Coexpression of podoplanin and Ki67 was only seen in a few basal keratinocytes in all stained tissue sections. In conclusion, we found different podoplanin expression patterns in inflammatory skin diseases suggesting different immunological regulatory mechanisms podoplanin is involved in.

038

FcgammaRIV Promotes, while FcgammaRIIB Protects from Autoantibody-induced Tissue Damage in Autoimmunity to Type VII Collagen

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Epidermolysis bullosa acquisita (EBA) is an autoimmune bullous disease mediated by autoantibodies against type VII collagen, the major component of anchoring fibrils of the dermal-epidermal junction. When passively transferred into mice, rabbit IgG against type VII collagen induces Fc-dependent activation of complement, the recruitment of leucocytes into the skin, and subepidermal blistering. Since co-expression of activating and inhibitory Fc gamma receptors (FcgammaRs) is believed to represent an immunoregulatory checkpoint by establishing a threshold for immune cell activation, we determined the role of different FcgammaRs in experimental EBA using knock out mice or function blocking antibodies. Mice lacking the common gamma chain of activating FcgammaRs were completely resistant to experimental EBA induction. Regarding the contribution of the 3 known activating FcgammaRs, inhibition of FcgammaRIV led to an almost complete protection from EBA induction, while FcgammaRI and FcgammaRIII deficiency had no or little effect on disease, respectively. In contrast, deficiency of inhibitory FcgammaRIIB led to an over 2-fold enhanced disease severity. We also observed that induction of EBA altered the ratio of expression of inhibitory and activating FcgammaRs towards the activating FcgammaRIV. Our observations suggest targeting of FcgammaRIV and FcgammaRIIB as potential therapeutic options in EBA.

039

The sebocyte-own corticotropin-releasing hormone system is an amplifier of inflammation

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Activation of the hypothalamic-pituitary-adrenal axis, which is the main adaptive response to chronic systemic stress, requires production of corticotropin-releasing hormone (CRH). CRH has also been detected in peripheral tissues, including the skin. Human sebaceous glands and cultured human SZ95 sebocytes express CRH, CRH-binding protein (CRH-BP) and CRH receptors (CRH-R) 1 and 2 at the mRNA and protein levels as we have shown by RT-PCR and immunohisto- and immunocytochemistry. CRH and urocortin, a peptide which shares a 45% homology with CRH, inhibit SZ95 sebocyte proliferation, which can be restored by α-helical-CRF, a CRH antagonist. CRH upregulates 3β-hydroxysteroid dehydrogenase/Δ^{5-4} isomerase mRNA levels and testosterone accordingly downregulates CRH-R1 and CRH-R2 mRNA expression in a negative feedback manner. Moreover, CRH enhances the basal synthesis, while antalarmin, a non-peptidic selective CRH-R1 antagonist, reduces the increased production of neutral lipids in SZ95 sebocytes. CRH also stimulates interleukin (IL)-6 and IL-8 release from SZ95 sebocytes but has no effect on IL-1α and IL-1β production or the IL1β-induced IL-8 release in these cells. Inflammation, such as acne, and proinflammatory signalling, such as UVB or the bacterial antigen MALP-2 amplify intracellular CRH levels, while dexamethasone inhibits the intracellular CRH synthesis in SZ95 sebocytes. No CRH release could be detected; CRH-BP expression was amplified in differentiating sebocytes of acne-involved skin. In conclusion, inflammatory signals can enhance intracellular CRH levels in human sebocytes leading to CRH-R1-associated lipogenesis and amplification of inflammation by secretion of proinflammatory cytokines. These events are sensitive to dexamethasone or specific CRH-R1 antagonists.

040

Enhanced NF-κB activation with NLRP3 activator correlates with disease activities in cryopyrin-associated periodic syndrome

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Cryopyrin-associated periodic syndrome (CAPS) is known as an autoinflammatory disease associated with mutations in NLRP3 and clinically characterized by urticarial rash. More than 50 mutations have been identified and most of them locate in exon 3. Those mutants induce spontaneous ASC-dependent NF-κB activation, that was associated with disease activities of the patients: e.g. an R260W mutant of NLRP3, which was identified in milder subtype of CAPS, showed lower NF-κB activation potential , whereas Y570C identified in the most severe cases with brain atrophy and episodes of epilepsy showed the strongest NF-κB activity. However, G755R located in exon 4 does not show spontaneous NF-κB activation, even though her clinical symptom is quite severe. To explore the discrepancy in a G755R mutant, 3 reported mutants outside exon 3 (G755R, G755A and Y859C) were cloned. G755A was identified in a typical CAPS patient. A patient with Y859C had only 1 episode of a transient rash and was noteworthy for the absence of fever. In the presence of R837, an NLRP3-inflammasome activator, a G755R mutant showed remarkably enhanced NF-κB activation comparable with Y570C, which level was higher than that of R260W. For 2 other mutations located outside exon 3, G755A showed slightly increased NF-κB activation with R837, but not Y859C. Thus, the enhanced NF-κB activation after stimulation with R837 correlates with disease activities, including mutations outside exon 3. We believe that, in addition to an excess production of IL-1β, enhanced NF-κB activation may contribute to disease-onset and the clinical manifestations of CAPS.

041

Impaired hapten sensitization in patients with autoimmune disease. Results from an experimental sensitization study.

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An inverse relation between contact allergy and autoimmune diseases has been suggested from epidemiological studies. The relation has not been investigated experimentally and the immunological mechanisms are unknown. We demonstrate in a controlled experimental sensitization study impaired senisitization to a strong hapten Diphenylcyclopropenone among 23 patients with psoriasis and 22 patients with diabetes type I compared to 23 healthy controls. Sensitization ratios were significantly lower in the psoriatic and diabetic groups 26 % and 36 % respectively compared to the healthy group 65%. Odds ratio for a patient with psoriasis or diabetes type I to become sensitized was 0.18 (95% CI 0.039 - 0.85) and 0.74 (95% CI 0.55 - 1.01) respectively. To investigate the possibility of active immunological down-regulation in the elicitation phase, mRNA was extracted from skin biopsies from challenge sites and gene expression profiles analysed with microarray technology. No difference in mRNA expression profiles were found between the groups, when comparing responders and non-responders individually. Furthermore no specific mRNA expression was found in the challenged skin of non-responders, indicating that non-respondence was not due to an active down-regulation. Collectively the results demonstrate impaired hapten sensitization among patients with two different autoimmune diseases and suggest that the immunological background for this is not to be found in the elicitation but more likely in the induction phase. The highly Th17 directed cytokine milieu of autoimmune patients may interfere with the differentiation of naïve T cells necessary for the contact allergic reaction; however, further studies are needed to clarify this.

042

Endocytosis of desmoglein 1, plakoglobin and IgG in skin of pemphigus foliaceous patients

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Pemphigus foliaceous (PF) is one of the two main forms of pemphigus and is characterized by circulating IgG to the desmosomal cadherin desmoglein (Dsg) 1 and subcorneal blistering of the skin. Previously we have shown that PF IgG induces aggregation of Dsg1, plakoglobin (PG) and IgG outside of desmosomes, visualized in immunofluorescence of PF patient skin as a granular IgG deposition pattern with few coarse IgG aggregates per cell. Older immunoelectron microscopic studies have suggested that in PF skin endocytosis of desmosomes takes place. The purpose of the present study was to verify this endocytosis and, if present, to investigate its relation with the IgG aggregates. We performed double immunofluorescence staining on 10 skin biopsies of six PF patients for IgG, Dsg1 and 3, desmocollin 1 and 3, PG, desmoplakin, plakophilin 3, and early endosomal marker 1 (EEA1). EEA1 did not colocalize with the IgG aggregates in the basal and suprabasal layers but did contact them in the upper spinous layers. In these same keratinocytes, endosomes were present in the cytoplasm and contained IgG, Dsg1 and PG but not other cadherins or plaque components. Endocytosis was present in lesional skin but not in non-lesional skin, situating it at a later stage in the pathogenesis. Our results confirm that endocytosis of IgG bound components occurs in PF patient skin. Based on the molecular composition of the endosomal cargo we conclude that these endosomes do not take up complete desmosomes but instead clear the IgG/Dsg1/PG aggregates from the cell membrane.

043

The involvement of miR-203 in NF-κB signalling

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MicroRNAs are small, non-coding RNAs that regulate gene expression post-transcriptionally and play important roles in various biological processes. We previously identified miR-203 as a skin- and keratinocyte-specific microRNA that regulates keratinocyte differentiation. NF-κB plays a central role in skin morphogenesis and homeostasis. Perturbation in its activity has been linked to developmental skin defects, inflammatory skin diseases and skin cancer. Using Human Signal Transduction Pathway Finder PCR Array, we found that overexpression of miR-203 in primary keratinocytes by transfection of a synthetic miR-203 precursor regulated several genes in the NF-κB pathway. Quantitative PCR analysis of NF-κB downstream genes in keratinocytes overexpressing miR-203 showed that IL-8, CCL-20 and IL-1α were suppressed by miR-203. In order to explore whether miR-203 modulates NF-κB activation also in stimulated cells, we overexpressed miR-203 in primary keratinocytes followed by treatment with TNF-α and TPA, well-known activators of the NF-κB pathway in keratinocytes. IL-8, CCL-20 and IL-1α were induced in stimulated keratinocytes and this induction was significantly suppressed by miR-203. To further investigate the role of miR-203 in regulating NF-κB signalling, we used bioinformatic tools to predict the potential target genes of miR-203 involved in NF-κB signalling pathways. We identified IL-8 as one of potential direct targets for post-transcriptional suppression by miR-203. In keratinocytes overexpressing miR-203, IL-8 protein production was significantly suppressed in culture supernatants, as determined by ELISA. Taken together, miR-203 suppresses several genes involved in NF-κB signalling, suggesting a potential role for miR-203 in keeping skin homeostasis and controlling inflammation.

044

The protein kinase C inhibitor sotrastaurin inhibits the chemokine CCL20 in primary human fibroblasts in vitro

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Sotrastaurin is a potent and selective inhibitor of the protein kinase C (PKC) isoforms θ, α and β, with lesser activity on PKCδ, ε and η. It has shown a clinical effect and histological improvement of skin lesions in a 2-week study in psoriasis patients. In addition to inhibiting T cell proliferation and cytokine release by T cells, sotrastaurin also has an effect on keratinocytes and macrophages in vitro. In the present study, we investigated the effect of sotrastaurin on cytokine and chemokine production, as well as proliferation, in primary human dermal fibroblasts (HDF). Cells were stimulated in microtiter plates in the presence or absence of graded compound concentrations with different proinflammatory stimuli alone or in combination (TNF-α, IFN-γ, IL-17, IL-1ß). After 24 hours, IL-6, IL-8, CCL2, CCL5, CCL20 and CXCL10 were measured in the supernatants by ELISA. In unstimulated cells treated with sotrastaurin, proliferation was determined after 72 hours by staining of cellular protein. Regardless of the stimulation conditions, sotrastaurin potently inhibited CCL20 secretion at clinically achievable skin concentrations (IC_{50} 0.1 - 0.5μM). CCL5 was inhibited only at micromolar concentrations, while IL-6, IL-8, CCL2 and CXCL10 were unaffected by sotrastaurin. Concentrations up to 10μM were not cytotoxic to HDF. The present study indicates that sotrastaurin, in addition to its immune modulatory properties has very selective effects on cytokine production in fibroblasts, suppressing the chemokine CCL20, which may thus inhibit recruitment of T cells to lesional psoriatic skin.

045

GED507L a new PPARγ agonist inhibits the inflammatory response in human skin and PBMCs: perspectives for the treatment of psoriasis

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Activation of peroxisome proliferator-activated receptors (PPARs) by corresponding ligands has been shown to regulate important functions in skin including proliferation and differentiation, as well as inflammatory responses. PPARγ is the PPARs members that antagonizes inflammatory signalling by interference with the NF-kB activation. Agonizing PPARγ pathway with pharmacological ligands has shown to be beneficial in the amelioration of symptoms in psoriasis. However, some studies have reported their notable cytotoxicity limiting their application. A novel class of PPARγ agonist has been designed to increase specificity of the ligand-receptor interaction associated with a low cytotoxicity. We investigated the effects of GED507L (0,01-0,5 mM) exerted on inflammatory response in: i) TNF-α-stimulated normal human primary keratinocytes (NHKs); ii) LPS-stimulated peripheral blood mononuclear cells (PBMCs) from psoriatic patients and healthy controls; iii) biopsies collected from plaque type psoriatic subjects. Our results demonstrated that GED507L significantly suppressed the expression of TNF-α, IL-6 and IL-8 induced by TNF-α at mRNA and protein levels in NHKs. GED507L influenced the NF-kB pathway by decreasing TNF-α-mediated cytoplasmic degradation of IkBα and by reducing nuclear accumulation of p65 subunit through PPARγ activation. The molecule inhibited the induction of the inflammatory keratin K6 up-regulated in psoriatic skin. In adddition we found a modulatory effect of GED507L on the release of TNF α, IL-12, IL-21 and IL-23 in skin biopsies and in LPS-stimulated PBMCs Our data demonstrated a strong anti-inflammatory activity of GED507L and suggested its possible role in the treatment of psoriasis.

046

The role of a novel PPARg agonist (GMG-43AC) in the control of sebogenesis and inflammatory processes: prospective for an innovating treatment of acne

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Increased sebum production and inflammation are accounted amongst the major factors involved in the pathogenesis of acne. A growing body of evidence indicates that peroxisome proliferators-activated receptor gamma (PPARg), one of the three PPARs isoforms, offers a promising target for the treatment of acne. PPARg controls lipogenesis in sebaceous gland cells and exerts anti-inflammatory activities in various cell types by inhibiting the expression of pro-inflammatory mediators, such as cytokines. We have investigated the capacity of a newly designed PPARg agonist (GMG-43AC) to exert anti-inflammatory and sebum regulating effects. To this end human primary keratinocytes (NHKs) were challenged with oxidative and biological stimuli such as hydrogen peroxide (H_2O_2) and LPS to induce pro-inflammatory cytokines expression. GMG-43AC inhibited significantly and dose-dependently the induction of TNFa and IL-6 at mRNA and protein levels in the non toxic interval of concentrations 10-500 μM. GMG-43AC at the same concentrations induced PPARg mRNA expression. The modulation of lipid synthesis by GMG-43AC was evaluated in SZ95 stimulated with the combination linoleic acid/testosterone. In SZ95 the increased PPARg mRNA expression by 1 mM GMG-43AC was associated with a significant reduction of the sebaceous lipids synthesis, as demonstrated with the assay of Nile Red incorporated into neutral lipids and by gas chromatography-mass spectrometry analysis. These results highlighted the ability of GMG-43AC to counteract inflammation and induced sebogenesis making it a potential new drug in the therapy of acne.

047

Myeloid cells, and not T cells, are the main source of TNF-a in plaque-type psoriasis

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The spectrum of tumor necrosis factor (TNF)-a-producing cells is not clearly defined in psoriasis. The elucidation of this question should allow us to better understand the mode of action, efficacy and, perhaps, also the risks of an anti-psoriatic therapy with TNF-a-antagonists. Using conventional immunofluorescence methods, we were not able to detect TNF-a in sections of lesional psoriatic skin, but by the application of a tyramide amplification system we obtained reproducible and firm stainings.TNF-a was exclusively found on dermal leukocytes coexpressing CD11c and HLA-DR and, to a lesser extent, CD163. This marker profile is consistent with that of mDCs and macrophages. Consistently, we found corresponding populations of TNF-a-producing mDCs and monocytes in unstimulated PBMCs of psoriatic patients. More importantly, their number closely correlated with disease activity. These myeloid PBMCs expressed CCR2, whose ligands CCL2, CCL7 and CCL11 were strongly upregulated in lesional psoriatic skin. In healthy persons, anti-TNF-a-stainings of skin and blood yielded essentially negative results. In vitro, we confirmed that TNF-a-antagonists are able to induce apoptosis in, as well as complement killing and antibody-dependent cellular cytotoxicity of TNF-a producing cell lines. *In vivo*, infliximab therapy reduced the number of TNF-a-producing cells in the peripheral blood of psoriatic patients 24 hours after administration.Our data strongly suggest that myeloid cells (dendritic cells, monocytes/macrophages) are the main source of TNF-a in stable plaque-type psoriasis. This highlights the importance of these cells in disease pathogenesis.

048

ROS-induced Activating Transcription Factor 3 (ATF3) Generates Solid Protection against Bacterial Endotoxins but Causes Severe, Interleukin 6-dependent Susceptibility to Bacterial Infections

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Systemic bacterial infections such as cellulitis generate severe oxidative stress and may result in severe shock. Fever and toxic symptoms result from TLR-signals like lipopolisacharide (LPS) that cause an inflammatory cytokine release syndrome. As activation of the negative transcription factor ATF3 provides protection against innate immune activation by LPS and LPS-induced death. ATF3 initiates a negative feedback loop in the NFkB signal pathway. Here, we found that oxidative stress, as it occurs during sepsis, strongly enhances ATF3 expression and production by LPS-triggered dendritic cells or peritoneal macrophages. *In vivo*, reactive oxygen species (ROS) stress resulting from glutathione depletion enhanced LPS-induced ATF3 4-fold, inhibited IL-6 mRNA and protein production >90% *in vivo*, and significantly decreased the risk of LPS-induced lethality. This protection was fully reversed by the ROS scavenger N-acetyl-cysteine and strictly dependent on ATF3-induction, as ROS stress affected neither cytokine production nor survival in ATF3-/- mice. We speculated that the increased awareness of ATF3-/- mice to innate signals such as LPS established solid protection against systemic infection: >90% of ATF3-/- mice survived conditions of bacterial infection after cecal perforation that were lethal in 100% of wild type (wt) mice. Depletion of gluthatione further increased susceptibility of wt mice to bacteriemia, whereas ATF3-/- remained unaffected. In contrast, ATF3-/-xIL-6-/- double knock out mice died as rapidly as wt mice directly showing that ATF3-mediated suppression of IL-6 caused susceptibility to bacteriemia. These insights are essential for the management of bacterial and fungal infections, especially in the ever increasing community of immune compromised patients.

049

The Activating Transcription Factor 3 (ATF3) critically determines the post-septic immune suppression (CARS) in humans

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Following systemic infections or trauma, patients are at high risk of severe secondary infections of bacterial, fungal or viral origin. This post-septic immune paralysis, termed CARS, is currently most relevant for sepsis-associated lethality. Yet, the molecular mechanisms underlying this phenomenon are elusive. ATF3 is the first transcription factor in the NF-kB signalling pathway induced after innate immune stimulation. Thereby, ATF3 negatively regulates the transcription of IL-6 and TNF. Analysing the role of ATF3 during CARS in humans, we first show a significant and close correlation of severely suppressed glutathione-levels with the strong induction of ATF3 and the loss of activation induced IL-6. We therefore speculated that ATF3 might be a key transcription factor responsible for the postseptic immune suppression and the increased susceptibility to opportunistic infections. To test this hypothesis we used CLP (cecal ligation and puncture), one of the best-established models of bacterial sepsis. We first induced sublethal CLP in wild type (wt) and ATF3-/- mice, to closely imitate the clinical conditions in mice. Subsequently, we challenged mice during the postseptic CARS phase with the fungal pathogen Aspergillus fumigatus, at doses that are non-pathogenic to healthy mice. Post-septic wt-mice rapidly succumbed to this sub-lethal pulmonary Aspergillus fumigatus infection. In sharp contrast, ATF3-/- mice had not only a significantly prolonged survival, 20% of these mice even survived this infection that was lethal in 100% of wt mice. Thus, ATF3 is a critical regulator of postseptic immune suppression, that determines susceptibility to and the course of opportunistic infections.

050

Anti-desmocollin autoantibodies in pemphigus herpetiformis and pemphigus vegetans: IgG antibodies to desmocollins 1-3 are the key factor for their characteristic phenotypes

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Recent progress in molecular biology has revealed that IgG autoantibodies in classical pemphigus react with desmoglein 1 (Dsg1) and/or Dsg3. Pemphigus herpetiformis and pemphigus vegetans are considered to be variants of pemphigus foliaceus and pemphigus vulgaris, respectively. Although some pemphigus herpetiformis and pemphigus vegetans cases have been reported to react with desmocollins (Dsc), the pathogenic role of anti-Dsc autoantibodies in both diseases is at present unknown. In this study, to further investigate IgG reactivity with Dsc in pemphigus herpetiformis and pemphigus vegetans, we examined immunological profiles by immunoblotting using epidermal extracts and cDNA-transfection method and ELISA for Dsc1-3. We collected 15 patients each of pemphigus herpetiformis and pemphigus vegetans, whose diagnoses were made by clinical, histological and immunological findings. By cDNA-transfection method, 13 cases of pemphigus herpetiformis and 10 cases of pemphigus vegetans showed IgG reactivity to Dsc1-3 in various patterns, while 20 classical pemphigus sera and 10 normal sera were negative. ELISA for Dsc1-3 confirmed the results in cDNA-transfection method. A few sera also reacted with Dsc by immunoblotting. These results indicate that Dsc1-3 are autoantigens specific for pemphigus herpetiformis and pemphigus vegetans, but not for classical pemphigus. Some sera did not react with Dsg, further suggesting a significant role of anti-Dsc antibodies in the pathogenecity of pemphigus herpetiformis and pemphigus vegetans. Anti-Dsc autoantibodies may reduce Dsc expression in keratinocytes, which results in characteristic phenotypes of these diseases. Furthermore, our studies demonstrated that cDNA-transfection method and ELISA are useful tools to detect anti-Dsc autoantibodies.

051

The expression and the role of thrombospondin-2 in systemic sclerosis

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Systemic sclerosis (SSc) is an acquired disorder which typically results in fibrosis of the skin and internal organs. Although the pathogenesis of this disease is still unclear, it includes inflammation, autoimmune abnormality, and vascular damage, leading to activation of fibroblasts and disturbed interactions with different components of the extracellular matrix, mainly collagen. Despite recent advances in understanding the regulation of collagen gene expression, the mechanisms responsible for the pathologic increase in the expression of collagen genes in SSc have not been elucidated. The thrombospondins (TSPs) are a family of multifunctional extracellular glycoprotein. The family consists of 5 subtypes and is classified into 2 subgroups: TSP-1 and TSP-2 are homotrimers consisting of three identical subunits, while TSP-3, TSP-4 and -5 (so-called cartilage oligomeric protein) forms homopentamers. TSP-1 has been reported to be overexpressed and contribute to autocrine transforming growth factor-β (TGF-β) signaling in cultured fibroblasts derived from SSc. Also, in a previous report, TSP-5 was shown to be increased in SSc fibroblasts. In this study, we found that TSP-2 was also expressed in cultured human dermal fibroblasts. Additionally, unlike TSP-1 and TSP-5, TSP-2 production was decreased in SSc fibroblasts both *in vivo* and in vitro. Accordingly, we tried to clarify the mechanisms of down-regulated TSP-2 in SSc fibroblasts, and discussed the role of TSP-2 in the pathogenesis of SSc.

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High Prevalence of HLA-Cw6*0602 irrespective of Phenotype in Childhood Psoriasis

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The heterogeneity of psoriasis is becoming increasingly evident and the strategy to stratify for subgroups such as childhood psoriasis is likely to prove rewarding in understanding the full spectrum of the disease. HLA-Cw*0602 is the candidate gene with the strongest genetic association to psoriasis but its mechanism of action is still unknown. We have examined and genotyped 246 children with onset of psoriasis before 15 years of age for HLA-Cw*0602. The phenotypic distribution in the group (18% guttate vs. 82% plaque) was surprisingly similar to the phenotypic distribution in our earlier examined patients with adult onset of psoriasis (22% guttate vs. 78% plaque). This phenotypic distribution, with plaque psoriasis as the main phenotype, is in accordance with earlier reported clinical studies of childhood psoriasis. Genotyping was performed by using the allele specific PCR phototyping technique described by Bunce et al., 1995. Irrespective of phenotype, we found significantly higher representation of HLA-Cw*06 in this paediatric cohort compared to patients with adult onset, 69% vs. 39%. In concordance with what has been reported the background HLA-Cw*0602 prevalence in matched healthy controls was 10%. This material shows that high prevalence of HLA-Cw*0602 in childhood psoriasis is primarily attributed to the early onset of disease and not by the guttate phenotype.

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Priming of adaptive immune responses by different subsets of skin dendritic cells

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Epicutaneous sensitisation in atopic dermatitis (AD) is thought to cause Th2 biased responses, possibly due to enhanced allergen penetration or an AD-specific microenvironment in the skin. We sought to investigate the effect of cytokine milieu during priming on the polarisation of adaptive immune responses and the capacity of different human cutaneous dendritic cell (DC) subsets to prime naïve T-lymphocytes. We were able to model priming of autologous T cells to the chemical contact sensitiser dinitrochlorobenzene (DNCB) comparing human epidermal (LCs) and dermal DCs (DDCs) and MoDCs in different in vitro conditions. Using flow-cytometry and Elispot assays we assayed the proliferation, phenotype and function of primed T-lymphocytes. The DNCB-specific responses peaked at day 17 post priming. Both LCs and DDCs potently activated T lymphocytes and induced proliferation and expression of skin homing markers. Although we found that both LCs and DDCs were able to induce expansion of antigen-specific IFN-γ producing cells and that a Th2 cytokine environment skewed T-cell priming outcomes towards Th2 profiles, LCs were significantly better inducers of Th2 and Th17 polarised cells ($p<0.01$). Despite the greater ability of LCs to polarize T lymphocytes towards Th2, DCs from both epidermal and dermal compartments sensitize to DNCB with a dominant Tc1/Th1 outcome. Thus the site of residence and also microenvironment of the priming cutaneous DC regulates the adaptive immune response outcome suggesting that differences in antigen penetration and tissue milieu in AD may both contribute to Th2 skewing.

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Heparin serves as a natural stimulant of inflammasome and exacerbates the symptoms of TRAPS

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Tumour necrosis factor receptor (TNFR)-associated periodic syndrome (TRAPS) is a rare autosomal dominant disorder characterized by recurrent episodes of fever, myalgia, abdominal pain, conjunctivitis and skin eruptions with mutations in the TNFRSF1A (TNFR superfamily 1A) gene. We saw a 16-year-old woman with a one-year history of wheal and episodic fever. Genetic evaluation revealed a missense mutation (p.T61I) in TNFRSF1A gene, leading to the diagnosis of TRAPS. Her mother and grandfather had the similar symptoms and the same gene mutation. On admission, she had an episode of shock and dyspnea when injected with even a small amount of heparin for venous lock. This urged us to investigate whether heparin is one of the stimulants for inflammasome. Peripheral blood mononuclear cells (PBMCs) from the patient and normal subjects were incubated with heparin at 1 unit/ml in the absence of LPS, and IL-1β concentration in the supernatants was measured. Heparin stimulated the patient's PBMCs, but not normal PBMCs, to produce IL-1β. ATP, instead of heparin, also induced the production of IL-1b by the patient's PBMCs, indicating that the abnormal TNFR signaling in the patient's PBMCs can replace LPS stimulation. To further examine the ability of heparin, THP-1 cells were cultured with heparin and/or LPS. In the presence of LPS, heparin enhanced IL-1β production in a dose dependent manner up to 1 unit/ml, which was inhibited by a caspase-1 inhibitor. These results indicate a novel finding that heparin is one of the natural stimulants to activate inflammasome.

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Mite allergen is a danger signal for the skin

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Atopic dermatitis (AD) is a highly pruritic chronic inflammatory skin disorder caused by multiple factors. Among them, house dust mite (HDM) allergens are important for the onset and exacerbation of AD. In airway allergy, HDM allergens activate innate immunity. However, there is limited knowledge regarding the activation of innate immunity by HDM in the skin. The inflammasome is a key regulator of pathogen recognition and inflammation, and can be activated by various stimuli. In this study, we investigated whether HDM allergens activate the inflammasome in human epidermal keratinocytes. HDM extracts from Dermatophagoides pteronyssinus (Der p) and Dermatophagoides farinae (Der f) activated caspase-1 and induced caspase-1-dependent release of IL-1β and IL-8 from keratinocytes, indicating that HDM activate the inflammasome in these cells. Release of IL-1β in response to HDM depended on cysteine protease activity. Group 1 HDM allergens (Der p 1 and Der f 1) also induced the release of IL-1β and IL-8 through their cysteine protease activity, but treatment of keratinocytes with recombinant group 2 allergens (Der p 2 and Der f 2) had no significant effect on IL-1β release. This is the first report showing that HDM allergens activate the inflammasome. In AD, disruption of the epidermal barrier facilitates HDM allergens to impact viable keratinocytes, resulting in inflammasome activation and IL-1b and IL-8 release. HDM allergen is a danger signal for the skin and HDM-induced activation of the inflammasome plays a pivotal role in the pathogenesis of AD.

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Regulatory mechanisms of collagen expression by interleukin-17 signaling in scleroderma fibroblasts

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Scleroderma, or systemic sclerosis (SSc), is a generalized connective tissue disease which is characterized by autoimmune abnormality, microvascular obliteration and increased deposition of extracelluar matrix (ECM) such as collagen, resulting in fibrotic lesions. The ECM synthesis and deposition are thought to be regulated by cytokines such as transforming growth factor (TGF)-b. Despite recent advances in understanding the regulation of ECM expression, the mechanisms responsible for the pathologic collagen accumulation in SSc have not been elucidated. Interleukin (IL)-17 is a proinflammatory cytokine produced by the Th17 CD4+ T cell lineage. Although IL-17 has been linked to the pathogenesis of various inflammatory and autoimmune diseases and serum IL-17 levels are reported to be elevated in SSc, the role of IL-17 signaling in the pathogenesis of SSc has been poorly understood. In this study, we found that cultured human dermal fibroblasts express IL-17 receptor A (IL-17RA), essential for biologcal activity of IL-17. The expression of IL-17RA in SSc fibroblasts was significantly decreased compared with normal fibroblasts. We then expected IL-17 signaling may play a role in the ECM accumulation in this disease. Based on the changes in the expression profile of ECM genes by IL-17 in vitro, we will discuss the possible role of IL-17 signaling in SSc.

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The Role of IL-17 in Different Clinical Subtypes of Psoriasis

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Psoriasis is a chronic, recurrent inflammatory skin disorder of unknown aetiology. Patients with psoriasis exhibit elevated levels of pro-inflammatory cytokines and a neutrophile and lymphocyte infiltration both in dermis and epidermis. Recent findings have revealed a potential role for IL-17 responses in the pathogenesis of psoriasis. 70 patients with psoriasis (30 psoriasis vulgaris, 20 guttate psoriasis, 20 pustular psoriasis) and 50 age- and sex- matched healty volunters were enrolled in the study. Serum IL-17 levels were examined by ELISA. Serum IL-17 levels of pustular psoriasis (9.6±12.6) were significantly higher than healthy controls (4.4±4.1) (p=0.02). Although serum levels of IL-17 in psoriasis vulgaris (6.2±5.6) were higher than healthy controls, the difference was not statistically different. The serum IL-17 levels in patients with guttate psoriasis (4.3±3.6) did not show a significant difference compared with healthy controls. When we evaluate psoriasis vulgaris patients according to the disease severity, the serum IL-17 levels were found significantly higher in patients with PASI≥10 (11,30±6,0) than in patients with PASI<10 (3,39±2,6) and healty controls (p<0.001). The Pearson correlation anlysis showed a positive correlations between the serum IL-17 levels and PASI. Our results suggests that IL-17 may play an important role in pustular psoriasis and severe psoriasis psoriasis.

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Anti-laminin γ1 pemphigoid mediated by IgA autoantibodies

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Anti - laminin γ1 pemphigoid is a novel autoimmune subepidermal blistering disorder. Clinically, it may resemble bullous pemphigoid, linear IgA bullous dermatosis or dermatitis herpetiformis. Immunologically, anti - laminin γ1pemphigoid is characterized by the development of IgG antibodies directed against basement membrane zone protein with molecular weight of 200kD. Here, we report a first case of anti-laminin g1 pemphigoid mediated by IgA antibodies with clinical features resembling pemphigus herpetiformis localized on traumatized areas. Histopathology of lesional skin showed dermal-epidermal separation with microabscesses composed of neutrophils in the dermal papillae. Direct immunofluorescence disclosed the presence exclusively of linear *in vivo* bound IgA along basement membrane zone. Using laser scanning confocal microscopy we found that *in vivo* bound IgA was localized above collagen IV and colocalized with laminin-332. Indirect immunofluorescence showed the presence of circulating IgA antibodies on the floor of artificial blister of salt split skin. Western immunoblot analysis using dermal extract confirmed the reactivity of circulating IgA antibodies with the 200kD antigen corresponding to laminin-γ1. Immunelectronmicroscopy disclosed the reactivity of circulating IgA autoantibodies within lower lamina lucida. To the best of our knowledge, this is the first case fulfilling immunopathological criteria for anti-laminin γ1 pemphigoid mediated by IgA antibodies with unusual clinical features.

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Differential skin expression of Caveolin-1 and Allograft-inflammatory factor-1 in chronic Graft-Versus-Host Disease suggests a specific role in the pathogenesis of Scleroderma

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The role of the immune system in the pathogenesis of Scleroderma is still unclear. Sclerodermoid-GVHD (Scl-GVHD) shows a tissue pathology similar to SSc providing a indirect evidence of this role. To determine the specificity of AIF-1 and caveolin-1 in the immunopathogenesis of skin fibrosis we analyzed their level of expression in 8 skin biopsies of scl-GVHD and 3 non scl-GVHD. Histopathological analysis confirmed intense tissue fibrosis in scl-GVHD without the vessel rarefaction typical of SSc whereas cGVHD was characterized by dense infiltrate and no skin fibrosis. Immunofuorescence studies followed by CLSM were conducted and quantitative analysis of fluorescence was determined by Image J software. AIF-1 expression was increased by 6.3 fold in Scl-cGVHD (±1.06; p= 0.0146) compared both with healthy and GVHD skin. The pattern of expression was mostly in perivascular and tissue infiltrating mononuclear cells, whereas microvascular endothelial cells did not show any AIF-1 expression despite what has been previously shown in SSc. Cav-1 immunofluorescence was decreased in Scl-GVHD (- 2.68 fold; p=0.0289), mostly due to lack of expression in stromal cells, since endothelial cells showed a conserved expression for Cav-1. AIF-1 expression in infiltrating cells correlated with the presence of tissue fibrosis and fibroproliferative vasculopathy in GVHD. The decreased expression of cav-1 is peculiar and specific of skin fibrosis regardless of its origin and therefore a good marker of tissue fibrosis. Transcriptome analysis of the same skin biopsies is on going to dissect the specific pathways associated with the different phenotypes.

060

Mesenchymal Stem Cells Exert an Inhibitory Effect on the Activation of Macrophages in vitro - Implications for the Non-Healing State of Chronic Venous Leg Ulcers

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There is accumulating evidence that activated macrophages play a major pathogenic role in the non-healing state of chronic venous leg ulcers (CVU). Previously; we found macrophages persist in high numbers and are highly activated in chronic venous leg ulcers. In addition, severe dermatoliposclerosis in the chronic venous leg ulcers leads to a progressive loss of the subcutaneous mesenchymal stem cell niche. Autologous or allogeneic transfer of mesenchymal stem cells represents a promising treatment which may control persistent macrophage activation and rescue the regenerative capacity of these difficult-to-treat wounds. We therefore studied the role of MSCs on macrophage activation using co-culture of bone marrow or adipose tissue derived MSCs with activated macrophages. Macrophages were effectively stimulated with the combination of LPS and IFNγ, leading to enhanced release of NO, IL-12 and TNFα. We found that the co-incubation with BM-MSCs resulted in a significant reduction of NO release from activated macrophages at the ratios (MSCs:macrophages) of 1:10, 1:50, 1:200 and 1:1000. While the suppression effect was only detected at the ratios (MSCs:macrophages) of 1:10 and 1:50 when AT-MSCs were applied. In the murine model of full-thickness excisional wounds in wildtype mice both BM-MSCs and AT-MSCs injected around the wounds significantly accelerated wound healing when compared with wounds injected with PBS. Collectively, these data show that MSCs exert a suppressive effect on macrophage activation, and *in vivo*, both BM-MSCs and AT-MSCs accelerated wound healing in wildtype mice. Thus, topical application of MSCs may qualify as a promising therapy in macrophage-dominated impaired wound healing.

061

Computational Analysis of sclerodermoid-GVHD and Scleroderma skin transcriptomes reveals the main pathways involved in the immunopathogenesis of skin fibrosis

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Scleroderma is a chronic disease involving autoimmune activation, fibroproliferative vasculopathy and tissue fibrosis. Microarray analysis of scleroderma skin biopsies identified an intrinsic gene signature associated with Scleroderma. Besides the putative role that can be hypothesized by the known gene product function, it is not clear which genes within this signature are specifically involved in the immunopathogenesis of Skin Fibrosis. Sclerodermoid GVHD (Scl-GVHD) is a form of chronic GVHD that shares with Scleroderma tissue fibrosis, induced by the immune trigger and the fibroproliferative vasculopathy, with the remarkable difference of increased angiogenesis, absent in Scleroderma. To identify which genes of the Scleroderma intrinsic gene signature are of potential importance in bridging the immune activation and the skin fibrosis we analyzed the level of expression of the scleroderma gene signature within scl-GVHD and non scl-GVHD transcriptomes, by pathway focused qPCR array. Of the 80 genes analyzed, 46 were differentially expressed in cGVHD biopsies and 34 remained peculiar of Scleroderma. 78.3% of the differentially expressed genes had a similar pattern of regulation in SSc. 25% were similarly expressed in both cGVHD variants, whereas 16.6% were specific of Scl GVHD. Remarkably, this analysis allowed to identify specific chemokines (CCL5, CXCL9-10-11) involved in the fibrotic versus non fibrotic response in GVHD and a specific modulation of WNT and Hedge-Hog pathway peculiar of Scleroderma and scl-GVHD skin biopsies. Identification of the source of l expression of these genes by immunohistochemistry will shed light in unraveling the events bridging immune activation and skin fibrosis.

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Topoisomerase I Gene Mutations In Patients With Systemic Sclerosis (Scleroderma)

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A hallmark of systemic sclerosis (SSc) is the presence of antinuclear antibodies. In 30-90% of patients these are antibodies directed against various epitopes of topoisomerase I. Neither etiology of the disease nor factors causing development of these antibodies are known. The aim of the study was to analyze the gene sequence of topoisomerase I in patients with systemic sclerosis. A total of 96 patients with SSc (including 45 patients with circulationg anti-topoisomerase I antibodies) were included into the study. All patients fulfilled the ACR (American College of Rheumatology) for the diagnosis of SSc. In all patients cDNA was isolated from peripheral blood mononuclear cells. The study was performed with the use of heteroduplex analysis and automated cDNA sequencing. The sequence of exons 20 and 21, which encode the active site of topoisomerase I was analyzed. In 3/45 (6.6%) Topo I(+) patients tree diverse mutations in Exon 21 were identified, in 22/45 of these patients (48,9%) mutations in exon 20 were observed. These abnormalities were not seen in Topoi(-) patients with SSc or healthy controls. We conclude that mutations in the gene, which encodes topoisometase I, may contribute to development of anti-topoisomerase I antibodies and possibly also to development of systemic sclerosis.

063

Therapeutic Inhibition of Hypoxia Prevents Psoriasis-like Arthritis in a Small Animal Model

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Auto-antibodies against Glucose-6 phosphate-isomerase (GPI) induce arthritis in mice that closely resembles human psoriasis arthritis (PsoA). Angiogenesis and hypoxia play a major role in organ-specific autoimmune diseases such as GPI triggered arthritis. Hypoxia induces angiogenesis via stabilization of the transcription factor hypoxia inducible factor (HIF)-1α and induction of pro-angiogenic mediators. The aim of our study was to analyze whether inhibition of hypoxia may protect from joint destruction. Methylene blue (Mb), a derivate of hemoglobin should inhibit hypoxia by interfering the nitric oxide activation synthase pathway and inhibition of nitric oxide synthase and guanylate cyclase. We injected GPI-serum or control-serum in mice to induce joint inflammation. After two days we started daily Mb-treatment (0,23mg/kg) or PBS-treatment. To investigate the effects of Mb-treatment, we measured ankle swelling and hypoxia in arthritic joints by [^{18}F]Fluoromisonidazole (FMISO) and positron emission tomography (PET). Additionally we analyzed mRNA expression of HIF-1α/2α, pro-angiogenic, and pro-inflammatory mediators as well as H&E-, and pimonidazol-stained slices of arthritic- and healthy joints. Mb-treatment nearly completely suppressed GPI-arthritis and protected mice from pannus formation and joint destruction. Investigating hypoxia *in vivo* using [^{18}F]FMISO-PET we detected a 55% reduction in tracer uptake four days after initiation of Mb-treatment. We performed histology and real-time PCR analysis (HIF-1α/2α, pro-angiogenic, and pro-inflammatory mediators) to correlate *in vivo* investigations with the molecular changes of PsoA. Thus, anti-hypoxic Mb-treatment is a powerful tool to suppress hypoxia-induced angiogenesis and to protect mice from joint destruction. [^{18}F]FMISO-PET provides a quantitative method to detect treatment responses in PsoA.

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ß2 integrin-dependent release of oxygen radicals from macrophages is required for TGF-ß1 activation in cutaneous wound repair

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Patients suffering from Leukocyte Adhesion Deficiency Syndrome type 1 (LAD1) with impaired ß2 integrin expression and function due to mutations in the gene encoding their common ß chain (CD18) present with spontaneous skin ulcerations and severe wound healing disturbances. In a model of full thickness excisional wounds we previously found that disruption of the ß2 integrin signaling pathway in CD18-/-, Vav3-/- and Rac2-/- mice results in delayed wound healing. This is due to impaired formation of the phagocytic synapse between apoptotic neutrophils and macrophages leading to impaired oxidative burst and reduced release of active TGF-ß1 at the wound site. However, the underlying mechanism is poorly understood. We here investigated whether the ß2 integrin-dependent release of ROS by macrophages upon phagocytosis of apoptotic neutrophils is responsible for TGF-ß1 activation during wound healing. In vitro co-culture experiments of wildtype macrophages with apoptotic neutrophils induced high amounts of active TGF-ß1 which were reduced by co-incubation with oxygen and nitrogen radicals scavengers. Notably, injection of the oxidative burst inducer Rotenone in wound margins of wildtype and CD18-/- mice enhanced the oxidative burst at wound sites and virtually rescued the wound healing defect of CD18-/- mice to wildtype levels. These results suggest that TGF-ß1 may be activated at wound sites by ROS and that in CD18 deficiency a reduced oxidative burst leads to reduced active TGF-ß1 release and eventually to impaired wound healing. Modulation of the oxidative burst in wound margins may be a promising therapeutic approach for LAD1 patients but also to prevent fibrosis.

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Curcumin protects from inflammatory autoimmune disease by inducing immuno suppressive DC and inhibiting Th1/Th17 responses

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Curcumin is a yellow pigment isolated from the rhizomes of the plant Curcuma Longa. Curcumin may possess anti-inflammatory activities and therefore is traditionally used for the treatment of inflammatory disorders in Asia. First, we investigated the activities of curcumin on the differentiation of mouse dendritic cells in vitro. We started by comparing gene expression of curcumin-treated DC or control DC after activation through TLR4 using PCR-Microarrays. Curcumin induced expression of genes of the TNF superfamily and NFkB inhibitory genes in DC and inhibited the production of inflammatory cytokines. To investigate the biological effect of curcumin in vitro, we isolated CD4$^+$ T cells from SJL mice immunized with PLP peptide in CFA. These CD4$^+$ T cells were activated with APC and PLP peptide. T-cells primed in the presence of curcumin showed significantly inhibited IFN-g and IL-17 expression and an increased expression of the Th2 cytokines IL-4 and IL-10. We next analyzed the effects of curcumin *in vivo*. Mice were fed with curcumin, immunized with PLP in CFA and pertussis toxin. Whereas control mice developed severe EAE, mice treated with curcumin remained healthy or developed only mild EAE. The protection from EAE by curcumin treatment was associated with a suppression of IL-12/IL-23p40 and subsequent Th1 and Th17 responses. In contrast curcumin treatment induced IL-4 and IL-10 in activated CD4$^+$ T cells. Even though the molecular mechanism, by which curcumin interacts with IL-12/IL-23p40 expression still requires further analysis, treatment with the natural extract curcumin is promising as therapeutic approach for inflammatory autoimmune disease.

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Fumarates improve Psoriasis and Multiple Sclerosis by inducing type II dendritic cells

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Fumarates improve multiple sclerosis and psoriasis, two diseases in which both IL-12 and IL-23 are responsible for pathogenic T helper (Th) cell differentiation. However, both diseases show opposing responses to most of the established therapies. First we show in humans that fumarate-treatment induces IL-4-producing Th2 cells *in vivo* and generates type II dendritic cells (DC) that produce IL-10 instead of IL-12 and IL-23. In mice, fumarates also generate type II DC that induce IL-4-producing Th2 cells in vitro and *in vivo* and protect mice from experimental autoimmune encephalomyelitis (EAE). Type II DC result from fumarate-induced glutathione-depletion, followed by increased hemoxygenase-1 (HO-1) expression and impaired STAT1-phosphorylation. HO-1-induction prevents IL-23p19 transcription without affecting IL-12p35, while STAT1 inactivation prevents IL-12p35 transcription without affecting IL-23p19. As a consequence, glutathione-depletion by small molecules like fumarates induces type II DC in mice and in humans that ameliorate inflammatory autoimmune diseases. This therapeutic approach improves Th1- and Th17-mediated autoimmune diseases such as psoriasis and multiple sclerosis by interfering with IL-12 and IL-23 production.

067

The role of chemerin, a new adipokine, in psoriasis

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Chemerin is a newly identified adipokine and an agonist of chemokine-like receptor 1 that is expressed by cells of the innate immune system. Chemerin stimulates chemotaxis of plasmatoid dendritic cells (pDC) and neutrophils, and high systemic chemerin level was found as an independent marker of the metabolic syndrome. Recently, strong expression of chemerin together with pDC and neutrophils in the dermis of early psoriasis lesions was reported. To investigate the association of chemerin with psoriasis, we examined whether circulating chemerin was elevated in psoriasis patients and whether it correlated with metabolic syndrome. Circulating chemerin levels were significantly elevated in psoriasis patients compared to those in chronic dermatitis patients and healthy controls. Serum chemerin levels did not positively correlate with body mass index, however, serum high chemerin level significantly correlated with hypercholesterolemia and hypertriglyceridemia in psoriasis patients by the logistic regression analysis. Interestingly, chemerin level showed a decrease after cyclosporine treatment, and the change in chemerin values was closely associated with alternations in PASI score. We also examined chemerin expression and chemerin mRNA levels in psoriatic lesions and healthy control skin. Expression of chemerin was observed in the epidermis of psoriatic or control skin. In contrast to the previous report, chemerin expressing cells were not detected in the dermis of active psoriatic lesions. Chemerin mRNA levels were mostly unchanged in the lesional skin compared with nonlesional skin from psoriasis patients. Systemic chemerin elevation may be relevant to the pathomechanism of psoriasis and metabolic syndrome risk factors.

068

Development of an ELISA for the specific detection of autoantibodies in anti-p200 pemphigoid

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Anti-p200 pemphigoid is a subepidermal blistering skin disease characterized by autoantibodies against a 200-kDa protein (p200) of the dermal-epidermal junction. Recently, the laminin γ1 chain has been identified as target antigen in this disease and the C-terminus was described as an immunodominant region of laminin γ1. Diagnosis of anti-p200 pemphigoid requires detection of serum IgG at the dermal side of 1 M salt-split skin by indirect immunofluorescence microscopy and labelling of a 200 kDa protein by Western blotting of dermal extract. However, preparation of dermal extract is not widely available limiting the possibility of diagnosing this disease to a few laboratories. In this study, we report on the development of a simple, sensitive and specific ELISA using a recombinant monomeric C-terminal fragment of human laminin γ1 (hLAMC1-cterm) expressed in E. coli. By this novel ELISA, serum reactivity with hLAMC1-cterm was detected in sera from 27 of 35 (77%) patients with anti-p200 pemphigoid, 6 of 101 (6%) with bullous pemphigoid, 0 of 10 with epidermolysis bullosa acquisita, 3 of 14 (21%) with anti-laminin 332 pemphigoid, 0 of 51 with pemphigus vulgaris, and 1 of 131 (0.8%) healthy volunteers. In order to explore the role of conformational epitopes and posttranslational modifications in eukaryotic cells, hLAMC1-cterm was produced in human HEK293 cells. However, using human cell-expressed hLAMC1-cterm, the sensitivity of the ELISA did not change. The novel ELISA using E.coli-expressed hLAMC1-cterm greatly facilitates the diagnosis of anti-p200 pemphigoid.

069

Human monoclonal antibodies bind desmoglein 3 epitopes shared by pemphigus vulgaris patient autoantibodies and synergistically induce loss of adhesion between keratinocytes

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Pemphigus vulgaris (PV) is a life-threatening autoimmune blistering disease of skin and mucous membranes associated with autoantibodies against the adhesion molecules desmoglein (Dsg)3 and Dsg1. We have previously isolated and cloned 16 Dsg-specific human monoclonal antibodies (hMabs) from 2 patients with active PV by using a method for efficient immortalization of IgG+ memory B cells. Most of the hMabs recognizing calcium-dependent conformational epitopes were mapped by a system based on domain swapped molecules between Dsg3 and Dsg2, which have similar structures but distinct epitopes. Specifically, 8 hMAbs reacted against the extracellular (EC) 2 subdomain, 1 with the EC3 and 4 against the EC5. The study of pathogenic effects of hMabs, assessed by in vitro keratinocyte dissociation assay and passive transfer in neonatal mice, showed a different capability to induce loss of keratinocyte adhesion. Furthermore, PV sera were able to block hMabs binding on Dsg3 indicating that the targeted regions were bound also by autoantibodies from different PV patients. Finally, hMabs, each displaying a very low fragmentation index by the in vitro dissociation assay, showed a synergistic pathogenic effect when used in combination in the same conditions. In conclusion, the cloned hMabs shared Dsg3 epitopes with PV sera and were able to mimic the combined effects of the polyclonal IgG from PV patients. They could thus represent valuable tools for studying the role of Dsg3 in epithelial cell adhesion and mechanisms of Dsg3 processing/presentation in PV pathogenesis.

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The vast majority of pemphigus vulgaris patients possess circulating autoantibodies reacting with an immunodominant epitope on desmoglein 3

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Pemphigus vulgaris (PV) is a potentially fatal autoimmune mucocutaneous disease associated with production of IgG autoantibodies to desmoglein 3 (Dsg3). The binding of autoantibodies to Dsg3 on keratinocytes leads to loss of intercellular adhesion and blister formation. We report the characterization of a Dsg3-specific human monoclonal antibody (hMab) previously isolated from a patient with active PV using a method for efficient immortalization of IgG+ memory B cells. The IgG1 hMab, termed PVA224, reacted with a conformational and calcium-dependent epitope on Dsg3 and induced a histological profile similar to human PV when injected into neonatal mice. PVA224 carried only a few somatic mutations (seven amino acid replacements in the CDRs of VH and VL) that will be analyzed by reversion to the germline to determine their contribution to binding. Of note, the vast majority of PV patients possessed circulating autoantibodies reacting with the same Dsg3 epitope recognized by PVA224, as determined by ELISA cross-competition assays. In particular, 48 out of 51 (94 %) PV patient sera and none of 40 control sera strongly inhibited the PVA224 binding to Dsg3. In addition, the ability to block PVA224 binding in PV patients fluctuated in parallel with disease severity during disease course, suggesting a role of this pathogenic hMab in monitoring PV disease. In conclusion, the pathogenic PVA224 hMab could represent a valuable diagnostic tool for disease monitoring, as well as a lead to design therapeutic approaches based on blocking peptides or anti-idiotypic antibodies.

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E6005, a Novel Topical Phosphodiesterase 4 Inhibitor, Ameliorated Pruritus and Inflammation in Mouse Atopic Dermatitis Models

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E6005, a novel Phosphodiesterase (PDE) 4 inhibitor with a new chemical structure, has been developed as a topical agent for atopic dermatitis (AD). The aim of this study was to assess the pharmacological potential of E6005 as a new therapeutic approach for AD *in vitro* and *in vivo* with a focus on the anti-pruritus effect. In vitro, E6005 showed a potent and selective inhibition of PDE4 with an IC_{50} of 2.8 nM, and suppressed production of a wide variety of cytokines from PBMCs (IL-2, IL-4, IL-12, IFN-γ and TNF-α). To assess the efficacy in AD, we studied the effect of topically applied E6005 on skin inflammation, pruritus and atopic dermatitis-like skin symptoms in mouse models. Topical application of E6005 decreased the ear thickening in chronic skin inflammation, accompanied by the inhibition of local cytokine/chemokine expression. Of interest, E6005 exhibited a suppression of scratching behavior in an allergic pruritus model immediately after a single application. In addition, the treatment with E6005 ameliorated atopic dermatitis-like skin symptoms in a mouse AD model. Finally, E6005 showed a lower emetic potential than the second generation PDE4 inhibitor cilomilast. These results indicate the therapeutic potential of E6005 for AD based on both anti-pruritus and anti-inflammatory effects.

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Modulation in the expression of substance P in the skin and brain of atopic mice during chronic mild stress

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Stress is known to worsen the symptoms of atopic dermatitis (AD). There is a bidirectional communication between the skin and the neuroendocrine system. Substance P is likely to play an important role in the development and pathogenesis of AD. We used NC/Nga atopic mice to examine a possible connection between chronic mild stress and changes in expression of substance P and its receptor (R) neurokinin (NK)1 in the skin and brain. The mice were divided into three groups (8 mice in each): SE (stressed eczema), NSE (non-stressed eczema) and SC (stressed control). Ears and brains of the mice were investigated by using RT-PCR and immunohistochemistry. In the skin, there was a decrease in the number of substance P immunoreactive nerve fibres, while the expression of NK1R immunoreactivity was highest, in the eczematous groups compared to the control group, SC. Using RT-PCR we found a high tendency to an increase in mRNA for NK1R in the skin of SE compared to the NSE mice. There was a tendency to an increase of the substance P immunoreactivity in the SE compared to NSE group in the prefrontal cortex. A decrease in substance P immunoreactivity in the hippocampus was found in the SE compared to NSE group. In the amygdala there was an increase in the substance P immunoreactivity in the SE compared to the SC group. An exposure to chronic mild stress in NC/Nga mice results in a modulation of expression of substance P in skin and brain regions related to stress.

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The new undecyl-rhamnoside (APRC11) inhibits P.acnes-induced inflammation in keratinocytes

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Acne vulgaris is a chronic skin disease of pilosebaceous glands in which inflammation plays a central role. In order to develop new therapies against the development of inflammatory events, we evaluated the effect of a new undecyl-rhamnoside, namely the undecyl-rhamnoside APRC11, on the keratinocytes inflammation and scarring markers. For this purpose, natural human keratinocytes taken from 6 healthy donors were first pre-incubated for 24 hours with increasing concentrations of APRC11(0.3 to 3 μM) or Zinc Gluconate (Zn, 15μM) which was used as reference molecule for its anti inflammatory activity. Then, keratinocytes were stimulated with *P.acnes* MF (Membrane Fraction) for 6 hours, in the presence of the same APRC11 concentrations or Zn. Different markers of inflammation or scarring were evaluated at protein level using ELISA tests and a Luminex array system, and at mRNA level using a Luminex-based Quantigene array system. Results showed a significant increase in IL-1α, IL-1RA, IL-6, IL-8 and MMP-9 expressions after *P.acnes* stimulation. Treatment with APRC11 down regulated the P.acnes-induced cytokines expression (IL-1α, -40%; IL-8, -26% and MMP-9, -38%) and up-regulated IL-1RA (+27%). These regulations were noted at both protein and mRNA levels. In conclusion, the new undecyl-rhamnoside APRC11 is able to inhibit the P.acnes-induced inflammation in natural human keratinocytes, confirming its interest in inflammatory acne and matrix remodelling.

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Expression of the 27kD and 90kD heat shock proteins in psoriatic skin

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Heat shock proteins (hsp) are a group of highly conserved proteins that are essential in cytoprotection, cell physiology, inflammation, and keratinocyte differentiation. Their role in psoriasis is largely unknown. In the current study a collection consisting of 30 archival samples from various stages and clinical variants of psoriasis and 9 samples of normal skin was investigated for the expression of hsp27 and hsp90 by immunhistochemistry. As in normal skin hsp27 in psoriatic epidermis was associated with keratinocyte differentiation. In addition psoriatic skin samples sometimes showed positive staining in the basal cell layer and in subcorneal areas of neutrophil aggregation. A striking finding in almost all samples was the specific loss of hsp27 in the characteristic dilated and tortuous capillaries of psoriatic papillae whereas endothelial cells of all other vessels of normal and psoriatic skin were regularly positive. Hsp90 was expressed within the entire epidermis and appendages with higher intensitiy in the basal layer without any apparent difference between normal and psoriatic skin. In contrast to normal skin hsp90 was found in all nucleated cells of psoriatic dermis including endothelial cells, leukocytes, and fibroblasts. Our findings indicate that hsp90 may be induced in psoriatic skin in response to inflammation. The differential expression of hsp27 in capillary versus postcapillary endothelium points to a role of hsp27 in angiogenesis and the endothelial pathology associated with psoriasis. Based on these findings further studies are warranted to investigate the functional role of hsp in psoriasis and the possible therapeutic use of hsp modifying agents.

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Modulation of autoantibody isotypes and Fc receptor expression by intravenous immunoglobulin

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High dose intravenous immunoglobulin G (IVIG) is used to treat refractory autoimmune diseases, including epidermolysis bullosa acquisita (EBA). EBA is characterized by autoantibodies to type VII collagen and blistering on skin and mucous membranes. We investigated effects of IVIG in immunization-induced EBA in mice. After disease manifestation, mice were treated with either methylprednisolone (MP, 20mg/kg/day i.p.) or IVIG (2g/kg once/week) for 4 weeks. While EBA progressed in untreated control mice, 31% of mice treated with MP, and 64% of IVIG-treated mice showed improvement. Compared to non-treated EBA mice, direct immunofluorescence (IF) microscopy showed reduced complement deposition in skin of IVIG-treated mice, while total immunoglobulin G (IgG) deposition was unaltered. By direct IF microscopy, IgG subclass analysis demonstrated reduced deposition of complement fixing IgG2 autoantibodies after IVIG compared to non-treated mice. To investigate a possible effect of IVIG on FcgR, leukocytes of IVIG-treated and non-treated EBA mice were evaluated by flowcytometry for expression of activating and inhibitory FcgRs. The number of FcgRIIB positive germinal centre B cells was increased and Gr-1 positive granulocytes showed a reduced FcgRIIB expression compared to non-treated EBA mice. In addition, FcgRIV expression on Gr-1 positive granulocytes was down-regulated by IVIG treatment and this reduction was more prominent than reduction of FcgRIIB. We demonstrate that IVIG is effective in experimental EBA, which is mediated by modulation of autoantibody-isotypes towards non-complement fixing autoantibodies and diminishing the expression of FcgRIV levels on effector cells. These findings may have implications for more specific treatment regimens of autoantibody-mediated diseases.

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The CD18 Hypomorphic Psoriasis Mouse Model - Insight into the Pathogenesis of a Complex Disease

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Psoriasis is a chronic disease affecting skin in 2-3% of the general population. We previously showed that the CD18^{hypo} PL/J mouse with a mutation resulting in a reduced expression of the common chain of $ß_2$ integrins (CD11/CD18) spontaneously develops a skin disease that closely resembles human psoriasis. Interestingly, when backcrossed onto the C57BL/6J background no psoriasiform dermatitis developed, suggesting that apart from the CD18 hypomorphic mutation a small number of modifier genes are required for the precipitation of the disease. Backcross analysis between susceptible CD18^{hypo} PL/J mice and the resistant CD18^{hypo} C57BL/6J strain and a genome-wide linkage analysis identified susceptible loci on chromosome 6 and chromosome 10. Using a congenic approach, we identified a 9-cM fragment on chromosome 10 with genes being responsible for the psoriasiform disease. Continuous backcrossing identified a 1.2cM fragment which - if intercrossed - resulted in the disease. As we recently found that regulatory T cells as well as activated macrophages play a central role in the psoriasiform disease, our efforts will now concentrate on identifying potential modifier genes which in conjunction with the CD18 hypomorphic mutation are responsible for the dysregulation of these cellular key players and the psoriasiform disease.

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Alterations in the structure and lipid composition of the stratum corneum in atopic dogs are normalized by food supplementation with essential fatty acids (Megaderm®)

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Whereas numerous reports have shown alterations of lipids in the human atopic skin, little information is available about the lipid composition and organization of the dog atopic skin. In the present study, the structure of the atopic dog epidermis was investigated by electron microscopy of biopsies, and the composition of free and protein-bound lipids of the stratum corneum was analyzed after tape stripping of non-lesional areas. The effect ESDR of a treatment of atopic dogs by food supplementation with a mixture of essential fatty acids (Megaderm®) was also studied on skin samples taken before and after treatment. Electron microscopy revealed that the non-lesional skin of atopic dogs exhibited an abnormal and largely incomplete structure of the lamellar lipids with little cohesion between the corneocyte strata. The stratum corneum of atopic dogs was characterized by an important heterogeneity in the lipid content of the successive layers. This feature was noticeable in the protein-bound lipids, and mostly in the omega-hydroxylated ceramides. Following oral treatment of atopic dogs with the mixture of essential fatty acids, the lipid content of the stratum corneum markedly increased and the distribution of the different classes of protein-bound lipids (cholesterol, fatty acids and ceramides) became much more homogenous in the successive layers of the stratum corneum. The study by electron microscopy confirmed that the organization of lamellar lipids was normal in the skin of treated dogs. Our results show that the dog could be considered as a suitable model for studies on atopic dermatitis.

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MN8001, a dendritic polyglycerol, inhibits type I allergic responses

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More than 20% of the world population suffer from type I allergic conditions such as atopic dermatitis, urticaria, or allergic rhinitis. Standard allergy treatments often fail to achieve symptom control, necessitating the development of novel drugs. Dendritic polyglycerols have been described to have anti-inflammatory properties. Here, we investigated the effects of the dendritic polyglycerol MN8001 on type I allergic responses using passive systemic anaphylaxis (PSA) in mice as a model. C57BL/6 mice were sensitized by intraperitoneal injection (i.p.) of IgE anti-DNP. After 24 h, the animals were subcutaneously injected with MN8001 (30 mg/kg bodyweight) or vehicle and PSA was elicited 10 min later by i.p. injection of DNP. 20 minutes after induction of PSA, vehicle-treated mice had a mean temperature drop of 3.5°C +/- 0.2 while that of MN8001-treated mice was only 1.6°C +/- 0.3, $p<0.005$. Allergic type I reactions are caused by mast cell degranulation, which results in the release of biologically potent mediators such as histamine, cytokines and proteases. To test whether MN8001 can inhibit mast cell degranulation in PSA, we measured mouse mast cell protease-1 (mMCP-1), which is known to directly correlate with mast cell activation status. Notably, mMCP-1 concentrations in serum of MN8001-treated mice were reduced by ~50% when compared to vehicle-treated animals (6.0±1.1 pg/ml vs. 11.8±2.2 pg/ml, $p<0.05$). Our results indicate that the dendritic polyglycerol MN8001 potently reduces allergic hypersensitivity reactions in mice. Additional investigations are needed to further identify the mechanism of action and the treatment potential of the substance for allergies in humans.

ABSTRACTS

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In situ Mycoplasma infection as aetiological factor in genital lichen sclerosus

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The pathogenesis of genital lichen sclerosus (GLS) is obscure: infections, inflammatory and autoimmun processes were most commonly implicated. Since the genital localization of GLS we examined, whether systemic *Mycoplasma* (*M.*) or in situ urogenital *M. hominis*, *M. genitalium*, *M. fermentans*, or *U. urealyticum* infections could induce chronic skin inflammation with sclerotic conditions. 21 male patients, who underwent therapeutic circumcision, have been selected for this study. 17 with GLS and 4 without GLS (WGLS) matched in age. (i) Preputial skin samples were histological examined. (ii) To determine prevalence of *M*. DNA we utilized the previously described method of Strömer *et al* for broad range real-time PCR (RT-PCR) identification of clinically important mollicute species on a Roche LC480 (Roche, Masnnheim, Germany). Skin samples were assayed for DNA using the QiaAMP DNA FFPE Kit (Qiagen, Hilden, Germany). Furthermore 14 GLS serum samples were evaluated also by: (iii) RT-PCR for general detection of M. DNA (MagnaPure Compact system - Roche, Masnnheim, Germany), (vi) and by *M. pneumoniae/genitalium* and *Borrelia burgdorferi* ELISAs. Skin samples from 13/17 ($p<0.0001$) GLS patients were *M*. RT-PCR positive, presenting a significant difference to WGLS group (0/4, $p<0.0002$). 9/14 cases showed serum M. RT-PCR positivity. *M. pneumoniae /genitalium* ELISA IgG positivity was detected in 4/14, IgM in 2/14 GLS cases without IgA antibodies. No *Borellia burgdorferi* seropositivity was observed. Our data indicate that chronic, latent genital *M*. infection could be a modifying factor of the local immune system and may contribute to development of GLS.

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Increase in IL-17A and IL-22 producing CD8 T cells in lesional psoriatic skin

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T cells that produce IL-17A and IL-22 have been suggested to play an important role in the pathogenesis of psoriasis. We determined the presence and distribution of such cells in situ as well as the frequency and heterogeneity of skin-derived CD4 and CD8 T cells that produce these cytokines. Double-stained sections from psoriatic and normal skin were analyzed by spectral imaging and in vitro-activated skin-derived T cells were analyzed by flow cytometry. We found that IL-17 was mainly expressed by mast cells and neutrophils and IL-22 by macrophages and dendritic cells, whereas only an occasional IL-17^{pos} T cell and no IL-22^{pos} T cells could be detected in psoriatic skin. Activation of skin-derived T cells in vitro, however, revealed that substantial percentages of cutaneous T cells were able to produce IL-17A or IL-22 or both. Remarkably, the percentages of CD8 T cells that expressed IL-17A and/or IL-22 (Tc17 and Tc22) were significantly higher in psoriatic dermis and even more pronounced in psoriatic epidermis than in normal skin. On the other hand, psoriatic skin and normal skin contained similar percentages of IL-17A^{pos} and/or IL-22^{pos} CD4 T cells (Th17 and Th22). Overrepresentation of Tc17 and Tc22 cells in psoriatic skin could result from a local disease related expansion and thus indicates a possible role in the pathogenesis of psoriasis. As IL-17A and IL-22 are crucial in psoriasis, also non-T cells that express IL-17A and/or IL-22 may represent potential targets for therapy.

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Comprehensive analysis of intact sebaceous lipids to investigate the fate of free fatty acids in human sebocytes

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Various long chain free fatty acids (FFA) have demonstrated a considerable lipogenetic activity in human sebocytes. However, little is known on the metabolic fate of exogenously added FFA and their capacity to promote sebaceous lipogenesis. To investigate how FFA are processed in human sebocytes to form sebaceous lipids, we exposed the SZ95 sebaceous gland cell line to exogenously supplied saturated (C16:0), monounsaturated (Δ9 C16:1, and Δ9 C18:1), and polyunsaturated (Δ9,12 C18:2, Δ9,12,15 C18:3 and Δ5,8,11,14 C20:4) FFA, each at the concentration 100 μM for 24 h. Lipid species were directly and simultaneously analyzed in crude cell extracts by RR-RP-HPLC/TOF MS, which allowed selecting and profiling the intact lipids belonging to their respective sebaceous classes, such as triacylglycerols (TAG), diacylglycerols (DAG), wax esters (WE), cholesteryl esters (CE), and FFA. Structure elucidation of individual neutral lipids was undertaken by MS/MS experiments. The RR-RP-HPLC/TOF MS and MS/MS analysis proved that the exogenously supplied FFA were uploaded into SZ95 cells and incorporated into the above neutral lipid classes as unprocessed acyl moieties. Moreover, exogenously added FFA underwent multiple metabolic processing, which included single and double desaturation steps, oxidation, and elongation by two carbon atoms units. The newly formed FFA were incorporated into neutral lipids and contributed to the multiplicity of acylglycerols (TAG and DAG), WE, and CE species induced by naïve exogenous FFA. Unequivocal identification and intracellular levels of species formed following lipogenic stimuli by FFA provided further insights into the enzymatic pathways concurring to the synthesis of sebaceous lipids in culture systems.

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Intense pulsed light's effect on inflammatory acne vulgaris is associated with alterations in TLR2 and IL-8 expression

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In acne vulgaris *P. acnes* binds to toll like receptor 2 (TLR2), activating the NFκB pathway resulting in the production and release of several inflammatory cytokines including IL-8. Intense pulsed light (IPL) is a broad-spectrum high intensity visible light source that has been said to be anti-inflammatory; however the mechanism is uncertain. This study aimed to determine whether IPL had any effect on well known markers of inflammation including IL-8 and TLR2. Twenty-nine patients with mild to moderate acne on their backs were treated with filtered IPL (peak emission at 530 nm) 4 times over 9 weeks. 4 mm punch biopsies of clinically uninvolved skin within the treatment area were taken at baseline, 2 days after the first treatment and 1 week after the final irradiation. There was a significant but small improvement in inflammatory lesion counts of 33.5% ($p = 0.003$). TLR2 and IL-8 RNA expression was evaluated in 11 cases via semi-quantitative PCR and visualised on 2% agarose gel. In 45.5% of cases, TLR2 and IL-8 were clearly down regulated, whereas the levels remained stable in 27.3% and increased in 27.3%. For each case, the trends noted in TLR2 expression were identical to the changes noted for IL-8. Using band intensities estimated through the AlphaImager® spot densitometry utility, there was a mean increase of 44.0% ± 79.79 in TLR2 and 3.24% ± 26.0 in IL-8 mRNA expression. Despite the clinical reduction in inflammatory lesion counts, IPL's anti-inflammatory effect may not be mediated via the TLR2-IL8 pathway.

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Licochalcone A protects against oxidative stress via activation of Nrf2

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Licochalcone A, a reversely constructed chalcone or retrochalcone, has previously been shown to possess strong anti-inflammatory properties. Licochalcone A inhibits the secretion of pro-inflammatory eicosanoids and cytokines. These anti-inflammatory effects are associated with the inhibition of NFκB signaling. In this study we focused on the anti-oxidative properties of Licochalcone A. We show that Licochalcone A protects against oxidative stress mediated by reactive oxygen species in in vitro experiments as well as *in vivo* after topical application on the skin. Licochalcone A inhibited the formation of reactive oxygen species in UVA-irradiated or hydrogen-peroxide treated human dermal fibroblasts as well as in pro-inflammatory activated neutrophils. Mechanistically, Licochalcone A induced nuclear translocation of the transcription factor Nrf2 in primary human fibroblasts, resulting in a many-fold increase in the mRNA and protein levels of the cytoprotective and anti-inflammatory enzyme heme oxygenase-I. Furthermore, Licochalcone A incubation increases the mRNA expression level of the glutamate-cysteine ligase regulatory subunit. Staining of intracellular thiols after Licochalcone A treatment revealed an elevated glutathione level in primary human dermal fibroblasts compared to non-treated control cells. These results correlated with *in vivo* experiments, showing a decrease in UVA-induced and ROS-dependent ultra-weak photon emission after topical application of Licochalcone A. We conclude from these data that Licochalcone-induced Nrf2 activation is an important contributor to cytoprotection in human skin cells.

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A possible role for LFA-1, CD39 and CD73 in regulatory T and B cells in psoriasis

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Regulatory T cells (Tregs) play a key role in autoimmune skin diseases such as psoriasis. The mechanisms by which Tregs mediate their function are not yet fully elucidated. Lymphocyte-function-associated protein-1 (LFA-1; CD11a/CD18), CD39 and CD73 on the surface of Tregs, are thought to play an important role. As regulatory functions were also recently attributed to B cells, we assessed the role for these markers in regulatory T and B cells in peripheral blood and skin of healthy donors and psoriatic patients. No difference was seen in frequencies of circulating CD4$^+$, CD4$^+$FOXP3$^+$, CD4$^+$CD25$^+$FOXP3$^+$, and CD19$^+$ cells between healthy and psoriatic patients, although a lower frequency of CD8$^+$ cells was observed in blood of psoriatic patients ($p=0.0841$). In contrast, a higher infiltrate of CD8$^+$ cells was detected in lesional psoriatic skin, tending to express more CD11a and CD18, as compared to non-lesional skin ($p=0.0547$; $p=0.0781$). The expression level of CD18 on CD19$^+$ B cells was lower in psoriatic patients as compared to healthy donors. Higher frequencies of CD39$^+$ and CD39$^+$CD73$^+$ T cells were observed in both healthy and psoriatic skin, as compared to the peripheral blood. Circulating Tregs showed a higher expression of CD11a in psoriasis ($p=0.03111$), without an increased CD18-expression. This suggests a role for CD11a in the dysfunction of Tregs in psoriasis. The altered expression of CD18 on B cells in psoriasis suggests a role for B cells in the pathogenesis of psoriasis. Further research on T and especially B cells is needed to elaborate these findings.

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TGF-β considered as an immuno-regulator in healthy controls becomes pathogenic in systemic sclerosis by regulation of IL-13 synthesis

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Systemic sclerosis (SSc) is an auto-immune disease characterized by skin fibrosis and internal organ dysfunction. Fibrosis observed in SSc patients is due to excessive collagen (type 1) deposition. It has been shown that TGF-β and IL-13 are closely involved in collagen production and elevated levels of these two cytokines have been observed in SSc serum. TGF-β activates directly collagen synthesis whereas IL-13 promotes fibrosis by stimulating TGF-β production. In the present study, we analyzed TGF-β effect on IL-13 expression by lymphocytes from SSc patients compared to healthy donors. Jurkat cell line was used as a Th2 model to study the TGF-β signaling pathway involved in IL-13 production. Analysis of IL-13 promoter activity, IL-13 mRNA and protein expression was studied in response to TGF-β exposure (5ηg/mL and 10ηg/mL), during 2 to 48hours. Our results show that TGF-β inhibits IL-13 production by 20 to 40% in normal T lymphocytes and in Jurkat cell line, as shown by downregulation of promoter activity, mRNA and protein expression level. By using specific inhibitors, we demonstrated that TGF-β inhibits IL-13 through activation of both MAPkinase (p38 pathway) and Smad pathways to induce repression of GATA-3 transcription factor. In contrast to Jurkat cell line and normal T lymphocytes, TGF-β induces an increase in IL-13 mRNA and protein production by 35 to 43% in SSc lymphocytes. TGF-β might be considered as an immuno-modulator in normal situation and conversely, as a potent immuno-activator in SSc pathological situation.

086

Participation of IL-13 in the cytokine network of psoriasis

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Recent genetic studies have shown that a variant in the IL4/IL13 region on chromosome 5 contributes to increased risk of psoriasis. This variant (rs20541) leads to nonsynonymous basepair change in the IL13 gene. Although IL-13 has traditionally been classified as a Th2 cytokine its role in psoriasis is unknown. IL13 mRNA ($n=38$, $p<0.01$) and protein levels ($n=12$, $p<0.01$) were increased in lesional psoriatic compared to uninvolved skin. Flow cytometric analysis of lesional skin lymphocytes demonstrated that the IL-13 was derived from CD4 and CD8+ T-cells (2-3% of total). There was increased expression of both chains of the IL-13 receptor (IL-4R and IL13-Rα1) in lesional compared to uninvolved psoriatic skin ($n=38$, $p<0.0001$, $p<0.05$, respectively) whereas IL13RA2, a soluble inhibitor of IL-13 was decreased ($n=38$, $p<0.05$). Immunohistochemistry and fluorescent microscopy revealed staining for IL-4R and IL-13Rα1 in psoriatic epidermis with intense foci of co-localization limited to few cells within the inflammatory infiltrate. These cells corresponded to monocytes as confirmed by flow cytometry. Using cytokine-stimulated post-confluent NHKs we observed a dose-dependent induction of IL4R and IL13RA1 with IFN-γ and IL-22 (>3-fold, $p<0.01$). IL-13 synergized with IFN-γ in the induction of CXCL9 and CXCL10 by NHKs ($p<0.001$, $p=0.08$, respectively $n=3$) whereas it suppressed IFN-γ- and IL-17-induced CCL20 and DEFB4 expression ($p<0.01$, $n=3$). Our results suggest that IL-13 plays a complex role in psoriasis acting both on the epidermis and the inflammatory infiltrate potentially leading to shifts in the polarization of the infiltrate through its activity on chemokine production.

087

Immunopathology of "neutrophilic" primary cicatricial alopecias

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Folliculitis decalvans and dissecting cellulitis are two clinically distinct cicatricial alopecias characterised by a predominantly neutrophilic infiltrate on scalp histology in association with hair follicle destruction and fibrosis. The pathogenesis of these disorders is unknown, but damage to epithelial hair follicle stem cells (eHFSC) located at the hair follicle bulge is thought to be a crucial pathogenic event. The aim of this study is to map the inflammatory cell infiltrate of the «neutrophilic» cicatricial alopecias and specifically to examine whether eHFSC damage and bulge immune privilege collapse play a role in disease pathogenesis. Paired frozen biopsies of lesional and un-involved scalp skin from affected patients ($n=10$), along with paraffin-embedded samples from patients ($n=12$) and normal controls ($n=15$) were examined. Immunohistochemical staining with CD1a, CD4, CD8, CD56, CD68, mast-cell tryptase, FoxP3, CD209, CD123, Ki67 and CXCR3 was performed and mapped to defined epithelial compartments of the hair follicle as well as the corresponding connective tissue sheath. Further, stains for apoptosis (TUNEL), immune privilege (MHC I and MHC II) and eHFSC (K15) were also examined. Semi-quantitative immunohistomorphometry at the bulge showed significant up-regulation of MHC class I ($p=0.007$), along with increased MHC class II+ cells, in lesional compared to uninvolved scalp skin. Further, keratin 15 expression was significantly reduced ($p=0.04$) in diseased tissue; whereas bulge Ki67+ and TUNEL+ cells were significantly more prominent. These results support the idea that bulge eHFSC damage is a key component in disease pathogenesis, and that bulge IP collapse is present in established disease.

088

Characterisation of T cell infiltration in guttate and chronic psoriasis

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T cell infiltration into the skin is associated with disease severity in psoriasis. A heterogeneous pattern of T cell infiltration, with CD8+ epidermal T cells and several different types of dermal CD4+ and CD8+ T cells has been proposed. The aim of the present project was to characterise the profile of T cells infiltrating into the skin during different clinical manifestations of psoriasis. The Stockholm Psoriasis cohort was utilised to collect skin biopsies from acute guttate and plaque psoriasis followed by FACS analysis of tissue derived cells and confocal microscopy of cryopreserved skin biopsies. T cell infiltration in epidermis was more pronounced during chronic as compared to guttate psoriasis. The number of T cells correlated to the epidermal hyperplasia in agreement with the notion that epidermal T cell infiltration drives the pathogenic process. Organised dermal lymphoid structures containing CD8+, CD4+ and FoxP3+ T cells together with dendritic cells were predominantly detected in dermis of chronic psoriasis. In chronic lesions the endothelium of dermal blood vessels frequently expressed peripheral node addressin (PNAd) which primarily if found on blood vessels in lymph nodes. One known function of PNAd is interactions with CD62L expressed on naive T cells as well as central memory cells. Our results indicate differential T cell infiltration in acute (guttate) and chronic (plaque) psoriasis. The presence of lymphoid blood vessels in the chronically inflamed skin could potentially allow different subtypes of circulating lymphocytes to enter the lesions as compared to normal skin.

089 [Oral 042]

The role of the receptor tyrosine kinase Axl in intercellular adhesion and signalling in cutaneous squamous cell carcinoma

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Axl is a receptor tyrosine kinase upregulated in many tumours including cutaneous squamous cell carcinoma (SCC) and breast cancer. Axl expression in cancer is associated with metastasis and poor prognosis. We hypothesized that Axl might play a role in intercellular adhesion which when disrupted leads to tumour progression and metastasis. To study this hypothesis, we established stable knock-down (KD) of Axl in cutaneous SCC and breast cancer cell lines overexpressing Axl. Subsequently, we studied colony morphology, intercellular adhesion and cell adhesion-related signalling pathways. We found that Axl KD led to formation of tighter colonies and increased strength of cell-cell interactions. Western blotting analysis and immunofluorescence staining of cell monolayers or organotypic cultures revealed that Axl KD in skin cancer cell lines caused re-distribution of cell junctional components such as alpha-catenin, p120-catenin, plakoglobin, ZO-1 and claudin-1 to the membrane (similar to control HaCaT keratinocytes). Live cell imaging revealed impaired migration of cells with Axl KD compared to controls. Furthermore, there was decreased nuclear localization and activation of beta-catenin and reduced phosphorylation of GSK3 in those cells suggesting that Axl plays a role in Wnt signalling. Our recent gene array data show changes in cell-matrix adhesion molecules that we are going to investigate further. We also observed that Axl expression correlates with putative cancer stem cell markers CD44 and ALDH-1 indicating that Axl might play a role in cancer stem cell resistance to chemotherapy. In conclusion, our results suggest that Axl is a promising target for future therapeutic intervention in skin cancer.

090 [Oral 043]

Defective granulation tissue formation in mice with specific ablation of integrin-linked kinase in fibroblasts - role of RhoA/ROCK signaling and TGFβ1 levels

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The extracellular matrix is a key regulator of cell functions, e.g. proliferation, differentiation and migration. Binding of matrix macromolecules to integrins initiates the assembly of large intracellular multiprotein complexes, the focal adhesions, which mediate cell-extracellular matrix communication. Integrin-linked kinase (ILK) is a central adapter recruited to integrin β1 tails in focal adhesions, connecting the outside environment to the actin cytoskeleton. Wound healing critically relies on mechanical activities of fibroblasts responding to forces transmitted across focal adhesions. Here, we report the generation of mice with inducible inactivation of ILK specifically in fibroblasts. Excisional wounds in these animals are characterized by absence of myofibroblasts, disturbed granulation tissue formation and strongly reduced wound contraction. Primary skin fibroblasts derived from such mice failed to secrete normal levels of TGFβ1 and showed strongly diminished αSMA expression, indicating that myofibroblast differentiation was hampered. This defect was rescued by exogenous TGFβ1, underscoring the importance of fibroblast-generated locally active TGFβ1 for myofibroblast function. ILK-null fibroblasts were also characterized by abnormal morphology due to impaired cell spreading. This was found to be due to enhanced RhoA/ROCK signaling. Addition of ROCK inhibitor Y-27632 not only restored cell morphology but also largely rescued TGFβ1 amounts in culture supernatants. We conclude that ILK is crucial for fibroblasts by limiting RhoA activity, which, if uncontrolled, precludes myofibroblast differentiation and function.

091 [Oral 044]

Transmembrane Collagen XVII Is Involved In The Glomerular Filtration Barrier Failure In Knock Out Mice

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Collagen XVII is a hemidesmosomal transmembrane component required for epithelial adhesion in skin. We have recently shown that collagen XVII is also expressed by podocytes, the visceral epithelial cells of the glomerulus. The role of collagen XVII in glomerular filtration barrier was further analyzed in collagen XVII knockout mice. Homozygous mice survived to birth, but had high neonatal mortality and growth retardation. As expected, all Col17-/- mice developed blisters and detachment of epidermis by applying mild mechanical trauma, non-pigmented hair growth and partial hair loss. Histopathology of skin showed subepidermal blistering, and electron microscopy revealed rudimentary and poorly developed hemidesmosomes, splitting of cutaneous basement membrane at the hemidesmosomal level and degeneration of basal keratinocytes. In addition to skin, the lack of collagen XVII caused several abnormalities in kidneys under physiological conditions. Autopsy of the Col17-/- mice revealed smaller and paler kidneys than those isolated from age matched control animals. In electron microscopy the number of glomeruli and the number and formation of podocytes and the foot processes was altered in mutant mice. Furthermore, a massive effacement of podocyte foot processes was detected, but no major slit diaphragm disruption. The glomerular basement membrane was detached especially on its endothelial side. Expression and localization of collagen IV was similar in control and mutant mice. We propose that collagen XVII is a novel component that attaches endothelial cells and podocyte foot processes to the glomerular basement membrane. It probably contributes to podocyte maturation and differentiation and may have a role in glomerular filtration.

092 [Oral 006]

Role of Caveolin-1 in Desmosomal Homeostasis and Desmoglein-Mediated Signaling

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Desmosomal cadherins are often found aberrantly expressed in various carcinomas. These cadherins can modulate signaling pathways directly relevant to epithelial cell proliferation and survival, however the mechanism by which these proteins could activate mitogenic signaling is not well understood. Using biochemical and immunological means, we demonstrate that desmogleins (Dsg) bound to and co-localized with caveolin-1 (Cav-1), the major protein of the specialized membrane microdomains caveolae. Caveolins have been implicated as modulators of signal transduction through the binding of key mitogenic signaling molecules to their scaffolding domains. Here we show by discontinuous sucrose-gradient ultracentrifugation the localization of Dsg with Cav-1 in the lipid raft fractions. Disruption of caveolae formation shifted Cav-1 and Dsg into non-raft fractions and compromised desmosomal cell-cell adhesion. Sequence analysis revealed that the intracellular cadherin segment of Dsg contain the putative Cav-1 binding motif. Using a cell permeable drug delivery competing peptide resembling the Cav-1 scaffolding domain, we showed that the peptide bound to Dsg further confirming a Dsg-Cav-1 interaction. Finally we demonstrate that Cav-1 modulates Dsg expression in the skin and may play an important role during the skin tumor development and the pathogenesis of pemphigus. In summary, the data presented here provide tantalizing clues that the mechanism by which Dsg mediate intracellular signaling may involve Cav-1.

093

Epidermal Tight Junction Maintains Calcium Distribution in Human Reconstructed Epidermis and Regulates the Epidermal Differentiation

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Tight junctions (TJs) among adjacent epithelial cells control paracellular permeability of solutes. Epidermal TJs are considered as restrictions on molecular movement to assist stratum corneum as secondary barrier in the skin. However, a role of TJ on molecular distribution in the epidermis is not closely studied. Calcium ion (Ca^{2+}), a well-known differentiation inducer for keratinocytes, distributes forming vertical gradient peaked at stratum granulosum. In this study, we applied sodium caprate (C10) which elicits dilations of TJ on human reconstructed epidermis, and investigated Ca^{2+} distribution in the epidermis. The localization of Ca^{2+} in the epidermis was observed by ion-capture cytochemistry and electron energy-loss spectroscopy of the transmission electron microscope. After C10 treatment, the epidermal Ca^{2+} localization was largely altered compared to the untreated epidermis. Ca^{2+}-containing precipitates appeared in intra- and extra-cellular spaces of the stratum corneum, and large clusters of Ca^{2+}-containing precipitates were occasionally observed in the stratum corneum and the stratum granulosum. Additionally, abnormal differentiation (e.g. parakeratosis) was also observed in the stratum granulosum. To confirm that these changes were caused by TJ disruption, we observed the structure of TJ strands by freeze fracture replica method, and measured trans-epidermal Ca^{2+} permeability by quantifying diffused Ca^{2+} through the epidermis. As a result, the TJ strands were fragmented and the Ca^{2+} permeability increased. These data suggest that the epidermal TJ maintains Ca^{2+} under the stratum corneum, and regulates the epidermal differentiation.

094

Phospholipid Scramblase 1 is Secreted by an Unconventional Mechanism and Interacts with the Extracellular Matrix Protein 1 in the Dermal Epidermal Junction Zone of Human Skin

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In an effort to define the biological functions of extracellular matrix protein 1 (ECM1) a yeast-two-hybrid screen was performed. This led to the identification of the type II transmembrane protein, phospholipid scramblase 1 (PLSCR1) as a binding partner. This interaction was confirmed by co-immunoprecipitation, and pull down experiments with GST-tagged ECM1a fragments identified that this interaction occurs within the tandem repeat region of ECM1a. Immunohistochemical staining revealed a partial overlap of ECM1 and PLSCR1 in human skin at the basal epidermal cell layer. Both proteins are deposited at the basal membrane in a dermal fibroblast dependent manner in human skin equivalents. Furthermore, immunogold electron microscopy of ultrathin human skin sections showed that ECM1 and PLSCR1 co-localize in the extracellular matrix. Using antibodies against ECM1 or PLSCR1 cross-linked to magnetic immunobeads we were able to prove the PLSCR1/ECM1 interaction in human skin extracts. Confocal microscopy on cultured keratinocytes demonstrated intracellular separation of both proteins with ECM1a in Golgi apparatus secretory vesicles and PLSCR1 mainly at the plasma membrane, thereby excluding intracellular interaction. Using different experimental set-ups we were able to exclude involvement of the Golgi apparatus, lysosomal transport, membrane translocation and palmitoylation as potential mechanisms for PLSCR1 secretion. However, methyl-β-cyclodextrin reduced the release of PLSCR1 from HaCat monolayer cultures. In summary, we here demonstrate that PLSCR1 interacts with the tandem repeat region of ECM1a in the dermal epidermal junction zone of the human skin and that PLSCR1 is secreted by a lipid raft-dependent pathway.

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Collagen deposition assessment in burn scar tissue using second harmonic generation and multi-photon microscopy

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High-resolution, high-contrast, three dimensional images of live cell and tissue architecture can be obtained using second harmonic generation (SHG), which comprises non-absorptive frequency changes in an excitation confocal laser line. SHG does not require any exogenous antibody or fluorophore labelling, and is capable of capturing images from unstained sections and of several key endogenous biomolecules, in a wide variety of species and from different types of processed tissue. Here, we examined normal control human skin sections and human burn scar tissues using SHG on a multi-photon microscope (MPM). Examination and comparison of normal human skin and burn scar tissue demonstrated clear fibrous dermal staining similar to dermal fibre collagen signal. Fluorescence-staining confirmed MPM-SHG collagen colocalisation with antibody staining for dermal collagen I but not fibronectin or elastin. Furthermore, we were able to detect collagen MPM-SHG signal in human frozen sections as well as in unstained paraffin embedded tissue sections. This same approach was also successful in localising collagen in porcine and ovine skin samples, and may be particularly important when species-specific antibodies may not be available. Taken together, our results demonstrate that MPM SHG-detection is a useful tool for examination of collagen architecture in human, porcine and ovine dermal tissue.

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Identification and characterisation of DSPIa, a novel isoform of human desmoplakin

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Desmoplakin is a ubiquitous component of desmosomes and desmosome-like structures, such as the cardiomyocyte area composita. Two major isoforms, desmoplakin I (DSPI) and desmoplakin II (DSPII) are encoded by alternative mRNA transcripts differentially spliced from the same gene. The resulting proteins are identical in amino acid sequence with the exception that DSPII contains only one third of the central alpha-helical rod domain present in DSPI. Here we describe a novel minor isoform of desmoplakin that is also produced by alternative splicing of the desmoplakin gene and that we name desmoplakin Ia (DSPIa). DSPIa is an alternatively spliced DSPI mRNA with a unique splice donor site that is 90% homologous to and downstream of the DSPII specific donor. The resulting DSPIa mRNA is in-frame and encodes a protein that has a central alpha-helical rod domain of intermediate size and that is 156 amino acids larger than DSPII and 443 amino acids smaller than DSPI. We demonstrate, through recombinant expression and short interfering RNA knockdown, that the DSPIa protein is readily detectable, albeit at substantially lower levels than the dominant isoforms, DSPI and DSPII. DSPIa mRNA has a similar tissue distribution to that of DSPI and of DSPII.

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The interstitial collagenases MMP-13 and MMP-14 are dispensable for the early onset of skin morphogenesis

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Connective tissue metabolism in skin is characterized by equilibrium of continuous synthesis and degradation of interstitial collagens e.g. the fibrillar collagen types I and III. These collagens are substrates of MMP-14 and MMP-13 the major collagenases synthesized by murine fibroblasts in vitro. MMP-14 deficiency *in vivo* results in death of mice by 3 weeks of age and several skeletal abnormalities, while MMP-13 deficiency has no obvious phenotype. We have analyzed the effect of ablation of MMP-14 on collagen metabolism *in vivo* and in vitro. Histological analysis did not display any abnormality in skin architecture and collagen distribution in MMP-14 knockout mice. In vitro, analysis of collagen expression in fibroblasts isolated from MMP-14 knockout animals showed accumulation of collagen type I as compared to control fibroblasts. Similarly, in human dermal fibroblasts silencing of MMP-14 resulted in an elevated collagen type I deposition on culture dishes. Nevertheless protein extraction of neonatal skin from MMP-14 knockout animals did not show any difference in collagen amount when compared to wild type animals. To investigate the role of MMP-13 in collagen metabolism, we generated mice carrying ablation of both genes. Surprisingly, mice depleted for both collagenolytic enzymes were born and recapitulate the MMP-14 knockout phenotype. Skin architecture and collagen distribution in these animals did not show any obvious difference or evidence for early fibrosis. In summary, our results show that ablation of both collagenases is compatible with birth and is dispensable for collagen metabolism in newly formed skin.

098

Epithelial-mesenchymal transition in skin fibrosis and tumor progression

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Epithelial-mesenchymal transition (EMT) is a process by which epithelial cells lose their epithelial characteristics and acquire mesenchymal-like phenotypes. EMT was initially described in early embryogenesis, however, we have recently revealed a role of EMT in the pathogenesis of postmenopausal frontal fibrosing alopecia. The aim of this study was to examine whether EMT took place also in systemic sclerosis and spindle cell squamous cell carcinoma (SCC). Immunohistochemisty using antibodies against EMT markers, Snail homolog 1 (SNAI1), Twist1 (TWIST1), and N-cadherin (CDH2), revealed that these proteins were expressed in the eccrine glands and hair follicles of patients with diffuse cutaneous SSc (dSSc) and spindle cell SCC cells. Real-time PCR analysis showed that SNAI1 mRNA expression was augmented in the skin of dSSc patients and spindle cell SCC. In a bleomycin injection-induced SSc model in mice, bleomycin promotes both Snai1 and collagen type 1 α1 (Col1a1) expression in the skin. To examine whether EMT inducer, TGF-β1, can evoke EMT in vitro, we cultured mouse keratinocyte cell line, PAM212 cells, in the presence of TGF-β1. TGF-β1 induced the spindle-shaped morphology of PAM212 cells and upregulated the expression of Snai1 and Col1a1, while it had no effect on other mesenchymal markers, indicating a partial recapitulation of EMT by TGF-β1 in vitro. These results suggest that EMT may be involved in the pathogenesis of SSc and spindle shaped SCC. A suppression of EMT, e.g. inhibition of SNAI1 and/or TGF-β1, may become a new therapeutic approach for SSc and spindle shaped SCC.

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Platelet-B16 melanoma interactions augment integrin-mediated tumor cell adhesion and promote lung metastasis formation

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Fundamental aspects determining the molecular basis for the reciprocal relationship between metastasizing tumor cells and soluble components of the coagulation cascade have recently become understood. However, the involvement of platelets, the cellular component of thrombus formation, in the pathogenesis of cancer metastasis is still poorly comprehended. Systemic platelet depletion before i.v. inoculation of luciferase-transduced murine B16 (B16-luc) into syngeneic C57BL/6 mice resulted in a more than 30 % decrease in micrometastasis as determined by bioluminescence analysis. While we found no significant platelet mediated effect on the growth of subcutaneously implanted B16 tumors, chemotaxis and migration, we determined an up to 50fold augmented adhesion of melanoma cells on immobilized platelets under static conditions and an up to 6fold increase in platelet-mediated B16 adhesion to endothelial cells under shear stress. Blocking mAb to alphaV integrin, highly expressed on 97,8% of B16 cells and blocking mAb to GPIIbIIIa, the most abundant platelet adhesion receptor, both significantly abrogated the platelet-mediated increase in in vitro B16 adhesion. Furthermore, systemic platelet depletion and an integrin αvβ3 antagonist synergistically decreased the B16 lung tumor burden up to 90% *in vivo*. Exploiting a model of intravital microscopy current experiments further elucidate the role of platelet-mediated B16 interactions with endothelial cells in the murine mesenteric microcirculation. Together, our results highlight a significant involvement of integrin-mediated interactions between platelets and tumor in the early phase of B16 lung metastasis and suggest that specific targeting of adhesion molecules involved in this process may represent a promising strategy for therapeutic intervention.

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Correlations of skin elasticity, fat mass and mimetic muscle function with sagging of human cheek

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Facial sagging is associated with aging, although the mechanism remains unclear. To clarify the mechanism, we examined the relationship of sagging severity at the cheek to skin elasticity, fat mass and facial muscle function. Faces of 108 healthy Japanese female volunteers (age range: 20s-60s) were photographed at the angle of 45 degrees. Standard scores of sagging severity were established by analyzing the photographs. We examined the correlations of sagging scores with skin elasticity measured with a Cutometer^{R}, fat content estimated by bioelectrical impedance analysis, and mimetic muscle function (lip sealing force) in middle-age female volunteers (age range: 30s-40s) with a wide range of sagging scores. Since the upper, lower and lateral areas in the cheek may independently show severe sagging, a six-grade score of sagging severity was separately established for each area. Scores in each area were significantly positively correlated with age (age range: 20s-60s). In middle-age volunteers, sagging scores were significantly negatively associated with skin elasticity. Body fat percentage was significantly positively correlated with sagging scores in the lower and lateral areas, though the correlation was weak in the upper area. Mimetic muscle function was significantly negatively correlated with sagging score at the upper area of cheek. This result led us to search for activators of myocyte function. We found that Sanguisorba officinalis L. extract could activate myocytes, in terms of respiratory activity measured with Alamar blue. In conclusion, cheek skin sagging may be associated with increased fat mass and functional decline of dermis and mimetic muscle.

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Increased Mechanical Tension by Injection of Cross-Linked Hyaluronic Acid Dermal Filler Restores Collagen-I Production in Naturally-Aged Human Skin

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During natural aging, human skin becomes thin and fragile. These changes largely result from fragmentation and reduced production of type I collagen (COL-I), which comprises the bulk of the dermal extracellular matrix (ECM). We hypothesize that COL-I fragmentation reduces mechanical tension within the ECM, thereby creating an environment that down-regulates COL-I production. Injection of cross-linked hyaluronic acid dermal filler (CL-HA) has been shown to stimulate increase mechanical tension within the dermis in photoaged skin. Therefore, we have examined the impact of increasing mechanical tension by injection of CL-HA, in aged (>80 years) human skin. Fibroblasts within CL-HA-injected dermis displayed a stretched morphology, indicative of increased mechanical tension. CL-HA stimulated expression of HSP47 and prolyl-4-hydroxylase (5-9-fold, N=10, $p<0.01$), two enzymes that are required for COL-I synthesis. CL-HA induced COL-I mRNA (6-fold, N=10, $p<0.01$) and protein (3-fold, N=10, $p<0.01$). Immunohistology revealed increased COL-I expression in fibroblasts. COL-I production is strongly regulated by the TGF-β pathway. Notably, CL-HA increased fibroblast expression of type II TGF-β receptor (TβRII, 3-fold, N=10, $p<0.01$) and its downstream effector CTGF/CCN2 (4-fold, N=10, $p<0.01$). Injection of CL-HA into dermal equivalents mimicked the effects observed *in vivo*, whereas injection of monomeric HA had no effect. CL-HA-induced COL-I production was blocked by TβRI kinase inhibitor. These data support the concepts that reduced dermal ECM structural integrity in aged skin down-regulates COL-I production, and aged skin retains substantial capacity to up-regulate COL-I synthesis. These findings provide rationale for therapeutic intervention to induce COL-I production to improve the health of aged human skin.

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Regulation of Cell Shape and Mechanical Tension in Aging Human Dermal Fibroblasts

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Skin aging is a complex process involving a series of cellular and extracellular matrix events. Recent studies indicate that both intrinsic aging and photodamage can lead to alterations of normal cellular behaviors. The important factors contributing to this process include degradation of extracellular matrix and a partial breakdown of focal adhesion complexes. This matrix damage is believed to lead to changes in mechanical tension and collapse of the cell shape of dermal fibroblasts. To better understand changes in cell shape and tension during the skin aging process, we developed a model to mimic these changes in vitro. It was observed that irradiation of human dermal fibroblasts with UVA leads to distinct changes in cell shape. A customized software program was developed to quantify various cell shape parameters. In addition, a novel CellDrum™ system was used for in-vitro measurements of mechanical properties of cell monolayers. This system allows for the evaluation of properties such as the rigidity and contractile strength of cells. The experiments showed reduced cell tension in cells from old donors compared to young donors, and UV treatment of these cells further decreased cell tension. In order to improve cell shape and mechanical properties in aged cells, we developed a technology that is a blend of novel plant extracts and bioactive synthetic molecules. It was observed that treatment of cells with this novel technology improves cell shape and mechanical tension in cells from older donors, suggesting that this technology may be beneficial in improving the appearance of aging skin.

ABSTRACTS

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Identification and validation of modulators of ECM1 expression in keratinocytes and reconstructed human skin equivalent

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The extracellular matrix protein 1 (ECM1) is an 85kDa secreted glycoprotein present in the dermal-epidermal junction (DEJ) zone of the human skin. It interacts with skin structural and extracellular matrix molecules (perlecan, laminin 332, fibulin-1C/D,-3) as well as dermal interstitial molecules (MMP-9, collagen type IV, FN, HA) providing a role in maintaining the functional integrity of human skin. Previous studies have shown that ECM1 expression is down regulated in intrinsically aged skin. Therefore, selectively enhancing ECM1 expression may be a potent way to maintain the integrity of the basement membrane and to prevent related skin disorders. The aim of this study was to identify new ingredients able to stimulate ECM1 production and to assess their putative beneficial effects on the DEJ zone in human reconstructed skin. Thirty compounds were screened, among which 6 revealed a significant stimulation of ECM1 expression in HaCat keratinocyte monolayer cultures. The effect of three actives on epidermal morphogenesis and basement membrane formation was studied in reconstructed human skin equivalents (HSE) by immunohistochemical analysis of proliferation, differentiation and markers of the basement membrane. Positive compounds could be candidates to fight intrinsic skin aging and its related negative effects.

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Short And Long Forms Of Latent TGF-Beta Binding Protein (LTBP)-4 Are Independently Regulated In Cultured Fibroblasts And Human Tissues

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Transforming growth factor (TGF)-beta is secreted and targeted into the extracellular matrix (ECM) in association with one of the latent TGF-b binding proteins (LTBPs). Activation of TGF-b from the latent complexes is a central regulatory step in TGF-b signaling. LTBPs target the growth factor into the ECM and expose it to activating mechanisms in specific ways. Disruption of LTBP-4 gene causes severe developmental abnormalities in both humans and mice. Transcripts for two N-terminally distinct LTBP-4 variants, LTBP-4S (short) and -4L (long), have been identified. In the current work, we have characterized the differences in the expression, processing, and ECM targeting of these LTBP-4 variants. Heart and skeletal muscle displayed expression of both variants. Interestingly, liver expressed mainly LTBP-4L and lung as well as small intestine LTBP-4S. This tissue-specific expression pattern was found to originate from control of transcription by two independent promoters. Accordingly, LTBP-4S and -4L proteins were secreted and processed differently. During secretion LTBP-4L was complexed with TGF-b1, whereas the majority of LTBP-4S was secreted in a free form. In addition, LTBP-4S was efficiently incorporated into the ECM, while full-length LTBP-4L was not readily detectable in the ECM. These data suggest that the functions of LTBP-4 are modified by tissue-specific expression of the two N-terminally distinct variants, which in addition exhibit significant differences in cellular processing and targeting. These features provide the basis for understanding the molecular diversity in LTBP-4 structure and function.

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Ultrastructural analysis of acantholysis in pemphigus foliaceus reexamined from the current perspective

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Pemphigus foliaceus (PF) is an auto-immune skin disease characterized by autoantibodies against desmoglein 1 and subcorneal blistering as a result of acantholysis. The exact mechanism how pemphigus antibodies induce acantholysis is not clear: theories mentioned are steric hindrance of desmoglein adhesion, desmoglein depletion of desmosomes by influencing assembly and disassembly, desmoglein compensation, disturbed cellular signalling, and keratin skeleton collapse. Original electron microscopy (EM) studies date back before these theories were formulated. In this EM study, we studied skin of PF patients to gain more insight into the mechanism of acantholysis considering the new theories. Three Nikolsky positive (N+) and two lesional biopsies from skin of PF patients were studied by light microscopy and EM. Subcorneal blistering was observed in two N+ biopsies and the lesional biopsies. At the granular and spinous layer we saw desmosomes torn off behind the desmosomal plaque, half-desmosome remnants, hypoplastic desmosomes and a reduction of the number of desmosomes in N+ and lesional skin. All biopsies showed intercellular interdesmosomal widening in the basal cell layer. Two N+ biopsies and the lesional biopsies showed a reduced number of desmosomes, and hypoplastic desmosomes in the basal cell layer. Keratin skeleton collapse was not observed. Steric hindrance does not explain the phenomenon of intracellularly torn-off desmosomes. The hypoplastic desmosomes and reduction of the number of desmosomes suggest that desmoglein depletion by disturbed assembly or disassembly of desmosomes is a major mechanism in the pathogenesis of PF. The pathology in the lower epidermis is suggestive for compensation by desmoglein 3.

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Structural diversity of human and mouse collagen XVII extracellular linker domain cause differences in shedding behavior

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The epithelial adhesion molecule collagen XVII represents a transmembrane component of hemidesmosomes. In our previous studies we have extensively analyzed ectodomain shedding of human collagen XVII within its extracellular linker domain NC16A. This is catalyzed by metalloproteinases of the ADAMs family and depends rather on structural molecule motifs than on specific amino acid sequences. Since the extracellular linker domain of human and murine collagen XVII shows high amino acid diversity, the goal of this study was to compare shedding of transiently transfected human and mouse collagen XVII constructs with defined deletions within their linker domain. In previous studies we have already analyzed ten human collagen XVII deletion constructs. Here, we investigated six linker domain deletion constructs of murine collagen XVII. Their normal membrane integration and Golgi transition was demonstrated by cell surface biotinylation and Endo H insensibility. The shortest deletion of human collagen XVII which resulted in non-shedding was a 20 amino acid deletion spanning Ale^{528} to Glu^{547}. In contrast, deletion of 20 corresponding amino acids in murine collagen XVII (Glu^{534} to Glu^{553}) do not result in decreased shedding. It revealed that the shortest deletion in murine collagen XVII which led to complete loss of shedding was a 32 amino acid deletion, from Lys^{513} to Ser^{544}. Secondary protein structure predictions of human and mouse linker domains indicated significant differences in their molecular structures. Our results suggest that human and murine collagen XVII molecules vary in their extracellular linker domain structure which in turn can alter regulation of shedding.

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Antifibrotic Effects of Dextran Sulphate on Keloid Fibroblasts

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Keloid is caused by abnormal wound healing process with excessive fibroblastic proliferation and overproduction of extracellular matrix. Despite numerous studies, the pathogenesis and optimal therapy have not been fully established. Dextran sulfate (DS) is sulfuric acid esters of polysaccharide, which has been used for treatment of hypercholesterolemia, atherosclerosis, and keloid (intralesional injection, 60 mg/ml, low molecular weight 3500-4000 with sulfur content 3-6%). DS has been shown to have various inhibitory effects on various cell types, including antiproliferative, anti-adhesive and anti-hyaluronidase effects. We sought to elucidate the antifibrotic effects of DS on normal fibroblasts (NF) and keloid fibroblasts (KF) and the mechanism involved. Our results showed that high molecular weight DS (molecular weight 500,000) at relatively low concentrations (25-100 μg/ml) displayed dose-dependent inhibitory effects on NF and KF in cell proliferation, migration, adhesion and gel contraction of the stressed delayed released fibroblast-populated collagen lattices. Compared with NF, DS showed a greater anti-proliferative effect on KF (DS 100 μg/ml, $p=0.047$). RT-PCR studies showed variable inhibitory effects on mRNA expression of TGF-β1, β2, β3 (TGF-β1 > β2 >> β3) but mild or no obvious inhibitory effects on MMP-1, collagen, fibronectin, and IL-6 mRNA expression. In conclusion, our study showed that DS at relatively low concentrations displays suppressive effects on cell proliferation, migration, adhesion, contraction of collagen gel, and mRNA expression of TGF-β of normal and keloid fibroblasts, and these results lend supports to the therapeutic effects of intralesional injection observed clinically.

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Kindlin-1 and -2 have overlapping functions in epithelial cells: implications for phenotype modification

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Kindlins are a novel family of intracellular adaptor proteins in integrin-containing focal adhesions. Kindlin-1 and -2 are expressed in the skin, but whether and how they cooperate in adult epithelial cells has remained elusive. Here, we uncovered the overlapping roles of kindlin-1 and kindlin-2 in maintaining epithelial integrity and show that the phenotype of kindlin-1 deficient cells can be modulated by regulating kindlin-2 gene expression, and vice versa. The experimental evidence is provided by use of human keratinocyte cell lines which express either both kindlins, just kindlin-1 or kindlin-2, or none of them. Double deficiency of kindlin-1 and -2 had dramatic negative effects on focal adhesion formation and actin cytoskeleton organization, but also on cell adhesion, proliferation, directional migration and activation of β1 integrin, whereas deficiency of one kindlin only showed variable perturbation of these functions. Cell motility and formation of cell-cell contacts were particularly affected by lack of kindlin-2. These results predict that kindlin-1 and -2 can functionally compensate for each other, at least in part. The high physiological and pathological significance of the compensation was emphasized by the discovery of environmental regulation of kindlin-2 expression. UVB irradiation induced loss of kindlin-2 in keratinocytes and had negative effects on kindlin-1 deficient cells. This first example of environmental regulation of kindlin expression has implications for phenotype modulation in Kindler syndrome, a skin disorder caused by kindlin-1 deficiency.

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Biosensors in monitoring concentration of anesthetics in tissues

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Nowadays, in the period of swift development of dermatosurgery and cosmeticology, actual is a problem of anesthetizing of operative interventions and small invasion manipulations with the clear monitoring of concentration and distribution of anethetic matters in tissues. During operating or other medical manipulations with the using of acesodynes, which in most cases are toxic, it is necessery to control strictly concentrations of aenestetics for an organism and fast of leading out due to physiology processes.Existing methodologies and apparatus are extraordinarily high-costs and bulky, and practically does not allow to conduct monitoring *in vivo*. An alternative to such methods is using of biosensory devices. They consider small invasion fiber-optics biosensores of reflacting type with a sensitive element on the butt end of general optical channel, which is entered directly in the tissues of organism. The nanocomposite membrane structure of photochromic biomolecules of bacteriorodopsin in porous polymeric or inorganic sol- gelnic matrixes will be used in a role of anesthetics element. Change of parameters of photocycle in membranes structures with bacteriorodopsin under the action of anesthetics is the basis in methodology of controling anestethic concentration. Besides of reversibility, high sensitivity peculiar for bacteriorodopsin is high quickness of response, due to the microporosity of matrixes, allowes to create small invasion biosensors of anestetics which work in the real mode of time.

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Differential expression of latent transforming growth factor β-binding proteins in intrinsically aged skin

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The dermal elastic fibre network imbues skin with its capacity to recoil. In intrinsically aged skin however, loss of this mechanical property contributes to an aged appearance. Elastic fibres are structurally complex, and as a consequence the effects of ageing on the composition of these macro-molecular assemblies remains poorly defined. In this study, we have used an Affymetrix® microarray-based approach to detect age-related changes in gene expression of elastic fibre-associated molecules. Skin biopsies (6mm diameter) were obtained from photoprotected buttock of three young (24-26 years) and three aged (74-75 years) male volunteers. Biopsies were bisected and processed for total RNA extraction, cDNA synthesis and hybridization (Affymetrix® U133A oligonucleotide array). A comparison of the gene expression profiles from young and aged individuals revealed that whilst the expression of the majority of elastic fibre components was unchanged with age, latent TGFβ-binding protein (LTBP)-2 was up-regulated ($p<0.05$) and LTBP-3 was down-regulated ($p<0.05$). Differential expression and translation of these genes was confirmed by quantitative real-time PCR and immunohistology. Whilst the functional implications of this altered expression profile remain to be determined, LTBPs-2 and 3 are thought to play important roles in controlling and maintaining elastic fibre deposition, structure and TGFβ signalling. As a consequence aberrant LTBP expression may influence both tissue mechanical properties and homeostasis in ageing skin.

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MMP-9, -10, and -26, and TIMP-1 expression in relation to filaggrin mutations in atopic dermatitis

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In addition to skin cancer and wound healing, matrix metalloproteinases (MMPs) have been implicated in atopic dermatitis (AD) related features, such as inflammation and processing of proteins needed for an intact epidermal barrier. A major component of the skin permeability barrier, filaggrin (FLG), is the strongest candidate gene for AD. We determined three common FLG mutations in 28 clinically moderate to severe AD patients and studied by immunohistochemistry the expression of MMP-9, -10, and more novel MMP-26 as well as TIMP-1 proteins in relation to FLG expression in AD. Only one of our 28 patients carried a FLG_S3247X mutation and two carried a FLG_R501X mutation. None of the patients carried a FLG_R2447X mutation or was a compound heterozygote. Our patient carrying a FLG_S3247X mutation had a distinct, faint and dotted immunostaining pattern of FLG both in the lesional and non-lesional skin, confirming the impact of FLG mutations to the skin epidermal barrier. The MMP profile reflected rather the histological features of the sample than the FLG mutation status. Basal and suprabasal keratinocytes expressed MMP-10 in 14/28 and MMP-26 in 15/28 of the AD samples, reflecting possibly the basement membrane disruptions. Even in non-lesional samples, MMP-10 (7/28), but not MMP-26, was demonstrated in keratinocytes. Inflammatory cells i.e. neutrophils (11/28) or macrophages (11/28) expressed MMP-9 and macrophages (11/28) MMP-10. Expression of TIMP-1 protein was encountered not only in the lesional AD samples but also in the non-lesional samples in macrophage/fibroblast-like cells.

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A potential role for oxygen in the photodegradation of fibrillin microfibrils by UVB radiation

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Exposure to ultraviolet radiation is thought to accelerate skin ageing via the activation and/or the enhanced expression of extracellular matrix (ECM) proteases. Whilst such enzyme-mediated pathways may play a role in photoageing, we have previously demonstrated that key ECM components (fibrillin microfibrils, fibronectin and type I collagen) are differentially degraded by UVB in enzyme-free environments. However, it remains unclear whether this UVB-induced damage occurs directly by photon absorption or indirectly via reactive oxygen species (ROS) intermediates. In this study we have characterised the effects of UVB irradiation in both ambient-oxygen and depleted-oxygen environments on microfibril structure. Aliquots of Cos-1 cell-derived microfibrils were adsorbed to hydrophilic mica substrates either: i) without any exposure to UVB radiation, ii) following exposure to 500mJ/cm^2 UVB radiation (TL12; 280-315nm) in ambient-oxygen conditions or iii) after exposure to the same UVB dose in an depleted-oxygen environment (achieved by bubbling with N_2 for 10min). Adsorbed microfibrils were subsequently imaged by atomic force microscopy. Relative microfibril flexibility (quantified via the angle between adjacent microfibril beads) was significantly increased (Mann-Whitney U test, $p = <0.0001$) following UVB exposure in both ambient oxygen and depleted-oxygen conditions (median angle: control = 170°; ambient-oxygen = 161°; depleted-oxygen = 165°). In the oxygen-depleted environment however, these UV-radiation induced structural changes were significantly abrogated (Mann-Whitney U test; ambient-oxygen vs. depleted-oxygen; $p = 0.0031$).These findings suggest that UVB-induced ROS play an important role in the photodegradation of ECM proteins in photoaged skin independent of protease activity.

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Loss of type VII collagen in cutaneous SCC increases cell invasion through TGF-beta signalling

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Mutations in the type VII collagen (ColVII) gene cause the severe blistering disorder, recessive dystrophic epidermolysis bullosa (RDEB), associated with increased risk of metastatic squamous cell carcinoma (SCC). We have previously shown that transient knock-down of ColVII (siCOL7) increased SCC migration/invasion, with disorganised epithelial differentiation and increased epithelial-mesenchymal transition. Increased expression of chemokines (CXC) and p-Smad2/3 was also observed in siCOL7 invading cells suggesting involvement of TGF-beta signalling. Furthermore, results from Illumina gene expression arrays demonstrated changes in TGF-beta targets. In this study, we explored further the role of TGF-beta in invasion of SCC cells with absent ColVII. Stable knock-down of ColVII was generated in SCC cell lines using a lentiviral shRNA system. Tracking of single cells by live cell imaging suggested that ColVII-knock-down cells (shCOL7) move faster, forming longer membrane protrusions, and create longer tracks when compared to control cells (shC). Interestingly, addition of a TGF-beta type I receptor inhibitor, SD-208, inhibited shCOL7 cell movement in monolayers and reduced the number of cell islands invading into 3-D collagen:matrigel gels. ColVII-knock-down cells showed increased phosphorylation of ERK1/2, downstream of the TGF-beta receptor complex. Moreover, shCOL7 3-D epidermal-dermal composites demonstrated increased expression of the TGF-beta-related proteins CTGF in the dermis and CLIC4 in epidermis compared to control. Immunostaining of RDEB SCC tumours suggested similar findings *in vivo*. Our results suggest that the TGF-beta pathway may play a critical role in migration and epithelial-mesenchymal interaction of SCC cells with absent ColVII providing a possible explanation for the accelerated metastasis in RDEB.

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Maturation and assembly of microfibrils in vitro

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Most extracellular matrix components form long-lived assemblies whose structures vary in remodelling and diseased tissues. Fibrillin-rich microfibrils, for example, are composite components of the elastic fibre system which confer elasticity in skin. Whilst ageing impacts greatly on this system, previous studies have demonstrated that a number of topical treatments, including retinoids, can stimulate deposition of fibrillin. However, it is unknown whether these interventions result in the formation of structurally and functionally competent microfibrils. Therefore, it is important to develop a model system with which to study microfibril maturation in response to exogenous agents. Here, we employ quantitative biochemical and ultrastructural approaches to characterise the ability of cultured human dermal fibroblasts (HDF) to synthesise mature microfibrillar assemblies.Microfibrils were isolated from HDF cultures at 1-, 2- and 3-weeks post-confluency using a well established bacterial collagenase and size exclusion chromatography approach. Microfibrillar components including fibrillins-1 and -2, MAGP-1 and decorin were identified by immunodetection and quantified by densitometric analysis. With the exception of fibrillin-2, these components accrued significantly with increasing time in culture (Mann Whitney U test: $p<0.05$: relative signal intensity (RSI) at week 1: fibrillin-1 $6.9x10^5$ (SD $5.1x10^5$); MAGP-1 $6.3x10^5$ (SD 6.2x104); decorin $6.3x10^5$ (SD $1.0x10^5$): RSI at week 3: fibrillin-1 $1.3x10^6$ (SD $2.1x10^5$); MAGP-1 $2.1x10^6$ (SD $2.6x10^5$); decorin $1.1x10^6$ (SD $4.8x10^4$). Therefore, given the observed increase in microfibril complexity with time in culture and the apparent reciprocal relationship between fibrillin-1 and -2 expression, we suggest that cultured HDFs may provide a robust experimental system with which to study microfibril maturation.

115 [Oral 039]

Integration of innate immune signalling pathways in epidermal barrier dysfunction and hyperkeratosis

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The signalling mechanisms activated in barrier defective epidermis are yet to be fully elucidated. Understanding of these mechanisms and how they result in hyperkeratosis will lead to better directed therapies for hyperkeratotic disorders such as the ichthyoses. We have modelled a severe epidermal barrier defect using siRNA knockdown of the principle gene mutated in lamellar ichthyosis (LI), Transglutaminase-1, in transformed rat keratinocytes and created an in vitro organotypic culture model that closely mimics the disease. A principle signalling pathway that was activated in this model was the NFkappaBeta pathway, mediated by constitutive degradation of IkappaBalpha, consistent with this, interleukin-1 alpha (IL1A) expression was markedly (30 fold+) increased. All LI patients tested had increased IL1A and treatment of wildtype organotypic cultures with IL1A was sufficient to induce hyperkeratosis. Treatment of disease mimic organotypic cultures with IL-1 receptor antagonist (IL1RA) led to a dose-dependent decrease in hyperkeratosis without a reduction in non-polar lipids in the cornified layer, which has the potential as a treatment to reduce scaling without the requirement to constantly apply emollients. IL1A blockade by IL1RA didn't prevent the IkappaBalpha. Kinases active in the Tgm1 siRNA expressing cells, downstream of IL1A and NFkB, were inhibited with IL1RA treatment, while activation of kinases upstream of NFkB and downstream of toll-like receptors was maintained. Based on these findings we propose a model in which there are two pathways upstream of NFkB, an IL1A mediated hyperkeratosis pathway, and a barrier dysfunction "sensor" pathway, mediated by Toll-like receptor signalling and constitutive IkappaBalpha degradation.

116 [Oral 054]

Thyrotropin-releasing hormone (TRH) controls mitochondrial function and biogenesis in human keratinocytes in situ

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Thyroid hormones can stimulate mitochondrial capacity and metabolic potential. Here, we have asked whether thyrotropin-releasing hormone (TRH), the key hypothalamic integrator of energy metabolism, directly modulates mitochondrial functions in normal human skin. After showing that human epidermis expresses TRH receptor protein, organ-cultured human skin was treated with TRH (5-100 ng/ml) for 12-48 hrs. While this did not alter epidermal morphology, TRH significantly increased immunoreactivity for the mitochondria-selective subunit I of respiratory chain complex IV (MTCO1). Since this suggested an up-regulation of mitochondrial biogenesis, transmission electron microscopy was performed. This revealed an increased number of perinuclear mitochondria in individual keratinocytes in TRH-treated epidermis. In human epidermal extracts, TRH significantly enhanced mitochondrial complex I and IV enzyme activity. This effect could be further increased by adding respiratory substrates (malate, glutamate). Furthermore, TRH treatment significantly increased the oxygen consumption of human skin in situ. TRH also significantly stimulated the transcription of several mitochondrial key genes (MTCO1, mitochondrial transcription factor A [TFAM], heat shock protein 60 [HSP60], peroxisome proliferator-activated receptor gamma coactivator alpha [PGC-1α] and brain and muscle ARNT-like protein 1 [BMAL1]) as well as the amount of synthesized mitochondrial DNA. These findings document complex stimulatory effects of TRH on mitochondrial function, and identify TRH as a potent novel neuroendocrine stimulator of mitochondrial activity and biogenesis in human skin epithelium in situ. Furthermore, we demonstrate that, contrary to conventional wisdom in mitochondrial research, human epidermis offers an excellent model system for dissecting neuroendocrine controls of human mitochondrial biology under physiologically relevant conditions.

117 [Oral 055]

p63 and p53 transcription factors co-regulate RUNX1 to control proliferation to differentiation transition in human interfollicular epidermis

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The interfollicular epidermis is continuously renewed thanks to a regulated balance between proliferation and differentiation. The transcription factor deltaNp63 plays a key role in the control of this process. It has recently been shown that deltaNp63 directly regulates the RUNX1 transcription factor expression in mouse keratinocytes and we have shown in the lab that RUNX1 is implicated in the proliferation to differentiation transition control in human keratinocytes. In this study, we investigated RUNX1 transcriptional regulation by deltaNp63 in human primary keratinocytes. We demonstrated that deltaNp63 directly binds two different RUNX1 regulatory DNA sequences and modulates its expression differentially in proliferative or confluent human primary keratinocytes. We also showed that this RUNX1 expression regulation by deltaNp63 is p53 dependent and that co-regulation of RUNX1 by p53 and deltaNp63 lies on differential binding and activation of RUNX1 regulatory sequences. Collectively, these data shed light on the role of p53 for interfollicular epidermal homeostasis regulation, in cooperation with deltaNp63, via the regulation of RUNX1 transcription factor expression.

118 [Oral 056]

Hornerin is a component of cornified cell envelopes which may contribute to atopic dermatitis epidermal barrier defect

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Although loss-of-function mutations in the filaggrin gene have been strongly associated with atopic dermatitis (AD), it has been suggested that other epidermal barrier related genes may account for the predisposition to the disease. A single nucleotide polymorphism within the gene encoding hornerin (HRNR) has recently been linked with AD susceptibility. HRNR shares numerous common features with filaggrin. Conflicting reports have been published concerning HRNR expression in the epidermis, and its role in epidermal barrier function is still unknown. To analyze HRNR expression and to characterize its function in human skin, we produced polyclonal anti-peptide antibodies. Immunohistochemical, confocal and immunoelectron microscopy analysis of normal skin sections showed that HRNR colocalizes with profilaggrin on keratohyalin granules in the upper granular layer. It was also detected in the entire cornified layer at the corneocyte periphery. Detected in western as numerous immunoreactive bands, HRNR was relatively insoluble and only extracted from normal epidermis with urea- and/or reducing agent-containing buffers. These data suggested that HRNR could be a component of cornified cell envelopes. To prove this hypothesis, we used cell envelopes purified from plantar stratum corneum. Immunodetection of proteolytic peptides and immunoelectron microscopy analysis confirmed the presence of HRNR in these structures. Moreover, in vitro assays with a recombinant form of HRNR showed that it is a substrate of transglutaminase 3. As a whole, these data demonstrate that HRNR is a component of cornified cell envelopes. Its reduced expression in AD lesions may contribute to the barrier defect observed in the disease.

119 [Oral 057]

Involvement of Proteinase Activated Receptor -2 on Mite-induced Atopic Dermatitis Model in Filaggrin Deficiency Mouse

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The barrier abnormality by a loss-of-function mutation in the gene encoding filaggrin (FLG) is an important factor in the pathogenesis of atopic dermatitis (AD). Recently, we have demonstrated that flaky tail (Flg^{ft}) mice, essentially deficient in filaggrin, denote human AD in the steady state and by topical application of Dermatophagoides pteronyssimus mite extracts that acts as a protease-activated receptor-2 agonist. PAR-2 is known to be involved in epidermal permeability barrier function and mediates itch in AD. However, the relevancy between PAR-2 activation and filaggrin deficiency has not been revealed. In this study, we utilized a newly developed specific PAR-2 antagonist agent on mite allergen-repeated exposure-induced AD model in Flg^{ft} mice. We found that PAR-2 was strongly expressed in keratinocytes of Flg^{ft} mice compared to control mice. Application of the PAR-2 antagonist improved the clinical manifestation of mite-induced AD model in Flg^{ft} mice. In addition, epidermal hyperproliferation and increased pH in accord with production of Th2 and proinflammatory cytokines of Flg^{ft} mice were inhibited by the PAR-2 antagonist. Moreover, total cells in skin draining lymph nodes, mite specific IgE, and transepidermal water loss were reduced in the PAR-2 antagonis-treated group. These results suggest that filaggrin-deficiency induces PAR-2 expression in keratinocytes, which further shifts the Th2 skewed condition and exacerbates AD.

120 [Oral 058]

IGFBP7 plays a critical role in the pathogenesis of psoriasis

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Psoriasis has been shown to result from combined immunological and epidermal defects. We recently demonstrated that IGFBP7 is downregulated in the psoriatic epidermis and regulates three processes typically abnormal in psoriatic keratinocytes: proliferation, apoptotic activity and differentiation. Since IGFBP7 was found to oppose psoriasis-associated abnormalities in epidermal cells, we hypothesized that IGFBP7 may play a role in the pathogenesis of the disease. To substantiate this hypothesis, we assessed the pathological features of three dimensional organotypic cell cultures following RNAi-induced IGFBP7 down-regulation. We observed that the absence of IGFBP7 expression in the stratifying epidermis was associated with marked with marked hyperkeratosis and acanthosis. Supporting a role for IGFBP7 in psoriasis, we demonstrated that down-regulation of IGFBP7 induces ERK1/2 phosphorylation in keratinocytes as seen in psoriasis. These results suggested that IGFBP7 may play a role in the pathogenesis of psoriasis. To assess this possibility, we treated SCID mice grafted with human skin in which psoriasis had been induced with NK cells for 10 days with (1) PBS (n=5), (2) dexamethasone (n=5; 2 μg/mouse/day) and (3) recombinant IGFBP7 (n=5; 2,4 μg/mouse/day). All mice treated with PBS developed psoriasis. In contrast, dexamethasone cured psoriasis in 5/5 of the mice. Recombinant IGFBP7 cured psoriasis in 3/5 mice with an additional mouse showing partial response. Taken altogether, our data position IGFBP7 as a key regulator of keratinocyte differentiation and proliferation and provide evidence for IGFBP7 contribution to the pathogenesis of psoriasis.

121 [Oral 059]

Deimination of filaggrin-2 increases its proteolysis by calpain I

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Filaggrin-2 (FLG2), a member of the S100 fused-type protein family, shares numerous common features with filaggrin, a key protein implicated in the epidermal barrier functions. Both display a related structural organisation, an identical pattern of expression and localisation in human epidermis, and proteolytic processing of a large precursor. Here, we tested whether FLG2 is a substrate of calpain 1, a calcium-dependent protease directly involved in filaggrin catabolism. In addition, deimination being critical for filaggrin degradation, we analyzed whether FLG2 deimination interferes with its proteolytic processing. To this aim, we first produced a recombinant form of FLG2 corresponding to subunits B7 to B10 fused to a carboxy-terminal His-tag. Incubation with calpain 1 in the presence of calcium induced a rapid degradation of the recombinant protein, and the production of several peptides, as shown by Coomassie blue stained gels and Western blotting with anti-FLG2 or anti-His antibodies. The degradation was not observed when a calpain 1-specific inhibitor was added. MALDI-TOF mass spectrometry confirmed this result and further evidenced the production of non-immunoreactive smaller peptides. The calpain cleavage sites identified by Edman degradation were shown to be frequent in the repeat-B domain. Moreover, immunohistochemical analysis of normal human skin revealed co-localization of FLG-2 and calpain 1 in the granular layer. Finally, the FLG2 deiminated by human peptidylarginine deiminases was shown to be more susceptible to calpain 1 than the unmodified protein. Altogether, these data suggest that calpain 1 is essential for the proteolytic processing of FLG2, and that deimination accelerates this process.

122 [Oral 060]

The Ubiquitous Dermokine Delta Directly Interacts With Rab5 And Modulates Its Activity In The Early Endocytic Pathway

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The Dermokine (Dmkn) gene is highly expressed by granular keratinocytes. Its expression leads to four families of proteins with as yet unknown functions. The secreted α, β and γ isoforms share an epidermis-restricted expression pattern, whereas the δ isoform is intracellular and ubiquitous. To get an insight into Dmknδ function, we performed yeast two-hybrid screening and identified the small GTPases Rab5 as partners for Dmknδ. The Rab5 proteins are known to regulate membrane docking and fusion in the early endocytic pathway. GST pull-down assays confirmed the direct interaction between Rab5 and Dmknδ. We also showed that Dmknδ was able to bind both inactive (GDP-bound) and active (GTP-bound) forms of Rab5 in vitro but preferentially targeted GDP-bound form in HeLa cells. Transient expression of Dmknδ in HeLa cells led to the formation of punctate structures colocalized with endogenous Rab5 and clathrin, indicating Dmknδ involvement in the early steps of endocytosis. Interestingly, Dmknδ expression rescued the Rab5S34N-mediated inhibition of endosome fusion. Moreover, Dmknδ caused the enlargement of vesicles positive for Rab5 by promoting GTP loading onto the small GTPase. Altogether, our data suggest that Dmknδ is involved in the early endosomal trafficking by activating Rab5 function. This work will be pursued by studying Dmknδ function and more widely the endovesicular traffic in the granular keratinocyte, which is a polarized cell with high secretory activity.

123 [Oral 061]

Insulin/IGF-1 signaling controls epidermal morphogenesis by regulating G2/M cell cycle progression

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The skin epidermal barrier protects the organism from external challenges and dehydration. The establishment, maintenance and restoration of this epithelia barrier is driven by a life long selfrenewal of epithelial progenitor cells. Recently, we identified insulin/IGF-1 signaling as key determinants of epidermal morphogenesis and proliferative potential. We observed a progressive decrease in the number of suprabasal layers in insulin receptor (IR), IGF-1 receptor (IGF-1R) or IR/IGF-1R (dko) knockout mice, which was first obvious at E16.5. Here we examined how IR/IGF-1R signalling regulates epidermal morphogenesis and proliferative potential. At E16.5 no difference was found in the number of BrdU positive cells, which is incorporated during S-phase of the cell cycle. However, the number of anaphase spindles was reduced both at E16.5 and in vitro in cultured keratinocytes in the absence of IR/IGF-1R signaling, thus suggesting a block in G2/M of the cell cycle. FACS analysis confirmed this arrest. Loss of IR/IGF-1R also increased expression of key cell cycle regulators, such as p53 and cyclin G1. The observed cell cycle arrest was also associated with increased activation of the stress kinase p38 MAPK, and its downstream targets stratifin (14-3-3s), both previously implicated in G2/M arrest. At the moment we are examining if these alterations are cause or consequence of the phenotypes induced by epidermal loss of IR and/or IGF-1R. In addition, we ask if the arrest is temporary or more permanent resulting in senescent cells. Finally, our findings may help to understand how increased IGF signalling drives carcinogenesis.

124 [Oral 062]

Hair follicle stem cell derived progeny drives sebaceous gland regeneration

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Mammalian epidermis comprises a highly dynamic and compartimised epithelium consisting of the interfollicular epidermis, the hair follicles (HF) and associated sebaceous glands (SG). Functional SG release sebum into hair shaft and are important for barrier aquisition and protection against pathogens and/or environmental assaults. The high cellular turn-over of the SG requires a constant replacement of cells.Currently, two hypothesis concerning the involvement of distinct stem cell (SC) or progenitor populations in the process of SG renewal are discussed: (1) residing unipotent progenitors located at the periphery of the SG are committed to sebaceous lineage. (2) SC or progenitors from different epidermal compartments migrate towards the SG and renew them. We have generated an inducible Cre-mouse model to map the fate of individual HFSC-derived progeny and to investigate sebaceous commitment *in vivo*. From these lineage tracing approaches we have learned that single progenitors emanated from the HFSC niche continuously drive SG regeneration. These cells exit their niche and transit through different epidermal progenitor compartments. Our data imply a certain hierarchy of multipotent keratinocytes contributing to epidermal homeostasis. Directing basal keratinocytes into sebocyte lineage by manipulating one of the crucial regulators of fate decision, Lef1, we could identify HFSC-derived progeny as cells of origin building up ectopically formed SG. Strikingly, new progenitor compartments adjacent to the de novo differentiating sebocytes were established.

125 [Oral 025]

Synthetic Peptide K5 Efficiently Detects in situ Transglutaminase1 Activity; Its Application for Diagnosis of Lamellar Ichthyosis

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Lamellar ichthyosis (LI) is a genetically heterogeneous, severe genodermatosis showing widespread hyperkeratosis of the skin. Transglutaminase 1 (TGase1) deficiency by TGase1 gene (TGM1) mutations is the most prevalent cause of LI. Screening of TGase1 deficiency in skin is essential to facilitate the molecular diagnosis of LI. However, cadaverine, the most widely used substrate for TGase activity assay, is not isozyme specific. Recently, a human TGase1 specific, highly preferred substrate peptide K5 (pepK5) was generated. To evaluate its potential as a diagnostic tool for LI, we performed pepK5 labeling of TGase1 activity in normal human and LI skin. Ca^{2+}-dependent labeling of FITC-pepK5 was clearly seen in the upper spinous and granular layers of normal human skin where it precisely overlapped with TGase1 immunostaining. Both specificity and sensitivity of FITC-pepK5 labeling for TGase1 activity were higher than those of FITC-cadaverine labeling. FITC-pepK5 labeling colocalized with involucrin and loricrin immunostaining at cornified cell envelope forming sites. FITC-pepK5 labeling was markedly reduced in LI patients with TGM1 mutations, but not affected in congenital ichthyosis patients without any TGM1 mutation. The present results clearly indicate that pepK5 is a powerful tool to distinguish LI patients with TGase1 deficiency from ichthyosis patients with other causative genetic defects. Furthermore, FITC-pepK5 labeling was negative in LI patients carrying TGM1 truncation mutations and partially abolished in the other LI patients harboring missense mutations. It might be possible to differentiate LI patients with nonsense/truncation mutations and those with missense mutations, although pepK5 fluorescence labeling is not completely quantitative.

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Skin cellular senescence expression control by a new orchid extract

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Cellular senescence plays an important role in skin aging. Active DNA replication S phase cells are characteristic of young cells. A β-Galactosidase staining Kit was used to histochemically detect senescence in young and old skin tissue. In vitro replicative senescence of human skin fibroblasts is performed from early stage passages (3P young cells) up to 22 passages (22P aged cells). Flow cytometry was used to analyse the difference of cell cycle stage distribution in young and aged fibroblasts population and in treated fibroblasts. The populations repartition in each cell cycle stage (G2+M; S, Go/G1) at 3P and 22P passage and with or without an orchid extract treatment were evaluated using MXP software. To investigate mechanisms induced during senescence developpement cell Cyclins profiles were determined using specific protein array. Vanda coerulea orchideaceae stem ethanol/water 90/10 purified extract was tested at 25µg/mL. *In vivo* beta galactosidase biomarker positive cells were found more numerous in aged skin and senescence-associated-β-galactosidase expression in human fibroblast cultures increased as a function of passage number. Young fibroblasts show 10.8% of G2+M, 4.55% S and 84.15 % G0/G1 respectively compared to 7.6, 1.2, 91.2 % in 22 passages fibroblasts. The 24 h Treatment with Vanda extract delays the entry in senescent state, 22P treated fibroblast population expressing 10.85% in G2+M phase, 4.7% S phase and 83.90% G0/G1 as in early passage young population. Cyclin E, which controls the G1 to S phase entry activation, was found decreased in old cells and, with cdk2, restaured by orchid treatment.

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Skin cells antioxidant and anti-inflammatory activities of phenanthrene constituants from Orchid

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Anti-inflammatory processes are known to be involved in skin ageing and PLA_2 enzyme supplies arachidonic acid to downstream COX-2 for the synthesis of PGE-2. New biologically active constituents from *Vanda coerulea* Griff.ex.Lindl (Orchidaceae), were isolated and structurally determined by 1D and 2D NMR spectroscopic spectroscopic studies. Biological screening on various extracts revealed that stem extract displayed in vitro DPPH / OH radical scavenging activity and inhibition of PGE-2 release from irradiated (UV_B 60mJ/cm^2) Normal Human Epidermal Keratinocytes (NHEK). Antioxidants experiments (DPPH, •OH/luminol chemiluminescence) and PGE-2 quantification (Elisa) were carried on 96-well plates with quercetol and indomethacine (1µg/ml) as positive references. COX-2 activity was measured using PGE-2 production Elisa test. Phytochemical analysis of stem extract yielded phenanthrene derivatives: imbricatin (1), methoxycoelonin (2), gigantol (3) and to a lesser content flavidin (4) and coelonin (5). They all displayed radical scavenging activities, compounds 1, 2 and 4, 5 being more potent free radical scavengers than (3). Major phenanthrene derivatives (1-3) were also able to inhibit PGE-2 release from irradiated (UV_B 60mJ/cm^2) NHEK with IC_{50} of 1.06µM for (1) and 4.36µM for (2). Major antioxidant phenanthrenes from Vanda coerulea stem extract showed also skin anti inflammatory properties by means of PGE-2 release inhibition on NHEK. Inhibition of COX-2 enzyme activity was found with (1) and (2) (IC_{50} 12µM and 8µM, respectively) but not with (3), confirming a selective biological interest of these secondary metabolites.

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Abnormal lipid composition of stratum corneum in haemodialysis patients

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The aim of the study was to analyse the lipid composition of the stratum corneum in patients undergoing haemodialysis and to evaluate its influence on the perception of uremic pruritus. 80 patients on haemodialysis (30 females and 50 males) in the age between 25 and 90 years (mean 59.9±15.6 years) were compared with 32 healthy volunteers (19 females and 13 males) in the age between 22 and 86 years (mean 59.7±16.0 years). Epidermis specimens were collected from the area of 1 cm2 on one lower leg by scraping and lipids were isolated using a two-step chloroform/methanol/water extraction. Lipid composition of the stratum corneum was assessed according to 3-step thin layer chromatography (TLC). The level of identified lipid classes was demonstrated as a percentage of the total lipids that were isolated. In addition, transepidermal water loss (TEWL), electric impedance of the stratum corneum, clinical severity of xerosis as well as the presence and intensity of pruritus using Visual Analogue Scale and a 4-point Itch Questionnaire was evaluated in each subject. All results were analysed statistically. Patients on haemodialysis in comparison to healthy controls showed significantly decreased level of cholesterol (22.5±8.0% vs. 28.5±9.7%, p=0.001) and triacylglycerols (6.5±5.8% vs. 10.5±6.4%, p=0.002) in the stratum corneum, while the content of ceramides was markedly increased (33.4±8.7% vs. 25.1±9.5%, p<0.001). However, there was no relationship between observed abnormalities in epidermal lipids and the degree of xerosis as well as the pruritus intensity.

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Siascopy detects intraindividual differences of skin ageing between sun-exposed and sun-protected skin

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To date, photoaging of the skin is hard to quantify in live patients. Sun light has a decisive influence on the content of collagen, haemoglobin and melanin of human skin. We report on the quantification of skin aging within individuals using the recently described SIAscopy method. SIAscopy (Spectrophotometric Intracutaneous Analysis) measures the reflectance of light by the chromophores collagen, hemoglobin and melanin in the skin with a depth up to 2mm. By SIAscopy, volunteers and patients were assessed at three different sun-exposed spots (forehead, cheek, back of the hand) and at a non-sun-exposed part of the body (buttocks), all axisymmetrically on both sides. The measurements were performed once for a total of 8 measurements per patient. A total of 79 subjects aged 18 to 81 years showed a significant difference between the sun-protected part of the body compared to sun-exposed parts. (Buttocks to back of the hand: melanin p<0.001; collagen p<0.001; haemoglobin values p<0.001. Buttocks to cheek: melanin values p<0.001; collagen values p=0.005; haemoglobin values p<0.001; buttocks to forehead: melanin values p< 0.0007; collagen values p=0.03; haemoglobin values p<0.001; all values Bonferroni-corrected). SIAscopy clearly distinguishes sun-protected skin intraindividually from sun-exposed areas of the skin by the quantification of the chromophores collagen, haemoglobin and melanin.

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Epidermal expression of host response genes upon skin barrier disruption in normal skin and uninvolved skin of psoriasis and atopic dermatitis patients

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Recent genetic studies have indicated that skin barrier abnormalities play a role in the pathogenesis of atopic dermatitis (AD) and psoriasis. Deficiencies in skin barrier formation or repair may expose epidermal cells to environmental stimuli such as microbial components which could in turn evoke a response that is shaped by the genetic background of the host. Here we investigated the effect of experimental skin barrier disruption on the expression of host defense genes in uninvolved epidermis of psoriasis and AD patients and healthy controls. Skin barrier disruption was induced by tape stripping of the stratum corneum or by sodium dodecylsulphate (SDS) application. Quantitative PCR was performed on epidermal sheets and immunohistochemistry on whole skin biopsies. Skin barrier disruption only marginally affected the mRNA expression levels of pattern recognition receptors (PRRs). However, mRNA and protein levels of antimicrobial proteins (AMPs) were strongly elevated and of similar magnitude in psoriasis and AD patients and healthy controls. In cultured keratinocytes from the three groups, Th2 cytokines partly reduced the Th1-cytokine mediated induction of several AMPs. We conclude that skin barrier disruption induces AMP expression similarly in AD and psoriasis patients without requiring transcriptional upregulation of PRRs. Our data indicate that the expression of AMPs in human epidermis is a complex process driven by many factors including skin barrier integrity, cytokine environment and genetic predisposition.

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Determination of Skin Aging In Vivo Using Multi Photon Laser Scanning Microscopy (MPLSM) in the Stratum Granulosum

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In the process of skin aging the growth rate of the epidermis decreases resulting in an increase of corneocyte size. Furthermore, light and electron microscopy revealed an enhanced keratinocyte size with in vitro aging. In this study we investigated the influence of aging on the size of keratinocytes of the stratum granulosum *in vivo*. Multi photon laser scanning microscopy (MPLSM) allows *in vivo* imaging without contrast agents due to the auto fluorescence of the keratinocytes. The endogenous fluorophores are NAD(P)H and flavin coenzymes (in glycolysis in cytoplasm and in citric acid cycle, β-oxiodation and oxidative phosphorylation in mitochondria matrix). Therefore, the autofluorescence of the cytoplasm of the keratinocytes allows a specific imaging of the cells. Automated image analysis can be used in order to obtain the area of a sufficient number of granulosum cells (Agran). The procedure is a combination of Voronoi sectioning and active contours. Young skin shows smaller cell areas in the stratum granulosum compared to the skin of elderly volunteers (+13%). Since keratinocytes grow slower with age they have more time to get wider in the stratum granulosum. This result supports the in vitro finding and explains the increased corneocyte size. Thus, Agran is a useful parameter to differenciate skin age *in vivo*.

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Stratum Corneum Depth and NMF Distribution Displayed Significant Differences Between Dry and Healthy Skin

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While Natural Moisturization Factors (NMFs) are essential for maintaining stratum corneum (SC) water content and hydration level, there has been little evidence regarding NMF variations in healthy and dry skin. To determine whether there is any difference in NMF levels between individuals with healthy skin and those with dry skin, sequential tape strips from 62 subjects with varying visual dryness grades were collected for protein, free amino acid (FAA) and NMF analyses. The results demonstrated that dry skin subjects had significantly more accumulated proteins which were stripped off by the tape strips. In addition, dry skin subjects showed significantly lower normalized FAA across the stripped SC depth (p=0.002 for peak), and the FAA peak appeared in the deeper layer of the SC in dry skin. Furthermore, HPLC analysis of pyrrolidone carboxylic acid (PCA), the prominent component of NMF, correlated well with FAA on the tape strips (R^2=0.73), and accounted for approximately 9% of total FAA regardless of skin condition. PCA also exhibited very similar SC distribution to FAA, and its level in dry skin was significantly lower compared to healthy skin (p=0.006 for peak). This suggested that profilaggrin break down occurs in the deeper SC, and the production of NMF (hence the profilaggrin expression) may be lower in dry skin subjects. These results confirmed that NMF levels are critical for skin conditions, and lower NMF production may be associated with dry skin. Therapies and moisturizers that help to maintain SC NMF level may be viable routes for dry skin management.

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ER signaling is activated to protect human keratinocyte from ER stress provoked by the environmental dose UVB

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Proteins are folded properly in the endoplasmic reticulum (ER). Various stresses such as hypoxia, ischemia, or starvation interfere the ER function leading to the ER stress defined by accumulation of unfolded protein (UP) in the ER. ER stress is avoided by UP response (UPR) and ER-associated degradation (ERAD). Signaling pathways to avoid UP are activated by 3 major ER molecules, ATF6, IRE-1, and PERK. We investigated the ER signaling in human keratinocytes irradiated by environmental dose ultraviolet B (UVB) using HaCaT cells. UVB irradiation at 20 mJ/cm^2 resulted in significant growth retardation and decrease of Ero1-Lα expression at 1 to 4 hours (h), indicating that UVB provoked ER stress in HaCaT cells. Expression of intact ATF6 was decreased and ATF6 was translocated into nuclei. Expression of XBP-1, a downstream molecule of IRE-1, and calnexin, an ER chaperone whose expression is regulated by XBP-1, and ubiquitination of UP were induced by 10 mJ/cm^2 UVB at 4 h. PERK that regulates apoptosis was not phosphorylated. These results demonstrate that UVB irradiation generates UP in HaCaT cells and UPR and ERAD systems are activated to protect cells from UVB-induced ER stress.

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Epimorphin-derived peptide antagonists remedy epidermal parakeratosis triggered by unsaturated fatty acid

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Unsaturated fatty acid affects calcium influx in keratinocytes and triggers the epidermal hyperplasia with perturbation of the keratinization program, which is supposed to be relevant to the acne and comedone formation caused by accumulation of excess sebum. Here, we show that the antagonistic peptide of epimorphin (EPn1: a circular compound composed of CGSIEQSC) clearly remedy the oleic acid-induced abnormal behaviors in epidermal keratinocytes. In culture, treatment with oleic acid augmented extracellular secretion of epimorphin in HaCaT keratinocytes and prevented the epidermal terminal differentiation including programmed cell death (anoikis) and the cornified envelope formation. Intriguingly, however, simultaneous addition of EPn1 peptide appeared to restore these keratinocyte differentiation programs. In the hairless mice skin, the topical application of oleic acid caused epidermal hyperplasia with the decrease of enucleation in outer epidermal layers, which was dramatically hampered by the administration of EP peptide. These results demonstrate that abnormal differentiation in the epidermal keratinocytes caused by unsaturated fatty acid is attributed to the overstimulation of epimorphin signaling and suggest the possible usage of EPn1 for the therapeutic purpose such as for acne and hyperkeratotic skin disease.

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Ameliorating effect of Yokukansan on atopic dermatitis-like lesions and scratching behavior in socially isolated NC/Nga mice

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Long-lasting itch is an intolerable sensation in all patients, especially in patients with serious atopic dermatitis. Irresistible itch leads to chronic, habitual scratching behavior, a vicious spiral called the 'itch-scratch cycle'. Effective antipruritic drugs are in high demand for many patients with atopic dermatitis. In the present study, the effect of Yokukansan, which is a traditional Japanese medicine, on the development of atopic dermatitis-like lesions and scratching behavior was investigated in socially isolated NC/Nga mice. Ten-week-old male NC/Nga mice were divided into three groups (n=5/group): control (nontreated), Yokukansan and fexofenadine. All mice were kept in isolation under conventional conditions for 6 weeks. Dermatitis score, scratching and grooming behaviors, and transepidermal water loss (TEWL) were measured once a week or in three weeks during the experimental period. N-methyl-D-aspartate (NMDA) receptor and glutamate transporter-1(GLT-1) mRNAs were evaluated on the terminal day of the experiment. Both Yokukansan and fexofenadine inhibited the aggravation of skin lesions and the increased in TEWL. However, scratching and grooming behaviors which are indices of itch sensation were decreased significantly after treatment with Yokukansan, but not affected by fexofenadine. Expressions of NMDA receptor and GLT-1 mRNA increased and decreased, respectively. Such abnormalities were also ameliorated by treatment with Yokukansan, but not affected by fexofenadine. These results suggest that Yokukansan ameliorates the aggravation of atopic dermatitis-like lesions and scratching behavior. In particular, the effect on itch sensation by Yokukansan may be related to the ameliorative effect against the dysfunction of NMDA receptor or GLT-1 in the skin.

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AQP3 expression in keratinocytes is involved in hyperplasia in atopic dermatitis

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Aquaporin-3 (AQP3) is a water/glycerol transporting protein expressed in keratinocytes of the epidermis. Previous studies have shown that AQP3-mediated water and glycerol transport is involved in keratinocyte migration and proliferation, respectively. AQP3 expression was also found to be increased in atopic dermatitis (AD) skin; however, the role of AQP3 in the pathogenesis of AD remains unknown. To test the hypothesis that AQP3 is involved in keratinocyte hyperproliferation during AD development, we used AQP3-deficient mice and human keratinocytes. In the murine AD model, which was generated by repeated applications of ovalbumin or hapten, AQP3 expression was remarkably increased in the wild-type AD epidermis. AQP3-deficient mice exhibited less severe epidermal hyperplasia and lower transepidermal water loss than wild-type mice. The number of proliferating cell nuclear antigen-positive cells decreased in AQP3-deficient AD epidermis compared to that in wild-type AD epidermis, suggesting the involvement of AQP3 in epidermal hyperplasia in AD. In human keratinocytes, overexpression of AQP3 by transfection of AQP3 cDNA facilitated proliferation and water/glycerol transport. Immunoblot analysis of various AD-related cytokines and chemokines indicated that AQP3 expression specifically increased by thymus and activation-regulated chemokine CCL17/TARC, which is known to be a Th2 chemokine and a specific marker for AD. Coincidentally, CCL17 treatment markedly enhanced keratinocyte proliferation. These results suggested that AQP3 is involved in the pathogenesis of AD development in which increased AQP3 expression was implicated in keratinocyte proliferation. AQP3 inhibition by topical drugs may be beneficial for the therapy of epidermal hyperplasia in AD.

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Appearance in Japanese Women Correlates with the Presence of Inosotides and Lysolipids, Both Significant Inhibitors of age-related NADH Oxidase Activity

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Accumulation of oxidative damage in proteins is considered to be a major contributor to skin deterioration during aging. Recent work has suggested that age-related NADH oxidase (arNOX), a significant generator of superoxide exterior to the cell, may contribute to accelerated apparent aging. Metabolic profiles from a cohort of 46 age-matched Japanese women aged 46 to 59 years, approximately half appearing younger (2 to 8.5 years, average 4.5 years) and half appearing older (5 to 11.3 years, average 6.2 years) than their chronological ages, were analyzed for levels of metabolites. Metabolites within all classes (lipids, carbohydrate, energy, amino acids, etc.) comprised the 471 profiled in this study. A statistical cutoff of $p \leq 0.05$ was employed to indicate significant metabolite differences. arNOX activity in serum and saliva was assayed from measurements of superoxide production based on the reduction of ferricytochrome c by superoxide as monitored from the increase in absorbance at 550 nm with reference at 540 nm. arNOX levels in the group appearing younger than their chronological age were lower than those in the older appearing group. Only a limited number of metabolites achieved strong statistical significance with metabolites related to lipid metabolism distinguishing the two groups. Elevated levels of inositides and lysolipids, both naturally-occurring arNOX inhibitors, were found in the younger looking group. Thus, apparent age appears to correlate closely with naturally-occurring inhibitors of endogenous circulating arNOX.

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Quantitative digital morphometry reveals decreased expression of desmoglein 3, but increased expression of desmoglein 2, in basal cell carcinomas

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Three isoforms, each in two splicing variants, of desmocollin (DSC1-3) and four isoforms of desmoglein (DSG1-4) are known in humans, each one arising from a different gene and having characteristic expression. In normal human epidermis DSG2 is expressed at the low level, and was reported to be restricted to the lowermost epidermis. DSG2 expression fades with keratinocyte differentiation, whereas DSG3 expression decreases somewhat gradually from the basal into the spinous cell layers. The aim of this pilot study was to examine expression patterns of both DSG2 and DSG3, adhesive molecules, in basal cell carcinoma (BCC) and to compare them with those in normal epidermis. Using immunoperoxidase staining on frozen tissues with monoclonal antibodies against human DSG2 and DSG3, we evaluated DSG2 and DSG3 expressions in specimens from 15 and 34 BCC patients, respectively. Intensities of positive immunostaining signals of these proteins in BCC-affected and BCC-free areas on serial sections were measured using digital microscopic image analysis with quantitative morphometric software. There was a significant overexpression of DSG2 and significantly lower expression of DSG3 in BCC tissue compared to normal epidermis. We found no significant correlation between DSG2 and DSG3 expression in BCC tissue, but there was a significant correlation ($r=+0.5868$) between those markers in non-BCC-affected epidermis. Our data might suggest that DSG2 and DSG3 indeed play different roles in proliferation and differentiation in BCC-free versus BCC-affected human epidermis. Seemingly, the expressions of DSG2 and DSG3 are coordinated in normal epidermis, but this apparent coordination is lost in BCC tissue.

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Identification and Characterization of MDR1 as a Sebum Secretion Transporter in Hamster Sebocytes

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Sebum secretion plays an important role in the formation of a physiological barrier formation on the skin surface. It is thought to be regulated by a holocrine mechanism that is associated with the apoptosis of sebaceous gland cells (sebocytes). On the other hand, ATP-binding cassette (ABC) transporters participate in the membrane transport of various substrates including lipids. However, whether apoptosis or ABC transporters are associated with sebum secretion is not well understood. In the present study, we examined the involvement of apoptosis or ABC transporters in sebum secretion in hamster sebocytes. The measurement of intracellular and extracellular triacylglycerols (TG), major sebum components, revealed that levels of extracellular TG increased along with the accumulation of intracellular TG during insulin-induced sebocyte differentiation. In addition, differentiated hamster sebocytes (DHS) exhibited apoptosis-independent phosphatidylserine exposure and tolerance against Ca^{2+} influx and apoptosis by the apoptosis inducer, A23187. Furthermore, transporter activity using an ABC transporter substrate, Rhodamine 123, was highly detectable in DHS rather than the undifferentiated sebocytes. An ABC transporter activator, BzATP, also enhanced not only transporter activity but also sebum secretion in DHS. Moreover, MDR1 (multidrug resistance 1)/ABCB1 was constitutively detectable, and both transporter activity and sebum secretion were suppressed by MDR1 antibody in the DHS. These results provide novel evidence that sebum secretion is mediated by MDR1 in an apoptosis-independent manner in DHS. Finally, these findings should help to accelerate the understanding of the functions of sebaceous glands, and may contribute to the development of therapies for skin diseases such as acne.

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Purification and Identification of Sphingomyelin Deacylase from Rat Skin

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Abnormal expression of sphigomyelin (SM) deacylase activity, that hydrolyzed sphingomyelin to sphigosylphosphorylchorine (SPC) and fatty acids, was reported in the epidermis of atopic dermatitis (AD) patients. SM deacylase is multifunctional enzyme influencing inflammatory systems and natural ceramide metabolism, which are considered to be etiologic factor of AD. Although SM hydrolyzing activity was certainly present, for many years, the enzyme itself was unidentified. To identify SM deacylase active protein we established suitable methods for measuring activity, enzyme purification, and protein identification. The specific enzyme activity was measured by tandem mass spectrometer by monitoring production of SPC. Solubilized proteins from the 10000 xg supernatant of various rat organs were assayed for SM deacylase activities, and we found that the activity was higher in the order of skin > lung > heart. No activity was detected in other organs. A protein spot on 2D-PAGE gel with strong correlation with SM deacylase activity was analyzed, and we identified the active protein as beta subunit of acid ceramidase (AC). Further, we found that SM deacylase activity was detected from AC beta subunit by reduction of recombinant with dithiothreitol. SM deacylase active fractions by isoelectoric focusing of reduced AC were well correlated with the distribution of AC beta subunit but not AC alpha subunit. These results may provide new insight to ceramide metabolism and its role in AD pathophysiology.

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Systemic recruitment of myeloid cells upon deletion of IKK2 from epidermal keratinocytes

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We have previously described the phenotype of mice with epidermis specific deletion of IKK2, the main regulator of NFkappaB activity. Four to five days after birth, these mice start to develop a skin disease that resembles human psoriasis and is characterized by a mixed inflammatory infiltrate and hyper proliferation of epidermal keratinocytes. We could also show that skin macrophages play a pathogenic role in the development of this inflammatory skin condition. Here we have asked by which mechanisms immune cells are recruited into the skin of these mice. We have found that, immediately after birth, IKK2 negative epidermal keratinocytes generate pro inflammatory signals that tailor the resulting skin inflammation specifically towards a myeloid cell dominated innate immune response. After birth, the first transcription dependent signal was the up regulation of members of the IL-19 family of cytokines which are known to mediate interactions between epithelia and monocytes/macrophages. Up regulation of IL- 19 and IL- 24 as well as phosphorylation of STAT3 in epidermal keratinocytes were detectable at P1 and preceded the first macroscopic and microscopic changes of the skin. This was followed by an increased production of other pro-inflammatory cytokines and of myeloid cell recruiting chemokines without regulation of lymphocyte directed chemokines. These mice showed signs of extramedullar myelopoiesis and an increased fraction of CD11b positive cells but no changes in B- and T- lymphocyte in the peripheral blood. Our results show that epidermal keratinocytes can control a systemic, myeloid cell dominated innate immune response by NFkappaB dependent mechanisms.

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The induction of mouse beta-defensin 3 expression in skin barrier disruption is mediated by TNF-alpha

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The physical permeability barrier and antimicrobial proteins of the innate immune system protect the skin against microbiological infection. We previously showed that expression of mouse beta-defensin-3 (mBD-3), which is an ortholog of human beta-defensin-2 (hBD-2), respectively, is stimulated by acute barrier disruption induced by tape-stripping or acetone treatment of mouse skin *in vivo*. Also, we found that mBD-3 mRNA expression is induced in primary mouse keratinocytes by treatment with tumor necrosis factor alpha (TNF-alpha) in vitro. We asked whether the induction of mBD-3 after skin barrier disruption depends on TNF-alpha signaling and whether TNF-alpha is the only cytokine mediating mBD-3 induction after skin barrier disruption. An anti-TNF-alpha antibody was injected in hairless mice for blocking TNF-alpha activity 24 hours before skin barrier disruption. Six hours after barrier disruption by acetone treatment, skin samples were harvested for mRNA isolation and for immunohistology. Realtime PCR analysis with mBD-3 specific primers revealed a significant induction of mBD-3 gene expression by skin barrier disruption compared to untreated controls. Mice injected with an anti-TNF-alpha antibody before barrier disruption showed a significant decreased in mRNA induction compared to mice without anti-TNF-alpha antibody treatment. Immunohistology using a specific anti-mBD-3 antibody confirmed these results on the protein level. In summary, we showed a crucial role of TNF-alpha in the induction of mBD-3 by skin barrier disruption. However, the anti-TNF-alpha pretreatment did not completely block the induction of mBD-3 expression. This might be a hint for an involvement of additional cytokines in the induction of mBD-3 by barrier disruption.

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Primary human keratinocytes and fibroblasts under hyperglycaemic conditions and of diabetic origin show reduced susceptibility to Cx43 targeting

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Connexin 43 (Cx43) is downregulated during early wound healing (WH) at cutaneous wound margins. Several mouse models and the dysregulation of Cx43 in diabetic chronic wounds indicate that this downregulation is important for WH. Therefore, Cx43 is a promising target to accelerate WH. We have previously shown that Cx43 targeting is beneficial to improve WH in infant and adult human keratinocytes and fibroblasts. However, cells isolated from diabetic patients, although showing no difference in Cx43 expression and localisation compared to non-diabetic cells, exhibit a reduced susceptibility to Cx43 targeting by Cx-mimetic peptide Gap27. Additionally, diabetic keratinocytes migrate significantly slower than non-diabetic keratinocytes. One possible reason for this "diabetic phenotype" might be the hyperglycaemia of diabetic donors. To investigate this hypothesis, we determined the behaviour of non-diabetic cells under hyperglycaemic conditions. We show here that cells under hyperglycaemia exhibit significant similarities to cells from diabetic origin. They exhibit higher fibronectin protein levels whereas there are only minor influences on Cx43. Hyperglycaemia in scratch wound assays leads to decreased migration rates. Application of Gap27 or Cx43-specific antisense oligonucleotides under these conditions does not result in accelerated migration although it does under physiologic glucose concentrations. These data show that the altered phenotype of cells of diabetic origin might be due to the hyperglycaemic conditions of the diabetic donors. This is also supported by the loss of "diabetic phenotype" during long-term culture of cells of diabetic origin in euglycaemic conditions.

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Involvement of DHCR24 in the physiology of epidermal keratinocytes in oxidative stress conditions

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Oxidative Stresses applied to keratinocytes perturb epidermal homeostasis and mechanisms are initiated to repair epidermal tissue. While earlier works focused on the damaging aspects of reactive oxygen species, it was discovered that hydrogen peroxide (H_2O_2) can act as a signalling molecule and be beneficial for skin wound healing. Because H_2O_2 induces a modified differentiation profile similar to the profile induce by cholesterol depletion of keratinocytes by methyl-b-cyclodextrin and because 3β-hydroxysteroid-Δ24 reductase (DHCR24), involved in cholesterol biosynthesis pathway is also implicated in the cellular responses to oxidative stress, we investigated functions of DHCR24 in the physiology of keratinocytes submitted to an oxidative stress. We show an increase of expression of DHCR24 in the suprabasal layers of epidermis by immunohistochemical labelling and in serum-free autocrine conditions where the confluence of cultured keratinocytes corresponds to a strong commitment toward epidermal differentiation. Interestingly, identical alterations in differentiation markers are detected in H_2O_2-treated cultures of keratinocytes, exactly as *in vivo* at the wound margin of injured skin. After an oxidative stress, keratinocytes reveal a decreased expression of keratin-10, fillagrin and loricrin, while the expression of keratin-14 and involucrin is not altered. This decrease of expression is amplified when DHCR24 is previously inhibited by siRNA. We show equally that the expression of miR-203, microRNA suspected in regulation of differentiation in keratinocytes, undergoes durable decreased expression after oxidative stress. Our results show that oxidative stress induced by hydrogen peroxide perturbs durably the process of differentiation in keratinocytes and suggest that DHCR24 moderates this perturbation in these conditions.

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CD44 regulates tight junction assembly and barrier function

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Upon barrier disturbance adult CD44 knock-out (KO) mice show delayed recovery of epidermal barrier function. This correlates with loss of apical polarization of lamellar body (LB) secretion. In simple epithelia tight junctions (TJ) are crucial for barrier function and regulate the polarized targeting of vesicles to either the apical or basolateral membrane. In addition, they are important for murine epidermal barrier function. Therefore we hypothesized that CD44 regulates TJ and associated cell polarity complexes, which in turn contributes to altered skin barrier function in CD44 KO mice. We show a delay in embryonic barrier formation associated with a loss of apical LB localization in CD44 KO mice which correlates with alterations in localization and/or expression levels of TJ proteins as well as the polarity protein Par3. Simultaneously, activity of the small GTPase Rac1, a major regulator of TJ barrier function, was reduced. Importantly, normalization of barrier function at E18.5 was paralleled by the recovery of these proteins. Tape stripping experiments revealed that loss of CD44 also affected TJ proteins upon induced disturbance of the barrier in adult mice. In primary keratinocytes, cell polarization and TJ barrier function was impaired in CD44 KO cells. An alteration of differentiation associated markers was also observed, but less pronounced and chronologically subsequent to alterations of TJ proteins. Together, the results reveal an important function for CD44 in the assembly and function of TJ, suggesting their involvement in the skin barrier phenotype of CD44 KO mice.

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Filaggrin mutations and their impact on stratum corneum lipid levels and skin physiology in patients with eczema

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Filaggrin (FLG) mutations cause ichthyosis vulgaris and are strong risk factors for eczema. Alterations in stratum corneum lipid levels, skin pH and trans-epidermal water loss (TEWL) are characteristic features of eczema. In order to examine the impact of FLG mutations on skin physiology parameters and their potential influence on ceramide levels, we investigated 49 German individuals with and without eczema and information on the two common FLG mutations R501X and 2282del4. Stratum corneum lipid analysis was carried out by high performance thin layer chromatography, and TEWL, erythema, skin hydration and pH were measured. In 27 individuals an additional 24-hour irritation patch test was performed.Eczema patients with FLG mutations (FLGmut) had significantly higher erythema than patients without FLG mutations. FLGmut individuals displayed significantly higher skin pH values than FLGwt individuals. The highest TEWL and lowest skin hydration values were observed in FLGmut eczema patients. FLGmut eczema patients also showed significantly lower ceramide 4 and significantly higher ceramide 7 levels as compared to both healthy control groups. However, ceramide 7 and ceramide 1 levels also significantly differed between FLGwt eczema and FLGwt controls. No significant differences were observed for ceramide 2, 3, 5 and 6. Our results provide preliminary evidence that the elevation of skin pH might be related to FLG status rather than eczema, and that FLG mutations are associated with increased TEWL and decreased skin hydration. Furthermore, they confirm previous findings of altered ceramide levels in eczema, which however appear to be due to mechanisms other than FLG.

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Enhanced contact allergen and UVB induced keratinocyte apoptosis in the absence of CD95/Fas/Apo-1

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FAS/ CD95/ Apo-1 is an ubiquitously expressed cell surface receptor involved in the initiation of programmed cell death. Its function in epidermal keratinocytes has been incompletely defined. Apart from its pro- apoptotic role, Fas has also been implicated in anti-apoptotic signaling in different systems. In skin, available evidence from in vitro studies points to important roles of Fas in the pathogenesis of contact dermatitis and in keratinocyte apoptosis induced by ultraviolet light. To elucidate the role of Fas in the epidermis *in vivo* and to test its requirement for DNFB induced contact dermatitis as well as for UVB induced keratinocyte apoptosis, we used conditional gene targeting technology to specifically delete the fas gene in the epidermis of C57/Bl6 mice. Unexpectedly we found that keratinocyte apoptosis induced by both a contact allergen and by UVB irradiation was significantly enhanced in Fas negative epidermis as shown by TUNEL staining, counting of sunburn cells and staining for active Caspase 3. In addition, the absence of Fas did not result in changes of the ear swelling response, the development of inflammation and of epithelial spongiosis in contact hypersensitivity, and Fas was not required for keratinocyte apoptosis following UVB irradiation *in vivo* and in vitro. Hence, our results demonstrate that, in the epidermis *in vivo*, Fas exerts anti apoptotic functions that outweigh its pro apoptotic role in contact hypersensitivity responses of the skin and in the tissue response of the epidermis to UVB irradiation.

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Impact Of Angiotensin II On Nhuman Keratinocyte Functions : Involvement In The Migration Process

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The tissue renin angiogenin system (RAS), which acts independently of the systemic RAS, is considered to play an important role in tissue repair in heart and kidney. Previous work documented the expression of angiotensin II receptors in the human skin. However, little data is available at present on the expression of the various components of the RAS in the human skin and on the functions of this cutaneous RAS. Using a model of human reconstituted skin on filters, we have shown that the key players of the SRA are induced during the in vitro maturation of the epidermis, ie renin, angiotensin II converting enzyme, and angiotensin II type-1 and type-2 receptors. This observation is in line with the distribution of the constituents of the RAS in normal skin, showing a stronger expression in the superficial layers than in the basal layers of the epidermis. Strikingly, the production of angiotensin II in the culture medium is negatively correlated with the age of the keratinocytes donor. The proliferation of keratinocytes is not modified in the presence of angiotensin II. Conversely, angiotensin II stimulates the migration of human keratinocytes, by an angiotensin II receptor-dependent process. In addition, the peptide induces a moderate increase of several proteins reflecting epidermal maturation (filaggrin, cytokeratines, involucrin). These results indicate that the RAS is expressed in human skin, and that it could control phenomena essential for the processes of cutaneous healing and repair.

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Activation of the Aryl Hydrocarbon Receptor In Human Keratinocytes and Relevance To Chloracne

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes chloracne in humans and is a potent activator of the arylhydrocarbon receptor (AhR). However, it remains unclear whether chloracne inducing potential of AhR agonists relates to binding affinity, duration of activation and/or down-regulation of the receptor. Currently, no in vitro skin model for chloracne exists but TCDD induces aberrant differentiation of epidermal equivalents with early induction of involucrin and a compacted stratum corneum. We studied the effects of TCDD and non-chloracnegenic agonists β-naphthoflavone (β-NF) and the physiological agonist 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE), which shows similar affinity to TCDD. β-NF showed ~2 fold AhR activation in keratinocytes using XRE-dependent Luciferase assay whereas ITE induced activity to a similar degree (~8 fold) as TCDD. Expression of CYP1A1 (utilised as readout of AhR activation) and AhR were determined by Western blotting. TCDD, ITE and β-NF induced CYP1A1 expression and degraded AhR to a similar degree. As expected, TCDD induced compaction of the stratum corneum in human epidermal equivalents. Notably, both β-NF and ITE induced similar changes. Reduced viable cell layers were seen with all 3 agonists and involucrin staining occurred in closer proximity to the basal layers suggesting early onset of terminal differentiation. We conclude that high and low affinity xenobiotic and physiological agonists induced activation and down-regulation of AhR in keratinocytes and induced similar phenotypic changes in epidermal equivalents. Therefore, these data suggest neither interaction with AhR or early induction of differentiation in epidermal equivalents correlate with the ability of AhR agonists to induce chloracne.

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Interactions between human primary keratinocytes and the dorsal root ganglion-derived neuronal cell line, the F-11.C.

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The epidermis can be considered as a true sensory tissue. Indeed, there's a dynamic communication between epidermal cells and the free nerves endings send by sensory neurons of the peripheral nervous system. However, little is known concerning the functional interactions between the sensory fibers and the keratinocytes constituting the epidermis. Therefore, it's difficult to reproduce these interactions in vitro. We developed an in vitro model based on the co-culture of human primary keratinocytes (KH) and a dorsal root ganglion cell line, the F-11. F-11 are classically used to mimic authentic, peptidergic, nociceptive neurons. We first describe the morphological and functional characteristics of F-11 in a basal keratinocyte medium (KM) and then analyze the influence of KH on these properties. We demonstrated that F-11 survive and differentiate well in the KM and that addition of neurotrophins was not necessary. Therefore, F-11 expressed sensory neuron markers and release SP and CGRP after activation by capsaicin. We noted that neuropeptide release could be obtained even at low calcium concentration and that axonal outgrowth was not influenced by the external Ca^{2+} concentration. These properties were reproduced when F-11 were cocultured with KH but KH had no significant influence on axonal development or neuropeptides release. Here, we described for the first time the culture of F-11 neurons with another cell type. This coculture model in which keratinocytes and neurons are maintained in low Ca^{2+} concentration may be useful to increase the in vitro alternative for studying and characterizing the close communication between keratinocytes and sensory neurons.

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Exposure to electromagnetic radiation alters skin homeostasis

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In this study we have looked at the effects of a strong acute electromagnetic radiation exposure (GSM basic, 900 MHz, SAR 2 mW/g, 6 hours) on a model of pigmented reconstructed epidermis. We have analyzed the variation in expression and localisation of various epidermal markers (stress, differentiation, proliferation) 2, 6 and 24 hours after exposure. By immunohistochemistry, no changes were found in the localisation of epidermal markers of differentiation/proliferation, presence of apoptotic cells and induction of p53 and Hsp70. By western-blot, Hsp70 was not found induced at 2 and 6 hours; caspase 3 was not activated at 2 and 6 hours but a slight increase was observed at 24 hours. A transient oxidation of proteins was detected 2 hours after exposure. A decrease at 2 and 6 hours was found in the levels of filaggrin, loricrin, cytokeratin 5 and involucrin. On the contrary, cytokeratins 14 and 10 were markedly increased at 2 hours followed by a decrease at 6 hours. The level of filaggrin was not fully recovered at 24 hours contrary to cytokeratins 5 and 10. Loricrin and cytokeratin 14 were markedly increased at 24 hours in possible correlation with the significant decrease in activity of the 20S proteasome. In conclusion, our data indicate that exposure to 900 MHz frequency do not seem to induce cellular stress but induce alteration of the epidermal barrier which may alter the protective capacity of the skin against external factors.

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High-resolution and three-dimensional analysis of atopic dermatitis affected stratum corneum using atomic force microscopy

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In the present study we used atomic force microscopy (AFM) to analyse the 3D topography of native stratum corneum (SC) obtained by tape stripping. AFM enables high resolution imaging of native human stratum corneum in a nanometric scale without any sample preparation. Therefore, an accurate calculation of the surface area and volume of single corneocytes is possible. Atopic dermatitis (AD), characterised by an impaired physical barrier results in an increased dehydration of the skin and thus to a shrinking of corneocytes. In comparison to the skin of healthy volunteers, AFM analysis revealed a dramatic change of the morphology of single corneocytes in skin samples obtained from AD patients. In AD affected skin, corneocytes are regularly decorated with cellular humps that are completely absent in healthy skin. These humps have an average height of 170 nm and a lateral extension of about 600 nm. Moreover, the volume and the surface of corneocytes in AD affected skin is significantly smaller than in healthy skin, indicating a cellular shrinking. In line with these findings intercellular gaps between single corneocytes are more prominent in atopic skin while they are barely visible in healthy skin. In conclusion, AFM gain access to the three dimensional morphology of the SC. The findings of changed corneocytes morphology in AD affected patients envision AFM as an innovative tool to investigate the dysfunction of the epidermal barrier in correlation to the severity of AD and to evaluate therapeutic interventions on the reconstitution of the SC toward a healthy morphology.

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A decline of TRPV6 function derives from a decrease of vitamin D activation and accounts for disturbed epidermal calcium gradient followed by skin barrier alteration in aged skin

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Epidermal Ca^{++} regulates keratinocyte differentiation and plays an important role in forming skin barrier. Because epidermal Ca^{++} gradient is restored with the recovery of skin barrier after its disruption, a disturbance in the recovery of Ca^{++} gradient delays skin barrier recovery. TRPV6 as a calcium ion channel, belonging to the superfamily of transient receptor potential channels constitutes an apical Ca^{++} entry mechanism in active Ca^{++} transport in the intestine, which has been also found in the epidermis of mammalian skin recently. TRPV6 modulates an influx of Ca^{++} into keratinocyte, and its expression is directly up-regulated by 1α, 25 dihydroxyvitamin D_3. Facial skin of aged humans showed the loss of epidermal Ca^{++} gradient, and the synthesis of cutaneous vitamin D3 was declined with aging. We hypothesized that this disrupted epidermal Ca^{++} gradient is caused by a decrease of TRPV6 function due to decreased vitamin D activation with aging. The mRNA expression of TRPV6 and vitamin D receptor (VDR) on fully aged skin (87 weeks old hairless mice) decreased; moreover their mRNA expression was not enough increased after 3-day low-dose (50mJ/day) UVB irradiation compared to young skin. Western blot for TRPV6 and VDR protein expression showed very similar results with mRNA. Topical ketoconazole, an inhibitor of 1α, 25 dihydroxyvitamin D_3, decreased TRPV6 expression even after UVB irradiation under immunohistochemical stain. In conclusion, a decline of TRPV6 function could be derived from decreased vitamin D activation and accounts for disturbed epidermal Ca^{++} gradient and altered skin barrier in aged skin.

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Epidermal Neuropilin 1 protects keratinocytes from UVB-induced apoptosis through regulation of Bcl-2

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Neuropilins (NRP1 and NRP2) are transmembrane receptors which regulate endothelial cell and neuron function. They are able to mediate signals from VEGFs and class 3 semaphorins. It was shown that NRP1 is also expressed on keratinocytes, but its function is still unclear. To elucidate the role of epidermal NRP1 *in vivo*, we generated epidermis-specific Neuropilin 1 deficient mice. These mice are viable and do not display obvious skin or hair defects. But we found that deletion of epidermal NRP1 leads to increased apoptosis after UVB irradiation in vitro and *in vivo*. There is a significant increase of active caspase 3 positive cells in the epidermis of K14Cre-NRP1 (-/-) mice 24h after irradiation. By Western Blot analysis we could show that NRP1 controls the expression of Bcl-2, a pro survival member of the Bcl-2 family. After irradiation the amount of Bcl-2 decreases in NRP1 deficient keratinocytes in vitro and *in vivo*. The amount of DNA damage in the irradiated skin of control and NRP1 deficient animals is equal. Therefore DNA repair mechanisms do not seem to be disturbed. We conclude that Neuropilin 1 is dispensable for normal skin development but has an important anti-apoptotic role in UVB response where NRP1 protects keratinocytes from apoptosis through modulation of Bcl-2.

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Improvement of skin barrier function by topically applying PPARa agonist

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Stratum corneum (SC) prevents the water transpiration from the body and the invasion of irritants as a skin barrier. Chronic skin barrier dysfunction is considered to be one of the causes of skin disorders characterized by itch and dry skin. HR-1 hairless mice fed a special-diet HR-AD are known to develop characteristic dermatitis demonstrating a variety of abnormality as follows: thickened epidermis, decreased dermal water content (DWC), increased transepidermal water loss (TEWL) and increased scratching behavior. In the past study, we showed that the skin penetration of exogenous substances was increased in HR-AD-fed hairless mice. Therefore, we considered that HR-AD-fed hairless mice were useful as a chronic skin barrier dysfunction model. Furthermore, we showed the decrease of intercellular lipid and abnormal expression of some factors which constitute SC in the model mice skin. Peroxisome proliferator activated receptors (PPAR) belong to the family of nuclear hormone receptor that regulates transcription of several genes involved in lipid metabolism. Recently, it has been reported that PPARa which is one of the subtypes also controls the expression of various SC components in epidermal homeostasis. In this study, we investigated the improvement of skin barrier dysfunction in this model by topical applying a PPARa agonist for 4 weeks. As a result, we showed the reduction of the skin penetration of substances and the recovery of expression of SC components in HR-AD-fed hairless mice. These results indicate that activation of intraepidermal PPARa is useful to improve skin barrier function.

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Toll like receptor 2 and 4 are expressed intracellularly in human keratinocytes

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Toll like recepetors (TLR) are pattern recognition receptors recognizing pathogen associated molecular pattern. Most of the TLR are expressed on the cell surface of immune cells, like lymphocytes, macrophages and dendritic cells, except TLR3, 7, 8 and 9 which are expressed intracellularly. Human keratinocytes constitutively express various members of the TLR family in particular TLR 2 and 4. TLR2 and 4 are known to recognize lipoteichoic acid and lipopolysaccharide, respectively. We investigated the expression of TLR in human keratinocytes by immunohistochemistry and fluorescence microscopy using specific antibodies. In contrast to the common model our results revealed an intracellular expression of both TLRs. TLR2 expression in healthy skin showed a staining of basal epidermis with a granular expression pattern. TLR4 expression was pronounced in the basal cell layers also, but in contrast to TLR2 the pattern was not granular but homogenous. We speculate that the uncommon subcellular localisation of TLR expression in keratinocytes may prevent the skin from sustained immune reaction as the surface of the skin is not sterile but in steady contact to microorganisms. In contrast immune cells are located in a sterile environment unless a pathogen has invaded the host. This concept is supported by the basal expression pattern which makes it likely that TLR activation necessitates the invasion of the epidermis by the pathogen.

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Identification of LCE6A, a New Member of the Family of Constitutive Proteins of the Cornified Envelope "Late Cornified Envelope"

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The most superficial epidermis layer, the cornified layer, is composed of corneocytes resulting from the transformation of the underlying granular keratinocytes. The cornified envelope is formed during cornification and replaces the plasma membrane of granular keratinocytes. It is made up of proteins covalently linked by transglutaminases and confers the corneocytes their rigidness and resistance, primordial to ensure the cornified layer its extreme strength. We recently described for the first time the transcriptome of granular keratinocytes by a large scale analysis. This study allowed the identification of more than 3,000 genes expressed by this cell type, among which LCE6A, coding for a new member of a family of constitutive proteins of the cornified envelope. Quantitative RT-PCR and immunohistochemical experiments demonstrated LCE6A is specifically expressed by granular keratinocytes in human epidermis. In vitro, LCE6A actually is a substrate for transglutamisases and in situ crosslink assays performed on human skin cryosections show that it is covalently linked to the cornified envelopes. It was recently demonstrated that the deletion of LCE3B and LCE3C was an important susceptibility factor for psoriasis. Moreover, the absence of the envelope proteins involucrin, periplakin and envoplakin alters the mechanical resistance of mouse cornified envelopes. These results suggest that variations in the protein composition of the envelope may weaken the corneocyte envelope.

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Natural PPARα agonist evaluation on in vitro and ex vivo models of Atopic Dermatitis

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Atopic Dermatitis (AD) is a chronic dermatosis involving skin barrier defects, altered immune response and exacerbated inflammation. Activators of PPARs (Peroxisome-Proliferator-Activated Receptors) have been recently described to be able to ameliorate inflammatory as well as barrier features of AD. An emollient containing 2% of patented Sunflower Oleodistillate (SO) has been specifically formulated to treat AD. SO is a natural PPARα agonist with demonstrated anti-inflammatory activities and ability to induce key epidermal lipids synthesis. In this study, we have evaluated SO and the emollient on in vitro and *ex vivo* models of inflammation and AD. The expressions of various markers were analysed by quantitative RT-PCR or ELISA assay. Inflammation was induced on reconstructed human epidermis by PMA. Emollient topical application inhibited expression of inflammatory mediators (ICAM1, IL1α, PGE2). Normal human keratinocytes were treated by IL4. SO was able to restore IL4-repressed gene expression of differentiation markers as filaggrin, involucrin, beta-glucocerebrosidase and sphingomyelinase. The cytokine environment of AD was mimicked on skin explants by treatment with TNFα, IL4 and LPS. Emollient topical application significantly inhibited the release of inflammatory mediators TSLP, TARC and RANTES as well as gene expression of kallikrein-7 and OSMR (involved in pruritus); furthermore gene expression of barrier markers as filaggrin, loricrin and beta-glucocerebrosidase were significantly enhanced. The beneficial effects on barrier, inflammation and pruritus of an emollient containing a natural PPARα agonist have been demonstrated on experimental models mimicking AD Th2 context.

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Regulation of epidermal differentiation evidenced by gene silencing of extracellular matrix protein 1

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Extracellular matrix protein 1 (ECM1) is an 85-kDa glycoprotein with wide tissue distribution, including the dermis and the entire epidermis. ECM1 plays a role in bone development, angiogenesis, and cancer biology. Its relevance to dermatology is highlighted by loss-of-function mutations in the ECM1 gene in lipoid proteinosis and autoantibodies to ECM1 in lichen sclerosus. Both diseases histopathologically show variable hyaline changes in the dermis as well as epidermal atrophy and hyperkeratosis, demonstrating that ECM1 contributes to both dermal and epidermal homeostasis. To explore the role of ECM1 in keratinocyte biology in more detail, we knocked down expression of ECM1 using small interfering RNA (siRNA) in HaCaT cells. We targeted exon 10 of ECM1 to ensure knockdown of all major splice variants of ECM1, including ECM1a and ECM1c (predominantly expressed in basal keratinocytes) and ECM1b (suprabasal keratinocytes). HaCaT cells were grown to 90% confluency in culture medium containing 1.8 mM calcium. After siRNA treatment, RNA was extracted and global gene expression was measured using Whole Human Gene Expression Microarray (Agilent). For epidermal differentiation, siRNA-treated HaCaT cells showed up-regulation of genes encoding keratin 1, S100A7 and calmodulin-like 5 proteins. For small proline-rich proteins (SPRRs), SPRR1A and SPRR1B (SPRR1 subfamily members) were up-regulated in siRNA-treated cells, while SPRR2A-F (SPRR2 subfamily protein) was down-regulated. Although previous studies identified some *in vivo* functional redundancy for ECM1 in epidermal differentiation, our findings indicate that there are specific processes of epidermal differentiation and cornification in which ECM1 plays an important role.

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Stress related overexpression of ABCG2 transporter in keratinocytes: a putative target to modulate the efficacy of photodynamic therapy

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Xenobiotic transporters of the ATP binding cassette protein superfamily play an important role in maintaining the biochemical barrier of various tissues, however the precise role of these transporters in skin is not known. Previously we have shown that the ABCG2 transporter is highly expressed in proliferating keratinocytes. However, abrogation of ABCG2 function using gene silencing and specific inhibition with the non-toxic fumitremorgin C analogue Ko-134 did not affect the proliferation of HaCaT keratinocytes, suggesting that this transporter is not essential for cell proliferation. Next, we aimed to investigate whether ABCG2 transporter contributes to cellular stress response in keratinocytes. Irradiating healthy human skin with ultraviolet light-B (UV-B) resulted in the induction of ABCG2 protein expression in the granular layer of the epidermis. In an in vitro experiment Ko-134 inhibition of ABCG2 in UV-B irradiated keratinocytes significantly decreased cell viability. These results suggested that ABCG2 contributes to cellular stress response in keratinocytes. As the extrusion of porphyrins is one of the known physiological functions of ABCG2, the transporter is a promising target molecule for improving photodynamic therapy applications based on porphyrin photosensitization. We demonstrated that specific inhibition of ABCG2 using Ko-134 significantly decreased cell viability in delta-aminolevulinic acid (DALA) sensitized HaCaT keratinocytes, suggesting that targeting of this xenobiotic transporter by specific inhibitors may modulate therapy efficacy in dermatological disorders, such as non-melanoma skin cancer, psoriasis and acne.

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Betaine increases tight junction integrity in epidermal keratinocytes

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Transepithelial electrical resistance (TEER) is used as a measure of tight junction integrity in vitro, and this study illustrates the effect of betaine, which is used as osmolyte to improve hydration status of skin, on epidermal keratinocyte tight junctions. Normal human epidermal keratinocytes were differentiated in cell culture inserts in vitro for 4 days and during the differentiation, TEER, as well as expression of tight junction proteins, occludin (OCLN), zonula occludens-1 (ZO-1) and claudin-4 (CLDN-4) were measured. Differentiated keratinocytes were treated from the outside with 10, 50, 100, 250 and 500 µM betaine, and effect on tight junctions was assessed with TEER at 1h, 12h and 24 h after test substance application. The keratinocyte TEER as well as the expression of CLDN-4 but not ZO-1 or OCLN, increased during the 4-day differentiation process, indicating that the model is suitable to study tight junctions in epidermal keratinocytes. Betaine induced increase in TEER in a time-dependent manner with maximum increase of 47 % at 24 h with 500 µM betaine. The study indicates that betaine improves tight junction functionality in the epidermis and may improve the hydration status of skin also by improving tight junction strength at granular layer of the epidermis.

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The p75 neurotrophin receptor is involved in early keratinocyte differentiation

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p75 neurotrophin receptor (p75NTR) mediates NT signals alone or as a co-receptor of the high affinity receptor Trk. While trk receptors stimulate proliferation and survival in human keratinocytes, the role of p75NTR in the skin is yet to be defined. We evaluated the expression and function of p75NTR in human keratinocytes. p75NTR was only expressed in the basal keratinocyte layer and confined to transit amplifying (TA) cells. Moreover, calcium treatment of subconfluent keratinocytes induced the up-regulation of p75NTR concurrently with the expression of the differentiation markers involucrin and citokeratin 10. p75NTR retroviral infection of stem cells induced a more differentiated phenotype with the same features of TA cells. On the other hand, when p75NTR was silenced, calcium treatment failed to induce differentiation in subconfluent keratinocytes, as shown by the absence of involucrin expression. By immunohistochemistry, p75NTR positive keratinocytes did not express the proliferation marker MIB-1. Consistently, in vitro data confirmed that p75NTR positive cells proliferated to a lesser extent, as compared to p75NTR negative cells. Because psoriasis is characterized by alteration of keratinocyte differentiation we evaluated the expression and function of p75NTR in this disease. p75NTR was absent in lesional psoriatic skin and p75NTR levels were significantly lower in psoriatic than in normal TA keratinocytes, as shown by western blotting and FACS analysis. Northern Blot revealed no modulation in mRNA of psoriatic keratinocytes compared to normal, suggesting that this receptor undergoes a post-trascriptional modulation. These results suggest that p75NTR acts as a "switch on-off" protein in keratinocyte differentiation.

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E-FABP mediates human keratinocyte differentiation

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Epidermal differentiation is controlled by increased intra-cellular calcium influx and is dependent, among other factors, by lipid metabolism. Epidermal fatty acid-binding protein (E-FABP) is a lipid carrier involved in keratinocyte differentiation and it is over-expressed in psoriasis. Psoriasis is characterized by defective differentiation and is associated with co-morbidities, including increased fat absorption. We evaluated the role of E-FABP in keratinocytes. We show by immunohystochemistry that E-FABP is localized in suprabasal layers of normal human skin, overlapping CK10 and involucrin distribution. Upon treatment of cultured human keratinocytes with calcium, E-FABP expression is upregulated from 48 up to 120 hrs. E-FABP levels are highest in confluent keratinocytes, similarly to CK10 and involucrin expression. E-FABP is mostly expressed in post mitotic cells from both normal and psoriatic epidermis, whereas it is nearly absent in stem and transit amplifying cells. Transfection of normal human keratinocytes with recombinant (r) E-FABP induces a marked overexpression of CK10, as compared to controls. When keratinocytes treated with rE-FABP are seeded onto dermal equivalents, they generate a complete epidermis. Nevertheless, rE-FABP+ model displays an increased number of corneocytes retaining the nucleus. Moreover, E-FABP+ model shows changes in the pattern expression of CK10, psoriasin and E-FABP itself that resemble those observed in psoriatic epidermis. These data suggest that E-FABP is important for keratinocyte differentiation and might be involved in the aberrant differentiation process of psoriasis.

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Ultraviolet A induced oxidation identifies distinct populations in azathioprine-treated primary human keratinocytes

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Treatment with azathioprine is related to a higher risk for the development of cutaneous malignancies. The thiopurines azathioprine, 6-thioguanine (6-TG) and 6-marcaptopurine (6-MP) are immunosuppressants and anti-inflammatory agents mainly used in organ transplantation and autoimmune disease. 6-TG, the active metabolite of azathioprine and 6-MP, integrates into DNA as a purine analog. Persistence of incorporated 6-TG, even after discontinuation of azathioprine, has been shown in skin. It is known that UVA interacts with 6-TG generating the mutagenic agents reactive oxygen species (ROS) and a fluorescent 6-TG photoproduct, 2-aminopurine-6-sulfonate. Therefore, in order to determine whether 6-TG shows a distinct distribution in azathioprine treated primary human keratinocytes after discontinuation of the treatment we used a ROS assay that allows the detection of a fluorescein derivative by flow cytometry. Flow cytometry analysis suggests that 6-TG treatment alone induces an increase in ROS over untreated cells. Furthermore, UVA-assisted induction of ROS allows the discrimination of two distinct sub-populations within these keratinocytes. Further analysis of keratinocyte sub-populations will yield better characterization and may allow the identification of keratinocyte stem cells such as label-retaining and slowly cycling cells.

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Deletion of the late cornified envelope genes LCE3B and LCE3C confers susceptibility to chronic hand eczema with allergic contact dermatitis

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Chronic hand eczema (CHE) is a common skin disease with a high disease burden. Its pathogenesis is multifactorial and involves both, endogenous predisposition and environmental triggers. According to current concepts, an impaired epidermal skin barrier function may be essential for the development of CHE. It may increase the penetration of allergens through the epidermis, reduce the resistance against irritant damage and is likely to be based upon genetically determined modifications in the structure of the cornified envelope. The aim of this study was to assess the role of a deletion in the late cornified envelope genes LCE3B and LCE3C in the development of CHE. In total, 153 German patients with clearly defined CHE subtype were screened for the LCE3C_LCE3Bdel-deletion by allele-specific polymerase chain reaction. The prevalence of this variant in CHE patients was compared to that in 268 healthy individuals. Overall allele frequency and number of deletion carriers did not differ in patients and controls. Nevertheless, when the deletion carriers were classified according to clinical subtypes a significant association of the LCE3C_LCE3Bdel allele with CHE due to delayed-type hypersensitivity became apparent. In the allergic contact dermatitis subgroup the number of deleted alleles and the frequency of individuals carrying at least 1 deleted allele prevailed [49/74 versus 25/74 alleles; p=0,0397; 95% confidence interval (CI) 0,99-3,17; 81% deletion carriers with at least 1 LCE3C_LCE3Bdel allele]. In conclusion, a deletion of late cornified envelope genes may contribute to manifestation and maintenance of CHE subtypes involving delayed-type hypersensitivity, i.e. allergic contact dermatitis.

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Effects of the re-innervation of organotypic skin explants on the epidermis

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The nervous system takes part in skin homeostasis and interacts with skin cells. In in vitro organotypic skin models, these interactions are lost due to absence of nerve endings.We have developed an in vitro organotypic skin model based on a re-innervated human skin explant using primary sensory neurons from the dorsal root ganglia of rats. After 10 days of co-culture of skin explant and neurons, a dense network of nerve fibers was observed. The epidermis and dermis presented many new structures that showed immunoreactivity to PGP9.5 and pan-neurofilaments antibodies. Epidermal thickness, cell density and quality were all higher when skin explants were cultured in the presence of neurons. Semaphorin 3a and number of proliferative epidermal cell were not modified in the epidermis. Nerve growth factor amounts were higher in the control skin explant. In vitro re-innervated skin explants appear to be more similar to normal *in vivo* skin than to non-re-innervated skin explants. Hence, this new model of co-cultured skin explants and neurons allows better epidermal quality and could be very useful for studies concerning interactions between the skin and its peripheral nervous system.

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Caspase-14 is required for filaggrin degradation to natural moisturizing factors in the skin

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Caspase-14 is mainly expressed in suprabasal epidermal layers and activated during keratinocyte cornification. Caspase-14 deficient mice display a reduced epidermal barrier function and an increased UVB radiation sensitivity. We found that although profilaggrin, a protein with a pivotal role in skin barrier function, is processed correctly to its functional filaggrin monomeric unit in caspase-14$^{-/-}$ mice, these mice accumulate proteolytic filaggrin fragments in the epidermis. We show here that the accumulation of these filaggrin fragments is due to a defect in filaggrin degradation in the cornified layers of caspase-14$^{-/-}$ skin. Consequently, this lack of normal filaggrin degradation results in a significant reduction in the levels of natural moisturizing factors, such as urocanic acid and pyrollidone carboxylic acid, in the skin from caspase-14 deficient mice as compared to wild-type mice. In addition, we demonstrate that caspase-14 can directly cleave the filaggrin monomer. Taken together, our data identify caspase-14 as a crucial protease in filaggrin catabolism.

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Development of a dynamic mathematical model of epidermis reveals critical importance of increasing the proportion of both cycling stem and transient amplifying cells, in the development of psoriatic plaques.

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Mathematic modelling allows exploration of the contribution of specific quantifiable parameters to processes such as epidermal homeostasis. We created a stochastic agent-based model which combines known kinetic parameters with histological features characteristic of normal and psoriatic epidermis. Iterative stimulation of the model allowed rigorous testing and establishment of robust boundaries including analysis of cell cycle time and the proportion of cells dividing, within which the model remained stable. In contrast to adjusting cell cycle times, the model showed a critical role for an initiating «cytokine» stimulus and a resulting increase in the proportion of actively dividing stem cells and the number of rounds of transient-amplifying (TA) cell division within 'uninvolved' epidermis. The model showed excellent concordance with existing data including an absolute increase in both the proliferative and differentiating compartments and a reduction in both total epidermal turnover time and transit time of differentiating cells. Adjusting cell cycle length within defined boundaries had no significant impact on the model. Importantly, the model remained stable and became independent of a continuous cytokine stimulus through production of «autocrine» factors. In summary, we have developed a stochastic model of epidermal homeostasis which has allowed us to create for the first time an in silico model of psoriasis development. The model has the potential to provide insight into psoriatic plaque development and the impact of treatment modalities.

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Sirt6 play an essential anti aging role in skin cells

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SIRT6 is a member of the Sirtuins genes family which regulates fundamental processes in aging and lifespan control. It has been shown that SIRT6 knockout mice display premature aging symptoms and that this effect is partly due to its role on the stabilization of the telomeric chromatin and to its implication on the NF-κB regulation pathway. Hence, we were interested in studying the impact of modulating SIRT6 on cell aging, using IV09.009, a selective SIRT6 inducer. Our studies of human fibroblasts and keratinocytes, aged in vitro by repeated subcultures, demonstrated that IV09.009 was able to increase SIRT6 expression in these cells. Furthermore, we were interested in investigating the involvement of SIRT6 towards telomeres. As the Telomere-Associated Protein TRF2 has an important role in telomeric protection, we evaluated this protein level. This study showed that long term application of SIRT6 inducer increased the expression of this protein, allowing a better stabilization of the chromosome ends. Moreover, we observed in aged fibroblasts an enhancement of Collagen I expression which indicates a better functioning suggesting an anti aging effect. Finally, SIRT6 implication in keratinocytes differentiation as another anti aging aspect, was demonstrated by IV09.009 effect on enhancing involucrin and transglutaminase-1 expression. These results confirm the important role of SIRT6 in cell differentiation and aging and that Sirt6 is a key target for anti aging approaches.

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Proteasome activation helps alleviate methylGlyoxal (MGO) induced ultrastructural damage in skin cells

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Previously we have shown beneficial effects of IV08.013, an inducer of proteasome activation, on cultured fibroblasts and keratinocytes. Presently, we examined the ultrastructure of human epidermal keratinocytes and fibroblasts, subjected to oxidative stress by MGO (0.2 and 0.4 mM for 24h), and subsequently treated with IV08.013 for 48 hours. We observed that effects of MGO stress was more pronounced in keratinocytes, which showed extensive cytosolic vesicules, evidence of ER stress, as well as altered keratin filament organization. Fibroblasts seemed more resistant to MGO induced damage compared to keratinocytes, but displayed hallmarks of ER stress. In both cell types, some cells were more damaged than others, possibly reflecting cell-cycle related susceptibility to stress. In general, IV08.013 treated cells displayed increased numbers of autophagic vacuoles, as well as healthier cellular organelles such as mitochondria and Golgi complex. However, some of the cells - both keratinocytes and fibroblasts - (presumably irreparably damaged) did not display signs of repair and recovery. Our preliminary observations also suggest that cells stressed with a higher dose of MGO show better recovery following treatment with IV 08.013, possibly due to a threshold effect on ubiquitination as well as induction of autophagy. These studies confirm the role of proteasome system on cell good functioning and its interest in the anti aging field

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Modulating Caspase-14 expression helps DNA protection and repair against UVB irradiation

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Caspase-14 was described to play a role in epidermal barrier formation, the upregulation of caspase-14 expression by UVB irradiation suggests its implication in the UV-stress response. Using IV08.003, which we developed as a caspase-14 inducer, we studied caspase-14 role in barrier recovery, as well as in UVB repair and protection. Human skin samples were submitted to repeated tape-stripping. Biopsies were then performed on stripped and adjacent undamaged area, and treated with IV08.003 for 72h. Our results showed better recovery of the cornified layer in treated biopsies. Moreover, cultured human keratinocytes (HK) were pre-treated with IV08.003 for 24h, and then irradiated with 50 mJ/cm2 of UVB. Expression of CPDs and of 8-Oxo-2′-deoxyguanosine (8-oxodG) were investigated 1 hour after UVB stress. This study showed a markedly reduced signs of DNA damage in IV08.003 pre-treated cells.Similarly, in skin biopsies where caspase-14 was pre-induced, we noticed fewer signs of UV stress and less CPDs staining, compared to control. Moreover, active caspase-3 level was also reduced in pre-treated biopsies. Interestingly, a reduction of DNA damage markers and active caspase-3 was also observed when HK and skin biopsies were first irradiated with UVB and then treated with IV08.003, indicating a repair effect. These results strongly suggest that enhancing caspase-14 is a relevant method for protecting the epidermis against UV irradiation and facilitating DNA repair after UV damage.

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Regulation of biological mechanisms by sucralfate, an actor of epidermal re-epithelization

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Wound healing is a multicellular process that, in skin, aims at barrier restoration. Epidermal regeneration is a crucial step in this process. It involves the combination of two cellular processes, proliferation and migration, temporally and locally orchestrated by specific molecular mediators (growth factors, cytokines, ECM, integrins and enzymes). Sucralfate, a basic salt of sulphated sucrose, has proven its efficacy in the healing process of skin wounds. The purpose of our study was to examine its action on proliferation and migration of human epidermal keratinocytes in culture and elucidate some of the molecular mechanisms involved. Normal human keratinocytes were submitted to 48h-treatment with increasing concentrations of sucralfate. Cell proliferation was assessed by the measurement of BrdU incorporation. HaCaT keratinocytes migration was analyzed with the Oris Cell System. Then we performed a HaCat culture with sucralfate to assay the expression of molecular mediators involved in wound healing at the transcriptional level with real-time RT-PCR and at the protein level by immunolabeling. Our results showed that sucralfate was able to stimulate cell proliferation and to enhance cell migration. We also showed that, following sucralfate treatment, the expression of hyaluronane synthase 2 (proliferation), MMP9 (migration) and activin A (cell migration and proliferation) was significantly induced with kinetic characteristics close to treatment with EGF or TGFb. Together, the present data obtained in vitro support the positive action of sucralfate on skin wound healing by improving epidermal re-epithelization through the specific regulation of the expression of molecular actors.

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Mitochondria boosters helps reducing cell oxidative stress induced by tobacco

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It is well documented that tobacco contributes to premature skin aging. Cigarette is considered as a key source of oxidative stresses, in addition to others stresses such as UV rays. In this study, we developed a method to examine the effects of cigarette smoke, as an oxidative stress inducer, on normal human keratinocytes. In parallel, we evaluated the effect of two active ingredients that boosts mitochondria, on helping reducing this stress. A preliminary study on keratinocytes morphology revealed that tobacco smoke induces characteristic stress damage within the first hour. Hence, the following tests were performed 1h after tobacco smoke stress. Our studies showed that ROS level increased significantly in cells exposed to tobacco smoke compared to unstressed control. This effect was modulated by IV08.001 or IV08.005 application, compounds demonstrated to protect and enhance mitochondria functions in cells. In stressed cells, the reduction of ROS level in smoke-exposed cells, treated with the compounds indicates that these cells were better protected from ROS damage. Moreover, HSP27 proteins studies revealed that smoke induced an increase in HSP27 expression, however, treated cells that were stressed, exhibited a reduced level of HSP27 expression compared to control, suggesting less stress in these cells. These results confirm the oxidative stress caused by cigarette smoke on the skin, that this stress affects mitochondria, and suggest that mitochondria boosters and protectors are of interest reducing smoke-induced premature skin aging.

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Evaluation of the features of skin aspect using in vivo confocal microscopy

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Skin aging and dryness depends on variety of different factors. In order to trace the stages of these changes, we investigated, through *in vivo* confocal microscopy (Vivascope® 1500), the following skin parameters, on the forearm: horny layer and epidermal thickness; granular cell morphology and organization. The number, height, and morphology of dermal papillae were investigated for skin aging changes. On the forearm, the thickness of the horny layer in normal, hydrated skin is around 8µm, while the thickness of the whole epidermis is around 55 µm. Corneocytes are well-organized, cohesive, and granular cells possess a cobblestone pattern. Keratinocytes in moisturized skin, on the other hand, are arranged in a regular honeycomb-like architecture. Moreover, the horny layer and epidermis are thinner than in normal skin, due to the excellent cohesion of cells. In dry skin, by contrast, architectural disruption of the epidermis is noticeable, and is accompanied by a thickening of the stratum corneum and epidermis. In aged skin, we observed a significant statistical correlation between age and thickness of the horny layer as well as with age and epidermal thickness. Indeed, with age, the thickness increased. Regarding dermal papillae, we found the number and the height in a reverse correlation with age. Dermal papillae became smaller, less defined, and irregular in shape. These results confirm that VivaScope® is useful and complementary to macroscopic observation in the evaluation of skin changes.

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Combining tyrosinase inhibitor and proteasome activator exhibits interesting whitening effect on skin. Histological and transmission electron microscopy studies

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Whitening products have become central in skin care research. Decreasing skin pigmentation involves mechanisms including inhibiting tyrosinase activity, as well as TRP-1 and TRP-2. Tyrosinase is the rate limiting enzyme, and TRP-1 (DHICA oxidase) and TRP-2 (Dopachrome tautomerase) are related gene products that are known to modulate the melanosomal matrix. In previous studies, we have showed that (IV08.013), a natural extract, exhibits whitening properties by inhibiting tyrosinase activity. Our studies have also revealed that (IV09.020), a compound that stimulates cell proteasome system, exhibits a lightening effect on *ex vivo* skin. As in silico simulations predicted synergy between these two approaches, we evaluated the whitening effect of (IV08.013) alone and in combination with (IV09.020) on human *ex vivo* skin. Examination of skin sections stained with Fontana-Mason (FM) revealed a noticeable decrease in melanin content in the epidermis, in both condition, compared to the controls. This decrease in melanin was more noticeable in the combination condition. Transmission Electron microscopy (TEM) revealed a lightening effect at the level of melanosomes in both conditions. Moreover, TEM study also showed that the combination of the two compounds resulted in lightest melanosomes, and melanophagosomes, confirming the synergy between the two. These studies support the interest of combining different mechanisms in order to decrease melanin expression in skin, and suggest that proteasome boosters are good candidate to combine with tyrosinase inhibiting extracts for whitening purposes.

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Studies of TRF2 telomeric protein role in aging

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Telomere shortening is one of the main characteristics of cellular aging. A complex of proteins called shelterin can bind directly to the TTAGGG repeats of telomeres, leading to their stabilization in T-loops. Among these proteins, TRF2 plays a central role as a binding platform for other telomere-associated proteins. Our previous studies have shown that positive modulation of TRF2 protein expression was associated with a decrease in the cellular senescence marker beta-galactosidase. In the present study, we further investigated the effect of TRF2 modulation in a model of accelerated, in vitro senescence induced by methylglyoxal (MGO). Morphological changes were observed in fibroblasts aged by replicative senescence in parallel to MGO-induced senescence. Fibroblasts were treated or not with the previously described TRF2 inducer (IV08.007). Morphological evaluation showed that TRF2-induced cells showed fewer signs of aging, compared to untreated cells. Aged phenotype was also associated with modifications of the cytoskeleton protein vimentin, observed in replicative and MGO-induced aging. Treatment with IV08.007 tended to limit the increase in vimentin expression in aged cells. Studies on aged fibroblasts using the senescence-associated beta-galactosidase (SABG) marker revealed, in TRF2-induced cells, a reduction in the number of SABG-positive cells. Our results confirmed that the TRF2 telomeric protein plays an important role in the appearance of a senescent phenotype, and suggest that modulation of TRF2 expression can be of interest in anti-aging applications.

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Impact of a lightly crosslinked PVP polymer on α-hydroxy, and b-hydroxy-acids on skin. Ex vivo skin model for structural studies

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Chemical peelings with alpha-hydroxy and beta-hydroxy acids are largely utilized for skin rejuvenation. We were interested in developing an *ex vivo* model to evaluate peeling products in early stage, distinguishing between the proper peeling effect and other skin damage signs. Using this model, we evaluated the exfoliating effect of glycolic acid and salicylic acid from lightly cross-linked PVP polymer (LPVP) gel formulations and compared them to simple solutions. Our kinetic studies of glycolic acid in LPVP gel included 5min, 10min, 1h, 2h, 3h and 4h. This study showed that 3h at least were necessary to alter the epidermis. Recovery was evaluated after 6 days. Then we proceeded on testing glycolic acid and salicylic acid in different LPVP gels. These studies showed that aqueous LPVP gel allowed better skin morphology whereas alcoholic LPVP gel seemed to alter aggressively the skin. They also showed that 50% glycolic acid in aqueous LPVP gel damaged less the skin compared to the alcoholic or aqueous solution. Concerning 10% salicylic acid, only the epidermis treated with salicylic acid in LPVP gel showed signs of regeneration after 6 days. Hence, with the developed protocol we were able to compare the impact of LPVP polymer on the different formulations containing alpha-hydroxy and beta-hydroxy acids, and to evaluate the degree of peeling or damage into skin.

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Mitochondria boosting exhibits rejuvenating effects on skin comparable to light-emitting diodes (LEDs). In vitro and in vivo studies.

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Interest in anti-aging approaches has grown significantly in recent years, among them mitochondrial boosting and light-emitting diodes (LEDs) known to target mitochondria. In this regard we were interested in evaluating the anti-aging effect of boosting mitochondria by cosmetic formulation, and to compare it to the bio-physical LED approach in in vitro and *in vivo* studies. Studies on cultured human fibroblasts and keratinocytes showed that boosting mitochondria activity by cosmetic compounds revealed anti-aging properties like enhancing cell fibronectin, collagen III, TG-1, and involucrin; and decreasing cellular ROS level, within 72h. Interestingly, similar effects are described for treating the skin with specific anti-aging LED light. For the *in vivo* double blind study, 12 volunteers applied a cream containing the mitochondria boosting cream formula or placebo on the forearm. Treated skin was compared to LED-exposed skin. Evaluation by *in vivo* confocal microscopy showed that, after 2 months, both booster-cream and LED similarly and significantly improved the skin structure. Interestingly, at day 28 significant improvement of many parameters of skin rejuvenation was seen only with the booster-cream. These parameters included epidermal thickness, granular cell shape and organization, dermal papilla morphology and embellishment. These results suggest that direct mitochondria boosters in formulated cream would act faster for anti-aging effect than LED.

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Fluorescein-Isothiocyanate Induced Sensitization Indicates a Damaged Skin Barrier in Transglutaminase 3 Knockout Mice

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Recently more structure proteins and enzymes have been identified as significant contributors to a normal skin barrier, which lack or damage results in defected skin functions, bent to enhanced percutan sensitization.The aim of this essay is to evaluate whether transglutaminase 3 (TGM3) knockout mice exhibit a defective skin barrier. Since we could not indentify significant physical differences between knockout and wild type (C57BL/6) mice indicating damaged barrier functions, we performed a fluoerescein-isothiocyanate (FITC) sensitization test. Mice aged 8-12 weeks, were sensitized by a 24h long occlusive application of FITC on day 1 and 8, were reexposed on day 15 and were monitored in 24h and 48h, by mouse ear swelling test, and histology. Out of the 38 TGM3 -/- mice 14(36%) exhibited a significant (>20%) ear thickening (ET) at 24h, and 31/38 (81%) at 48h. In the control group 0/ 22 (0%) mice at 24h and 5/22 (17,8%) at 48h had significant pathology. The extent of ET among TGM3 -/- mice (n=38) was 13,98% at 24h, and 47,10% at 48h, displaying a significant difference to the wild type animals (n=22), where ET was 7.03% at 24h ($p<0,05$) and 14,64% ($p<0,001$) at 48h. While the histopathological signs of skin inflammation in TGM3 knockout mice were more conspicuous, in the number of infiltrating mastocytes no significant difference was found. Our data prove that TGM3 -/- mice display a significantly enhanced skin sensibilization rate to FITC. This observation indicates a defected skin barrier in these animals showing that TGM3 is a key enzyme of the functional skin barrier.

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The structure and regulation of miR-203 gene in keratinocytes

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Recently, our group identified a keratinocyte-specific microRNA, miR-203, and showed that this microRNA is crucial for keratinocyte differentiation. To date little is known about the factors that are responsible for preferential expression of miR-203 in the skin as well as about its structure, biogenesis and turnover in keratinocytes. To get insights into the structure of the miR-203 gene, we used 5´RACE to identify the transcriptional start site. To learn more about its transcriptional regulation, we cloned the putative promoter region of miR-203 and identified several AP-1 transcription factor binding sites. We therefore investigated the influence of the AP-1 complex and the MAPK signaling pathway on the expression of miR-203. Chemical inhibition of the MAPK pathway and knock-down of AP-1 members showed that the expression of miR-203 is negatively regulated by the EGF-induced MAPK signaling pathway and by JNK, a regulator of AP-1. These results suggest that the activation of AP-1 by the MAPK pathway inhibits the expression of miR-203 and thus prevents differentiation of keratinocytes. Interestingly, the miR-203 gene resides in a classical CpG island, suggesting that it is regulated by epigenetic mechanisms such as DNA methylation. To get insight into the long term regulation of miR-203, we are currently profiling the CpG methylation pattern of the miR-203 gene locus. Preliminary data from bisulfate-converted DNA show that the miR-203 gene locus is partly methylated in PBMCs, which do not express miR-203. Thus, methylation represents a putative mechanism to silence miR-203 in cells other than keratinocytes.

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Adipose tissue-derived mesenchymal cells support more efficiently than dermal fibroblasts the growth of keratioNcytes through secretion of KGF-1 and PDGF-BB

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Epidermal organization and homeostasis are regulated by mesenchymal influences through paracrine actions. Until today, dermal fibroblasts are used as the classical feeder layer to support keratinocyte growth in vitro, and in skin substitutes used to cover burns and non-healing wounds. We examined the potential use of human adipose tissue-derived mesenchymal cells (ADMC), a novel source of mesenchymal stem cells, instead of dermal fibroblasts in 2D and 3D keratinocyte (primary and HaCaT) cultures. We show that ADMC induce cell proliferation through induction of cell cycle progression, as well as migration of keratinocytes more efficiently than fibroblasts. This effect does not require cell contact and is, at least partially, mediated by KGF-1 and PDGF-BB, which are more prominently expressed in ADMC than in fibroblasts. Furthermore, replacement of fibroblasts by ADMC in the dermal compartment of organotypic cultures leads to an artificial epidermis with a structure resembling more to that of normal skin, concerning cytokeratins 14, 1 and 10 expression. However, in the presence of ADMC cytokeratins 5 and 19 are abnormally highly expressed in all epidermal layers compared to fibroblasts. In conclusion, ADMC could serve as supportive cells for primary keratinocyte cultures, while due to their high abundance and the great cell yield obtained during isolation compared to dermal fibroblasts, they comprise an interesting cell source, with potential aspects for clinical use in artificial skin substitutes.

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Comparative analysis of esterase activity in reconstructed human skin models and excised human skin

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To date, reconstructed 3D models of human skin and epidermis have mainly been characterized regarding their barrier function and validated for the use in in vitro tests for skin corrosion and skin irritation. A great deal less is known about their metabolic capacity concerning activation of prodrugs and toxification or detoxification of chemicals and drugs. In this study, we aimed to compare the esterase activity in commercially available reconstructed human skin and epidermis models and excised human skin. Metabolite profiling of the double ester prednicarbate by HPLC-UV and determination of enzyme kinetic parameters Vmax and KM using fluorescein diacetate as a model substrate was performed in parallel. After 24 hours prednicarbate exposure, prednisolone was detected as the main metabolite, however, the relative amount ranked as: full-thickness (epidermis and dermis) skin model ~ epidermis model > excised human skin. The similar results for full-thickness and epidermis models can be explained by the higher esterase activity in human keratinocytes as compared to fibroblasts which contribute very little to the total activity. The formation rates of fluorescein fitted the Michaelis-Menten model. In accordance with prednicarbate metabolism, Vmax of fluorescein diacetate cleavage was highest in full-thickness skin models and lowest in excised human skin. KM values did not markedly differ between the test matrices. In conclusion, our results indicate that reconstructed human skin models may be useful to quantitatively address esterase activity of native human skin, although an increased activity compared to normal human skin should be taken into account.

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The weak rate of sphingolipid biosynthesis shown by basal keratinocytes isolated from aged versus young donors is fully rejuvenated after treatment with peptides of a potato hydrolysate

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A new study was carried out to bring more informations on the effect of the potato hydrolysate Lipidessence®. Basal keratinocytes were established in culture from freshly excised skin samples of two groups of 5 donors, a young one (25 to 36 year-old) and an aged one (59 to 70 year-old). The results showed a downward trend in the content of all lipid fractions in untreated keratinocytes of aged donors as compared to young ones. We found major differences between young and old donors in the response of keratinocytes to Lipidessence® treatment. Whereas the lipid content of cells from young donors increased either moderately or actually decreased in some cases versus untreated controls, the lipid biosynthesis was strongly stimulated in aged donors' keratinocytes whose lipid contents globally became close to those found in young donors. However, the changes elicited by Lipidessence® treatment were not seen at the same extent extensive for all lipid classes. Cholesterol increased up to threefold and alpha-hydroxy fatty acids were augmented up to sevenfold, whereas the increase in normal fatty acids was quite moderate. In sphingolipids labeled by incubation of keratinocytes in culture medium containing [^{14}C]-serine, ceramides and glucosylceramides in cells from aged donors showed the highest uptake of radioactivity, with somewhat less incorporation in sphingomyelin and gangliosides. Therefore, it seems that Lipidessence® has a much more potent stimulatory activity on the lipid biosynthesis of basal keratinocytes of aged donors, thereby normalizing the cellular lipid content that obviously decreases along with ageing.

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The use of a precursor as a source of exogenous bio-available linoleic acid: assessment of the skin metabolism and of the nourishing effect of a cream containing it

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Skin aging is associated with lipid content modifications, such as age-dependant decrease in epidermal linoleic acid. Here, we used a prodrug approach providing the linoleoyl monoglycerate (LMG), that could undergo biotransformation into free linoleic acid, under epidermal esterase action. Using *ex vivo* skin model and radiolabeled [C14]-linoleyol glycerate we investigated the skin metabolism of 0.5 to 2% LMG emulsion, topically applied. We demonstrated the prolong release of exogenous linoleic acid into the skin from LMG. After 24h, the 1% emulsion gave the highest percentage of enzymatic release. As it was partial, we studied the ability of LMG and free linoleic acid to stimulate some epidermal differenciation markers involved in the barrier function against water loss. The keratinocyte expression of Tansglutaminase 1, ABCA12 transporter and beta-glucocerebrosidase were induced, with a higher effect of the free form. Moreover, we analyzed de novo synthesis of epidermal lipids, in human skin explants, treated or not by the formulated LMG in association with intermediate size hyaluronate fragments (HAFi). Evaluation was performed using the incorporation of the radioactivity into acetate and specific lipids were analysed on chromatographic system. We showed a significant increase in the three classes of cutaneous lipids, cholesterol, ceramides and fatty acids. Together, these data demonstrated the interest of using the prodrug technology to liberate, exogenous linoleic acid to compensate the endogenous decrease with aging, and hydrating glycerol into the skin. Moreover, this study has demonstrated the nourishing effect of a cosmetic product containing the association HAFi-LMG.

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The tight junction protein, claudin-7 is increased in photoaged human epidermis

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Epidermal tight junction (TJ) plays an important role as a barrier which protects the skin against water loss and penetration by pathogens. Human skin is subject to intrinsic and extrinsic ageing which both cause a reduction in skin barrier function. The major environmental insult to human skin is chronic sun exposure, termed photoageing. In this study, we investigate whether the TJ protein claudin-7 is altered in intrinsic and extrinsic skin ageing. Punch biopsies (6mm diameter) were taken from healthy Caucasian volunteers (age range, 18-30 and 65-75 years; n=8 per group) from photoprotected hip and upper inner arm and from photoexposed forearm. Cryosections (10μm) were prepared, stained with a polyclonal antibody to claudin-7 (Zymed Labs) and visualised using an FITC-conjugated secondary antibody. Staining intensity was quantified using ImageJ software (NIH). Immunofluorescence confirmed the epidermal expression of claudin-7 in the granular layer of the epidermis. Image analysis shows a significant difference in the expression pattern between photoprotected (hip, upper inner arm) and photoexposed (forearm) sites (repeated measures ANOVA; $p = 0.018$ and $p = 0.019$ respectively). However, no significant changes observed between young and aged photoprotected buttock (unpaired Student's t-test; $p = 0.397$). This study indicates that claudin-7 is up-regulated in chronically photoaged human skin *in vivo*. Further work is required to look at the effect of acute UVB exposure on claudin-7 expression in vitro and to clarify the mechanisms underlying these observations.

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Phospholipase C signalling in cutaneous squamous cell carcinoma

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Phospholipase C (PLC) is an enzyme that triggers cell growth, differentiation and migration through activation of pathways like protein kinase C (PKC). Recent work from our group showed that knock-down of type VII collagen (ColVII), the main component of anchoring fibrils, in SCC cell lines leads to increased expression of an isoform of phospholipase C, PLC-β4, previously known to be expressed in neural tissue. In this study, we used siRNA technology to examine the function of PLC-β4 in cutaneous SCC. Immunostaining of a panel of SCC samples revealed expression of PLC-β4, mainly in poorly differentiated tumours. Organotypic 3-D cultures generated with SCC cells with ColVII knock-down (siCol7) and control (siC) cells demonstrated that PLC-β4 and PKCα are both localised to the basal layer in control and have homogenous expression throughout the epidermis in siCol7 cultures. Successful knock-down of PLC-β4 (siPLC-β4) was achieved in a cutaneous SCC cell line. A time-course analysis by immunoblotting demonstrated potent inhibition of PLC-β4 expression up to 6 days post-transfection. Expression of PKCα was also decreased in siPLC-β4 cells up to 4 days post-transfection. High proliferative activity, characteristic of SCC, is associated with ERK activation and siPLC-β4 cells had decreased phosphorylation of ERK1/2. In a scratch assay of confluent cells, siPLC-β4 cells display markedly decreased migration compared to control. Experiments in progress include 3D cultures to examine the role of PLC-β4 in differentiation, invasion and calcium mobilization assays. The data, so far, suggest that PLC-β4 may be an important player in the migration of aggressive/poorly-differentiated SCC.

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The role of cell polarity in epidermal morphogenesis and homeostasis

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Cell polarity is crucial for tissue morphogenesis and maintenance by regulating a variety of functions, such as barrier function, migration and cell shape. Atypical protein kinase C (aPKC) is a key player in the regulation of polarity, although most of its functions have been studied in lower organisms or in mammalian simple epithelial cells. To examine how polarity regulates the formation and maintenance of a stratifying epithelium, we inactivated aPKCλ in the epidermis and its appendages using the K14-Cre recombinase (aPKCλ$^{epi-/-}$). Loss of aPKCλ resulted in a hyperthickened interfollicular epidermis, enlarged sebaceous glands, misshaped hair follicles and keratinocyte cell shape changes. Murine hair follicles undergo constant cycles of degeneration, rest and growth. In contrast, aPKCλ$^{epi-/-}$ hair follicles (HF) failed to enter resting phases of the hair cycle. This was associated with increased proliferation *in vivo* and vitro and a loss of label retaining cells in the HF bulge. Detailed immunohistochemical and FACS analysis revealed major alterations in HF differentiation and stem cell markers in the absence of epidermal aPKCλ, suggesting that changes in cell fate underlie some of the observed phenotypes. Loss of aPKCλ resulted in altered expression of several components of the Hedgehog pathway, which might provide a molecular explanation how aPKCλ regulates epidermal morphogenesis and maintenance. Overall, our analysis indicates a key role for aPKCλ in the regulation of stem/progenitor cell proliferation, differentiation and maintenance of stratifying epithelia.

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Analysis and Partial Characterization of Murine Filaggrin-2

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Expression of the "S100 fused-type" family is upregulated during formation of the outermost epidermal cell layer, the stratum corneum. Here a member of the murine S100 fused-type proteins, the profilaggrin-related filaggrin-2, was partially analyzed on mRNA and protein level. It shows a highly conserved organization similar to all known S100 fused-type proteins on genomic, mRNA and protein level. The 5′ and 3′ BALB/c mouse mRNA sequence was verified by RACE and results in a 2362 amino acid long protein. The characteristic S100/EF-hand-domains at the N-terminus are followed by 14 repeat domains of 73-80 amino acids in length flanked by a spacer sequence related to the profilaggrin B-domain and the C-terminus. To compare the murine to the human expression pattern different tissues were analyzed by RT-PCR, immunohistochemistry, and Western Blot. The murine filaggrin-2 shows expression patterns on mRNA and protein level similar to the human filaggrin-2 and was detected in the upper cell layers of skin at different body locations, tongue, esophagus, and forestomach. Cultured BALB/c keratinocytes showed an elevated filaggrin-2 mRNA expression upon $CaCl_2$-stimulation whereas barrier disruption had no significant effect on either mRNA or protein expression. The presence of multiple bands lower than the expected full length protein in SDS-treated skin extracts indicates a processing of filaggrin-2 as already known for profilaggrin. Since the expression of murine filaggrin-2 is regulated differentiation-dependent as also known for the human homologue the protein might be involved in terminal differentiation and may even have filaggrin-supporting function during this process.

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Profilaggrin constitutively provides a basal antimicrobial reservoir for the skin

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The stratum corneum of the outermost skin layer employs numerous antimicrobial peptides to protect from microbial infection. However, it remains unclear which molecular components maintain a basal and constitutive reservoir of antimicrobial activity. We previously revealed that protein fragments of hornerin and filaggrin-2, two members of the S100 fused-type protein family, possess strong antimicrobial activities. Fragments of profilaggrin, another member of the S100 fused-type protein family, might be a novel source of the antimicrobial barrier function of the skin. Seven different peptides, representing parts of the N-terminal domain, the C-terminal region, and two fragments located within the repetitive regions were recombinantly expressed. Radial diffusion assays showed that some of these fragments exhibited antimicrobial activity against the tested microbes, including E. coli, S. aureus, P. aeruginosa and C. albicans. As filaggrin occupies up to 10% of total protein of the cornified cell envelope in the stratum corneum, our results suggest that profilaggrin may constitutively provide a basal antimicrobial reservoir for the skin to fight micro-organisms at the earliest stage of infection.

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Epidermal Calcium Concentrations in a Murine Atopic Dermatitis Mouse Model Visualized by Fluorescence Lifetime Imaging

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Calcium is a major player of regulation of keratinocyte differentiation and proliferation. It is involved in establishing barrier function of skin which is, in part, maintained through Ion-selective tight junctions localized in the Stratum granulosum (SG), the cornified envelope in the Stratum corneum (SC), and the extracellular lipid matrix of the SC. Earlier experiments showed a calcium gradient in normal skin increasing from the basal layer to its peak in the Stratum granulosum and an abrupt drop in the lower Stratum corneum. Atopic dermatitis is associated with an impaired epidermal barrier function, increased epidermal proliferation and changes in differentiation of epidermal keratinocytes. Several reports indicate changes of epidermal calcium and an influence of external calcium on eczema. We here are using a defined inducible murine atopic dermatitis model, OVA, to assess changes of the epidermal calcium distribution compared to normal skin by using two-photon fluorescence lifetime imaging microscopy (FLIM). We reproducibly induce an eczema as evidenced in gross morphology (erythema and scaling), a broadened epidermis in standard histology (H&E), and function (increased TEWL). Our first results in FLIM-experiments show increased calcium values throughout a major part of the broadened epidermis with a less steep increase, where normal, untreated murine skin shows a narrow epidermis with lower calcium values overall, increasing towards the SG. Our ongoing investigation will further detail the epidermal calcium distribution, and its dependence on the state of the eczema, be it acute or chronic, and on its treatment.

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Skin barrier disruption caused by insect bites selectively upregulates skin's antimicrobial protein expression

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Insect bites lead to toxic and allergic reactions, inflammation, itch, and pain. Infections occur only occasionally, though bites disrupt the skin barrier and may lead to invasion of bacteria into the skin. We ask whether antimicrobial proteins (AMPs) may be responsible for the low rate of infections. We determined the expression of human beta defensin (hBD)-2 and -3, human neutrophil peptide (HNP)-1-3, RNase 7, psoriasin (S100A7), and cathelicidin LL-37 by immuno-histochemistry in skin samples obtained from insect bite lesions. Skin explants injured by pinpricks served as control. H&E staining after insect bites revealed an infiltrate of lymphocytes, neutrophils, eosinophils, and mast cells in the dermis. In the epidermis adjacent to the insect bite channel moderately increased staining for hBD-2 and -3, and RNase 7 was found, whereas a pronounced increase of psoriasin expression was noted. Within the insect bite channel and in the adjacent dermis, but not within the epidermis, we found an intense staining for LL-37 and HNP-1-3, both mainly derived from neutrophils. In bullous insect bite reactions staining for LL-37 and HNP 1-3 was found within the blister fluid. In the pinprick controls induction of hBD-3, RNase 7, and LL-37 occurred. In summary, we found upregulation of hBD-3, RNase 7, and LL-37 in insect bites and in pinpricks probably due to barrier disruption. The induction of psoriasin may be caused by the insect poison. This data indicates that AMPs protect skin against infections after skin barrier disruption caused by stinging and pinpricks.

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Lipid Signaling Through Liver X Receptor Alters Functions of Dendritic Cells

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Dendritic cells (DC) respond to changes in their lipid environment by changing their gene expression and immunophenotype. Some of these changes are mediated via the members of the nuclear receptor superfamily such as Peroxisome Proliferator Activated Receptors (PPARs), Retinoic Acid Receptor (RAR) and Vitamin D Receptor (VDR), that were found to support a tolerogenic DC phenotype.The Liver X Receptor (LXR) is also a nuclear receptor of oxysterols and as such regulates gene expression in response to changing lipid environment. In our work we found that LXRs are present and can be activated throughout human dendritic cell differentiation in monocyte derived DCs as well as in blood derived DCs. Administration of LXR specific natural or synthetic activators induced target gene expression accompanied by increased expression of several DC maturation markers such as CD80 and CD86. In mature DCs upon ligation with TLR4 or TLR3 ligands LXR activation also augmented the production of inflammatory cytokines, underpinned by prolonged NFkB signaling. The overall outcome of such activation of the LXR programmed DCs resulted in an increased capacity to activate $CD4^+$ T cell proliferation. Supporting such an inflammatory role we found that LXR positive DCs are present in reactive lymph nodes from patients with tuberculosis and sarcoidosis and was present also in DCs of tumor associated lymph nodes. We propose that activation of LXR represents a novel lipid-signaling paradigm that alters the inflammatory response of human DCs, therefore the role for LXR activation of DCs *in vivo* calls for further studies.

193 [Oral 028]

Interferon γ represses cancer cell proliferation through Tumor Necrosis Factor Receptor 1-mediated cell cycle arrest

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Even though most cancer immunotherapies are based on cytotoxic cells, an increasing number of data show that successful cancer immunotherapy depends on interferon γ (IFNγ)-producing T cells, either $CD8^+$ T cells or T helper 1 (Th1) cells. Mice that develop endogenous cancer due to aberrant p53 and Rb regulation (RIP1-Tag2 mice) undergo multistage carcinogenesis due to T antigen 2 (Tag2) expression in pancreatic β cells. Treatment of RIP1-Tag2 mice with antigen-specific Th1 cells doubled lifespan by arresting tumor growth, angiogenesis and tumor cell proliferation without causing cytotoxicity. Importantly, prevention of tumor development strictly required both IFNγ and TNFR1-signaling, as shown by antibody blocking or knockout mice like RIP1-Tag2xTNFR1-knockout (TNFR1ko) mice. Based on these findings, we investigated the direct effects of IFNγ and TNF on Tag2-expressing cancer cells from normal RIP1-Tag2 mice, on TNFR1-deficient and STAT1-deficient cancer cells from RIP1-Tag2xTNFR1ko and RIP1-Tag2xSTAT1ko mice in vitro. We analyzed the effects on proliferation, cell cycle arrest and apoptosis. In RIP1-Tag2 tumor cells, both IFNγ and TNF strongly suppressed proliferation, surprisingly without inducing apoptosis. Instead, both cytokines induced cell cycle arrest. As expected, IFNγ failed to induce cell cycle arrest in STAT1koxRIP1-Tag2 cells, while TNF failed to induce cell cycle arrest in TNFR1ko cells. Surprisingly, IFNγ-mediated cell cycle arrest also required TNFR1 expression, as TNFR1ko cells resisted to IFNγ-induced cell cycle arrest. Detailed analysis, including gene arrays in knockout cell lines, revealed that IFNγ induces TNF production by cancer cells, that arrest cell cycle through autocrineTNFR1-mediated signals.

194 [Oral 029]

Cross-talk of novel phenotypes of MDSC and regulatory T cells (Tregs) during the melanoma growth

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Myeloid derived suppressor cells (MDSC) comprise a phenotypically heterogeneous population of cells ($CD11b^+$, $Gr\text{-}1^+$), which can be found in tumor-bearing mice and in patients with cancer. Though several reports suggest that MDSC suppress the activity of T cells during tumor growth, the exact mechanism of their suppressive function is still unclear. To investigate the further mechanisms of suppressive function of MDSC, we first investigated the regulatory molecules (B7H1, B7H3, B7H4) on MDSC during ret melanoma growth. FACS analysis reveals that only tumor-derived $CD11b^+Gr\text{-}1^+$ cells highly express these regulatory molecules. To analyze the suppressive function of MDSC, we isolated $CD11b^+$ cells by MACS and co-cultivated them with syngeneic $CD4^+$ T cells and allogeneic bone marrow derived dendritic cells. Interestingly, tumor-derived $CD11b^+$ cells suppress the proliferation of $CD4^+$ T cells, which is abrogated by blocking of B7H1 molecules. Moreover, to assess the "cross-talk" of $CD4^+CD25^+Foxp3^+$ T cells (Tregs) and MDSC, we examined the MDSC from Treg-depleted and non-depleted tumor-bearing mice. After *in vivo* depletion of Tregs by intraperitoneal injection of PC61, the expression of B7H-families is significantly downregulated on $CD11b^+$ cells isolated from tumors. Furthermore, though there was no significant differences in the suppressive function of MDSC isolated from Treg-depleted and non-depleted mice, the IL-10 production from MDSC isolated from Treg-depleted mice was significantly downregulated compared to non-depleted mice. Our results suggest that tumor infiltrating $CD11b^+$ cells highly express B7H regulatory molecules in the existence of Tregs in tumor microenvironment, and that these MDSC suppress T cell proliferation during melanoma growth.

195 [Oral 030]

Treg deficient in IL-10 do not suppress the CHS reaction due to a lack of adenosine production

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Transferred $CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg) from wild type (wt) mice suppress the elicitation phase of murine contact hypersensitivity (CHS) reactions. In contrast, Treg isolated from IL-10 deficient ($IL\text{-}10^{-/-}$) mice fail to reduce the CHS reaction. Recently we have shown that hapten application activates wt Treg *in vivo* via ATP, resulting in degradation of extracellular ATP into immunosuppressive adenosine. Therefore, adenosine is a key mediator of Treg induced suppression. When measuring adenosine production of $IL\text{-}10^{-/-}$ Treg by HPLC, we recorded significantly lower amounts of adenosine as compared to wt Treg. Using the flow chamber to assess the adherence of $CD4^+$ effector T cells on endothelial cells, mimicking inflammatory reactions, we show that wt Treg become activated via the P2X7 receptor and prevent the adherence of $CD4^+$ T cells to activated endothelial cells through adenosine and A1 adenosine receptors. In contrast, the $IL\text{-}10^{-/-}$ Treg failed to block adherence of effector T cells to the endothelium. Analyzing the calcium flux in Treg, which accompanies signal transduction through the P2X7 receptor, we showed significantly reduced calcium concentrations in $IL\text{-}10^{-/-}$ Treg as compared to wt Treg after stimulation with ATP. In aggregate, our data indicate that the defect of $IL\text{-}10^{-/-}$ Treg in suppressing CHS reactions is not attributed to the lack of IL-10 production. In contrast we show that $IL\text{-}10^{-/-}$ Treg have a defect in reacting to ATP and in producing adenosine. Thus these data have to be taken into account when using $IL\text{-}10^{-/-}$ Treg in assessing Treg-mediated suppression in other disease models.

196 [Oral 031]

Over-expression of epidermal protease-activated receptor-2 leads to spontaneous pruritic skin lesions in mice

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Epidermal expression of protease-activated receptor-2 (PAR-2) is increased in various inflammatory skin diseases with pruritus such as atopic dermatitis. Moreover, local application of PAR-2 agonists induces itch in patients with atopic dermatitis. These indicate a major involvement of PAR-2 in skin inflammation and itch associated with atopic dermatitis. However, the precise mechanism how epidermal PAR-2 is involved in inflammation and itch has not been clarified as of yet. Therefore, we investigated the role of PAR-2 in inflammation and pruritus by behavioral and histological analyses, immunohistochemical staining, double-immunofluorescence, and quantitative polymerase chain reaction in transgenic mice that over-express PAR-2 in keratinocytes. Our data show that PAR-2 over-expressing mice spontaneously develop eczematous skin lesions in ears accompanied by enhanced scratching behavior. The histological analysis of the lesional skin revealed epidermal acanthosis and inflammatory infiltrates in the papillary dermis. Immunofluorescence labeling of PGP9.5 has shown increased nerve fiber sprouting in transgenic mice as compared to wild-type mice. The up-regulated expression of nerve growth factor (NGF) was determined by quantitative polymerase chain reaction. Together, our results suggest that over-expression of epidermal PAR-2 leads to atopic dermatitis-like skin inflammation accompanied by enhanced scratching behavior, increased release of NGF and nerve sprouting. Thus, antagonizing PAR-2 may be beneficial for the treatment of inflammatory and pruritic skin diseases such as atopic dermatitis.

197 [Oral 032]

Optical Imaging (OI)- and Positron Emission Tomography (PET)-investigations reveal different migration and activation patterns of T cells after either intra-venous or intra-peritoneal administration

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Specific T cell based immunotherapy for cancer or autoimmune diseases require suitable administration routes to ensure appropriate T cell homing to the target site. The aim of our study was to analyse differences in $CD4^+$ T helper (Th1) cell homing and bio-distribution after intra-peritoneal (i.p.) and intra-venous (i.v.) administration by PET and OI. Ovalbumin (OVA)-T cell receptor (TCR) transgenic $CD4^+$T cells derived from DO.11.10 mice were cultured together with irradiated antigen presenting cells, OVA peptide, CpG 1668, anti-IL-4, and IL-2 to generate Th1 cells. OVA-Th1 cells were labelled with 0.7 MBq $[^{64}Cu]$PTSM or with Cy5 vibrant dye solution. $[^{64}Cu]$PTSM labelled OVA-Th1 cells were followed *in vivo* for up to 48 hours, and Cy5-labelled OVA-Th1 cells for up to 4 weeks. We injected 10^7 $[^{64}Cu]$PTSM- or Cy5-labelled OVA-Th1 cells into naive mice and analysed their migration properties using PET/ CT and OI. Additionally, we investigated $[^{64}Cu]$PTSM-/Cy5-labelled OVA-Th1 cells by bio-distribution and flow cytometry (FACS). After i.p. injection OVA-Th1 cells accumulated into the perithymic-, inguinal-, and axillary lymph nodes, as well as into the mesenteric lymphatic tissue even after 4 weeks. After i.v. administration OVA-Th1 cells migrated predominantly into the lung, liver and spleen. Interestingly, only after i.v. administration, OVA-Th1 cells migrated into the bone marrow. PET and OI data were confirmed by FACS- and bio-distribution analysis. Thus, significant differences exist in T cell migration and bio-distribution after either i.v. or i.p. administration. These surprising findings are relevant for T cell based specific immunotherapy.

198 [Oral 033]

Reciprocal regulation of IL-10 and IL-17 in human Th17 cells

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Th17 cells have emerged as a new T helper cell lineage involved in the clearance of extracellular bacteria and fungi. A dys-regulated Th17 response, however, can induce severe tissue destruction and autoimmunity. Therefore, mechanisms must be in place to shield the host from immune-mediated damage. We demonstrate that human Th17 cells transiently produce the anti-inflammatory cytokine IL-10 upon stimulation. Interestingly, IL-10 expression was accompanied by reciprocal down-regulation of IL-17, leading to a functional regulatory Th17 cell phenotype after the peak of the effector response. The ability of Th17 cells to express IL-10 was, however, restricted to certain antigen specificities. *Ex vivo* isolated C. albicans specific Th17 cells could not produce IL-10 in comparison to S. aureus specific Th17 cells. This was due to differential priming requirements of these Th17 cell sub-populations. IL-1beta instructed naïve T cells to develop into a pro-inflammatory non-IL10 expressing Th17 cells subset. Th17 cell priming with S. aureus, however, was not IL-1beta dependent, leading instead to the generation of IL-10 producing Th17 cells with self-regulatory activities. Our results identify pathogen dependent differential priming requirements for human Th17 cells and demonstrate that IL-1beta is a molecular switch for determining a functional memory for IL-10 expression. This has important consequences for the physiological termination of pro-inflammatory immune responses and the limitation of bystander damage in certain pathogen microenvironments. Targeting IL-1beta early in the differentiation process of Th17 cells might therefore represent a promising therapeutic strategy to confer anti-inflammatory properties to these culprits of autoimmune diseases.

199 [Oral 034]

CD8+ T cell migration to hapten exposed skin sites requires CD4+ help in a murine model

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The relative contribution of CD4+ and CD8+ T cells to contact hypersensitivity (CHS) responses has been much debated. In an adoptive transfer model of CHS, we investigated the role of the respective T cell subset during the effector response. Magnetic bead separated CD4+ and CD8+ T cells from oxazolone (OXA) sensitized C57BL/6 mice were transferred into syngeneic RAG-/- mice. Ear swelling, inflammatory cell recruitment, the expression of inflammatory mediators, and the extent of cell apoptosis in ear and lymph node tissue were analyzed at 24 hours after OXA challenge. The CD4+ T cell reconstituted RAG-/- mice developed a potent CHS response to topical OXA, including ear swelling, immune cell infiltration, and expression of inflammatory cytokines and chemokines. Adding CD8+ T cells to the CD4+ T cell transfers further amplified the CHS response, including the recruitment of plasmacytoid dendritic cells, the expression of T cytotoxic/helper type 1 inflammatory mediators, and the induction of apoptosis in the epidermal layer of the skin. Unexpectedly, in the absence of CD4+ T cells, CD8+ T cells were not sufficient to mediate the effector phase of CHS in the RAG-/- host environment. These data show that CD4+ T cells can elicit strong CHS responses, which CD8+ T cells may further amplify. Conversely, in the absence of CD4+ T cells, fully differentiated CD8+ T cells fail to migrate to the skin and mediate CHS. The results shed new light on the relative importance of CD4+ and CD8+ T cell interaction during the effector phase of CHS.

200 [Oral 035]

Effect of IL-10-modulated tolerogenic DC in a humanized mouse model of lethal GvHD

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Tolerogenic dendritic cells play a critical role in induction and maintenance of peripheral tolerance. We have previously demonstrated in vitro that IL-10 modulated human dendritic cells (IL10DC) display a tolerogenic phenotype and induce anergic regulatory $CD4^+$ and $CD8^+$ T cells (iTreg). iTregs might be exploited therapeutically for suppression of severe immune responses including allergies, autoimmunity or transplantation rejections. As a preclinical screening system for the *in vivo* efficacy of tolerogenic IL-10DC, we choose a model of graft versus host disease (GvHD) in humanized NOD-Scid mice. Transfer of human PBMC into newborn NOD-Scid mice resulted in lethal GvHD within 2-3 months, characterized by growth retardation, weight loss, inflammatory reaction of the skin and multiple organs and death of animals. Treatment of GvHD with a single or multiple doses of unloaded IL-10DC, did neither prevent growth retardation, nor affect symptom-free time and overall survival of the animals to a significant extent. Human IL-10DC, which express CXCR4 but reduced levels of CCR7, did reach secondary lymphoid tissue as demonstrated by recovery from spleen and lymph nodes. Organ infiltration (lung, skin) by human cells and the percentage of human $CD4^+$ in spleen, lymph nodes and peritoneum were comparable in animals treated with IL-10DC and controls. In contrast, multiple treatments with IL10DC resulted in a decrease of infiltrating $CD8^+$ T cells. These data show, that in vitro generated human IL-10DC without additional antigen loading are able to reach secondary lymphatic tissues in NOD-SCID mice but do not modulate experimental lethal GvHD to a significant extent.

201 [Oral 106]

E-cadherin on epidermal γδ T cells is an inhibitory receptor

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Dendritic epidermal T cells (DETCs) are γδ T cells that reside in the murine epidermis and play important roles in immunoregulation, tumor surveillance, and wound healing. DETCs constitutively express a homophilic adhesion molecule, E-cadherin and a receptor for E-cadherin, $\alpha_E\beta_7$ integrin. Functional differences between E-cadherin-mediated and $\alpha_E\beta_7$ integrin-mediated adhesion of DETCs to E-cadherin-expressing epidermal keratinocytes have not been determined. In this study, we analyzed roles of E-cadherin and $\alpha_E\beta_7$ integrin in activation of DETCs. Both E-cadherin and $\alpha_E\beta_7$ integrin were expressed on resting DETCs, but only E-cadherin was down-regulated upon activation. Short-term adhesion of resting DETCs to E-cadherin-expressing keratinocytes was primarily mediated by $\alpha_E\beta_7$ integrin. When DETCs were cultured on recombinant E-cadherin-coated plates, binding of E-cadherin on DETCs to plate-bound E-cadherin was observed 24 h but not 1 h after the initiation of culture. Blocking of interactions between $\alpha_E\beta_7$ integrin and E-cadherin inhibited killing of transformed keratinocytes by DETCs. Cytokine production by DETCs in response to suboptimal TCR cross-linking was augmented by co-ligation of $\alpha_E\beta_7$ integrin. Therefore, $\alpha_E\beta_7$ integrin on DETCs acts as a co-stimulatory receptor. In contrast, co-ligation of E-cadherin on DETCs inhibited activation of DETCs by TCR cross-linking. These results suggest that E-cadherin on DETCs is an inhibitory receptor and that E-cadherin-mediated adhesion of resting DETCs to normal keratinocytes in uninflamed skin regulates activation thresholds of DETCs to prevent self-reactivity. Down-regulation of E-cadherin after activation may be essential for DETCs to exert effector functions through $\alpha_E\beta_7$ integrin-mediated adhesion to stressed, damaged, or transformed keratinocytes.

202 [Oral 108]

Mast cells play a key role in host defense against lethal herpes simplex virus infection

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Mast cells are known as immunoregulatory cells in both innate and adaptive immune responses. Several lines of evidence indicated that mast cells played significant roles in host defense against bacteria. However, the protective role of mast cells in viral infections is unknown. Here, we have examined the role of mast cells in herpes simplex virus (HSV) infection. In our previous report, mast cell-deficient $Kit^{W/W\text{-}v}$ (W/W-v) and wild-type (WT) mice were intradermally infected with HSV-2. Surprisingly, HSV-induced zosteriform lesions in W/W-v were much severer than those in WT, and the mortality in W/W-v was significantly higher than that in WT. We extensively assessed whether the different responses specifically reflected the lack of mast cells in W/W-v, rather than other c-kit-related differences, using W/W-v reconstituted with bone marrow-derived mast cells (BMMCs) from WT. Nine weeks after intradermal transfer of BMMCs into W/W-v, reconstituted mice were intradermally injected with HSV. The severe mortality in W/W-v was completely recovered by intradermal reconstitution of BMMCs. In addition, HSV titers at HSV-infected skin lesions in W/W-v were higher than those in WT. We next examined the cytokine production by BMMCs via HSV-infected keratinocytes in vitro. TNF-α was significantly increased in BMMCs treated by supernatants of HSV-infected keratinocytes. Unexpectedly, in the number of $CD8\alpha^+$ $CD205^+$ dendritic cells, HSV-spesific CTLs and regulatory T cells in the lymph nodes, there was no significant deference between W/W-v and WT. Thus, our findings suggest that mast cells play a key role in host defense at HSV-infected sites through TNF-α production.

203 [Oral 111]

Dietary vitamin D3 protects against bacterial skin infection

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In skin infection vitamin D metabolism is activated to induce expression of the antimicrobial peptide cathelicidin, as well as CD14 and TLR2, thus enabling increased response to microbes. The aim of this study was to assess if exposure to vitamin D3 is physiologically relevant for increasing resistance to infection. Human keratinocytes (NHEK) stimulated in culture with 100nM 25-D3 for 24h showed 150-fold higher cathelicidin mRNA and 25-fold increased CD14, but when co-stimulated with a low-MW-(<10kDa)-fraction of S.aureus (SA113), expression further increased for cathelicidin (510-fold) and CD14 (190-fold). There was no increase with the low MW SA fraction alone, indicating a synergistic effect of 25-D3 and SA. Functional relevance was seen in a bacterial killing assay in which lysates from NHEK co-stimulated with 25-D3 and SA most efficiently killed S. aureus-deltaMPRF(3-fold decrease in SA growth). Based on these observations we wished to develop a mouse model to test the importance of vitamin D *in vivo*. Mice lacking the 1α-hydroxylase enzyme CYP27B1 were fed a special chow devoid of vitamin D3 for 3 weeks to achieve vitamin D deficiency (n=7), and were then compared to CYP27B1-/- mice receiving vitamin D (2200 IU/kg) in their diet (n=6). 3 days after injection sc. with 10^7CFU of Group A streptococcus NZ131, mice lacking vitamin D in their diet developed significantly larger skin lesions (30.4±14.95mm vs. 5.72±1.12mm, $p<0.05$) and had more bacteria (2-fold increase, $p<0.05$) than when receiving vitamin D. Our results show that vitamin D3 intake may protect from bacterial infection in vitro and *in vivo*.

204 [Oral 113]

Innate signals of non-pathogenic bacteria induce IL-10 producing dendritic cells, regulatory Th cells and clear atopic dermatitis

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Immune homeostasis at surface organs is maintained by non-pathogenic bacteria. These have also been successfully administered for therapy of inflammatory diseases of the gut. As topical application to treat skin diseases has not been reported, we performed a prospective, double-blind, placebo controlled trial to investigate the therapeutic potential of non-pathogenic, gram-negative bacterium Vitreoscilla filiformis (Vf) applied to atopic patient skin. Vf lysate mediated reduction of SCORAD was highly significant. To unravel underlying mechanisms, consequences on the most important surface organ immune sentinels, the dendritic cells (DC), were analyzed. While different Vf preparations uniformly lead to DC maturation, purified Vf LPS induced little IL-10 and high levels of IL-12p70 that was dependent on TLR4. In contrast, DC stimulated with complete Vf lysate produced little IL-12 but large amounts of IL-10. Most importantly, IL-10 production was not dependent on TLR4 but on TLR2 signalling suggesting the presence of a dominant immuno-modulatory TLR2 ligand. Indeed, priming naïve Th cells with Vf stimulated DC induced an IL-10++ Th cell phenotype. Functional assays were carried out demonstrating that IL-10++ Th cells strongly suppressed T effector cells. This indicates that effective treatment of atopic dermatitis by application of non-pathogenic bacteria is the result of i) TLR2 dependent IL-10 induction in DC, ii) priming of IL-10++ regulatory Th cells, and iii) the consecutive suppression of cutaneous effector T cells. Our data demonstrate a new therapeutic strategy for inflammatory skin diseases and point towards a general principle of how non-pathogenic bacteria stabilize the immune barrier of surface organs.

205 [Oral 010]

Macrophages as sentinels directing the quality of skin repair

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Substantial evidence in different model organisms indicates that the immune system is of primary importance in determining the quality of the repair response, including the extent of scarring as well as the restoration of organ structure and function. However, the relationship between repair and the immune response is complex and not completely understood. Indeed, there is evidence for both negative and positive roles. To unravel the dual role of the innate immune response in diverse repair mechanisms, we developed a novel mouse model that allows conditional depletion of macrophages during the sequential stages of the wound healing response. Depletion of macrophages restricted to the early stage of the repair response (inflammatory phase) significantly reduced the formation of a vascularized granulation tissue and impaired epithelialization as well as wound closure kinetics. Furthermore, differentiation of macrophages towards an M2-phenotype was attenuated. However, these wounds revealed minimal scar formation. In contrast, depletion of macrophages restricted to the consecutive mid stage of the repair response (phase of tissue formation) resulted in severe hemorrhage in the wound tissue. Under these conditions, transition into the subsequent phase of tissue maturation and wound closure did not occur. Finally, macrophage depletion restricted to the late stage of repair (phase of tissue maturation) did not significantly impact the outcome of the repair response. These results demonstrate for the first time that macrophages exert distinct functions during the diverse phases of skin repair, which are crucial to control the natural sequence of repair events.

206 [Oral 019]

TGF-β and IL-10 are critical regulators of Langerhans and dendritic cell function in vivo

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TGF-β and IL-10 are immunoregulatory cytokines that suppress dendritic cell (DC) maturation in vitro. TGF-β is also required for Langerhans cell (LC) development and TGF-β-deficient mice lack epidermal LC. Whether TGF-β and IL-10 govern LC and DC homeostasis and function *in vivo* remains elusive. To this aim, we generated mice with a DC-specific knockout of, respectively, the TGF-β and IL-10 receptor (DC-TβR1^{del} and DC-IL10R^{del} mice). While initial LC seeding occurred in DC-TβR1^{del} mice, the cells disappeared from the epidermis within a week after birth. TβR1-deficient LC matured spontaneously and gained migratory potential, based on increased expression of MHC-II, co-stimulatory molecules and CCR7, and down-regulation of E-cadherin. In contrast, the frequency and phenotype of (Langerin^{+}) dermal DC was unaffected. In the absence of LC, low-dose contact hypersensitivity (CHS) in DC-TβR1^{del} mice was diminished, but restored to wild-type at a higher hapten dose efficiently targeting TβR1-deficient dermal DC. IL-10R-deficient DC retained their immature phenotype *in vivo*, but produced elevated levels of proinflammatory cytokines following in vitro stimulation. While IL-10-deficient mice develop enhanced CHS, induction of CHS in DC-IL10R^{del} mice was indistinguishable from controls at 24h post hapten challenge. In contrast, ear swelling was increased at 48h. Adoptive T-cell transfer experiments revealed that T-cell reactivation and not sensitization by IL-10R-deficient DC leads to enhanced CHS. Our data identify TGF-β as a critical factor controlling LC homeostasis in the steady-state and, on the other hand, demonstrate that IL-10 signaling in DC is essential to prevent an exaggerated effector T-cell response in the skin.

207 [Oral 003]

The antimicrobial peptide murine beta defensin-14 induces immunosuppression via switching CD4+CD25- cells into regulatory T cells

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Ultraviolet radiation (UVR) suppresses the adaptive immune response in an antigen-specific fashion via induction of CD4+CD25+ regulatory T cells (Treg). In contrast, the innate immune response appears to be induced by UVR through the release of antimicrobial peptides (AMPs) in the skin. These diverse effects may contribute to a protective mechanism after UVR, by protecting the skin from microbial attacks on the one hand, but taming T cell-driven pathologic reactions on the other hand. Hence, we asked whether AMPs can contribute to photoimmunosuppression. Recently, we observed that the UVR-regulated murine beta defensin (mBD)-14 increased the expression of the Treg specific marker Foxp3 and CTLA-4 in CD4+CD25- T cells. To investigate the biological relevance of this finding, CD4+CD25- T cells were incubated with mBD-14 and injected into naïve C57BL/6 mice before sensitization with 2,4-dinitrofluorobenzene. 5 days later ear challenge was performed. In contrast to untreated cells, the contact hypersensitivity (CHS) response was significantly reduced upon injection of mBD-14-treated cells. Direct injection of mBD-14 into naïve mice also abrogated the CHS response. The suppression could be adoptively transferred into naïve recipients in an antigen-specific fashion, indicating that Treg were developed in the mBD-14 injected donors. mBD-14 did not suppress sensitization in interleukin (IL)-10 knockout mice, suggesting that the immunosuppressive effect of mBD-14 is mediated via IL-10. Through its immunosuppressive capacity mBD-14 might be able to cope with a microbial attack without inducing an inflammatory reaction.

208 [Oral 005]

The role of basophils in skin Th2 response using newly generated basophil specific conditional depletion model

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Basophils have recently been reported to elicit antigen (Ag) presenting in a certain condition. However it remains unknown whether basophils play a central role in the induction of cutaneous Th2-type immune responses. We have previously reported that HS4 region of IL-4 gene is essential for basophils to produce IL-4. Taking advantage of this system, we have newly generated BaS TRECK transgenic (Tg) mice in which a diphtheria toxin (DT) receptor is knocked-in to the HS4 region, and analyzed the role of basophils in cutaneous immune responses.We initially confirmed that basophils in BaS TRECK Tg mice were completely depleted by treatment with DT. We then evaluate the effect of basophil-deficiency on single hapten challenge-induced classic contact hypersensitivity (CHS) and repeated hapten exposure-induced chronic CHS responses. The classic CHS response as a representative of delayed-type hypersensitivity was comparable between DT-injected BaS TRECK Tg mice and wild-type mice, suggesting that basophils are dispensable for CHS. On the other hand, in chronic CHS model as a representative of cutaneous Th2 skewing responses, skin inflammation was significantly decreased in BaS TRECK Tg mice. In addition, basophil-GFP mice showed that basophils were accumulated in the neighbors of T cells in the skin lesion of repeated CHS responses. These results indicated that activated basophils might interact with T cells to induce or maintain Th2-type skin responses. These data suggest that basophils play a central roll in skin Th2 response and might be a novel key factor in the pathogenesis of Th2-type cutaneous immune responses.

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Sensitization to Dermatophagoides pteronyssinus and Tyrophagus putrescentiae in a group of cats and dogs owners

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The frequency of allergic diseases is growing and this phenomenon is being recorded all over the world. Mites are more frequently identified as factors provoking and inducing the symptoms of allergy.This work was aimed to determine a level of sensitisation to particular protein fractions Dermatophagoides pteronyssinus-LD and Tyrophagus putrescentiae –TP in a group of 15 dog owners and 9 cat owners. In the group of cat and dog owners serum was collected and frozen in order to determine a level of specific IgE directed against antigens DP and TP. Extracts of purified mite bodies after standarization were separated, Western-Blotting and incubation with antibodies of 2nd order. Obtained results were compared with standard of mass particles of Biorad company and based on it. In the group of dog owners 23 % shown a positive reaction to all determined TP protein fractions, none for DP, appropriately 22% and 0% for cat owners. Average quantity of allergens causing allergy of a single person amounts to almost 3 and oscillates from 0 to 6 for TP, from 0-5 with average above 3 for DP for dog owners, from 1-6 (above 3) for TP and from 0-5 (above 3) for DP in a group of cat owners. The DP antigen which makes allergic most often is 45.7 kDa (over 89% cat owners and 77% dog owners), for TP 78 kDa (89% cat owners) and 45.7 kDa (62% dog owners). Long lasting exposure to mite allergens may cause allergic diseases, including bronchial asthma.

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Human Demodex mite extracts stimulate an immune response in vitro

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Demodex mites (n=10 per treatment) were obtained by standardised skin surface biopsies from a rosacea patient and mechanically lysed in cell medium. The immortalised human sebaceous gland line SZ95 was employed to determine the immune response to Demodex mite extract on both gene and protein expression levels, at 4 h and 24 h post exposure. A cell stimulant and untreated control cells served for comparison. The expression of genes CAMP, KLK5, SERPINB3, IL6 and IL8 were assessed by quantitative real time PCR. The protein expression level of the antimicrobial peptide cathelicidin (LL-37) was determined by Western blot analysis. No significant change in mRNA levels for CAMP, KLK5 or SERPINB3 were found in response to Demodex mite extracts at the 4 h and 24 h time points. At 24 h a significant increase in mRNA expression levels of IL8 (2000 fold) and IL6 (20 fold) was found, along with increased expression of LL-37 by cells treated with Demodex extract. Demodex mite extracts stimulate a sebocyte immune response by increasing antimicrobial peptide synthesis and pro-inflammatory chemokine and cytokine levels. These events may cause the perifollicular neutrophilic infiltrate demonstrated histopathologically in patients with papulopustular rosacea.

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Withdrawn

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Inhibition of H1-receptors does not aggravate sepsis in mice

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Sedating and non-sedating H1R antihistamines and H2R-blockers are widely used for the treatment of allergies, insomnia and motion sickness, or the prevention and treatment of gastric ulcers, respectively. However it has been shown that histamine receptors are also involved in innate immune responses to bacteria and that pharmacological blockade in patients can negatively affect the outcome of severe bacterial infections. To characterize the contribution of the individual histamine receptors in such infections in more detail, we induced septic peritonitis in H1R -/- or WT mice, and in C57BL/6 mice treated with 1st generation H1R antihistamine, H2R antagonist, H3R/H4R antagonist, second generation H1R antihistamine, or vehicle. While H1R-/- mice as well as C57BL/6 mice receiving the second generation H1R antihistamine desloratadine did not show any differences in morbidity and mortality in this model as compared to WT or C57BL/6 mice treated with vehicle, significant increases in morbidity were observed for all other treatment groups as compared with vehicle. These findings indicate that histamine contributes to some extent to optimal host responses in mice with septic peritonitis by receptors other than H1R and that pharmacological blockade of histamine receptors may impair innate immune responses. In the model used here, which mimics perforated appendicitis in humans, modern second generation antihistamines can be considered as safe medications without any relevant effects on morbidity and mortality, probably because of their higher H1R specificity.

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Expression of pattern recognition receptors in normal and inflamed human epidermis: upregulation of dectin-1 in psoriasis

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Human epidermis plays an important role in host defense by acting as a physical barrier and signaling interface between the environment and the immune system. Pattern recognition receptors (PRRs) are crucial to maintain homeostasis and provide protection during infection, but are also causally involved in auto-inflammatory diseases. This study aimed to investigate the epidermal expression of PRRs and several associated host defense molecules in healthy human skin, psoriasis and atopic dermatitis. Microarray analysis and qPCR were performed on epidermal sheets and immunohistochemistry on whole-skin biopsies of normal skin and chronic psoriasis and atopic dermatitis lesions. In vitro, primary human keratinocytes were stimulated with cytokines mixtures and PRR ligands. Many PRR genes are transcribed in normal human epidermis. Only a few genes were differentially induced in psoriasis (CLEC7A (dectin-1), TLR4 and MRC1) or atopic dermatitis (MRC1, IL1RN and IL1β) compared to normal epidermis. A remarkably high expression of dectin-1 mRNA was observed in psoriatic epidermis and this was corroborated by immunohistochemistry. In cultured primary human keratinocytes, dectin-1 expression was induced by interferon-γ, interferon-α and Th17 cytokines. Keratinocytes were unresponsive, however, to dectin-1 ligands such as β-glucan or heat-killed Candida albicans, nor did we observe synergy with TLR2/TLR5 ligands. In conclusion, many PRRs and other host defense genes are expressed in human epidermis, and RIG-like helicases, TLR2 and TLR3 are the most abundant PRRs. Upregulation of dectin-1 in psoriatic lesions appears to be controlled by psoriasis-associated cytokines. Its role in the biology of skin inflammation and infection remains to be explored.

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Lesional skin of patients with chronic atopic dermatitis shows increased proportions of IL-31 positive T cells and a Th2/Th22 dominance

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IL-31 plays an important role in pruritus, a prominent feature of atopic dermatitis (AD). Because IL-31 is mostly produced by T cells, local T cell responses may be responsible for the increased level of IL-31 mRNA observed in AD lesions. We therefore investigated whether lesional AD skin contains an increase in IL-31 producing T cells by determining the cytokine profile of the infiltrated T cells. T cells were isolated from chronic AD lesions (>3 months), peripheral AD blood and healthy skin. After in vitro stimulation with PMA/ionomycin, the intracellular expression of IL-31, IFN-γ, IL-13, IL-17 and IL-22 was measured using flow cytometry. Lesional AD skin contained significantly higher percentages of IL-31-producing T cells compared to autologous blood and healthy skin (CD4: AD skin 9.9%; AD blood 1.2%; healthy skin 1.3% and CD8: 12.7%; 1.8% and 1.7% respectively). This may be due to local activation and subsequent expansion of these T cells, causing overproduction of IL-31 and consequently increased pruritus in AD. The majority (>77%) of the IL-31 positive T cells did not co-produce any of the other cytokines under investigation, and may thus represent a distinct T cell subset. The remaining IL-31 positive T cells predominantly co-produced IL-22 and/or IL-13 and only rarely IFN-γ or IL-17, showing a closer association with the Th2/Th22 cytokine profile. In comparison to healthy skin, the AD lesional skin cytokine milieu was generally more shifted towards a Th2/Th22 cytokine profile, disputing the widely accepted view that chronic AD lesions are dominated by a Th1 response.

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The role of OX40 ligand and OX40 receptor in atopic dermatitis

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The interaction between OX40 ligand (OX40L) and OX40 receptor has been suggested to be a novel pathogenetic factor in atopic dermatitis (AD). Therefore, the purpose of this study was to investigate the expression and significance of OX40L and OX40 in the lesional and nonlesional skin of 17 patients with AD using immunohistochemistry and cultivation of peripheral blood mononuclear cells (PBMCs). Control group consisted of 10 patients with psoriasis vulgaris. The staining intensity of OX40L and the number of $OX40^+$ cells in the dermis were significantly greater in the lesional skin than in the healthy-looking skin both in AD ($p<0,001$) and psoriasis ($p=0,01$ and $p<0,001$ respectively), but neither molecule correlated markedly with the clinical severity of AD. The stainings were slightly more intense in AD than in psoriasis. LAD-2 human mast cell line stimulated with phorbol myristate acetate (PMA) expressed OX40L. A cell membrane preparation from non-stimulated or PMA-stimulated LAD-2 cells was incubated with PBMCs in the presence or absence of blocking monoclonal antibodies (up to 30 μg/ml) towards OX40L, CD30L or ICAM-1. The PBMC proliferative response of 3 AD patients was strongly increased by PMA-stimulated LAD-2 membranes, but it was inhibited by 42-89% in the presence of 30 μg/ml anti-OX40L. This inhibition was comparable with that by anti-CD30L and anti-ICAM-1. In summary, OX40L and OX40 receptor are strongly expressed in the lesional skin and the blocking of their interaction may provide opportunities for drug development in AD.

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Differentiation of MUTZ-3 cells to Langerhans-like cells and integration into a three-dimensional full-thickness skin model

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Here we show that MUTZ-3 cells, derived from a CD34+ human acute myeloid leukemia, can be differentiated into Langerhans-like cells in the presence of a cytokine cocktail including GMCSF, TGFbeta1 and TNFalpha. As differentiation markers served the expression of langerin (CD207), CD1a, CCR6, Birbeck granules and the ability to antigen internalization. The aim of the present study was to integrate differentiated MUTZ-3 cells (MUTZ-LCs) into a three-dimensional fullthickness skin model consisting of primary human keratinocytes and fibroblasts. Keratinocytes were seeded on top of a dermal equivalent composed of fibroblasts seeded into a collagen matrix (Henkel AG & Co. KGaA, Düsseldorf). After 24h, MUTZ-LCs were seeded on top followed by a second keratinocyte seeding 24h later. After further 24h, the models were lifted up to the airliquid interface (ALI). Histological evaluation performed after 10-12 days featured a fully stratified epidermis with all characteristic epidermal strata. The identification of MUTZ-LC within the full-thickness skin model was performed by immunohistochemical stainings against langerin. Langerin-positive cells were detected suprabasally within the epidermis indicating that keratinocytes and/or fibroblasts provide environmental conditions for long-time maintenance of MUTZ-LCs. This skin model provides a tool to further investigate the interaction between Langerhans-like cells and other skin cells and particularly learn more about the cutaneous immune response.

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Foxp3+ regulatory T cells control inflammation in the murine model of atopic dermatitis

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The role of Foxp3+ regulatory T cells (Treg) in the pathogenesis of atopic dermatitis (AD) is controversial. We analysed how depletion of Treg cells in conditional knockout mice (DEREG) influenced the outcome of the inflammatory response in the mouse model of AD. AD-like skin inflammation was induced by repeated exposure of shaved and tape stripped skin to topical ovalbumin. Treg cells were depleted during the last week of challenge in DEREG mice, which express diphtheria toxin (DT) receptor-EGFP fusion protein under the control of the foxp3 locus. Blood, skin and draining lymph node samples were collected for analysis. Depleting Treg cells by DT in the DEREG mice resulted in a significantly exacerbated immune response compared to the wild type mice, including increased inflammatory cell infiltration and enhanced cytokine expression in the skin as well as elevated IgE levels in the serum. These results suggest that Foxp3+ Treg cells are critically involved in the regulation of AD-like skin inflammation.

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Decreased TNF-α synthesis by macrophages restricts cutaneous immunosurveillance by memory CD4+ T cells during ageing

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There is an age-related increase of cutaneous malignancy and infection. However, the exact mechanism of the decline in immunity in the skin of old subjects is unknown. To address this, we induced cutaneous delayed type hypersensitivity responses to either candida (n=74), tuberculin purified protein derivative (PPD)(n=52) or varicella zoster virus (VZV)(n=26) antigens in young and old subjects. Old individuals showed significantly lower clinical responses and decreased antigen-specific CD4+ T cell infiltration after injection. This was unrelated to expression of CCR4, CLA or CD11a on T cells or their physical capacity for migration. Instead, there was significantly reduced expression of E-selectin, ICAM-1 and VCAM-1 on dermal endothelium at the site of antigen injection in old subjects. The cytokines TNF-α and IFN-γ that are pre-requisite for endothelial activation were significantly reduced in old donor skin. In particular, TNF-α was produced by CD163+ macrophages in the skin of the young, but was absent in these cells from old volunteers. Collectively we show that decreased recall responses to bacterial, fungal and viral antigens in the elderly are due to decreased conditioning of the skin microenvironment by "innate" macrophage-derived cytokines and not a systemic T cell defect per se. The resulting decrease in immunosurveillance by T cells may contribute to the increased incidence of cutaneous malignancy and infection during ageing

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Formation of DC-Treg aggregates depends on adenosine

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$CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg) suppress the sensitization phase of murine contact hypersensitivity reactions (CHS) by blocking the activation and antigen-presenting capacity of dendritic cells (DC) in the draining LN. This suppression is dependent on the close interaction between DC and Treg and the formation of gap-junctions. To further elucidate the underlying mechanisms, we set up antigen-non-specific co-cultures of DC and Treg in vitro. Using video microscopy we show formation of large DC-Treg clusters within 2h. In contrast, DC established hardly any stable contacts with polyclonal $CD4^+$ T cells. Life images sampled over 2h demonstrated fast movement of the DC in the cocultures, resulting in frequent contacts with the rather immobile Treg. Next we analyzed the role of adenosine, which is produced by the Treg via the ectonucleotidases CD39 and CD73, in the aggregation between DC and Treg. We detected, that inhibition of adenosine production by the CD39-blocking agent POM-1 abolished the formation of clusters between DC and Treg significantly. This consequently led to a reduced formation of gap junctions and resulted in abrogation of DC-Treg interaction. In aggregate our data indicate that the formation of antigen-non-specific aggregates between DC and Treg, which is one prerequisite for Treg to exert immunosuppressive actions on DC, is dependent on adenosine produced by Treg.

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Tissue derived ATP activates $CD4^+CD25^+FoxP3^+$ regulatory T cells and stimulates its own degradation to immunosuppressive adenosine during contact hypersensitivity reactions

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Intravenously injected naïve $CD4^+CD25^+FoxP3^+$ regulatory T cells (Treg) suppress the elicitation phase of hapten induced contact hypersensitivity (CHS) reactions. To determine the underlying mechanisms of suppression, we first compared the activation status and the suppressive capacity of the Treg before injection and after injection and elicitation of a CHS reaction. We show by flow cytometry and by in vitro suppression assays that naïve Treg become activated in the blood (upregulation of CD69) and demonstrate augmented suppressive capacity after i.v. injection. Further, we recorded elevated levels of ATP in the blood after hapten application to the skin, and blocking of the ATP-specific P2X7 receptor on Treg before injection completely abrogated their suppressive function. This clearly indicates involvement of ATP and P2X7 receptors in activation of Treg *in vivo*. When analyzing the fate of the tissue-derived ATP, we found that ATP was degraded to adenosine by the ectonucleotidases CD39 and CD73 expressed by Treg. Our data obtained in the CHS model further indicate that Treg-derived adenosine engages with A1 receptors expressed by the vascular endothelium, leading to reduced expression of the adhesion molecules E- and P-selectin. Thus, effector T cells are unable to migrate from the blood to the tissue and as a consequence, the CHS response is suppressed. These data, in addition to defining a novel mechanism of Treg mediated immune suppression, elucidate a novel nucleoside-mediated regulatory feedback loop, in which the pro-inflammatory "damage associated molecule" ATP activates immunosuppressive cells, preventing exacerbated inflammation.

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Depletion of $DEC205^+$ dendritic cells by a specific single chain fragment variable (scFv) toxin

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$CD8^+$ $DEC205^+$ $CD11c^+$ Dendritic cells (DC) are able to crosspresent antigens via MHC-I and are involved in the induction of $FoxP3^+$ Treg. In order to study their function in more detail, we set out to devise a novel tool to deplete this DC subset *in vivo*. We generated a single-chain fragment variable (scFv) specific for the murine DEC205 surface antigen (CD205). This scFv was fused to Pseudomonas aeruginosa Exotoxin A (ETA), an inhibitor of the cellular protein synthesis, leading to induction of apoptosis. The respective DNA sequences were cloned into a bacterial 6xHis- and c-myc-tag containing vector and the bacterial expressed recombinant scFv fusion proteins were affinity-purified. As controls, a nontoxic DEC205-specific scFv and a β-Gal specific scFv fused to ETA were generated. To assess targeting capabilities of the cloned constructs, cultivated DC were incubated with scFv and stained thereafter with myc-tag specific antibodies. Evaluation via fluorescence microscopy showed effective binding of the DEC205 specific scFv constructs. To reveal functionality of the anti-DEC205-ETA scFv, DC were incubated with graded doses of the scFv and induction of apoptosis was analyzed. Here a significant depletion of $DEC205^+$DC was achieved by treatment with anti-DEC205-ETA scFv but not with control scFv. Specificity of the toxic effect was validated by incubation of $DEC205^-$ fibroblasts with anti-DEC205-ETA scFv that did not lead to apoptosis. Thus, our data show that anti-DEC205-ETA scFv is an efficient tool for the depletion of $DEC205^+$ DC and further experiments will reveal the role of this DC subpopulation in tolerance and immunity.

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Intradermal CpG treatment reduces lung inflammation but induces IFN-γ mediated airway hyperreactivity in a murine model of natural rubber latex allergy

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Asthma and other allergic diseases are continuously increasing causing considerable economical and sociological burden to the society. Hygiene hypothesis proposes that lack of microbial Th1-like stimulation during early childhood leads to increased Th2-driven allergic disorders later in life. Immunostimulatory CpG-ODN (Cytosine-phosphate-Guanosine Oligodeoxynucleotide) motifs are candidate molecules for immunotherapeutic studies as they have been shown to shift the Th2 response toward Th1 direction and reduce allergic symptoms. Using natural rubber latex (NRL) -induced murine model of asthma, we demonstrated that intradermal CpG administration with allergen reduced pulmonary eosinophilia, mucus production and Th2-type cytokines but unexpectedly induced airway hyperreactivity (AHR) to inhaled metacholine, one of the hallmarks of asthma. We found that induction in AHR was dependent on STAT4 but independent of STAT6 signalling. CpG treatment increased production of IFN-γ in the airways and shifted the ratio of $CD4^+/CD8^+$ T cells towards $CD8^+$ dominance. By blocking soluble IFN-γ with neutralizing antibody, AHR diminished and the $CD4^+/CD8^+$ ratio returned back to $CD4^+$ dominance. These results indicate that increased production of IFN-γ in the lungs may lead to severe side effects such as enhancement of bronchial hyperreactivity to inhaled allergen. This should be taken into consideration when planning prophylaxis treatment of asthma with intradermal CpG injections.

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Biodegradable Polylactic Acid Particles for Transcutaneous Drug Delivery and Skin Cell Targeting

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In our previous studies we have shown that biodegradable polylactic acid (PLA) and biocompatible polystyrene (PS) particles preferentially accumulate in hair follicles, where release of the incorporated lipophilic compounds or translocation of intact particles to the viable epidermis occurred, respectively. In this study, we aimed to investigate the suitability of polymeric particles for the transcutaneous delivery of peptides and Langerhans cells (LCs) targeting. We applied HIV-p24-loaded PLA and PS particles on human skin explants and studied particle penetration in hair follicles (HFs), particle translocation into the epidermis and their ability to induce the maturation of LCs. We found that both PLA and PS particles released the surface-adsorbed HIV-p24 peptide after their internalization by LCs in vitro hereby inducing the expression of maturation markers. Upon topical application on the skin surface, particles preferentially accumulated in HFs openings and penetrated in 52±11% of HFs. PS but not PLA particles were found to be associated with LCs after 16h of incubation on excised human skin. Nevertheless, partial maturation of LCs (e.g. up-regulation of CD83 but not of CD80) was observed after treatment with both PS and PLA p24-loaded particles. Thus, both particle types may allow the transcutaneous delivery of adsorbed peptides. However, while PS-particles targeted LCs upon translocation across the skin barrier, PLA particles accumulated and released the HIV-p24 peptide in the HFs. Both mechanisms, i.e. transcutaneous delivery of drug-loaded particles and particle-based delivery of incorporated drugs, may open interesting new strategies for skin cell targeting and transcutaneous vaccination.

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IL-33 and its receptor complex are upregulated in atopic dermatitis

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IL-33 is a recently described cytokine which belongs to the IL-1 family and is known to amplify Th2 response. IL-33 signals through the IL-1 receptor-related protein ST2 and IL-1 receptor accessory protein IL-1RAcP. To explore the role of IL-33 and its receptor complex in atopic dermatitis (AD), we characterized their expression profiles in lesional and nonlesional AD skin. The expression of IL-33 was also investigated in vitro. Skin biopsies were taken from either lesional or nonlesional skin of AD patients. In addition, clinically healthy back skin of atopy patch test-positive patients was treated with topical application of house dust mite in petrolatum. The expression of IL-33, ST2 and IL-1RAcP was studied by real-time PCR. To further investigate the cellular source of IL-33, human dermal fibroblasts were stimulated with proinflammatory, Th2- and Th1- cytokines. mRNA expression of ST2 and IL-1RAcP was upregulated in lesional skin of AD patients when compared to nonlesional skin. Both IL-33 mRNA and ST2 mRNA levels in the human skin biopsies were significantly induced after allergen application. In vitro studies showed that cultured human dermal fibroblasts produce high levels of IL-33 mRNA when stimulated with a combination of TNFα and IFNγ. These findings suggest that IL-33-ST2 interaction is important in the pathogenesis of AD, especially in the induction of acute lesions.

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Prevention of carcinoma development by tumor-specific Th1 cells

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Most cancer immunotherapies rely on CD8-positive cytotoxic T cells (CTL), capable of causing either tumor cell lyses or apoptosis. Due to tumor escape mechanisms like tumor-induced antigen-specific T cell tolerance, CTL often fail to efficiently eradicate tumors *in vivo*. Interferon-gamma (IFNγ)-producing $CD4^+$ T cells (Th1 cells) are incapable of directly recognizing MHC-class II-negative cancer cells. Surprisingly, various studies showed that adoptive transfer of Th1 cells can circumvent tolerance induction and efficiently arrest tumor growth *in vivo*. To elucidate the mechanisms underlying tumor growth arrest by Th1 cells, we investigated the development of islet carcinomas, resulting from aberrant Rb and p53 expression in a model tumor with well-described multistage carcinogenesis. Tumor-specific Th1 cells reduced cell proliferation in a strictly IFNγ-dependent manner *in vivo*, without causing cell death or apoptosis. Simultaneously Th1 responses preserved the normal structure, antigen-profile and functions of the tumor-prone, transgenic islet cells. Expression of the classical islet cell differentiation marker insulin (ins) and glucose transporter 2 (Glut2) revealed that in Th1-treated mice ins and Glut2 remained fully preserved. In sharp contrast, islet cells from sham-treated mice grew 5-10 times more rapidly, lost their normal structure, acquired a malignant antigen-pattern, and completely lost the typical islet cell functions. Sham-treated cancer cells showed a 50 % reduction of the differentiation marker ins and complete loss of Glut2. Thus, Th1 immunity can not prevent tumors through cytotoxic effects or apoptosis. Th1 immunity induces potent cytostatic effects that prevent carcinoma development in cancer-prone precursor cells, a mechanism that underlies interferon-induced tumor dormancy.

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A functional role of stimulator of interferon gene (STING) in antiviral response of human epidermal keratinocytes

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The innate immune system is the first line of defense against microbial pathogens, and the production and secretion of type I interferons (IFN) plays an important role of the innate response to RNA viruses. Retinoic acid-inducible gene-I (RIG-I) is a cytoplasmic protein that recognizes viral double-stranded RNA to induce IFN response. In previous studies, we demonstrated that RIG-I expression in human epidermal keratinocytes (HaCaT) is regulated by cytokine stimulation and UVB exposure. On the other hand, a novel protein called STING (stimulator of interferon gene) has been proposed as an important signaling adaptor protein that functions downstream of RIG-I signaling. STING was localized to the outer membrane of the mitochondria and the endoplasmic reticulum, and detected in a variety of human tissues including heart, spleen, kidney, placenta, lung and peripheral leukocyte. In this study, we identified the up-regulated STING expression by cytokine stimulation in HaCaT cells, indicating that STING protein may function as the innate immune system defense in the skin. Furthermore, we analyzed the interaction between RIG-I and STING in a sufficient antiviral response of human keratinocytes.

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PPAR-alpha deficiency triggers allergic contact dermatitis by affecting regulatory T cells through lack of IL-2

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The aim of our work was to decipher the cellular basis of the pro-inflammatory skin phenotype of PPAR-alpha deficient mice. After challenge with a contact allergen, contact hypersensitivity reaction was increased and prolonged in PPAR-alpha deficient mice when compared to wild type mice. Numbers of T-lymphocytes in the skin of PPAR-alpha deficient mice were increased and showed enhanced expression of the activation marker CD25 when compared to controls. After antigen challenge, percentages of Treg in the blood, the skin draining lymph nodes and the skin were decreased in PPAR-alpha deficient mice when compared to controls. Moreover, PPAR-alpha deficiency impaired the production of IL-2 in lymph nodes, whereas production of TGF-beta remained unchanged. Injection of PPAR-alpha deficient mice with IL-2 restored the size of the Treg population in the skin draining lymph nodes of challenged mice. *In vivo* induction of Treg from wild type $CD4^+CD25^-$ T-cells was impaired when adoptively transferred into PPAR-alpha deficient mice as compared with wild type mice and reversed by injection of IL-2. Furthermore, PPAR-alpha deficient Treg exhibited impaired suppressive capacity when compared to wild type Treg in mixed leukocyte reactions. Co-adoptive transfer of both $CD4^+$ T cells and Treg confirmed poor suppressive capacity of PPAR-alpha deficient Treg *in vivo* presumably due to reduced IL-10 and perforin/granzyme B. Injection of IL-2 fully restored the expression of perforin in PPAR-alpha deficient mice but partially the expression of granzyme B. In conclusion, PPAR-alpha deficiency aggravates skin contact hypersensitivity by affecting Treg function through lack of IL-2.

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The expression of dual specificity phosphatase 1 is dysregulated in psoriasis vulgaris

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The dual specificity phosphatase 1 (DUSP1) is an important negative regulator of p38 MAPK activity. The purpose of this study was to investigate DUSP1 in psoriasis. The experimental setup included in vitro culturing of normal human epidermal keratinocytes (NHEKs), punch biopsies from nonlesional and lesional skin from patients with psoriasis or nickel-induced allergic contact dermatitis, and RT-qPCR. We found that the DUSP1 mRNA expression was significantly induced in NHEKs in response to stimulation with IL-1beta (36 fold) or TNF-alpha (9 fold). For both stimuli the DUSP1 mRNA expression peaked after 1 hour and returned to baseline after 3 hours. Using chemical inhibitors, we demonstrated that the IL-1beta-induced expression of DUSP1 was mediated through the p38 MAPK-MSK1/2 signaling pathway. Both the IL-1beta level as well as the activity of the p38 MAPK-MSK1/2 pathway are increased in psoriasis. Nevertheless, in chronic plaque-type psoriatic skin from 17 patients we found that 12 patients displayed a decreased expression of DUSP1 mRNA whereas 5 patients showed an increase. The overall difference was a significant reduction of approximately 26 percent. In contrast, in skin biopsies from 9 patients with a positive nickel patch test after 72 hours, we found a 1.7 fold increase in the expression of DUSP1 mRNA. Thus, it appears that the apparent compensatory upregulation of DUSP1 as seen in nickel-induced allergic contact dermatitis is deficient in psoriasis. Dysregulation of DUSP1 is therefore likely to contribute to the ongoing inflammatory response seen in psoriasis.

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Development of a human dermis equivalent model incorporating dermal macrophages

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The reconstruction of human skin in vitro by a three-dimensional skin culture system has already been significantly improved by incorporating Langerhans cells and dermal dendritic cells. Yet, skin culture systems still suffer from the lack of the third immune cell subtype, i.e. dermal macrophages. Monocyte-derived dermal-type macrophages (MDdM) were seeded into dermis equivalents and cultured for 1 additional week without adding exogenous cytokines. Their phenotypic stability and distribution within the 3D culture were analyzed by immunolabelling on histological sections. After 1 week inside the dermis equivalent, MDdM expressed CD14, CD163, CD209 and HLA-DR and lacked CD1a/c. In addition, MDdM released IL-10, but no TNFα, when stimulated by LPS. We successfully integrated stable and functional MDdM into dermis equivalents. Because there is increasing evidence that dermal macrophages and dendritic cells co-exist in a dynamic equilibrium, our model opens the possibility of studying the relationship and potential cross-talk between the two cell types. Such interactions between dermal macrophages and dendritic cells need to be clarified in certain infectious diseases, where they elicit particular immune responses in reaction to bacterial or viral pathogens. Finally, with the aim of finding new treatments for inflammatory skin diseases, we wish to use our model to find ways to specifically target dermal macrophages and stimulate their release of anti-inflammatory IL-10.

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An unexpected twist in alopecia areata pathogenesis:Are NK cells protective, and CD49b+ T cells pathogenic?

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Natural killer (NK) cells have become a recent focus of interest in alopecia areata (AA) research. To further investigate their role in an established mouse model of AA, lesional skin from older C3H/HeJ mice with AA was grafted to young C3H/HeJ female mice, and NK cells were depleted by continuous administration of rabbit anti-Asialo GM1. As expected, this significantly reduced the number of pure NK cells in murine skin, as assessed by NKp46 quantitative immunohistochemistry. Quite unexpectedly, however, the onset of hair loss in C3H/HeJ mice was accelerated, rather than retarded. NK cell depletion was accompanied by a significant increase in the number of perifollicular CD49b+T cells in the alopecic skin of anti-Asialo GM1-treated mice. These findings underscore the need to carefully distinguish in future AA research between pure NK cells and defined subsets of CD49b+ lymphocytes, since they may exert diametrically opposed functions in hair follicle immunology and immunopathology.

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Upregulation of TLR2 expression in keratinocytes in psoriatic skin can be caused by cytokines

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The innate immune system supports the physical skin barrier in antimicrobial defense. Human keratinocytes constitutively express various members of the toll-like receptor (TLR) family, important for innate immunity including TLR2. TLR2 recognizes pathogen associated molecular pattern of gram-positive bacteria such as lipoteichoic acid. Its expression has been shown to be upregulated in keratinocytes of psoriatic skin. TLR4 recognizes lipopolysaccharide, a cell wall component of gram-negative bacteria. Conflicting data exist about the expression of TLR4 in psoriasis. We asked whether TLR2 and TLR4 expression is stimulated by cytokines in human keratinocyte culture in vitro. The expression of human TLR2 and TLR4 was assessed by realtime PCR analysis and subsequent gel electrophoresis for verification. For stimulation experiments we used cytokines which have been described to be involved either in Th1 or Th2 dominated immune responses: Interleukin (IL)-1 β, IL-4, IL-5, IL-6, IL-13, IL-15, IL-17, IL-18, IL-20, IL-31, Interferon (IFN) γ, tumor necrosis factor (TNF) α and tissue derived growth factor α. We found a significant upregulation of TLR2 expression in keratinocyte cell culture by stimulation with IL-1 β and IFN γ. This induction was potentiated when stimulation with both cytokines was performed. Stimulation with interleukin 4 and 5 also resulted in a slight upregulation of TLR2. TLR4 expression was not stimulated by the cytokines tested. As IFN gamma is a typical Th1 cytokine it may be responsible for the induction of TRL2 expression seen in psoriatic skin.

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Adjuvant activity of Gram-positive bacteria on grass pollen results in the induction of inflammatory Th1 responses and reduced IL-10 production

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Recently, it has been established that pollen grains contain Th2-enhancing activities besides allergens. The aim of this study was to analyze whether pollen carry additional adjuvant factors like microbes and which immunologic effects they may exert. Timothy pollen grains were collected, disseminated on agar plates, and the growing microorganisms were cultivated and defined. Extracts were performed from selected microbes, physically inactivated, and used to determine their influence on immune cells. A complex mixture of bacteria and moulds was detected on grass pollen. Besides Gram-negative bacteria that are known to favor Th1-directed immune responses, molds were determined being sources of allergens themselves. Therefore, we focused on Gram-positive bacteria which were found in high numbers, e.g. Bacillus cereus, Bacillus subtilis, and Bacillus sphaericus. Contact of immature dendritic cells (DC) from grass pollen allergic donors with most of these inactivated bacteria induced their maturation similar to LPS as measured by flow cytometry and by their production of IL-6 and IL-12p40. When CD4+ T cells were stimulated with mature autologous DC, which had been pulsed with Gram-positive bacterial extracts plus grass pollen allergen, a reduced production of IL-4, IL-5 and IL-10 and an enhanced production of IFN-gamma was observed compared to T cells which were stimulated with allergen-pulsed DC alone, while proliferation was not affected. Our data indicate that grass pollen are colonized by several microorganisms that influence the immune response differently. Gram-positive bacterial components may serve as adjuvants by augmenting DC maturation and inflammatory Th1 responses while reducing tolerogenic IL-10 production.

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TLR3 mediated cytotoxicity in keratinocytes is inhibited by co-stimulation with oligodeoxynucleotides

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TLR3 is an important sensor of viral infections and injury of self in keratinocytes, and modulation of TLR3-induced responses may be of clinical relevance. Stimulation with the TLR3-ligand polyI:C induces a toxic effect, shown by up-regulation of high mobility group protein B1 and reduced responses in a MTT- assay. We analyzed the proinflammatory and cytotoxic effects of polyI:C in keratinocytes, and found these to be TLR3 dependent, demonstrated by the use of siRNA for TLR3. Interestingly, co-stimulation with oligodeoxynucleotides inhibited all polyI:C induced effects. This inhibition was mediated by competition of endocytic uptake of polyI:C and oligodeoxynucleotides. With these findings we see a promising potential for oligodeoxynucleotides in the treatment of both viral skin infections and inflammatory skin disorders.

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Vaccination of Cutaneous T Cell Lymphoma with a Telomerase-specific Peptide

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Primary cutaneous T-cell lymphoma (CTCL) is a non-Hodgkin lymphoma with slow progression to advanced stages despite therapy. The enzyme telomerase is critically involved in tumor cell immortalization. Due to its relatively specific expression in a broad range of tumor tissues, telomerase is an attractive target for tumor therapy. In this study, we present an immunotherapy approach to induce T cell specific immune responses against telomerase expressing cancer cells. CTCL patients were vaccinated with a telomerase specific peptide using GM-CSF as adjuvant. Objective tumor-response was assessed by standardized whole body photography. The T cell response was assessed by proliferation assay and measurement of cytokine-production upon antigen stimulation. Immunohistochemistry and immunofluorescence was performed on tumor tissue samples. 6 patients have completed the vaccination. 4 patients showed no objective tumor response and 2 patients even showed progression of the tumor lesions. Positive DTH reactions to the vaccinated peptide was detected in 2/6 patients. A telomerase-specific T cell immune response was seen in 1/6 patient in vitro. In summary, no clinical response to the vaccination was detected although two patients showed a positive DTH reaction and a positive proliferative response was observed in one patient. It remains to be assessed whether the poor efficacy of the vaccination may be due to the immunosuppressive environment observed in CTCL.

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Differential expression pattern of antimicrobial peptides in nasal mucosa and secretion

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The intact nasal barrier is a prerequisite for a functioning defense of the upper airway system. Antimicrobial peptides (AMP) are known to play an important role in maintaining barrier function and until now there is little available data about AMP in respect of nasal mucosa. This study was addressed to gain further insight into the differential AMP expression and secretion pattern according to defined anatomical regions of the nose. ELISA was applied to quantify concentrations of RNase-7, psoriasin, hBD-2, hBD-3 and LL-37 in nasal secretions of 20 healthy volunteers and immunohistochemistry was used to detect the local cellular sources of AMP in the vestibulum nasi and the mucosa of the turbinates. Expression of RNase 7 and psoriasin could be detected in all nasal secretions, whereas LL-37 was only detected in 16, hBD-2 in 5 and hBD-3 in 6 specimens. In the vestibulum nasi luminal cell layers could be demonstrated as cellular sources for hBD-3 and RNase 7, whereas psoriasin was found in all layers of the stratified squamous epithelium. LL-37 was only detected in one sample. In turbinate biopsies hBD-3 and LL-37 were detectable in the epithelium, stroma cells and submucosal glands, RNase 7 was only present in submucosal glands. These data demonstrate that the epithelium of the nose contains a chemical defense shield through the expression and secretion of various AMP. Our results could be the base for further investigations, concerning inflammatory and autoimmune diseases of the nose and paranasal sinus in respect of dysregulated AMP pattern.

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Production of LL-37 during herpes simplex virus types 2 infection in human keratinocytes enhances HIV susceptibility in Langerhans cells, but not in Dendritic cells

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Recent studies have shown that patients who were infected with HSV-2 had three times the risk of acquiring HIV. Since Langerhans cells (LC) were thought as initial cellular targets in sexual transmission of HIV, we tested whether HSV-2 affected HIV susceptibility in LC. To assess the direct effects, monocyte-derived LC (mLC) were exposed with HSV-2 before HIV infection. HIV infection was analyzed by HIV p24 intracellular staining. Interestingly, HSV-2 did not directly affect HIV susceptibility in mLC. As for the indirect effects, we tested whether antimicrobial peptides (AMPs), including αDefensin 5, 6 and βDefensin 1, 2, 3, 4 and LL-37 affected HIV susceptibility in LC because it has been reported AMPs affected HIV susceptibility. Surprisingly, only LL-37 dramatically enhanced HIV susceptibility in mLC, whereas other AMPs did not. On the other hand, HSV-2 infection in keratinocytes increased the expression of vitamin D receptor and the production of LL-37. In contrast, LL-37 decreased HIV susceptibility in monocyte-derived DC (mDC). To assess the physiological effects, we used *ex vivo* skin explant model. Epithelial sheets were incubated with HSV-2 before HIV infection, and then floated. The emigrating LC from HSV-2 treated epithelial sheets significantly enhanced HIV infection compared with LC from non treated epithelial sheets.Regarding mechanisms, LL-37 induced significant up-regulation of cell surface CD4 and CCR5 on mLC, whereas it induced down-regulation of cell surface DC-sign on mDC. Furthermore, in mLC, LL-37 significantly decreased an innate HIV suppressor factor, TRIM5α. Given these data, LL-37 produced from HSV-2 infected keratinocytes enhances HIV susceptibility in LC.

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UV-induced tolerance to a contact allergen is impaired in polymorphic light eruption

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Polymorphic light eruption (PLE) is a common skin disorder provoked by exposure to ultraviolet radiation (UVR). Its clinical symptoms resemble those of a contact allergic reaction. PLE is generally considered a T-cell-mediated autoimmune reaction towards a yet unidentified antigen formed in the UVR-exposed skin. Predisposition to such an immune reaction may result from aberrant epitope formation, increased immune reactivity to a universal epitope, or from diminished propensity to UVR-induced immunosuppression or to the induction of tolerance. In a study comprising a total of 24 PLE patients and 24 healthy sex- and age-matched controls, we found that both groups demonstrated similar immunosuppression of contact sensitization to diphenylcyclopropenone by prior exposure to solar-simulating UVR. However, only one out of 13 PLE patients (8%) vs. 6 out of 11 controls (55%) that had been immunosuppressed by UVR exhibited a state of immunotolerance towards the same allergen after one year ($p=0.023$). We conclude that the impaired propensity to UVR-induced allergen-specific immunotolerance may promote recurrent PLE.

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The caspase-5 expression is upregulated in psoriasis

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Caspase-5 belongs to the family of inflammatory caspases, which activates the pro-inflammatory cytokines IL-1b and IL-18. The purpose of this study was to: 1) characterise caspase-5 expression in psoriatic skin, 2) investigate caspase-5 mRNA induction in cell cultures, 3)investigate the signalling pathways involved in caspase-5 mRNA induction. RNA and protein was purified from paired biopsies from nonlesional and lesional psoriatic skin and used for RT-PCR and Western blotting. Cultured human keratinocytes and PBMC´s from healthy donors were stimulated with TNF-α, IFN-γ, IL-17a, IL-4 or LPS. In other experiments, PBMC´s were pre-incubated 45 minutes with inhibitors of the p38 MAPK or NF-kB signalling-pathways before LPS stimulation. We found a 20.0 fold upregulation ($p<0.05$) of caspase-5 mRNA and a 1.4 fold increase in caspase-5 protein ($p<0.05$) in lesional psoriatic skin compared with nonlesional skin. In in vitro PBMC cultures, LPS induced a 20 fold ($p<0.05$) increase in caspase-5 mRNA after 12 hours stimulation, whereas IFN-γ induced a 3.6 fold ($p<0.05$) increase in both kerationcyte and PBMC cultures. In PBMC´s, pre-incubation with a NF-kB-inhibitor significantly reduced the stimulatory effect of LPS. In conclusion, both caspase-5 mRNA and protein are significantly upregulated in lesional psoriatic skin compared with nonlesional skin. In vitro studies of keratinocytes and PBMC´s reveal that IFN-γ induces caspase-5 mRNA in both cultures, and that caspase-5 mRNA can be induced through the NF-kB signalling pathway. These findings suggest, that both keratinocytes and infiltrating leucocytes contribute to the maintenance of the positive feedback-loop between IFN-γ and caspase-activated proinflammatory cytokines found in psoriasis.

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Frequency and function of $CD4^+CD25^+FOXP3^+$ regulatory T cells in atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease resulted partly of type I and type IV hypersensitivity reactions triggered by T helper 2 (Th2) cells. The explanations behind the Th2 dominance in the disease are still controversial. We addressed the question whether the Th2 cell domination is associated with a reduction and/or functional impairment of $CD4^+CD25^+FOXP3^+$ regulatory T cells (Treg) essential for the maintenance of Th1- Th2 balance. Peripheral blood of AD patients with severe clinical symptoms and extremely high serum IgE levels (>2000 U/ml) were investigated. Flow cytometry was utilised to determine the percentage of $CD4^+CD25^{bright}FOXP3^+$ Tregs and $CLA^+CD4^+CD25^{bright}FOXP3^+$ Tregs in the samples of AD patients and healthy controls. For detection of suppressor activity of $CD4^+CD25^+$ Tregs, they were cocultured with $CD4^+CD25^-$ effector T cells, and for T cell stimulation, anti-CD3/CD28 microbeads were applied alone or with Staphylococcus Enterotoxin B (SEB). The proliferation of the cells was measured by EZ4U colorimetric cell proliferation assay. Significantly increased number of $CD4^+CD25^{bright}FOXP3^+$ Tregs and also of $CLA^+CD4^+CD25^{bright}FOXP3^+$ Tregs were found in the peripheral blood of AD patients compared to healthy individuals. The degree of suppressor activity of $CD4^+CD25^+$ regulatory T cells were decreased in both anti-CD3/CD28 and SEB stimulated subgroups compared to control. Our data suggest coincidently with the literature that the pathogenesis of AD cannot be explained with the absence of $CD4^+CD25^+FOXP3^+$ Treg cells, moreover the number of these cells is increased in the peripheral blood. At the same time the function of Tregs may be impaired.

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Immature 6-sulfo LacNAc (slan)-expressing dendritic cells display a unique capacity of Fc gamma RIII-mediated binding of immune complexes

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The ability of immune complexes to induce potent humoral immune responses has long been known. However, to our knowledge no comparative data of the capacity of immune complex binding of different dendritic cell (DC) subtypes have been generated. We have previously described 6-sulfo-LacNAc expressing DCs (slanDC) within human blood which stand out by their high level expression of the low affinity Fc gamma receptor III (CD16). SlanDC are highly proinflammatory, serve as the major and early source of IL-12, IL-1 beta and TNF-alpha and can become inflammatory dermal dendritic cells in psoriasis. Comparative binding studies of IgG complexes revealed a much higher capacity of slanDC to bind immune complexes compared to $CD1c^+$ DCs, pDCs and monocytes. Blocking experiments further revealed that the selective expression of CD16 by slanDC enabled this outstanding capacity. CD16 also proved critical for slanDC to phagocytose IgG-opsonized sheep red blood cells. Finally, a higher proliferation of specific T cells was observed when antigen was targeted to CD16 on slanDC. During maturation expression of CD16 is rapidly lost by the action of metalloproteinases while maturation markers such as CD86, CD80, CD83 and MHC class II are upregulated. Taken together the expression of CD16 equips immature slanDC with a unique capacity to capture immune complexes for the subsequent presentation of antigen to T cells. Given the fact that slanDC can become inflammatory dermal DCs, the high capacity to capture immune complexes adds an important function to these cells in terms of bridging innate and adaptive immunity.

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Analysis of interleukin-7 receptor-a gene single nucleotide polymorphism in Behcet's diseases

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Behcet disease (BD) is an inflammatory skin disease affecting various organs. Interleukin-7 (IL-7) functions as a T cell proliferation molecule. The association of IL-7 receptor (IL-7R) a single nucleotide polymorphism (SNP) has been reported in several diseases.To elucidate the participation of IL-7Ra gene in BD, we investigated IL-7Ra SNP frequencies of BD patients and healthy controls. There were no significant differences in the IL-7R (-1085) SNP genotype and allele frequencies between BD patients and healthy controls. There was a significant difference in the IL-7Ra (-449) SNP genotype frequencies between BD patients and controls. No significant relationships were observed between IL-7Ra (-1085, -449) SNP genotype frequencies in patients with or without skin involvement, arthralgia, oral aphtha, genital ulcers, and between complete and incomplete types of BD, respectively. However, a significant difference was observed in the IL-7Ra (-1085) SNP genotype frequencies between ocular involvement and non-involvement ($p<0.001$). These results suggest that IL-7Ra may therefore be associated with the pathogenesis of BD in terms of ocular involvement. Further experiments will be required to elucidate the role of IL-7 signaling in the pathogenesis of BD.

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Induction of allergen tolerance in birch pollen allergic patients by specific immunotherapy is established by differentiated cellular and humoral immune responses

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Clinical efficacy of allergen specific immunotherapy (SIT) is well accepted; however, the underlying immune mechanisms leading to allergen tolerance are still focus of intensive research. In a long-term study we characterized allergen-specific T cell frequencies and serum antibody levels in birch pollen allergic patients (n=15) receiving SIT over a 3-year period. Peripheral blood T cell subsets were quantified by ELISPOT (Th2, Th1, type 1 regulatory T (Tr1) cells) or FACS analysis ($CD4^+CD25^+CD127^{low}$ regulatory T cells). Finally, birch pollen-specific IgE and IgG4 serum concentrations were determined. Interestingly, results showed distinguished immune reaction patterns at different time points of the 3-year treatment. During the induction phase, SIT-treated allergic subjects developed an increase of allergen-specific Tr1 cells, which was maintained over the first year of SIT and led to a decreased Th2/Tr1-ratio. In contrast, the number of $CD4^+CD25^+CD127^{low}$ regulatory T cells remained unchanged. However, allergen-induced increases of the different Bet v 1-specific T cell populations ceased to appear in the subsequent course of SIT. Of note, while there were no substantial alterations in Bet v 1-specific IgE serum levels, a progressive increase of allergen-specific IgG4 antibody concentrations was seen over the whole observation period. In summary, we here show that SIT is accompanied by a transient increase of allergen-specific Tr1 cells, followed by reduced numbers of both Bet v 1-specific Th2 and Tr1 cells, while allergen-specific 'blocking' IgG4 antibodies continue to rise. These data suggest a differential impact of cellular and humoral immune parameters on the development and preservation of allergen tolerance.

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Analysis of the frequency of regulatory T cells in recovered SJS/TEN patients

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Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening adverse drug reactions. SJS/TEN is thought to be mediated by imuunological responses including cytotoxic T cells, and soluble factors, such as soluble Fas ligand and granulysin. Moreover, previous papers have reported a relationship with SJS/TEN and regulatory T cells (Treg). Treg expresses chemokine receptors required for skin homing, such as CLA and CCR4. Thus, we aimed to investigate the frequency of Treg and changes in Treg surface markers in SJS/TEN. In this study, SJS/TEN patients and healthy controls were included. The expression of surface markers (CCR4, CCR5, CCR6, CCR10, CLA, CTLA-4 and CD45RA) in Treg in peripheral blood was analyzed using FACS. The frequency of Treg ($CD4^+CD25^{high}$) cells in $CD4^+$ T cells from patients with SJS/TEN (6.47±3.53%, n=6) were significantly increased from those of healthy subjects (1.47±1.70%, n=12)($p<0.01$). However, there was no difference in surface markers expression between SJS/TEN and healthy control. Previous studies reported that the function and frequency in Treg on TEN at the resolution stage was normal. However, our results showed the frequency of Treg with SJS/TEN at the resolution stage was elevated in comparison with healthy control group. Our data also, suggested that the frequency of Treg in recovered SJS/TEN patients could increase to compensate for their declined function.

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Differential expression of interleukin-1 receptors in normal and psoriatic T lymphocytes

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According to our previous results regulatory T cells are functionally defective in psoriasis, however, the reasons are unknown. In this study we investigated the possible role of interleukin-1 receptors in psoriasis pathogenesis, with emphasis on regulatory and effector T cells. Effector ($CD4^+CD25^-$, Teff) and regulatory ($CD4^+CD25^+CD127^-$, Treg) T cells were isolated from normal and psoriatic blood. Expression of type 1 (signal-transmitting) and type 2 (decoy) interleukin-1 receptors, and IL-1R antagonist (IL-1RN) mRNAs were determined by real-time RT-PCR. Cell surface, intracellular and soluble protein expression was investigated by flow cytometry and ELISA. Upon CD3/CD28 activation, IL-1R1 mRNA expression was increased in normal Teff and Treg. Interestingly, while CD3/CD28 activation did not influence IL-1R1 expression in psoriatic Treg, activation induced stronger IL-1R1 mRNA expression in psoriatic Teff compared to normals. Expression of decoy receptor (IL-1R2 and soluble IL-1R2) mRNA was elevated in activated normal Teff and Treg. Psoriatic Teff and Treg, however, more rapidly and more intensively increased their IL-1R2 and sIL-1R2 expression. Expression of IL-1 receptor antagonist (IL-1RN) was moderately increased upon activation in both normal effector and regulatory T cells. However, expression was markedly downregulated in psoriatic Treg, and highly activated in psoriatic effector cells. IL-1R2 protein expression was constant in normal cells, but significantly increased in activated psoriatic effector cells compared to regulatory T cells. Our data suggest that differential expression of genes in the interleukin-1 pathway in normal and psoriatic T lymphocytes may underline some functional differences in these cells, and may have a role in psoriasis pathogenesis.

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The mandatory role of PI3-kinase pathway in IL-4 and IL-13 production by basophils stimulated with IgE and antigen

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Basophils are potent IL-4-producing cells and may therefore contribute to the process of polarizing immune responses and allergic skin responses. We have shown that the PI3-kinase pathway is a crucial signal pathway for IL-4 production by IL-3-stimulated basophils. Stimulation with IgE + antigen (Ag) also activates basophils to produce IL-4 and is thought to activate a signal pathway different from IL-3 stimulation. In this study, we investigated whether PI3-kinase pathway regulated IgE+Ag-induced IL-4 production by murine basophils in vitro and *in vivo* experimental systems. Freshly isolated and cultured murine basophils were enriched for CD49b (DX-5)-positive cells and CD117 (c-kit)-negative cells, respectively, with auto-MACS. The enriched cells produced substantial levels of IL-4 in response to IgE + Ag. Treatment of basophils with PI3-kinase inhibitor, LY294002 and rapamycin or cyclosporine A significantly inhibited IL-4 release from IgE + Ag-stimulated basophils. Interestingly, these signal inhibitors also suppressed IL-13 production in basophils. Mice sensitized with i.v. injection of TNP-specific IgE displayed the ear swelling after s.c. administeration of TNP-OVA. Rapamycin and cyclosporin A, which strongly suppressed IL-4 and IL-13 production by basophils, downmodulated the ear swelling. Our results indicate that PI3-kinase signaling is a critical pathway in both IL-3- and IgE+Ag-induced IL-4 production by basophils, and suggest that IgE-mediated allergic inflammation might be controlled by PI3-kinase signaling via the induction of IL-4 and IL-13 production.

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IL-20, IL-21 and p40: Potential Biomarkers of Treatment Response for Ustekinumab

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Biologic agents targeting specific cytokines in the inflammatory cascade are effective in the treatment of psoriasis.The purpose of this study was to investigate the molecular effects of the biologic agent ustekinumab in lesional psoriatic skin and to identify potential biomarkers of treatment response for ustekinumab. Biopsies from 12 patients with psoriasis were examined before and 4, 28, and 112 days after the onset of ustekinumab therapy. Cytokine mRNA expression was measured by quantitative reverse transcription-polymerase chain reaction. The expression of the following genes were investigated: p40, p19, IL-17A, IL-17C, IL-20, IL-21, IL-22, IL-1β, IL-6, IL-8, TNF-α, IFN-α, β-defensin, IRF-7, IL-23R, and IL-12Rβ1. The clinical changes were detected by Psoriasis Area and Severity Index score. At baseline, the mean PASI score for all patients was 15.2 and there was no difference between responders and non-responders. Six patients were clinical responders with a mean baseline PASI score of 17.2 and a mean percent improvement of 91.1 (range 71.5-100; SEM= 3.1) at day 112. No or little change in PASI was seen in the non-responders. At baseline IL-20, IL-21, and p40 mRNA expression in lesional psoriatic skin was significantly upregulated by a factor 3, 2, and 2 respectively, in non-responders compared with responders. No significant differences were seen at treatment start in the expression of the other genes studied between responders and non-responders This study suggests p40, IL-20 and IL-21 as potential biomarkers of treatment response for ustekinumab based on their significantly upregulation among non-responders.

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IFN-γ induces bulge immunoprivilege collapse and may cause epithelial stem cell exhaust

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The hair follicle (HF) bulge immunoprivilege (IP) may serve to protect the vital epithelial HF stem cells (HFSCs) from (auto)-immune-mediated attacks, ensuring ongoing cyclic HF-regeneration. Primary cicatricial alopecias (PCA) are inflammatory hair disorders that result in HF-destruction as a consequence of irreversible HFSC damage and depletion. Therefore, we investigated bulge IP-characteristics of the HFSC-rich HF-bulge by quantitative immunohistochemistry, qPCR and microarray-analysis. These studies revealed high expression of the immunoinhibitory surface molecule CD200, of proopiomelanocortin, indoleamine-2,3-deoxygenase, HLA-E, and very low expression of MHC-II and b2-microglobulin, on normal human bulge HFSC. This indicates that these HFSCs indeed enjoy relative IP in-situ. Immunohistochemistry of paired biopsies from lesional and non-lesional scalp skin from PCA-patients, suggested IP-collapse accompanied by downregulation of K15 and CD200 and upregulation of b2-microglobulin, MHC-I and -II, and vimentin expression in the bulge of PCA specimens. This was confirmed by microarray analysis. Therefore, we next assessed the effects of the recognized IP-collapse-inducing cytokine, IFN-γ, on human cytokeratin-15-(K15)-promoter-driven GFP+HFSCs. IFN-γ first up-regulated K15 and K15-promotor-driven GFP expression on the gene and protein level in-situ and in-vitro. Subsequently, however, IFN-γ decreased proliferation, enhanced sensitivity to cytotoxic agents, impaired colony-forming-capacity of K15-GFP+ human HFSCs, induced a fibroblastoid morphology, and CD68 expression, and downregulated the HFSC markers K15, b1-integrin and CD200. These findings suggest that IFN-γ can induce HFSC-depletion in PCA by promoting progenitor cell differentiation and/or epithelial-mesenchymal-transition (EMT), besides the induction of bulge IP collapse. Together, these events may lead to cytokine-induced HFSC exhaust as a key element in the pathobiology of PCA.

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Expression and function of the natural cytotoxicity receptor NKp46 on circulating malignant CD4⁺ T lymphocytes of Sézary syndrome patients

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The natural cytotoxicity receptors NKp30, NKp44, and NKp46 were identified as activating receptors mainly expressed by NK lymphocytes. Here we show that peripheral blood malignant CD4⁺ T lymphocytes from patients with Sézary syndrome, an aggressive form of cutaneous T cell lymphoma, express NKp46 at their cell surface. Although NKp46 does not behave as an independent functional receptor, its engagement provides a strong inhibiting signal on the malignant T lymphocyte CD3-induced proliferation. We show that this inhibition is correlated to a decreased phosphorylation of the CD3ζ chain associated to NKp46 and/or the TCR/CD3 complexes. Our results reveal that, in addition to KIR3DL2/CD158k expression, NKp46 could represent an additional marker on the circulating malignant T lymphocytes of Sézary patients, and that its aberrant expression could participate to the pathophysiology of the disease.

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The contact allergen 2,4-dinitrochlorobenzene does not affect microRNAs' -155, -125b and -146a expression in skin explants, keratinocytes and monocyte derived dendritic cells

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MicroRNAs are small non-protein coding RNA-transcripts that control gene expression in a post-transcriptional manner, affecting several cellular processes including development, differentiation and disease. MicroRNAs are crucially important in the immune system and their deregulation leads to inflammatory diseases. Recent evidence showed miR-155, miR-146a and miR125b to be deregulated in inflamed skin. In particular miR-155 is critical in antigen presentation by dendritic cells; miR-146a is transcribed by NFkappaB and miR-125b silences TNF-alpha. Thus, these microRNAs are implicated in processes that take place in the sensitization process of allergic contact dermatitis (ACD), when the Langerhans cells (LC) migrate from the epidermis to present the hapten to T-cells. In this project, these microRNAs' expression profiles were investigated using an ACD model which involved the application of the allergen 2,4-dinitrochlorobenzene (DNCB) onto skin tissue *ex vivo*, human primary keratinocytes and monocyte derived dendritic cells (MoDCs) in vitro, in order to elucidate their role in ACD initiation. Migration experiments showed consistent LC depletion after DNCB application (N=8). However, qPCR data from DNCB treated skin explants showed variable expression of miR-155, -125b and -146a (31.9±55.5, 2.3±1.3, -1.2±2.5 fold difference respectively). In DNCB stimulated MoDCs, the microRNAs expression was variable between different volunteers (N=8) and exposure time (6, 24 and 48h). Primary human keratinocytes expressed the aforementioned microRNAs but failed to show any significant modulation in their expression (-0.7±1.6 miR-155, -0.01±2.6 miR-125b, 2.9±4.0 miR-146a). In conclusion, this data shows that miR-155, miR-125b and miR-146a don't play an important role in skin sensitization to DNCB.

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The serum- and glucocorticoid-inducible kinase 1 is critical for early but dispensable for delayed type mast cell responses

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Mast cell activation determines IgE mediated immediate type allergic reactions and also some prototypic T cell mediated contact-hypersensitivity-responses (CHS). It was still undefined whether early and delayed mast cell activation are consecutive events or independently regulated. Among others, crosslinking of FcεRI receptors activates phosphoinositol-3 (PI3)-kinase. PI3-kinase is known to activate the serum- and glucocorticoid-inducible-kinase 1 (SGK1). We aimed to investigate the role of SGK1 for early and delayed mast cell responses. SGK1 knockout (sgk1-/-) and wild-type mice were sensitized with DNP-specific-IgE and challenged with DNP to elicit anaphylaxis. Anaphylaxis with a fast decline in body temperature (1,74±0,41°C) was detected in sgk1+/+ mice while sgk1-/- mice showed no anaphylactic reaction. Degranulation as detected by release of β-hexosaminidase was significantly reduced in bone marrow derived mast cells (BMMC) from sgk1-/- mice. In mast cell dependent CHS to TNCB the ear swelling was significantly impaired in sgk1-/- only during the early response (4 and 8 hours) whereas no difference in CHS responses was detected at 24 hours. In agreement with these data we could show that SGK1 is crucial for early cytokine release, but not for delayed type cytokine secretion by BMMC. In conclusion, our data demonstrate for the first time that consecutive early and delayed type mast cell activation pathways can be regulated independently even following the same stimulus. Based on this specificity, targeting SGK1 may be highly promising to treat immediate type allergic responses while sparing critical delayed type mast cell responses.

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Streptococcal extract-induced activation of CLA+ T cells and epidermal cells coculture in psoriasis: gene expression, protein expression and in vivo hyperplasia capacity

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Psoriasis is associated with streptococcal throat infections and increased occurrence of such infections in patients. Up to know it has not been clarified whether Streptoccocal extract can induce T-cell dependent keratinocyte activation, Th17/Th22 cytokine production and *in vivo* epidermal hyperplasia in psoriasis. We have generated a coculture system comprising circulating CLA+/CLA- memory T cells and autologous epidermal cells to test streptococcal extract activation activity in patients with psoriasis and healthy individuals. The results show for the first time that streptococcal extract induces a strong activation of the autologous coculture of circulating CLA+ T cells together with epidermal cells in cells from psoriatic patients. No activity was present with CLA negative cells from psoriatic patients or in healthy subjects. Streptococcal extract induced IL-17, IL-22, IP-10, IL-8, IFN-g, and ICAM-1 gene expression only with psoriatic CLA+ T cells and autologous epidermal cells, either from lesional, and interestingly from non-lesional psoriatic skin. Gene expression was confirmed at protein level. Coculture supernatants of CLA+ T cells and epidermal cells activated with extract induced significant epidermal hyperplasia and lymphocyte infiltration when injected intradermally into mouse skin *in vivo*, as well as keratins 16 and 6 at the gene expression. The study shows that Streptococcal extract preferentially induces keratinocyte and CLA+ T cell (Th1, Th17, Th22) activation and *in vivo* epidermal hyperplasia This *ex vivo* approach might contribute to clarify the role of streptoccocal infections in early molecular mechanisms of psoriasis development.

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Systemic IL-4 treatment suppresses IL-23 and abrogates inflammatory responses in T cell mediated delayed type hypersensitivity

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Over decades, the scientific debate on T-cell mediated delayed-type hypersensitivity reactions (DTHR) was focused on Th1/Tc1 and Th2/Tc2 cells. IFN-γ producing Th1 cells were considered to be the primary effector cells and IL-4 producing Th2 cells to be the counter-regulator of DTHR responses. Indeed, systemic IL-4 treatment was shown to reverse established DTHR. More recently, experimental evidence on the role of Th17/Tc17 in DTHR increased, but the role of the Th17 sustaining IL-23, especially in the context of successful IL-4 treatment, remained elusive. We used a model of TNCB-induced DTHR. TNCB challenge in sensitized mice induced ear swelling and skin inflammation. Interestingly, the inflammatory infiltrate was dominated by both Th17 cell-associated IL-23 and IL-17. Systemic IL-4 treatment of these mice resulted in reduction of ear swelling and normalized skin morphology. Moreover, we found that IL-4 therapy selectively suppressed cutaneous IL23A mRNA expression. In agreement with the critical role of IL-23 in sustaining Th17 responses, efficient IL-4 treatment also suppressed IL17A mRNA in mouse ears. To further analyze the selective suppression of IL-23 by IL-4, bone marrow-derived dendritic cells (BMDC) were allowed to mature in an IL-4 dominated milieu. In these BMDC IL-4 suppressed IL-23 while promoting up regulation of IL-12. Unraveling the molecular mechanism, we found that IL-4 exerts its regulatory effect in a STAT6 dependent manner. Our findings not only assign cutaneous DTHR as IL-23 and Th17 dependent. We also demonstrate a novel strategy to selectively target IL-23, which might be beneficial in the treatment of Th17-mediated autoimmune diseases.

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Hidradenitis suppurativa and innate immunity: a target for zinc gluconate

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Hidradenitis suppurariva (HS) is a chronic, suppurative, inflammatory skin disease that affects mainly apocrine glands. Our hypothesis is that the development of nodules could be related to abnormal innate immunity at the site of nodules. Twelve patients with HS (Hurley stage I, II) with a minimum of two closed nodules in usual sites and a disease progressing for at least 6 months were included in the study. Two biopsies were performed: on a closed inflammatory nodule and healthy adjoining skin. The patients were then treated for 3 months with zinc (Rubozinc) dose of 90 mg daily (modulator of innate immunity). A new biopsy was then performed on the same nodule. Two biopsies from abdominal plasties were used as controls. The innate immunity markers (TLR2, 3, 4, 7, 9, ICAM1, IL-6, TNF-a, IL-10, a-MSH, TGF-b, b-defensins 2 and 4, IGF-1) were studied by immunohistochemistry. We observed a significant decrease in the expression of the innate immunity markers in healthy skin and more strongly in inflammatory nodules of HS compared with control skins, associated with an increase in IL-10. After zinc treatment, we noted a significant increase in the expression of all the innate immunity markers with more than 50% regression of the inflammatory lesions in 7 patients. Our study demonstrates for the first time that a deficit of innate immunity in the usual sites of HS could explain the development of chronic inflammatory nodules in this illness. These abnormalities are partially reversed by zinc with a partial regression of nodules.

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IL-1F5, F6, F8, and F9: a novel IL-1 family signaling system that is active in psoriasis and promotes keratinocyte antimicrobial peptide expression

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Four new members of the IL-1 family have been identified: pro-inflammatory IL-1F6, -F8 and -F9 and the IL-1R6(RP2) receptor antagonist IL-1F5. These constitute a novel IL-1 signaling system that is poorly understood in skin. Because over-expression of IL-1F6 in IL-1F5 knockout mice leads to a psoriasiform phenotype and these two cytokines are over-expressed in psoriatic skin, we hypothesized that these IL-1 members play key roles in psoriasis pathogenesis. To test our hypothesis, we assessed expression of all IL-1 cytokines in healthy control (NN), uninvolved psoriasis (PN) and psoriasis plaque (PP) skin using QRT-PCR. Expression of IL-1F5, -F6, -F8, and -F9 were increased 2-3 orders of magnitude in PP vs. PN skin ($p<0.001$,all), which was supported immunohistologically. Moreover, treatment of psoriasis with etanercept led to significantly decreased IL1-F5($p=0.009$), -F6($p<0.0001$), -F8($p<0.0001$) and -F9($p<0.0001$) mRNAs, concomitant with clinical improvement. Similarly increased IL-1F5, -F6, -F8 and -F9 was seen in the involved skin of KC-Tie2 mice. Suggestive of importance in the epidermal compartment, treatment of normal human keratinocytes (NHK) with IL-1α and TNF-α induced 4 to 10-fold increases in IL-1F5, -F6, -F8, and -F9 mRNA. The effect of IL-1F8 on the expression of antimicrobial peptides by NHK and reconstituted human epidermis (RHE) was striking. Treatment of RHE with IL-1F8 increased mRNA expression of HBD2 (18-fold,$p=0.0001$), HBD3 (2.3-fold,$p=0.024$) and CAMP(5.6-fold,$p=0.03$) and protein secretion of HBD2 (6-fold,$p=0.008$) and HBD3 (2.6-fold,$p=0.007$). Taken together, our data suggest important roles for these novel cytokines in the pathogenesis of psoriasis and identify these peptides as potential targets for antipsoriatic therapies.

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Effect of chronic mild stress on serotonergic expression in skin and brain of a NC/Nga atopic mouse strain

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There is a bidirectional interaction between the neuroendocrine and immune systems in atopic dermatitis with stress being a worsening factor. The impact of chronic mild stress on the expression of serotonin (5-hydroxytryptamine; 5-HT), 5-HT1A, 5-HT2A receptors (R) and serotonin transporter protein (SERT) in the eczematous skin and brain of atopic NC/Nga mice was investigated. The mice were divided into three groups, stressed eczematous (SE), non stressed eczematous (NSE) and stressed control (SC). Biopsies were analysed by immunohistochemistry. There was an increased number of 5-HT containing dermal mononuclear cells in the eczematous skin compared to the control, with a tendency to more 5-HT positive cells in SE compared to NSE group. There was an epidermal immunoreactivity for 5-HT1AR and 5-HT2AR, where the intensity for 5-HT1AR was highest in the SE and NSE groups compared to SC, and intensity and fraction for 5-HT2AR were highest in SE and NSE compared to SC, with a recorded difference between SE and NSE. 5-HT2AR expression was also seen on nerve bundles, their number and staining intensity being decreased in the SE compared to NSE. SERT immunoreactivity was found on nerve bundles with a decreased number in the SE group compared to NSE and SC. In the CA1 area of hippocampus, there was an increased number of cells immunoreactive for 5-HT2AR between SE and NSE groups and also between SE and SC. There is a modulation of the expression of 5-HT receptors in the eczematous skin and brains of the atopic mice during chronic mild stress.

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Human skin and hair follicle versus nasal polyp organ culture as clinically and physiologically relevant model systems for studying normal human connective tissue-type and mucosal-type mast cell biology in situ

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Given the key role of mast cells (MCs) in normal epithelial biology as well as in allergic diseases, it's biologically and clinically important to characterize both normal connective tissue-type (CT-MC) and mucosal-type MCs (MT-MCs). Since MCs functions mainly depend on their local tissue environment, it's more important to investigate MCs within their natural tissue habitats than in isolation. Here, we show how this can be done, using three human tissue organ-culture assays. Since the connective tissue sheath (CTS) of hair follicles (HFs) represents a compartment rich in CT-MCs, while nasal polyps (NPs) offer an accessible source of MT-MCs. HFs, NPs, or full-thickness scalp skin were organ-cultured for up to 6 days in the serum-free medium. All tissues maintained viability and normal morphology during organ-culture. MCs were readily detectable by histochemistry and Kit/tryptase-immunohistology. By quantitative (immuno-)histomorphometry, the total number of Kit+ cells in the CTS of HFs significantly increased over time, whereas that of tryptase+ (i.e. mature) MCs remained largely unchanged, documenting that the CTS is an important reservoir of skin MC progenitor cells. NPs showed significantly increased number of both tryptase+ and Kit+ cells, suggesting that NP also contain resident progenitor cells, which continue to differentiate into mature MCs. MC secretagogues (substance P and cannabinoid receptor-1 antagonist) induced MC degranulation in HFs, skin and NPs, documenting that organ-culture conditions perfectly preserved MC differentiation and activation. These assays offer instructive, clinically relevant tools for developing novel pharmacological strategies to selectively manipulate human CT-MCs and MT-MCs activation and/or maturation in situ.

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Filaggrin Knockout Mice as a Tool for Understanding Percutaneous Antigen Exposure in Barrier-disrupted Skin

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Recent reports have revealed that the loss-of-function mutations in filaggrin gene (FLG) cause the keratinizing disorder, ichthyosis vulgaris, and are major predisposing factors for atopic dermatitis (AD). Filaggrin is a major constituent in stratum corneum (SC). Previous epidemiological studies have suggested the importance of filaggrin for skin barrier formation and that its deficiency might result in increased entry of external antigens. To better understand processes of percutaneous antigen exposure and pathogenesis of AD, we generated filaggrin knockout (KO) mice. Natural moisturizing factors, derivatives of filaggrin degradation, were markedly decreased in SC of filaggrin KO mice. Surprisingly, transepidermal water loss, the most widely used measure for skin barrier function, was not enhanced. Increased outside-to-inside permeability was confirmed by topical application of fluorescent substances, however, and susceptibility of filaggrin KO mice to irritant contact dermatitis demonstrated the critical role of filaggrin in skin barrier function. The flaky tail (ft/ma) mice carry 1-bp deletional mutation in FLG, which arose spontaneously in the 'matted' background. Importantly, analyses for ft/ma mice revealed that it had reduced, but clearly detectable amounts of mature filaggrin protein, indicating that these mice are not deficient for filaggrin. Long-term observation revealed that whereas ft/ma mice developed dermatitis spontaneously, filaggrin KO mice did not cause any cutaneous manifestation under SPF conditions. Our filaggrin KO mice, purely deficient for filaggrin, should provide a valuable tool to evaluate filaggrin function and to assess immunological consequences after percutaneous antigen exposure via epidermal barrier-disrupted skin, particularly in the context of AD.

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Differential capacity of human skin dendritic cell populations to polarize naïve T cells into IL-17, IL-21 and IL-22 producing cells

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Accumulating evidence now points to an important contribution of T cell-derived IL-17, IL-21 and IL-22 cytokines in skin immune homeostasis as well as many proliferative skin disorders. We therefore wondered whether the cytokine-producing T lymphocytes could be primed and biased by the different subsets of human skin DCs, i.e. epidermal Langerhans cells (LCs) and dermal DCs (DDCs), consisting of at least two migratory $CD1c^+CD14^-$ and $CD1c^{+/-}CD14^+$DC subsets. DCs were purified using antibody-coupled magnetic beads following a 2-day migration from separated human epidermal and dermal sheets. DCs were then co-cultured with allogeneic naïve T cells and cytokine secretion was explored using both ELISA protein assay and intracellular cell staining. Results showed that neither of skin DCs could induce substantial IL-17 production by naïve T lymphocytes. However, LCs and $CD1c^+CD14^-$DDCs were able to induce naïve $CD4^+$T lymphocytes to secrete both IL-21 and IL-22 cytokines; LCs being far more efficient in this process. Interestingly, the majority of IL-21 or IL-22 secreting $CD4^+$T lymphocytes did not co-express IFN-γ (Th1 cytokine), nor IL-4 (Th2 cytokine). As they don't express IL-17 either, they may be therefore considered as distinct T helper subsets. Furthermore, both expression of ICOS-L co-stimulatory molecule on LC and presence of serum in the culture medium negatively regulated "Th21" production. Neither of IL-17, IL-21 nor IL-22 was detectable in supernatants from naïve $CD8^+$T lymphocytes primed by any skin DCs. These results add new knowledge on human skin immunology and mostly offer new targets for the treatment of inflammatory skin disorders.

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Mast cells and neutrophils are major producers of IL-17A in psoriasis

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IL-17A produced by T cells is thought to play a central role in the pathogenesis of psoriasis. Recent work has shown that antibodies specifically targeting the IL-17 pathway are highly effective in treatment of psoriasis. Previous studies indicated that psoriasis lesions contain increased percentages of T cells capable of producing IL-17A after *ex vivo* stimulation. Using dual-color immunofluorescence, we observed that IL-17A secreting CD4+ and CD8+ T cells are increased in psoriasis lesions compared to normal appearing skin. However, the major producers of IL-17A in psoriasis lesions were mast cells expressing tryptase and chymase (MC-TC cells). Mast cell IL-17A colocalizes with chymase and tryptase in some granules, but is also found in chymase- and tryptase-negative granules. In well developed psoriasis lesions, neutrophils located in the epidermis, stratum corneum, and superficial dermis also contain significant amounts of IL-17A. These observations suggest a major role for activated mast cells and neutrophils as producers of IL-17A in psoriasis, with significant implications for pathogenesis and pharmacologic management of this disease.

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Necrotic Melanoma Cells Induce Il-1B Secretion Through Nalp-3 Inflammasome Activation

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Chronic inflammation is associated with an increased risk of carcinogenesis. The role of pro-inflammatory cytokines such as IL-1b in the pathogenesis of cancer is still controversial. IL-1b mediates acute inflammatory responses and provides a link between innate and adaptive immunity. Induction of IL-1b secretion is controlled by a cytosolic complex of proteins known as the inflammasome. This multiprotein complex is composed of a NALP family member, ASC and caspase-1 and/or -5. Necrotic cells are often found in tumors, including melanoma, and they occur as a consequence of chaotic growth and as a result of radio/ chemotherapy. Necrotic cells are able to induce immune responses. Furthermore, melanomas are known to be infiltrated by antigen presenting cells (APCs). The aim of our work was to assess the ability of necrotic melanoma cells to elicit an innate immune response through IL-1β secretion by APCs. To address this question, primary monocytes and the monocytic cell line THP-1 were exposed to necrotic melanoma cells in vitro. Interestingly, a high inflammasome-mediated IL-1b secretion was observed after a 24-hr exposure to necrotic melanoma cells. We observed that IL-1b secretion is dependent on Nalp-3 inflammasome and requires internalization of the necrosis products. Heat-treatment of necrotic melanoma cell preparations abrogated IL-1b secretion by APCs, suggesting a protein nature of inflammasome-ativating agent(s). Taken together, our data show that necrotic human melanoma cells are highly potent inducers of pro-inflammatory responses through Nalp-3-inflammasome-induced IL-1b secretion. Investigations aiming i) identifying the inflammasome-activating agent(s), and ii) characterizing upstream events, including possible receptors, are underway.

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Anti IL-12 / IL-23 p40 therapy inhibits epidermal activation in uninvolved psoriatic skin

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Cumulating data point towards "pre-psoriatic" alterations in uninvolved psoriatic skin, such as aberrances in innate immunity and lipid biosynthesis. Despite the fact that ustekinumab (anti-p40) successfully targets the IL-23/Th17 axis in lesional skin, little is known about targeting constitutively expressed or induced IL-23 in uninvolved skin. We investigated the effects of ustekinumab therapy on selected biomarkers of epidermal activation in tape stripped (TS) uninvolved psoriatic skin. The selected markers of epidermal activation (and barrier function) included psoriasin (s100A7), hBD2, LL37, GATA3 and NGF. Biopsies were taken from uninvolved and TS-skin from ten patients with psoriasis prior to treatment with ustekinumab and again after 4 and 16 weeks of treatment. Blood was drawn for measuring circulating levels of IL-12, -17 and -23 in serum. Mean PASI improvement was 50% at week 4 and 75% at week 16 of treatment. Ustekinumab increased the baseline level of expression of GATA3 mRNA in uninvolved skin, whereas it progressively blocked TS-induced upregulation of s100A7, LL37 and NGF but not hBD2 mRNA. Serum levels of IL-12, IL-17 and IL-23 remained stable during treatment and elevated compared to healthy controls. Our results show that ustekinumab blocks early activation of „pre-psoriatic" uninvolved skin, preventing triggering of new psoriasis lesions. This mechanism of action together with the long half life may explain the prolonged clearance of psoriasis seen with ustekinumab treatment.

262 [Oral 078]

In vivo education of UVR-induced regulatory T cells to inhibit the elicitation of contact hypersensitivity

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Ultraviolet radiation induced regulatory T cells (UVR-Treg) inhibit the sensitization but not the elicitation of contact hypersensitivity (CHS) when injected i.v, because UVR-Treg express lymph node, but not skin homing receptors and thus migrate into the lymph nodes but not into the skin. It was shown that the homing receptor expression and the migration of UVR-Treg can be altered by tissue-specific antigen presenting cells in vitro. This is also possible *in vivo* since i.v. injection of dinitrofluorobencene (DNFB)-specific UVR-Treg into DNFB-sensitized mice inhibited the elicitation of CHS, provided the UVR-Treg were activated by an epicutaenous DNFB boost on the abdomen of the recipients before ear challenge. The presence of Langerhans cells (LC) appears to be essential for the boosting effect, since it was lost upon depletion of LC, as demonstrated in langerin diphtheria-toxin receptor knock in mice treated with diphtheria toxin. Finally UVR-Treg were induced in already DNFB-sensitized mice by painting DNFB onto UVR-exposed back skin. Those mice responded to a DNFB challenge with a pronounced ear swelling response. However, when these mice were in addition exposed to an epicutaneous DNFB boost on the flank before ear challenge, the CHS response was significantly suppressed. This is the first demonstration of *in vivo* induction of UVR-Treg and their *in vivo* education to inhibit the elicitation of CHS. This may have major implications on strategies trying to utilize Treg not only for the prevention but also for the treatment of immune-mediated disorders.

263 [Oral 079]

Calcioptriol cream as a prophylactic treatment for polymorphic light eruption

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Polymorphic light eruption (PLE) is suggested to be caused by resistance to UV-induced immunosuppression and simultaneous immune reactions against skin photo-neoantigens. Similar to UV exposure, vitamin D analogues such as calcipotriol have been reported to cause immunosuppression as demonstrated by suppression of *in vivo* contact hypersensitivity reactions. We therefore performed a randomized double-blind placebo controlled study to investigate the preventive effect of topically applied calcipotriol cream in PLE patients. Thirteen PLE patients (10 women, 3 men; mean age, 37 years) were pre-treated at two symmetrically located test areas on body sites prone to disease either with calcipotriol cream or placebo cream twice daily for seven days before the start of phototesting with solar simulated UV radiation. Pre-treated test sites were assessed for minimal erythema dose, and then photoprovocation was performed with repeated daily near erythemal irradiation on 4 consecutive days. Photoprovocation provoked PLE lesions in 12/13 (92%) patients. Pretreatment with calcipotriol cream significantly delayed the appearance and/or severity of PLE symptoms in 10 (77%) patients and completely prevented symptoms in 2 (15%) patients. The administration of a specific PLE scoring system with a maximum of 12 (composed of ratings for affected area, infiltration, and itching) revealed a statistically significant ($p<0.002$) reduction of symptoms of more than 40% throughout the observation period starting at 48h until 144h post photoprovocation. Visual evaluation and reflectance spectroscopy showed reduced erythema but increased pigmentation at calcipotriol-treated sites in phototesting. These results suggest a therapeutically beneficial role for calcipotriol cream as a prophylactic treatment for PLE.

264 [Oral 080]

UVB-induced CD1a$^+$ Langerhans' cell trafficking occurs in histidinaemic human skin lacking urocanic acid

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Ultraviolet-B radiation (UVB)-induced Langerhans' cell (LC) trafficking from mammalian epidermis is a putative step in photoimmunosuppression. Epidermal trans-urocanic acid (trans-UCA) is formed from histidine by the deaminating action of histidase and isomerises to cis-UCA on UVB exposure. In mice, topical application of cis-UCA reduces epidermal LC numbers by >50%. To explore the role of UCA in UVB-induced epidermal LC trafficking in humans we studied the skin of subjects with histidinaemia, a rare (1 in 11,000) genetic condition characterised by defective histidase activity and a lack of UCA. Histidinaemic (n=6) and healthy age, sex and skin-phototype matched control (n=6) subjects were recruited. Skin UCA levels were quantified by HPLC. 24h after exposure to a range (20-200 mJ/cm^2) of broadband UVB (TL-12; 280-315nm) doses, visual minimal erythemal doses (MED) were determined for each subject and CD1a$^+$ LC enumerated in epidermal sheets taken from the 200 mJ/cm^2 irradiated site. Control subjects had normal levels of trans-UCA (mean±se; 9.8±0.6 nmol/cm^2) which was not detectable in histidinaemic skin. Despite age, sex and phototype matching, the mean histidinaemic MED was lower than in controls ($p=0.04$; 42±7 and 74±11 mJ/cm^2 respectively). Mean CD1a$^+$LC density per mm^2 in non-irradiated histidinaemic and control skin was similar (985±44 and 950±38, $p=0.56$). After UVB exposure there was a similar percentage reduction in CD1a$^+$LC observed in histidinaemics and controls (44±4% and 36±2% respectively, $p = 0.17$). These findings demonstrate that the presence of UCA is not essential for UVB-induced LC migration from the human epidermis.

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Fractionated Illumination at low Fluence rate Photodynamic Therapy in mice

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Photodynamic therapy (PDT) for actinic field cancerization is effective but painful. Pain mechanisms remain unclear but fluence rate has been shown to be a critical factor. Lower fluence rates also utilize available oxygen more efficiently. We investigated PDT effect in normal SKH1-HR mice using low and high fluence rate aminolevulinic acid (ALA) PDT and a fractionated illumination scheme. Six groups of six mice with different light treatment parameters were studied. Visual skin damage was assessed up to 7 days post PDT. Fluorescence and reflectance spectroscopy during illuminations provided us with real time information about PpIX photobleaching. A novel dosing approach was introduced in that we used a photobleaching percentage instead of a pre-set fluence. Data show similar total and maximum damage scores in high and low fluence rate groups. Photobleaching of PpIX in the low fluence rate groups shows a trend towards more efficient photobleaching. Results indicate that low fluence rate PDT is as effective as, and more efficient than high fluence rate PDT in normal mouse skin. Low fluence rate PDT light protocols need to be explored in human studies in search for an effective and well tolerated treatment for actinic field cancerization.

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Melatonin differentially regulates antiapoptotic mechanisms on a wide range against UV-induced damage in a human full skin model in vitro

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Melatonin has been recognized as a protective agent in many conditions related to oxidative stress such as neurodegenerative diseases, sepsis and aging. These processes are supposedly mediated through melatonin`s scavenging properties and antiapoptotic activity. Here, we investigated the effect of wide range (25-600 mJ/cm2) UVB/UVA-radiation on apoptosis in a human full skin model by involvement of p53 protein, caspases-dependent apoptotic pathways, activation of DNA repair enzyme poly(ADP-ribose) polymerase (PARP), induction of oxidative stress, and the protective effects of melatonin. Dose- and time-dependent structural disturbances were found as sunburn cells and cleft formation increased significantly in the epidermis at 200 mJ and 75 mJ at 24 and 48 h post UV-exposure, respectively. Further analysis revealed apoptotic events such as up-regulation of p53 visible after 24 h at 300 mJ while melatonin revealed a significant 40% and 94% decrease after 24 and 48 h. Concurrently, melatonin down-regulated TUNEL positivity reaching levels of 63% and 370% at 300 mJ after 24 and 48 h, respectively, compared to control samples without melatonin. Moreover, melatonin reduced UV-mediated down-stream activation of intrinsic apoptotic pathway by suppression of casp-9 and casp-3 as well as subsequent down-regulation of PARP. Immediate significant consumption of catalase as UV-induced oxidative stress event was observed directly upon UV irradiation and melatonin significantly counteracted this consumption by 25%. In conclusion, melatonin showed precise evidence for strong protection against UV-induced damage by reducing a wide range of apoptosis- and DNA-damage-related events and by up-regulating the antioxidative enzyme capacity in human full skin.

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Withdrawn

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Topical aminolevulinic acid- photodynamic therapy (ALA-PDT) for acne induces apoptosis of sebocytes and down-regulates TLR-2 and TLR-4 expression of sebocytes

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Photodynamic therapy (PDT) is a popular therapeutic method for inflammatory acne. Although acne PDT is widely performed, little is known of its exact therapeutic mechanism. In this study, we aimed to evaluate the efficacy and safety of PDT on acne and discover its mode of action. PDT was performed in 12 patients with mild to moderate acne. Clinical efficacy was assessed by counting the acne lesions and measuring the sebum output before and after PDT. Punch biopsies were obtained from the perilesional sites, before and after 3 sessions of PDT, where TUNEL stain and immunohistochemical staining for Fas, TLR-2 and TLR-4 was performed. There was significant reduction in the number of inflammatory acne lesions after PDT and also a decrease in sebum output. An increase in TUNEL and Fas positive sebocytes were observed after PDT. The expression of TLR-2 and TLR-4 in the sebaceous glands and epidermis decreased by 40% and 30% respectively, after PDT. Our results show that apoptosis of the sebaceous glands is associated with the improvement of acne by PDT. An extensive follow up should be made in future to measure the long term effects of PDT

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Narrow-band ultraviolet B induces significant changes in composition of keratinocytes lipids

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UV light triggers a variety of biological responses in irradiated keratinocytes that might be associated with global perturbation of their lipidome. However, lipids that are specifically affected and the exact molecular mechanisms involved remain poorly understood. We performed a study to characterize time-dependent changes of the lipidome of cultured keratinocytes induced by narrow-band ultraviolet B (NB-UVB) irradiation. Immortalized human keratinocytes (HaCaT) were cultured under standard conditions, irradiated with NB-UVB light (311 nm) at 400 and 800 mJ/cm^2 and collected 1, 2, 3, 6, 12 and 24 h later for lipid extraction. Lipid extracts were separated on silica plates in chloroform/ethanol/water/triethylamine (35:40:9:35) and in n-hexane/ethylacetate (5:1) followed by quantitative shotgun lipidomics analysis. Irradiation with 800 mJ/cm^2 of NB-UVB altered morphology and lipidome composition of HaCaT cells. Ceramide content increased two-fold 6- and 12-h postirradiation with 800 mJ/cm^2, followed by threefold increase in triacylglycerols (TAGs) that peaked at 24 h. In addition, we observed marked increase of various phosphatidylcholine and phosphatidylethanolamine ethers, whereas phosphatidylcholine-species with short-chain fatty acid moieties decreased. The abundance of other lipid species was altered to lesser extent or remained unchanged. NB-UVB affected the cellular lipidome of keratinocytes in strictly apoptosis-specific manner.

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Effects of Low Level Light on Hair Papilla Cells: Implications on Hair Growth Promotion

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Cycling of hairs is maintained by perpetual dermal-epidermal interaction between dermal papilla cells (DPCs) and overlying keratinocytes. Conditions that disrupt the balance will lead to hair loss, causing clinical alopecia. Various modalities have been introduced to treat alopecia based on the knowledge of follicular homeostasis and pathophysiology. Among which low level light therapy (LLLT) has gained increasing popularity. Light emitting diode (LED), when designed as a LLLT, is well tolerated by biological tissues and has been used as a safe therapeutic device. Recently, related products are proven to be beneficial in promoting hair growth, without knowing the actual working mechanism. We hypothesize low level light may promote hair growth by means of activating DPCs. To investigate the mechanisms governing follicular growth after LED treatment, we harvested DPCs from rat vibrissa follicles and irradiated them with LED of specific wavelength. In comparison with the untreated group, irradiated DPCs have a distinct higher rate of proliferation and enhanced cellular viability. Also, the treated group has a significant higher proportion of cells in S/G_2M phases of cell cycle. These results may be explained by increased ERK1/2 (extracellular signal-regulated kinase-1 and -2) and Akt (protein kinase B) phosphorylation in DPCs after irradiation. In clinical aspects, increased numbers of DPCs can result in prolonged anagen phase and enlarged terminal hair caliber, forming "healthier" thick hairs. Our observation shows that LED treated DPCs have strengthened proliferative and survival signals, which may contribute to the clinical effects of low level light on hair growth.

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Prevention of UVB Radiation-induced Epidermal Damage by Expression of Heat Shock Protein 70

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Irradiation with UV light, especially UVB, causes epidermal damage via the induction of apoptosis, inflammatory responses, and DNA damage. Various stressors, including UV light, induce heat shock proteins (HSPs) and the induction, particularly that of HSP70, provides cellular resistance to such stressors. The anti-inflammatory activity of HSP70, such as its inhibition of nuclear factor kappa B (NF-κB), was recently revealed. These in vitro results suggest that HSP70 protects against UVB-induced epidermal damage. Here we tested this idea by using transgenic mice expressing HSP70 and cultured keratinocytes. Irradiation of wild-type mice with UVB caused epidermal damage such as induction of apoptosis, which was suppressed in transgenic mice expressing HSP70. UVB-induced apoptosis in cultured keratinocytes was suppressed by overexpression of HSP70. Irradiation of wild-type mice with UVB decreased the cutaneous level of IκB- α (an inhibitor of NF-κB) and increased the infiltration of leukocytes and levels of pro-inflammatory cytokines and chemokines in the epidermis. These inflammatory responses were suppressed in transgenic mice expressing HSP70. In vitro, the overexpression of HSP70 suppressed the expression of pro-inflammatory cytokines and chemokines and increased the level of IκB-α in keratinocytes irradiated with UVB. UVB induced an increase in cutaneous levels of cyclobutane pyrimidine dimers and 8-hydroxy-2′-deoxyguanosine, both of which were suppressed in transgenic mice expressing HSP70. This study provides genetic evidence that HSP70 protects the epidermis from UVB-induced radiation damage. The findings here also suggest that the protective action of HSP70 is ediated by anti-apoptotic, anti-inflammatory, and anti-DNA damage effects.

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Ultraviolet Radiation And Leucocytes Function In An Experimental Model

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Skin is a major target of oxidative stress due to reactive oxygen species (ROS) that originate in the environment, produced especially by ultraviolet radiation (UVR), and in the skin itself. The study estimated, in psoriasis patients, the phagocytosis and ROS production after whole blood-exposure to UVR. Heparinized venous blood was taken from 20 patients with psoriasis. Blood samples were exposed to UVR for 5, and 15 min. in a dark room by means of an 80 W biological quartz lamp placed at a distance of 20 cm from the sample. The pre-irradiated state served as control. The activation of oxidative metabolism during phagocytosis and ROS formation were evaluated by nitroblue tetrazolium dye reduction test (NBT%). ROS reduced NBT dye to nitroformazan. In psoriasis patients, the leukocytes phagocytosis was reduced in basal conditions, but NBT tests increased to 7.53 ± 4.68%, and 5.33 ± 2.30% over the initial value (4.25 ± 3.02%), respectively with 77.17%, and with 25.4% correlated with the blood UVR time exposure. These high levels of NBT tests showed an increase of phagocytosis, the granulocytes oxidative metabolism activation induced by UVR, and a lot of ROS production. In psoriasis skin patients there is an infiltrate with leukocytes which can produce ROS after UVR exposure and can modulate the local inflammation. Experimental model can be used to quantify the leukocytes oxidative metabolism after UVR blood exposure. The obtained results can indirectly explain the modifications of lesions and the beneficial effects after UVR skin contact, in psoriasis patients.

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Comparison of ALA and MAL uptake in keratinocytes

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5-aminolevulinic acid (ALA) and methyl-ALA (MAL) are widely used as prodrugs, which are metabolized to the photosensitive compound protoporphyrin IX (PpIX) which is an efficient photosensitizer in photodynamic therapy (PDT). In this study, we analyzed the uptake mechanism of ALA and MAL in two keratinocyte cell lines. Cells were incubated with 1.8 mM ALA or MAL and competitive uptake inhibitors for 30 min. PpIX was measured fluorimetrically after 2 h. Inhibitors used were β-alanine, (S)-SNAP-551 and different amino acids. Both, β-alanine and (S)-SNAP-5114, inhibited the ALA-uptake completely, but did not reduce the uptake of MAL. For MAL-uptake, cysteine and histidine showed the strongest inhibitory effect, reducing the PpIX amount by 50%, but did not have the same inhibitory effect on the ALA-uptake. The results indicate that ALA and MAL are taken up by different transporter systems. Further, to analyze the origin of the painful sensation many patients experience during the irradiation phase, we searched for compounds released by the irradiation of keratinocytes after loading with PpIX. We found that ATP was secreted upon irradiation at an amount that was dependant on the concentration of PpIX formed after ALA or MAL incubation. Inhibiting the uptake of MAL with cysteine reduced the ATP release. The amount of ATP released was sufficient to stimulate sensory nerve endings through the activation of P2X receptors.These results indicate that ATP released upon irradiation from keratinocytes preloaded with PpIX is at least partly responsible for the painful sensation felt by many patients during PDT irradiation.

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Modification of microRNA expression by baicalin in UVB irradiated mouse skin using a microRNA microarray

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MicroRNAs (miRNAs) are non-coding RNA molecules of 21 to 24 nt that regulate the expression of target genes in a post-transcriptional manner. Evidence indicates that miRNAs play essential roles in embryogenesis, cell differentiation, and pathogenesis of human diseases including skin cancer. To assess the effects of baicalin on UVB-mediated miRNAs expression changes in mice skin. We analyzed the miRNA expression profiles in 3 pairs of UVB irradiated mice, baicalin treated irradiated mice, and untreated mice. Real-time RT-PCR analysis was used for confirm the miRNA expression changes. TargetScan and GO-analysis were employed for the prediction of miRNA targets. 3 miRNAs are down-regulated and 3 miRNAs are up-regulated in UVB irradiated mice compared with untreated counterpart. Differentially expressed miRNAs were predicted to have some relationships with photocarcinogenesis, hypomethylation and apoptosis. 3 miRNAs are down-regulated and 1 miRNA are up-regulated in baicalin treated irradiated mice compared with UVB irradiated mice. Differentially expressed miRNAs were predicted to have some relationships with DNA repair signaling. MiRNAs were potentially involved in the pathogenesis of photodamage, and differentially expressed miRNAs in baicalin treated group may help to treat and prevent UV-induced dermatoses.

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Protective Effect of Baicalin on Multiple Ultraviolet B Exposure-mediated damages in C57BL/6 mouse skin

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Multiple exposures to solar ultraviolet (UV) radiation cause critical damages that may lead to the development of several cutaneous disorders including skin cancer. Protection against sun-induced damage is therefore a highly desirable goal. Chemoprevention via plant-based agents may be a useful approach for the management of UV-induced neoplasia. In this study, we assessed whether Baicalin afford protective effect on multiple UVB exposure-mediated damages in C57BL/6 mice skin and the underlying mechanisms. C57BL/6 mice were topically pretreated with baicalin (1 mg/cm^2 skin area/mouse/100 μL acetone) and were exposed to UVB 30 minutes later (180 mJ/cm^2, on alternate days ×10 exposures). The animals were sacrificed 24 h after the last UVB exposure. Skin edema, histopathology changes, Ki-67, PCNA, COX-2 were assesed to determine the multiple UVB induced photodamage. Multiple UVB exposures to C57BL/6 mice resulted in an increase in skin edema and hyperplasia, topical application of baicalin prior to UVB radiation resulted in a significant inhibition of Ki-67, PCNA and COX-2, these protective effects of baicalin are supposed to inhibit UVB-induced skin carcinogenesis. Based on this data, we suggest that baicalin could be developed as an agent for the management of conditions elicited by multiple UV exposure including skin cancer.

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Two weeks of holiday sun exposure significantly enhances the pigment protection factor (PPF) in Polish children

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Solar UVR is the most important environmental factor on human health. Most human photobiological research has been with acute erythemogenic doses of UVR in healthy adult volunteers. However, we lack data on skin responses to natural solar exposure in children; this is important because childhood sunburn is a risk factor for melanoma. Thus, the aim of our study, within the EC-funded ICEPURE project, was to assess changes in parameters that determine individual skin sensitivity to the sun in children. The study group was 32 Caucasian children (mean age 8.9 y, phototypes I-IV) on a 2-week summer camp holiday (23rd June - 6th July 2009) at the Baltic Sea (Sztutowo 54°N). The children were advised to use sunscreens according to their usual habits. In each child, redness (erythema), pigmentation and pigment protection factor (PPF) were measured by a UV-Optimizer at six locations (forehead, upper arm, lower arm, back, buttock, dorsal hand) before the holiday and 24h after returning home. Erythema on the back and hand increased significantly after the camp, whereas pigmentation increased on the back, upper arm, lower arm and hand. The PPF increased significantly on the same five sites. There were no changes on the unexposed buttock skin. The lack of change in forehead erythema and pigmentation probably results from protection by a fringe and face being a site where sunscreens are more commonly applied. Nonetheless, there was a perhaps surprising significant increase in PPF. Overall, we show adaptive photoprotection with limited cumulative erythema.

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Erythemal UVB radiation alters the expression of Vitamin D receptor in the human skin with leading to photoadaptation

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UVB is one of the major factors involved in photocancerogenesis as well is required for vitamin D production. As there are discrepant results on the role of Vitamin D receptor (VDR) in skin oncogenesis, thus the aim of the study was to assess the expression of VDR in UVB exposed skin . The study consisted of 4 groups of 10 healthy individuals who were whole-body UVB irradiated for 10 days with 0.7 MED on each occasion, or whole-body irradiated as before followed by a single high dose of UVB (10x10 cm), or were irradiated only with a single dose of UVB (3 MED) on the small area of the body, or were unirradiated. Skin biopsies were taken in all participants and VDR expression was assessed (Western blot). The VDR expression was significantly higher in 3 MED UVB-irradiated group when compared to unirradiated skin ($p<0.05$). In the volunteers who were whole-body irradiated for 10 days followed by a single 3 MED UVB the VDR expression was relevantly lower than in group of 3 MED ($p<0.05$). No changes in VDR expression were found in group of volunteers exposed only to suberythemal UVB doses. The repeated low doses of UVB protect against the effects of an erythemal UVB dose on VDR expression what may be the next proof of the organism's ability to develop photoadaptation.

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Phototherapy with narrowband ultraviolet B enhances serum concentration of 25-hydroxycholecalciferol in psoriatic patients

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The narrowband ultraviolet B radiation (UVB-NB; 311-313 nm) is used for phototherapy of skin diseases. The ultraviolet B radiation leads to the change of accumulated in the skin 7-dehydrocholesterol into previtamin D, which is later converted by the thermal energy into cholecalciferol and hydroxylated in liver. Over 90% of daily vitamin D demand is produced in the skin under sunlight. The aim of the study was to determine the serum 25-hydroxycholecalciferol (25OH) concentration changes in the patients irradiated with UV-NB. The study consisted of 47 psoriatic patients in mean age of 41 y.o. The course of 20 irradiations with UVB-NB was performed in each patient. The vitamin D serum concentration (RIA method) was measured 3 times, before treatment, after 10 and after 20 irradiations. The mean serum concentration of 25OH before treatment was 27ng/ml; 39 ng/ml after 10 irradiations and 44.3 ng/ml after 20 irradiations. 10 and 20 exposures to UVB-NB caused significant increase in 250H serum concentration when compared to the baseline results ($p<0.001$ for both comparisons), however we observed no statistically significant difference between serum concentration of this parameter in the measurements after 10 and 20 irradiations. We found positive correlation between 250H serum concentration after final exposure and cumulative UVB-NB dose ($R=0.09$; $p<0.001$). The highest increase in 25OH serum level in psoriatic patients is observed after first 10 irradiations whereas next 10 exposures did not raise it so effectively what might testify to the occurrence of photoadaptation phenomenon.

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A multifunctional plant extract helps prevent photoaging

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Photoaging is the main cause of premature skin aging. UVA radiation generates reactive oxygen species (ROS) that denature proteins, cause DNA mutations and deregulate gene expression in the dermis. Solar elastosis is a sign of photoaging in which non-functional elastin deposits accumulate. Elastin expression increases while macrophages and neutrophils secrete elastases. We first observed that, while UVA radiation rapidly induced MMP-1 and elastin mRNA in cultured dermal fibroblasts, Lysyl oxidase-Like (LOXL) mRNA remained unchanged. Since LOXL normally creates cross-links that confer their mechanical properties to elastic fibers, increasing LOXL could be a way to turn elastin accumulation into functional fibers. We thus searched for a global protective agent that would inhibit elastase activity, increase LOXL expression and display good radical scavenging properties. We identified a plant extract that decreased the free radical DPPH by 60% when used at 2%, inhibited human neutrophil elastase activity by 46% and doubled LOXL mRNA expression in cultured fibroblasts. Moreover, the extract inhibited type IV collagenase (MMP-2) activity by 30% and macrophage elastase (MMP-12) activity by 65%. When applied on confluent fibroblasts in vitro for 5 days, the extract also boosted collagen deposition. LOXL labeling was more intense and appeared as droplets on thin fibrils. A double labeling with fibrillin-1 or elastin is required to confirm that these latter are elastin-associated microfibrils. These in vitro results suggest that the aforementioned plant extract used alone or in combination with sunscreens could be an effective protective agent to help prevent photoaging.

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High SPF sunscreen for children intolerant skin protects from UV deleterious effects on DNA and immune system

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Ultraviolet radiations (UVR) result in a number of alterations in the skin: DNA lesions (dimers), alteration of p53 tumor suppressor, photo-immunosuppresion (PIS) by phenotype alteration of Langerhans cells (LC). All together, these effects can contribute to cutaneous carcinogenesis. A high SPF sunscreen was specifically formulated for children intolerant skins. In addition to mineral sunblocks, it contains zinc gluconate; alpha-tocopherol (anti-oxidant with UVR-protective effects on p53 and PIS); Aloe Vera (with immuno-protective properties by preserving LC and inhibiting IL10 secretion after UVB) and Sunflower Oleodistillate (SO, which has hydrating and anti-inflammatory properties).HaCaT cells, pre-treated or not with SO, were subjected to UVR. IL6 and IL8 release were measured by ELISA assay. Skin explants topically treated, or not, with the high SPF sunscreen, were irradiated by UVA+B. Fluorescent immunostaining of CPD (pyrimidine dimers), CD1a (LC) and p53 were realised.SO was able to modulate significantly the release of IL6 and IL8 induced by UVR. The sunscreen totally protected skin explants from UV-induced DNA damage as none pyrimidine dimer was detected. The sunscreen limited the raise in p53 positive cells induced by UVR (protection by 96%); and was able to counteract the decrease in epidermal LC induced by UVR (protection by 113%). Exposure to sunlight during childhood is a risk factor for skin cancer such as melanoma. That's why children need highly efficient sunscreen offering surface as well as depth protection. This high SPF mineral sunscreen answers this need with active ingredients providing UV protection at two levels: immune and cell defenses.

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Narrow-band UVB alters the expression of cathelicidin and human β defensin 2 in skin lesions of psoriasis and atopic dermatitis

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Narrow-band UVB (NB-UVB) is routine treatment for psoriasis (PS) and atopic dermatitis (AD), but its effect on antimicrobial peptides is not well documented. We studied the effect of NB-UVB on lesional expression of cathelicidin and human β defensin 2 (HBD2), which can act as proinflammatory mediators, as well as on serum 25-hydroxyvitamin D (25OHD) concentration. Six whole body NB-UVB treatments were given for 18 PS patients, 18 AD patients, and 15 healthy subjects. Respectively, skin biopsies were taken from seven, eight and seven subjects out of them. Antimicrobial peptide expression was examined by quantitative real-time PCR and serum 25OHD by radioimmunoassay. Before NB-UVB treatment cathelicidin ($p<0.05$) and HBD2 ($p<0.001$) expression were significantly elevated in PS lesions compared to the skin of healthy subjects. Six NB-UVB exposures (12.3 SED) markedly increased cathelicidin expression while HBD2 expression decreased statistically significantly. In AD lesions, the slightly elevated levels of cathelicidin increased further, whereas the significantly ($p<0.05$) elevated HBD2 levels decreased. At the onset the median 25OHD concentration was 39.6 (range 15.6 - 58.5) nmol/L in PS, 29.5 (range 15.1 - 53.2) nmol/L in AD and 48.8 (range 35.2 - 112.4) nmol/L in healthy subjects. Six NB-UVB treatments increased serum 25OHD concentration statistically significantly, by 24.3 nmol/L in PS, by 27.1 nmol/L in AD and by 17.0 nmol/L in healthy subjects. We conclude that small doses of NB-UVB increase cathelicidin and decrease HBD2 levels in healing PS and AD skin lesions. These effects might be mediated by improved vitamin D balance.

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The dynamics of hyaluronan secretion in ultraviolet B irradiated rat epidermal keratinocytes

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Peri- and extracellular hyaluronan (HA) has profound effects on the migration, proliferation and differentiation of keratinocytes. Its metabolism in the dermis and epidermis is partly regulated by ultraviolet B (UVB) radiation, which makes it an indispensable target in current dermatological research.To elucidate the dynamics of hyaluronan secretion in cultured rat epidermal keratinocytes (REK), cells in monolayers were exposed to 2.5-20 mJ/cm² of broadband UVB-irradiation and organotypic 3D-cultures similarly to 20 or 30 mJ/cm² of UVB. Accumulation of HA and the transcript levels of assorted genes were quantified at various timepoints. Additionally, different inhibitors were applied to unravel the intracellular signaling pathways involved. Moderate UVB-irradiation caused a dose-dependent increase in total HA. This response was clearest in the monolayers, but a slight induction was also seen in the organotypic cultures. Blocking the intracellular kinase MEK1/2 in the monolayers partially reversed the effects of UVB. On the mRNA-level, hyaluronan synthases (HAS1-3) were dynamically regulated with HAS1 showing the earliest induction after just 2 h. HAS2 and HAS3 had similar profiles with fluctuating upregulation between 8-36 h. A slight induction in the HA-receptor CD44 was seen at 24-36 h after an initial decline. Our results demonstrate that hyaluronan production specifically and rapidly responds to UVB-exposure in both the monolayers and the organotypic culture model. The data imply that acute irradiation activates signalling routes involved in the regulation of HAS-gene transcription, and that the MEK-ERK -pathway might be essential for the response.

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Successful phototherapy of polymorphic light eruption patients is associated with a recruitment of mast cells into the skin

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Resistance to UVB-immunosuppression is thought to be one reason for the symptoms of polymorphic light eruption (PLE) following UV exposure. While UVB photohardening is used for prophylactic therapy in PLE, little is known on how this affects the immune system of patients. Dermal mast cells mediate UVB-immunosuppression and are important regulators of UVB-inflammation. However, the role of mast cells in PLE is not known. We investigated the effect of photohardening in PLE patients on the prevalence of dermal mast cells, as well as Langerhans cells (LC) and skin-infiltrating T cells. Biopsies from five female PLE patients (39±17 years) were taken at four time points: (1) early spring before photohardening, (2) late spring after photohardening, (3) summer, and (4) winter. Giemsa staining identified mast cells, while immunohistochemical staining for CD1a and Langerin identified LC. CD4, CD25, and Foxp3 antibodies identified helper, activated and regulatory T cells, respectively. Sections from 17 PLE patient samples and 11 controls were analysed. A significantly lower mast cell density in the papillary (but not reticular) dermis was observed in PLE patients compared to controls. Photohardening with 311nm UVB significantly increased dermal mast cell densities in PLE patients (by 55%; $p<0.05$). Indeed, phototherapy restored dermal mast cell densities to levels similar to that of controls. Photohardening decreased LC densities in the epidermis (by 35%; $p<0.05$) but did not alter the CD4+, CD25+ or Foxp3+ T cell numbers in PLE skin. Our results suggest that successful phototherapy of PLE patients involves UVB-induced dermal mast cell recruitment.

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Proliferative potential and stage of cell cycle determines keratinocyte susceptibility to UV-induced apoptosis

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Keratinocyte apoptosis occurs in response to ultraviolet (UV) radiation, allowing elimination of DNA damaged cells and thereby prevents UV-induced tumour formation. Understanding why some cells appear differentially susceptible to apoptosis may provide insight into individual variation in the apoptotic response and whether external factors may be able to alter the balance of apoptosis. Live cell imaging techniques and flow cytometry were utilised to investigate the differential susceptibility of keratinocytes at different stages of the cell cycle, and the apoptotic affect of UV on cells of different proliferative potential was analysed. Fresh human foreskin was irradiated ex-vivo with narrowband UVB at physiological doses, and the whole epidermis was then analysed to elucidate which cells underwent apoptosis. Proliferating cells were defined as those with a bright anti-α6-integrin signal, and were categorised into putative stem or TA cells according to expression of anti-transferrin (CD71). All experiments were repeated using skin derived from different donors on at least 3 occasions. Epidermal cells in G2M were significantly more likely to undergo apoptosis than cells in G1 or S phase following UVB irradiation ($p<0.01$). In freshly isolated epidermis a median of 8% of apoptotic cells were α6-integrin+ but CD71 - (a putative stem cell population), with apoptosis occurring in a median of 13% of stem cells, 20% of TA cells, and 9% of differentiating cells. In conclusion, we show that cell cycle is important in determining apoptosis susceptibility, and that stem and TA cells are sensitive to UV-induced apoptosis.

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Photohardening restores the impaired neutrophil responsiveness to chemoattractants in patients with polymorphic light eruption

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A failure to induce immune suppression after UV exposure has been implicated in the pathogenesis of polymorphic light eruption (PLE). This immunological resistance has been linked to an impaired neutrophil infiltration into the skin following UV exposure. Therapeutic photohardening can restore this abnormal neutrophil infiltration in PLE skin, and is thought to be responsible for the prophylactic efficacy. The aim of this study was to elucidate the pathogenic mechanism of the described neutrophil deficiency in PLE. Peripheral blood neutrophil responses to the chemoattractants leukotriene B4, LTB4 and formyl-methionyl-leucyl-phenylalanin, fMLP were investigated in vitro.Samples from 10 PLE patients (9 female; 1 male; mean age 37 years; age range, 27 to 51 years) before and after 6 to 9 weeks of photohardening therapy (311-nm UVB or PUVA) were assessed. Flow cytometry was used to measure the changes associated with neutrophil activation. We found a significantly reduced neutrophil responsiveness to LTB4 and fMLP in PLE patients ($p < 0.01$), which was restored to normal levels after phototherapy. Indeed, PLE neutrophil responsiveness to these two chemoattractants after (but not before) phototherapy was similar to that of age- and sex-matched healthy control subjects. This indicates that an abnormal chemotactic potential of neutrophils is a crucial factor in the pathogenesis of PLE. Normalization following photohardening may therefore account for the therapeutic efficacy by restoring UV-induced neutrophil skin infiltration.

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Patients with polymorphic light eruption have decreased levels of serum 25-hydroxy vitamin D: epiphenomenon or pathogenic factor?

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Polymorphic light eruption (PLE) is a very common disease with an incidence of up to 20 percent in young women between the ages of 20 and 40. The exact cause of the disease remains to be determined, however, an abnormal immune regulation with resistance to UV-induced immune suppression and simultaneous autoimmune reactions to photo-induced neo-antigens have been implicated. Vitamin D3 has been linked via RANKL to the induction of T regulatory cells which is thought to play a role in the modulation of immunity following UV exposure. We therefore analysed 25-hydroxy vitamin D (25-OH-VitD) serum levels by enzyme immunoassay in 24 PLE patients during different seasonal time points over the year: (1) early spring before photohardening, (2) late spring after photohardening therapy, (3) summer, and (4) winter. We found that PLE patients had significantly decreased 25-OH-VitD levels, which were below normal limits throughout the year (mean values around 13 to 14 ng/ml), compared to age- and sex-matched control subjects (30 to 60 ng/ml). Prophylactic phototherapy with narrowband UVB (311nm) delivered for 6 to 9 weeks, 2 to 3 sessions per week, significantly increased (by nearly 50%; $p<0.000001$) 25-OH-VitD serum levels in treated PLE patients. While low 25-OH-VitD serum levels in PLE patients as an epiphenomenon cannot be excluded, another study from our laboratory shows that topical pretreatment with the synthetic vitamin D3 analogue calcipotriol, can be used to treat PLE patients. These studies indicate that low vitamin D levels may play an important role in the pathogenesis of PLE.

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2,4,6-octatrienol, a polyunsaturated alcohol, counteracts molecular markers of skin cell senescence

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Parrodienes are synthetic congeners of psitaccofulvins a mixture of polyenals contained exclusively in Ara Macao red plumage. They possess a polyene structure and alcohol functional group and share some structural features with carotenoids, natural precursors of retinoids. Considering that both retinoids and carotenoids have been reported to repair UV-induced skin damage and to prevent aging phenomenon, we selected 2,4,6-octatrienol, one of the main components of parrodiene family, to study its ability to counteract photo-accelerated cellular senescence, a phenomenon highly related to organism aging and tumor progression. For this purpose we adopted an experimental model based on single exposure of human dermal fibroblasts to 8-methoxypsoralen plus + ultraviolet-A-irradiation (PUVA) previously reported to activate a premature cell senescence characterized by an imbalance of cell antioxidant defence system. PUVA-photoactivated fibroblasts grown in the presence of 2,4,6-octatrienol are partly, but significantly rescued from the features of cellular senescence phenotype, such as cytoplasmic enlargement, expression of senescence-associated-b-galactosidase and of matrix-metalloproteinase-1. The mechanisms by which 2,4,6-octatrienol exerts its "anti-aging" effect does not involve a direct scavenging of PUVA-induced reactive oxygen species, but a synergistic up-modulation of different components of cell antioxidant network, such as catalase or reduced glutathione, a preservation of cell membrane lipids such as phospholipids, or cholesterol, and the activation of intracellular pathways, linked to cell proliferation and anti-inflammatory response. Moreover, 2,4,6-octatrienol reverted p21 expression in PUVA-treated fibroblasts. Our data suggests the usefulness of 2,4,6-octatrienol as innovative molecule to counteract aging phenomenon.

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UVB-311nm advances the clearance of psoriatic lesions in ustekinumab-treated patients

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Although the IL-12/23 blocking antibody ustekinumab exhibits high efficacy in psoriasis, there remain about 30% of patients who do not reach a PASI75% reduction upon treatment. Therefore additional means of improving overall antipsoriatic response are needed in those patients. Similar to treatment with other biologics, one possibility is the combination with conventional systemic drugs, such as etretinate, methotrexate, and/or phototherapy. In a half-side comparison study we assessed whether additional 311nm-narrowband-UVB phototherapy (311nm) could add to therapeutic efficacy of ustekinumab. Ten patients (5F/5M; mean age, 57 years; mean PASI, 13.4) with moderate to severe plaque-type psoriasis were enrolled and received ustekinumab 45 or 90mg s.c. depending on body weight (below or above 100kg) at week 0 and 4. The patients were additionally treated for 6 weeks 3 times per week on a randomly selected body half (left or right side, except the head) with 311nm and monitored weekly by half-body PASI. In one patient, study participation was stopped after 2 wks of 311nm due to a herpetic eruption. During the 6-week irradiation regimen, 311nm significantly bolstered the therapeutic response. At wk6 PASI75% reduction was achieved in 7/9 (78%) vs. 1/9 (11%) comparing UV-irradiated vs. non-irradiated body halves. The mean PASI reduction was 82% vs. 52% ($p<0.005$; paired t-test). At wk12 4/9 (44%) patients continued to have a better response on UV-irradiated body halves. This indicates that 311nm may be useful to improve the therapeutic outcome in psoriasis treated with ustekinumab, particularly in patients not satisfactorily responding to biologic treatment alone.

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Differential health gain of summer sunlight exposure in people of different skin types

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Public health policy advises limiting summer sunlight exposure, but provides no specific advice for darker-skinned people. This study assessed the relative vitamin D gain on following UK sunlight exposure recommendations in White Caucasians and South Asians. During wintertime (negligible ambient UVB), 86 volunteers (n=70 skin-type II, 16 skin-type V), 20-60y, Manchester, UK, received a simulated summer's sunlight exposures, comprising 1.3SED, 3xweekly for 6wks, wearing T-shirt and UV-opaque shorts. Circulating 25(OH)D was evaluated by HPLC (n=60xII, 10xV). A 10x10cm^2 area of one buttock was left exposed (n=10xII, 6xV) and underwent spectrophotometric measurements. At course end, exposed and unexposed skin received an acute 2xMED challenge, with 24h measurements. Vitamin D status improved post-course, with significant difference in 25(OH)D gain between types II (mean 10.2, SD7.5ng/ml) and V (4.6, 1.9), $p<0.05$. Baseline L* (white-black differentiation) differed between groups, reflecting higher pigmentation of V, $p<0.01$. The exposures darkened the skin, decreasing L* by 6.2(5.3), $p<0.001$, with 6% and 24% reduction in II and V respectively, and with strong negative correlation between L* decrease and 25(OH)D gain (R^2= 0.94, 0.92; $p<0.001$). The simulated summer conveyed photoprotection, UV-treated skin demonstrating lower A* values (erythema) than UV-protected skin after challenge with 2xMED, $p<0.05$, with 14% erythema protection in II and 50% in V. Thus, the same summer exposure conditions cause approximately half the 25(OH)D gain in darker than fair skin-types, with greater pigmentation gain contributing to this. More specific advice on sunlight exposure may be required for people of skin type V living at higher latitude.

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Little Effect on p63 but Significant Effect on miR-21 and miR-125b by NB-UVB Phototherapy on Psoriatic Lesions

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Psoriasis is an inflammatory skin disease in which dysregulation of p63, a member of the p53 family and crucial for skin development and maintenance, has been shown. Though currently incurable, many therapies are available including narrowband ultraviolet B (NB-UVB) phototherapy. To further elucidate the role of p63 in psoriasis and increase our understanding of the mechanisms of phototherapy, we studied the effects of NB-UVB treatment on p63 expression. Expression of p53 was also studied due to its functional role in the response of skin to UV. In addition, we investigated expression of miR-203, miR-125b and miR-21, as these microRNAs are p63 and/or p53 regulators and their involvement in psoriasis pathogenesis has previously been suggested. Skin biopsies from 12 psoriasis patients were collected before, during and at the final session of phototherapy. Real time RT-PCR and immunohistochemistry showed that epidermal p63 mRNA and protein levels were not significantly affected following phototherapy, whereas a significant increase in p53 mRNA expression and protein accumulation was found. NB-UVB treatment also significantly affected expression of miR-21 and miR-125b, whereas individual clinical improvement seemed related to p53 status only. Our results indicate that even though NB-UVB phototherapy causes diverse molecular changes, induction of p53 is pivotal for successful treatment of psoriasis, and unresolved p63 abnormality in the treated epidermis of psoriasis patients further indicate a role for p63 in psoriasis.

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Histidinemic mice are hypersensitive to ultraviolet B radiation-induced DNA damage

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Histidinemic mice carry a mutation in the gene encoding histidase, the enzyme that produces urocanic acid (UCA). The mutation is associated with greatly reduced histidase activity, UCA content and UVB absorption capacity of the stratum corneum. Here, we compared histidinemic mice and wild-type mice with regard to their sensitivity to ultraviolet (UV) B irradiation. The back skin of adult mice was shaved and exposed to 250 mJ/cm^2 UVB. Newborn mice were sacrificed and exposed to 25 mJ/cm^2 UVB either with or without prior topical application of UCA. DNA damage was quantified by ELISA for cyclobutane pyrimidine dimers. Histidinemic mice accumulated significantly more DNA damage than wild-type mice. Exogenous UCA reversed the UVB-photosensitive phenotype of histidinemic mice and increased UVB-photoprotection of wild-type mice. These results suggest that UCA protects mammalian skin against UVB radiation.

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UV-B irradiation induces epidermal up-regulation of heparanase expression and activity

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Heparan sulphate glycosaminoglycan (HSPG) is an abundant component of cell surfaces and extracellular matrix, and is mainly located in the basement membranes. It plays many roles such as in cell proliferation and differentiation, or in cell-matrix adhesion and assembly via protein interactions. It has been shown that HS expression decrease during ageing. Because UV-B irradiation from sun exposure is one of the most influent factor that promote skin ageing and because modulation of HSPG expression could be explained by changes in expression and function of its degrading enzyme, heparanase (HPSE) expression and activity were investigated after UV-B irradiation in normal human epidermal kératinocytes (NHEK). NHEK and reconstructed epidermis were submitted to increasing doses of UV-B. HPSE mRNA levels were measured using real time PCR and total enzymatic activity was quantified using microtiter-based assay in human keratinocytes in culture. Expression and distribution of HPSE were also performed by immunohistochemistry on reconstructed epidermis. Our results showed that both HPSE mRNA level and total enzymatic activity were increased by UV-B in a time and dose-dependent manner in keratinocyte cultures. Protein expression of HPSE was also up-regulated according to the increasing dose of UV-B in reconstructed epidermis. In addition, we observed an intra-cellular localization of HPSE in all viable cell layers in both reconstructed epidermis and normal human epidermis. Increase of HPSE expression and activity in the epidermis after UV-B irradiation could contribute to skin photo-ageing.

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The role of vascular endothelial cells in PDT using porphyrin pre-cursors

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Using ALA we have shown in numerous studies that the efficacy of ALA-PDT is significantly improved by applying light fractionation. In pre-clinical models, PDT efficacy using MAL is unaffected by adopting this approach. Our previous results show acute edema formation following ALA-PDT suggesting a role of endothelial cells. Cryosections of hairless mouse skin 4h after topical MAL or ALA application were stained with CD31. Co-localization with PpIX fluorescence was investigated using spectral imaging under a confocal microscope. Images were analyzed using the single value decomposition algorithm to confirm the presence of PpIX. This approach was used to analyze intra-vital confocal microscopy images of mouse skin chambers. We investigated the PpIX fluorescence distribution in the vasculature in-vivo during PDT. Fluorescein exclusion studies were performed to investigate the vascular response during illumination. PpIX fluorescence at depth is found in cryo-sections 4 hours after of ALA and PpIX is co-localized with vascular endothelial. The application of MAL shows less fluorescence at depth and less PpIX localized in the skin vasculature. Intra-vital microscopy shows PpIX fluorescence in tissue and endothelial cells/vasculature deep in mouse skin (even below the cutaneous muscularis). PDT studies show that there is a significant vascular response to ALA-PDT in the micro- vasculature close to the base of the epidermis that is absent during PDT with MAL. These results illustrate the significant vasculature response following ALA -PDT and have clear implications for optimizing porphyrin pre-cursor PDT.

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Comparison of a mitochondrial and a nuclear CC to TT transition in human skin cells

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CC to TT transitions in mitochondria and nucleus result from photo-induced cyclobutane pyrimidine dimers (CPDs). This damage is repaired by nucleotide excision repair (NER) in nucleus, but not in mitochondria. In this study we established an assay to quantify a CC to TT transition in mitochondrial DNA at bases 591/592 and then compared levels with a CC to TT transition in the p53 gene (codons 281/282). Fibroblasts and keratinocytes were isolated from human skin samples (n=7). DNA was isolated and then analyzed using probe-based allele-specific quantitative real-time PCR. Copy numbers of mutated and not mutated DNA were determined for both positions to calculate mutation levels of mitochondrial and nuclear CC to TT transitions respectively. The p53 mutation was measured in four fibroblast cell cultures (1,81‰ - 6,46‰) and three keratinocyte cell cultures (5,81‰ - 13,44‰). In contrast to these findings the mitochondrial CC to TT transition was only quantified in one fibroblast sample (0,163‰) with levels remaining stable over five passages. Compared to this, our previous work showed that levels of the p53 CC to TT transition significantly declined during cell culture. Surprisingly, incidence and levels of the mitochondrial transition were much lower compared with the p53 transition. As CPDs remain stable in mitochondria and are removed in nucleus, we assume that these comparatively low mutation levels are caused by a replication disadvantage of mitochondrial DNA containing CPDs. This replication disadvantage might be an important mutation preventing mechanism and replace NER in mitochondria.

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UVB induces HIF-1α in keratinocytes in two sequential phases

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Hypoxia in the skin is thought to play an important role in various changes that have long-term effects, such as chronic degenerative changes, inflammation, photoaging and cancer. Vascular endothelial growth factor (VEGF) has been shown to be crucial player in these processes. Hypoxia inducible factor-1 (HIF-1) in a key regulator of the expression of VEGF which is also known to be affected by ultraviolet radiation. We studied the impact of a single UVB irradiation on the level of HIF-1 in keratinocytes by Western blots at various time points after irradiation. The physiological importance was confirmed by measuring downstream target gene expressions by quantitative real-time PCR. UVB treatment resulted in an initial quick decrease of HIF-1a that was followed by a subsequent prolonged increase. These changes followed a strict timeline and were dose-dependent. PI3K/AKT pathway is potentially involved in this response, so we set out to examine the mechanism underlying the upregulation of HIF-1α upon UVB irradiation. No change was observed in the total level of AKT after UVB, however, its phosphorylation level was found to be markedly higher. In accordance with these observations, wortmannin, an inhibitor of PI3-kinase effectively blocked the increase in HIF-1α. Similarly to previous findings, UVB irradiation increased VEGF and HO-1 mRNA levels determined by quantitative real time PCR. It is shown that changes in HIF-1α expression lead to a change in VEGF expression after UVB. It is presumed that UVB effects in the skin are partially mediated by the PI3K/AKT pathway.

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Evaluation of the impact of 810 nm laser diode irradiation on pigmented keratinocyte cultures' structure and apoptosis

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Laser light is widely used for removal of dark hair from fair skin taking advantage of the localized thermolysis of pigment-rich structures. We used pigmented human epidermis cultures to evaluate the impact of 810 nm laser diode light on the tissue structure. In vitro reconstructed epidermis of phototype VI was subjected to variable doses of the laser radiation whereas ultraviolet light was used for irradiation of control cultures at „sunburn" range (UVB+A 50 and 100 J/m²). The cultures were studied with light and electron microscopy at 24h after exposure. Qualitative and quantitative analysis showed a dose-dependent induction of morphological changes. Baseline TUNEL reactivity was observed on nuclear chromatin in normal non-irradiated tissue and was probably due to DNA breaks existing naturally or induced through tissue sectioning. TUNEL reaction could be quantified with immunogold. The impact of UV irradiation was mostly focused on DNA alteration leading to selective cell apoptosis whereas laser exposure resulted in extensive necrosis of the lower part of the cultured epidermis. Lesions generated by the laser light were due to energy absorption by melanin contained in the basally located melanocytes. Superficial epidermal layers containing only sparsely distributed melanosomes were not visibly impacted by the laser treatment. Further studies are required to establish the outcome of depilatory laser treatment on tanned skin of phototypes I to IV, where increased number of melanosomes is likely to persist within the upper epidermal layers.

297 [Oral 072]

Matrix Metalloproteinase (MMP)-7 Activates Heparin-binding EGF-like Growth Factor in Squamous Cell Carcinomas Complicating Recessive Dystrophic Epidermolysis Bullosa

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Tumour specific expression of matrix metalloproteinase (MMP)-7 has been noted in the cutaneous squamous cell carcinomas (SCC) in recessive dystrophic epidermolysis bullosa (RDEB) patients. Here, we have examined the potential role of MMP-7 in shedding of heparin-binding epidermal growth factor-like growth factor (HB-EGF) in RDEB-associated and sporadic SCCs. Tissue microarrays consisting of RDEB-associated SCCs (n=20), non-EB SCCs (n=60) and Bowen's diseases (n=28) were immunostained for MMP-7, CD44 variant 3 (CD44v3) and HB-EGF. Shedding of HB-EGF was studied in vitro using two cutaneous SCC cell lines. Immunohistochemical analysis showed that HB-EGF is absent in tumour cells when MMP-7 and CD44v3 co-localize, and that the absence of HB-EGF was more pronounced in RDEB-associated SCCs than in non-EB SCCs. The loss of HB-EGF in MMP-7 – CD44v3 doublepositive areas was interpreted to indicate shedding and activation of HB-EGF and it was also detected in Bowen's disease indicating its importance in the early phase of SCC development. Specific knockdown of MMP-7 expression in human cutaneous SCC cells by siRNA inhibited shedding of HB-EGF and resulted in diminished activation of epidermal growth factor receptor (EGFR) and ERK1/2, and in reduced proliferation of SCC cells. These findings provide evidence for the role of MMP-7 in promoting the growth of cutaneous SCCs via shedding HB-EGF, and identify EGFR signalling as potential therapeutic target in RDEB-associated and unresectable sporadic cutaneous SCCs.

298 [Oral 073]

Tumor suppressor activity of the developmental factor IRF6 in squamocellular carcinomas

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The transcriptional regulator IRF6 is linked to the control of craniofacial development and epidermal proliferation. We have recently demonstrated that IRF6 is a component of a regulatory feedback loop that controls the proliferative potential of epidermal cells, being transcriptionally activated by DNp63 and inducing its proteasome-mediated downregulation, thereby limiting keratinocyte proliferative potential. Given the involvement of p63 gene in carcinogenesis, we have hypothesized that IRF6 could be also involved in skin carcinogenesis. Therefore, we analyzed IRF6 expression in a large series of squamocellular carcinomas finding strong downregulation of IRF6 that correlated with their invasive status. IRF6 downregulation seems to be mediated by methylation on a CpG island located in its promoter region. Exogenous expression of IRF6 in SCC cell lines induced reduction in proliferation and colony formation. To identify the molecular mechanisms regulating IRF6 potential tumor suppressive activity, we performed gene expression analysis in primary human keratinocytes after siRNA-mediated IRF6 depletion.Interestingly., we found up-regulation of cell cycle related genes and down-regulation, among the others of 14-3-3sigma (stratifin) expression. 14-3-3sigma protein is also involved in skin differentiation and is known to be activated by different kinds of DNA damage agents and to induce cell cycle arrest and tumor suppression by hampering G2 to M progression and by inhibiting Akt kinase. We have found that IRF6 directly binds to 14-3-3sigma promoter and induces its transcription. Our data highlights a novel function for the developmental regulator IRF6 as tumor suppressor in skin carcinogenesis identifying 14-3-3sigma as one of potential mediators.

299 [Oral 074]

Identification and characterization of SerpinA 1 as a Novel Biomarker for Progression of Cutaneous Squamous Cell Carcinoma

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The incidence of keratinocyte-derived nonmelanoma skin cancers is increasing globally due to cumulative recreational exposure to sunlight. No specific molecular markers are available for the early detection and progression of the invasive types of the cutaneous squamous cell carcinoma (SCC). In this study we have pinpointed the role of SerpinA1 in cutaneous SCC. Affymetrix based expression profiling of SCC cell lines (n=8) revealed high expression of Serpin Peptidase Inhibitor Clade A Member 1, also known as alpha 1-antitrypsin or alpha 1-proteinase inhibitor, compared to normal epidermal keratinocytes (n=5). Analysis with quantitative RT-PCR showed that SerpinA1 mRNA was markedly up-regulated in 8 cutaneous ($p=0.03$), 40 head and neck SCC cell lines ($p<0.001$), 4 cutaneous SCC tumors and 64 head and neck SCC tumors ($p<0.001$), as compared to corresponding normal cells and tissues. Western immunoblotting revealed production of SerpinA1 protein by all skin SCC cell lines (n=8), but only in 1 out of 5 normal keratinocyte strains. The highest SerpinA1 expression was observed in high grade head and neck SCCs (Grade 3), tumors of the larynx, and tumors with metastasis to lymph nodes. Immunostaining of tissue arrays with 148 samples revealed tumor cell-specific expression of SerpinA1 in 19 of 36 actinic keratosis, 22 of 29 Bowen's disease, 67 of 71 sporadic SCCs and in all RDEB-associated SCCs (n=12). Taken together these in vitro and *in vivo* data provide evidence for an important role of SerpinA1 in progression of cutaneous SCC, suggesting it as an attractive diagnostic biomarker and therapeutic target.

300 [Oral 075]

Endothelial cell-specific RAGE signaling sustains inflammation and promotes tumor-angiogenesis

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Chronic inflammation plays a pivotal role in the development of cancer. However, the mechanisms that sustain a tumor-promoting microenvironment remain largely elusive. Previously, we have shown that feed-forward signals downstream of the receptor for advanced glycation end-products (RAGE) can fuel chronic inflammation, creating a microenvironment that promotes tumor formation. Mice deficient for RAGE are resistant to DMBA/TPA-induced skin carcinogenesis and show severely reduced inflammatory response to treatment with TPA accompanied by impaired infiltration with subsets of innate immune cells and impaired upregulation of pro-inflammatory genes. Interestingly, tumors in RAGE-deficient mice showed severely reduced vascular density and impaired perfusion using confocal microscopy and perfusion-ultrasound-sonography. Furthermore, aortic rings explanted from RAGE-deficient mice were characterized by impaired microvessel outgrowth and reduced activation of Rho, Rac, and Cdc42 as well as MMP2 and MMP9 expression revealing an important role of RAGE signaling in endothelial cell migration and endothelial progenitor cell recruitment. In order to elucidate an endothelial cell-specific role of RAGE signaling *in vivo*, we generated wildtype bone marrow chimeric Tie2 promoter-driven RAGE-deficient mice (RageΔend). RageΔend show a significantly altered inflammatory response to TPA treatment characterized by impaired immune cell recruitment in the presence of epidermal hyperplasia thereby partly phenocopying non-conditional RAGE-deficient mice. In conclusion, we provide direct genetic evidence for a novel endothelial cell-specific function of RAGE signaling in driving the strength and maintenance of an inflammatory reaction during tumor-promotion and in promoting tumor angiogenesis thereby bridging the gap between inflammation and cancer.

301 [Oral 076]

Tumor derived CCL20 production critically contributes to angiogenesis and tumor progression

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Activation of the EGFR/Ras-signaling pathway is a crucial step in the malignant transformation of a wide variety of tumors. Modulation of gene expression by EGFR/Ras promotes cell proliferation and survival. Here, we demonstrate that the activation of Ras regulates chemokine expression in a dichotomic manner with an inducible set demonstrating pro-tumor and a repressible set showing anti-tumor properties. In particular, tumors enhance angiogenesis by upregulating the production of CCL20 through the activation of the EGFR/Ras-signaling pathway. *In vivo*, the chemokine CCL20 is over-expressed in melanoma, breast cancer, colon cancer, and head and neck squamous cell carcinoma in correspondance to increased ERK phosphorylation. Moreover, CCL20 expression is correlated to pT and pN status of tumor patients using large scale tissue microarrys and CCL20 expression negatively correlated to patients' survival in breast cancer. Its specific corresponding receptor, CCR6, is abundantly expressed on endothelial cells in vitro and *in vivo*. Activation of CCR6 signaling in endothelial cells induces cell migration, in vitro-repair and leads to enhanced vessel formation. *In vivo*, CCL20 specifically induced increased vascularization of Matrigel plugs in wildtype mice, which was abrogated in CCR6-deficient mice. Furthermore, CCL20-expressing B16F10 melanomas showed significantly decreased tumor growth and vascularization in CCR6-deficient compared to wildtype using flat panel volume computertomography. Collectively, our findings identify a novel chemokine-driven mechanism of tumors to promote angiogenesis and progression.

302 [Oral 077]

Polo-like kinase 1 inhibitors hold potential as targeted therapies for squamous cell carcinoma

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Squamous cell carcinoma (SCC), the most frequent neoplasm with malignant potential, burdens high risk groups such as immunosuppressed patients and those with recessive dystrophic epidermolysis bullosa, with increased incidence, metastasis and mortality. As yet, a specific therapy for SCC remains an unmet clinical need. We used integrative in vitro and *in vivo* expression profiling followed by high-throughput RNAi screening to identify genes essential for SCC cell growth and survival. Cell viability following gene knockdown was assessed by the MTS assay. RNAi screening of 22 upregulated genes identified by expression profiling revealed several potential targets, of which polo-like kinase 1 (PLK1) produced the most potent decrease in SCC keratinocyte viability (40% ±2.7% of negative control siRNA), an effect not seen in normal human keratinocytes. This result was confirmed in vitro and *in vivo* using PLK1 inhibitors: BI2536 and GW843682X decreased viability (9.5% ±2.8% and 46.4% ±4.1% of control, respectively) and produced a potent cell cycle arrest (2.5-fold increase in G2/M population) followed by apoptosis (5-fold increase in cleaved nucleosomes detected by ELISA) in vitro. *In vivo*, six cycles of intratumoural injection of BI2536 in SCC xenografts resulted in a marked reduction in tumour volume (10 day post-treatment average volume 201.7 ± 26 mm^3 vs 440.5 ± 74.3 mm^3 in control) and virtual ablation of tumour keratinocytes. We have shown efficacy of PLK1 inhibition in vitro and *in vivo* and propose that PLK1 inhibitors represent a viable strategy for the treatment of cutaneous SCC.

303 [Oral 016]

XPC silencing in normal human keratinocytes increases reactive oxygen species and triggers metabolic alterations that drive the formation of squamous cell carcinomas

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Genomic mutations, Warburg effect, and alterations in the levels of reactive oxygen species (ROS) are consistently observed in a variety of cancers. However, the inter-relationships among these factors and their impact on the neoplastic process remain poorly understood. We took advantage of the intrinsic genomic instability arising in the nucleotide excision repair disease xeroderma pigmentosum C (XPC) to look at underlying molecular mechanisms. Here we show that shRNA-mediated knockdown of XPC in normal keratinocytes (KC) leads to the activation of the NADPH oxidase 1 which, in turn, results in 1) increased reactive oxygen species (ROS), 2) metabolic alteration through reduction of mitochondrial function but increased glycolysis, as defined by decreases in the NADH dehydrogenase subunit 1 and cytochrome c oxidase subunit III; and increases in proteins involved in glucose uptake and lactate generation. We also detected high levels of basal cell layer markers keratin 14, α6 and β1 integrin in epidermis reconstructed with XPC-deficient KC, which manifested epithelial hyperplasia with large and frequent extension of rete pegs, compared to control epidermis. These effects were also dependent on NOX1 activation. Furthermore, XPC-deficient KC formed SCCs in immunodeficient mice, but NOX1 inhibition blocked this effect. Our results revealed that up-regulation of NOX1 alone has no effect on the induction of tumors, whereas NOX1 overexpression in XPC-deficient KC has a synergistic effect on mitochondrial dysfunction and tumor formation. Our data indicate that NOX1 activation in cells lacking XPC contributes to high susceptibility to carcinogenesis and this may involve NOX1-mediated alteration of mitochondrial function.

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Calcineurin and ATF3: opposite role in keratinocyte cancer development versus senescence

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Calcineurin inhibitors like cyclosporin A (CsA) are the cornerstone of immunosuppressive regimens for organ transplant recipients. Squamous cell carcinoma (SCC) of the skin is a major complication of treatment with these drugs, with a 65-100 fold higher risk than in the normal population. By contrast, the incidence of basal cell carcinoma (BCC), the other major keratinocyte-derived tumour of the skin, of melanoma and of internal malignancies increases to a significantly lesser extent. Here we report that genetic and pharmacological suppression of calcineurin/NFAT function promotes tumour formation in mouse skin as well as in xenografts, in immune compromised mice, of H-ras^{V12} expressing primary human keratinocytes or keratinocyte-derived SCC cells. Calcineurin/NFAT inhibition counteracts p53-dependent cancer cell senescence and associated decrease in tumourigenic potential. ATF3, a member of the "enlarged" AP-1 family, is selectively induced as a consequence of calcineurin/NFAT inhibition, both under experimental conditions and in clinically occurring tumours, and increased ATF3 expression accounts for the observed suppression of p53-dependent senescence and enhanced tumourigenic potential. Thus, intact calcineurin/NFAT signalling is critically required for p53 and senescence-associated mechanisms against keratinocyte tumour development.

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The production of gangliosides in actinic keratosis, basal cell carcinoma and squamous cell carcinoma

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Gangliosides modulate several cellular functions: cell proliferation, intercellular adhesion, signal transduction, apoptosis, angiogenesis. The analysis of gangliosides production in benign and malignant non-melanoma cutaneous tumors. The present study included specimens like: 16 actinic keratosis, 23 basal cell carcinoma, 37 squamous cell carcinoma, 17 normal skin. The analysis of gangliosides included : Svennerholm method, Folch partition, thin layer chromatography and resorcinol reaction for quantitative analysis. In actinic keratosys, gangliosides concentrations in tumor, peritumoral tissue and normal tissue are almost equal. (t=0,274, p>0,05 in tumoral tissue; respectively t =0, 813, p>0,05 in peritumoral tissue compared to normal skin). In basal cell carcinoma, gangliosides concentrations has statistically significant increases in tumor compared with normal skin. (t= 2,376, p<0,05). The gangliosides values for peritumoral tissue are not different of the values for normal tissue. (t = 0,891, p>0,05) In squamous cell carcinoma, gangliosides concentration has statistically significant variations both in tumor and peritumoral tissue compared with normal skin (t = 5,171, p<0,05, respectively t = 4,437, p < 0,05). The composition and production of gangliosides is altered in non-melanoma tumors. The production of gangliosides can be used as a criteria for the differentiation of benign and malignant proliferations and also for, potentially metastatic lessions.The quantification of gangliosides in correlation with other histological markers can be the scientific support for modern treatment in cutaneous cancer.

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Skin cancer occurrence on antimetabolites Azathioprine and Mycophenolate mofetil

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Renal transplant recipients develop 60-fold to 100-fold more squamous cell carcinoma of the skin. For some time it has been argued azathioprine may be deleterious due to photosensitzation of the skin to UVA and direct DNA damage enabled by UVA in comparison to other antimetabolites such as mycophenolate mofetil. In this retrospective study of the University Hospital in Zurich the intention was to clarify whether and how many cases of epithelial skin cancer occurred in renal transplant patients. The following inclusion criteria had to be met: first kidney transplantation only, immunosuppression for at least 7 years of which at least 70% of the time with azathioprine or mycophenolate. Based on patient records from the Dermatology Department of the University Hospital in Zurich, we identified 101 eligible patients for our study. Thereafter, patients were contacted by phone to find further treating physicians. These doctors were asked to provide us with all findings of previous skin biopsies. Out of 50 patients treated with mycophenolate, 36% had developed at least one squamous cell carcinoma of the skin. 56.8% of 51 patients treated with azathioprine developed at least one squamous cell carcinoma of the skin. Further analyses for confounders will be presented.

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Squamous cell carcinoma of the skin shows a distinct microRNA profile modulated by UV radiation

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Cutaneous squamous cell carcinoma (SCC) is the second most common skin malignancy in the general population with ultraviolet radiation as most important risk factor. Immunosuppression dramatically increases SCC as seen in organ transplant recipients (OTRs) with a 60-100 fold increased SCC incidence, making it the most common malignancy in these patients. Recent work has revealed the existence of a class of small non-coding RNA species known as microRNAs, which have critical functions across various biological processes. We investigated the expression of four selected microRNAs in cutaneous SCC: miR-21, miR-184 for both of which oncogenic properties have been reported, miR-203 as keratinocyte-specific microRNA, and miR-205 as antagonist of miR-184. Using RT-PCR we measured expression of these microRNAs in SCCs of OTR and immunocompetent patients. We found increased expression of miR-21 and miR-184, decreased levels of miR-203 and no difference in miR-205 expression between SCC and normal skin. There were no differences between OTR and immunocompetent patients. We further investigated the influence of UV radiation on expression of these microRNAs in normal human keratinocytes and found significant increases in miR-21, miR-203 and miR-205 expression after UVA radiation, while UVB induced miR-203 expression, decreased expression of miR-205 and had no influence on miR-21 levels. Moreover, UVA/UVB influence on miRs expression did not depend on cyclo-oxygenase activity. Taken together, our results show that miR-21, miR-203 and miR-184 expression are altered in cutaneous SCC. We show that UV radiation impacts microRNA expression pattern, suggesting a possible early role for these miRNAs in SCC development.

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Inflammation as a driver of Squamous Cell Carcinoma of the skin

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Squamous cell carcinoma (SCC) is a common skin neoplasm characterized by infiltrative, destructive growth and metastasis. SCC is the most common neoplasm in organ transplant recipients on long-term immunosuppression and occurs 60-100 fold more frequently than in the general population. In this study, we present the receptor for advanced glycation end products (RAGE) and its ligand S100A8/9 as a link between inflammation and the development of human SCC. It was recently reported that RAGE deficient mice are resistant to DMBA/TPA-induced carcinogenesis and exhibit a severe defect in sustaining inflammation. Moreover, a relation between RAGE expression and the inflammatory proteins S100A8 and S100A9 was proposed. We therefore investigated the role of RAGE and the S100 proteins in the development of human SCC. RAGE, S100A8 and S100A9 were transcriptionally upregulated in SCC with respect to normal epidermis and in SCC of OTR with respect to SCC of IC patients. We were able to induce the proliferation of human SCC-derived keratinocytes by exposure to exogenous S100A8/9 which in turn was abolished by directly blocking RAGE. The migratory activities of normal and SCC-derived keratinocytes were also increased upon exposure to S100A8/9. We hypothesize that RAGE and its ligand S100A8/9 contribute to the development of human SCC by modulating keratinocyte growth and migration, thus linking inflammation and cancer development. These processes do not seem to be impaired by profound drug-mediated immunosuppression in OTR.

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The relationship between 24-hour urine melatonin level and skin Cancers

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It has been postulated that in industrialized societies rapid light exposure at night by suppressing melatonin production poses a new risk for the development of breast cancer and perhaps other cancers like skin cancers as well. Therefore we decided to compare 24 -hour urinary melatonin level in skin cancers with control group. In this Case - Control study (studied group comprised 70 patients with skin cancer and control group comprised 70 healthy volunteers) we matched each group according to age and sex.After we collected testimonial from persons that attended in study, information of each person including: History of smoking, amount of daily sleeping, daily exercise and BMI recorded in a questionnaire that was admitted by a Dermatologist. The subjects collected the total volume of urine excreted during a 24-hour period. Melatonin in urine was calculated using ELISA method. Then data was analyzed using SPSS software, t, Manvitni, Kruskal Wallis and chi-square tests. Average and mean of 24 -hour urinary melatonin level in case group was 15.9 ± 8.1 and 15.5, and in control group was 52.3 ± 50.4 and 47.4, that test showed significant deference between two groups.($Z=8.42$, $P<0.001$) In this case - control study, there was a significant relationship between skin cancers and low level of 24- hour urinary melatonin level that can set forth role of melatonin in prevention or treatment of skin cancer.

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Calcipotriol reduced induction of MMP-9 and MMP-13 in human squamous cell carcinoma cell line

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VitaminD is a well-known, potent regulator of cell growth, differentiation, cell death, tumor invasion and angiogenesis. Production of MMP-9 and MMP-13 by tumor cell may facilitate tumor growth, invasion and metastasis. We aimed to investigate whether calcipotriol can suppress the expression of MMP-9 and MMP-13 in human SCC cell line [DJM cells] and to examine the mechanism of modulation of MMP-9 and MMP-13 by calcipotriol in TNF-α treated DJM cells. The protein and mRNA levels of MMP-9 and MMP-13 were examined with ELISA and real-time PCR, respectively. Activation of signaling cascades was assessed by utilizing several inhibitors of signaling molecules, and by Western blot analysis. Twelve samples from patients with actinic keratosis, bowen disease and SCC were stained with MMP-9 and MMP-13 antibodies. Production of MMP-9 and MMP-13 markedly increased when the cells were treated with TNF-α. Calcipotriol suppressed the production of MMP-9 and MMP-13 significantly in a dose dependent manner at the protein and mRNA levels. Induction of MMP-9 and MMP-13 by TNF-α was partially dependent on NF-κB. Suppression of MMP-9 by calcipotriol was dependent on ERK, while the suppression of MMP-13 was dependent on p38. Calcipotriol partially inhibited activation of NF-κB by TNF-α, which may contribute to the suppression of MMP-9 and MMP-13.

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Membrane-type-3 matrix metalloproteinase (MT3-MMP) functions as a matrix composition -dependent effector of MT1-MMP activity and tumor cell invasion

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Membrane-type matrix metalloproteinases MT1- and MT2-MMP promote tumor cell invasion through basement membranes and collagen type I-rich tissues, whereas the functions of MT3-MMP in tumor invasion have remained less clear. Interestingly, MT3-MMP has been reported to be overexpressed in human melanoma selectively in the most aggressive nodular type tumors. We demonstrate here that MT3-MMP inhibits MT1-MMP-driven melanoma cell sprouting and single cell invasion in three-dimensional collagen, thus resulting in restricted, yet MT1-MMP-dependent expansion of compact cell colonies. In WM852 cells that have been originally isolated from nodular human melanoma, endogenous MT3-MMP expression was associated with rapid fibrin invasion and unexpectedly limited MT1-MMP-driven collagen invasion, both of which were reversed by siRNA-mediated MT3-MMP gene silencing. Consistent with such reverse matrix composition-dependent MT3-MMP functions, MT3-MMP overexpression reduced collagen invasion in parallel with increased fibrin invasion of Bowes cells with superficially spreading melanoma origin. Rather than altering MT1-MMP transcription, catalytically active MT3-MMP interacted with MT1-MMP thus impairing the proinvasive MT1-MMP activity in collagen. While MT3-MMP, unlike MT1-MMP, is not universally overexpressed in primary melanoma, it was significantly upregulated in pathologic specimens of human melanoma metastasis to lymph nodes. MT1-MMP-MT3-MMP interactions thus seem to provide tumors with a novel mechanism to restrict local cell invasion but allow nodular tumor cell growth in collagen-rich tissues such as skin, while simultaneously promoting invasive spread into distant sites.

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Characterization and prognostic value of distinct Merkel Cell Polyomavirus molecular features in tumor and non tumor specimens from patients with Merkel Cell carcinoma

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Merkel Cell Polyomavirus (MCPyV) is associated with Merkel Cell carcinoma. MCPyV causal role was highly suggested by monoclonal integration of its genome and expression of the viral large T antigen in MCC cells. We investigated and characterized MCPyV molecular features in MCC, respiratory, urine and blood samples from 33 patients by quantitative PCR, sequencing and detection of integrated viral DNA. We examined associations between either MCPyV viral load in primary MCC or MCPyV DNAemia and survival. Patients with MCC containing above 1 copy MCPyV per cell had longer survival in complete remission than patients with less than 1 copy per cell (34 vs 10 months, $P = 0.037$). PBMC contained MCPyV more frequently in patients sampled alive with disease than in patients in complete remission (60% vs 11%, $P = 0.00083$). The detection of MCPyV in at least one PBMC sample during follow-up was associated with shorter overall survival ($P=0.003$). Sequencing of viral DNA from MCC and non MCC samples characterized common single nucleotide polymorphisms defining 8 patient specific strains. However, specific molecular signatures truncating MCPyV LT were observed in 8/12 MCC cases but not in respiratory and urinary samples from 15 patients. New integration sites were identified in 4 MCC cases. Finally, mutated-integrated forms of MCPyV were detected in PBMC of two patients with disseminated MCC disease, indicating circulation of metastatic cells. We conclude that MCPyV molecular features in primary MCC tumor and PBMC may help to predict the course of the disease.

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P53 mutation at early stages of keratinocyte transformation impairs TGFbeta sensitivity by downregulating IKKa expression

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It has been proposed that actinic keratosis (AK) and squamous cell carcinoma (SCC) are merely stages in evolution of a continuous process. Cancer-related molecular alterations are found in both AK and SCC. In particular dysregulation of the p53 pathway appears to be an early event in SCC carcinogenesis, together with disruption of TGFbeta tumor suppressive activity.We have previously described that IKKalpha is a DNp63 transcriptional target acting as a component of TGFbeta tumor suppressive pathway and that its downregulation in SCC progression correlates with the loss of TGFbeta anti-proliferative activity.The aim of our study was to determine when IKKa downregulation occurs in skin carcinogenesis and its relation with disruption of p53 pathway. Therefore, we analyzed the expression levels of IKKa and p53 in skin biopsies from patients affected by lesions at different stages of keratinocyte transformation. We observed that downregulation of IKKa expression already occurs at early stages of transformation (AK stage I) correlating with mutant p53 accumulation in transformed keratinocytes.To study the molecular mechanisms underlying this observation we used a SCC cell line carrying thermosensitive p53. Induction of p53mutant conformation by temperature shift leads to downregulation of IKKa expression at both protein and mRNA level suggesting that mutant p53 may interfere with DNp63-mediated IKKa induction.These results suggest that p53 mutation may interfere with TGFbeta tumor suppressive pathway by inhibiting IKKa transcription in transforming keratinocytes. Increased knowledge of the interplay between p53, p63, IKKa and TGFbeta pathway will help in the identification of innovative therapeutic targets.

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Complement Factor H and Complement factor H-like protein 1 in Cutaneous Squamous Cell Carcinoma

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The incidence of cutaneous squamous cell carcinoma (SCC) and its precancerous forms is increasing globally. Inflammation is a typical feature of cutaneous SCC. We have investigated the expression of complement system inhibitors in cutaneous SCC cell lines (n=8) and in normal human epidermal keratinocytes (n=5) by Affymetrix based expression profiling and quantitative real time RT-PCR (TaqMan). In addition, expression of complement inhibitors was examined in tissue arrays generated from cutaneous SCCs (n=65) and actinic keratoses (n=37) by immunohistochemistry (IHC). The mean expression level of complement inhibitors Complement factor H (CFH) and Complement factor H-like protein1 (FHL-1) mRNAs was increased up to 12-fold and 24-fold, respectively, in SCC cells compared to normal keratinocytes. The expression of CFH and FHL-1 mRNAs was also significantly higher in SCC tumors (n=5) than in normal skin (n=2) (p=0.047). IHC of SCC tissue arrays revealed that CFH was specifically expressed by tumor cells and the staining intensity increased during tumor progression from premalignant lesions (actinic keratoses) to invasive SCCs. The production of CFH and FHL-1 by SCC cells was up-regulated by by inflammatory cytokines IL-1β and IFN-γ at mRNA and protein level, as detected by Western blotting. Complement factor 3 (C3) cleavage product C3c was detected in media of SCC cells and in tumor tissue indicating CFH and FHL-1 activity. These results show that cutaneous SCC cells express inhibitors of the complement system, which may play a role in SCC progression. These results suggest complement inhibitors as potential diagnostic markers and therapeutic targets in skin SCCs.

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A study on the prognostic value of clinical and histopathological features of dermatofibrosarcoma protuberans in Korean patients

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Dermatofibrosarcoma protuberans(DFSP) is a rare spindle cell tumor with locally aggressive characteristics. Only few studies on the epidemiology of DFSP in Asian have been studied, and racial difference of the tumor has not been reported yet. The purpose of the study was to evaluate the epidemiological, clinical and histopathological characteristics of DFSP in Korean patients and determine the prognostic factors that affect disease-free survival. We conducted retrospective review of patients diagnosed with primary or recurrent DFSP between 2000 and 2009 at Seoul National University Hospital and Boramae Hospital. Patient, tumor and treatment factors were analyzed for local recurrence-free survival. Pathologic slides were reviewed in available cases and classified into subtypes. We analyzed data for 65 patients, of whom 36 (55.4%) were female. The mean age at disease onset was 34.4 years with 50% aged between 20 and 40 years. Involved sites were most often on trunk (66.1%). Among them, 58 patients underwent a radical excision and 6 (9.2%) patients experienced local recurrence during 3.6 years of follow-up. Three cases (5%) had visceral metastasis, and 2 of them died of the disease. The recurrence-free survival was significantly related to the microscopic margins (P=0.01) and level of tumor invasion (P<0.005) in univariate analysis. On histopathologic study of 31 cases, subtypes included conventional (17 cases), with myxoid degeneration (4 cases), Bednar (3 cases), myoid (5 cases) and fibrosarcomatous change (2 cases). c-kit staining for mast cell count showed negative correlation with mitosis and tumor size (P<0.05).

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Investigation of VEGF signaling on human angiosarcoma using a novel experimental mode

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Among human neoplasm, angiosarcoma is one of the most life-threatening. The limitation of experimental model of angiosarcoma has resulted in the delay of the clarification of its biology and the development of effective therapeutic procedures. So far we have succeeded in establishing a novel experimental model of human angiosarcoma. Newly established human angiosarcoma cell line, HAMON, expressed endothelial cell surface markers such as CD31, VEGFR2 and Tie2. HAMON formed irregular vessel-like structures in tube formation assay, and had tumorigenic potency in NOD/Scid mice. Alongside it, human angiosarcoma tissue has been serially passaged in NOD/Scid mice and is available as *in vivo* experimental model. Although VEGF is assumed to be a key molecule for angiosarcoma biology, actual impact of VEGF signaling on angiosarcoma has not been fully elucidated. In this study, we investigated VEGF signaling on angiosarcoma and performed therapeutic experiments targeting VEGF signaling with recombinant anti-human VEGF antibody (bevacizumab) and receptor tyrosine kinase inhibitor (sunitinib). HAMON expressed not only human VEGF and VEGFR2 but also phosphorylated VEGFR2 constantly. Therapeutic experiment with bevacizumab reached growth inhibition both in vitro and *in vivo*, suggesting that VEGF positively regulated angiosarcoma growth. On the contrary, sunitinib treatment led to the increase of VEGF expression and tumor growth, implying that blocking of VEGR2 resulted in compensatory VEGF production and led to the promotion of angiosarcoma tumor growth. These results suggest that VEGF signaling on angiosarcoma is autoregulated through autocrine or paracrine loop and that the inhibition of VEGFR2 is insufficient to control angiosarcoma.

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Keratinocyte Growth Factor Specific Gene Expression Signature Associates with Reduced Invasiveness of Cutaneous Squamous Carcinoma Cells

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We have studied the role of keratinocyte growth factor (KGF, FGF-7), a fibroblast-derived keratinocyte mitogen and differentiation factor, in squamous cell carcinoma (SCC) of the skin. The levels of KGF mRNA determined by quantitative RT-PCR were similar in cutaneous and head and neck SCCs (HNSCCs) (n=58), as in corresponding normal tissue samples (n=12). The expression of KGF receptor (KGFR) mRNA was lower in cutaneous SCCs and HNSCCs (n=58) than in normal tissues (n=14). Expression of KGFR mRNA was similar or higher in cutaneous SCC cell lines (n=7) than in normal keratinocytes, and the levels were reduced by 24-h treatment with KGF. Gene expression profiling (Affymetrix) of three cutaneous SCC cell lines treated with KGF for 24 h revealed a specific gene expression signature with down-regulation of various genes up-regulated in SCC cells compared to normal epidermal keratinocytes, including matrix metalloproteinase-13 (MMP-13). KGF induced rapid activation of ERK1/2 in SCC cell lines (n=4) and resulted in ERK1/2-dependent down-regulation of MMP-13 expression, determined with quantitative RT-PCR and immunoblotting. KGF treatment also resulted in reduced invasion of SCC cells through collagen. These results show that cutaneous SCC cells are responsive to KGF, resulting in down-regulation of KGFR, in specific gene expression signature and in reduced invasion capacity of the cells. These results suggest that KGF does not promote progression of cutaneous SCC, but rather suppresses the malignant phenotype of cutaneous SCC cells by inhibiting the expression of several genes specifically up-regulated in SCCs, including MMP-13.

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Topical treatment with imiquimod suppressed UVB-induced skin carcinogenesis in a mouse model

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Constitutive activation of Stat3 has been found in a wide spectrum of human malignancies including skin cancer. Critical role of Stat3 signaling in the skin carcinogenesis was demonstrated by our previous studies. K5.Stat3C mice, in which active form of Stat3 is expressed in the skin, developed skin tumors with a shorter latency compared with wild-type mice by DMBA / TPA treatment or UVB irradiation. Their tumors rapidly progressed to squamous cell carcinoma (SCC), indicating that Stat3 activation contributed to early development of cancer. In our previous study the majority of K5.Stat3C mice showed precancerous lesions, which resemble the histological features of human actinic keratosis, on the ears by UVB irradiation. The lesions were soon converted to SCCs by continuous UVB irradiation. Imiquimod, a small molecule toll-like receptor 7 antagonist, has been used for the treatment of skin cancers/precancerous conditions, including basal cell carcinoma, Bowen's disease, Paget disease, and actinic keratosis. Here, we examined whether topical imiquimod treatment affected the progression of UVB-induced cancer in the ears of K5.Stat3C mice. Topical imiquimod 5% cream treatment onto the right ears, during the progressive stage of UVB-induced carcinogenesis, markedly inhibited the growth of cancers, compared with that in the left ears. Histopathological examination revealed that many inflammatory cells including plasmacytoid dendritic cells infiltrated beneath the epidermis with nuclear atypicality, suggesting an anti-cancer effect of the innate immunity. Thus, K5.Stat3C mouse represented not only a skin cancer-prone mouse model, but also provided a platform to study the anti-cancer mechanism of imiquimod.

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Expression of human constitutive photomorphogenic protein-1 in melanocytic and non-melanocytic skin lesions

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Consitutive photomorphogenic protein (COP1) is a key regulator in light dependent plant development. COP1 is highly conserved across species and was also detected in mammalian cells. We have recently found that human COP1 (huCOP1) plays an important role in the UVB-induced signalling of human keratinocytes as a negative regulator of p53. Since p53 plays an important role in photocarcinogenesis we studied the topographical expression profile of huCOP1 and p53 proteins in human skin using tissue microarray technique, huCOP1 and p53 immunohistochemistry with digital scanning microscopy. Normal and most perilesional epidermis exhibited consistent huCOP1 expression in basal keratinocytes with a cytoplasmic dominance, p53 expression in normal epidermis was strictly restricted to basal keratinocytes. Among non-melanocytic lesions, acanthomas showed similar huCOP1/p53 topography, in actinic keratoses huCOP1/p53 expression was extended parallel with hyperproliferation of basal keratinocytes. Bowen's keratoses did not alter the pattern of huCOP1. In basal and squamous cell carcinoma focal huCOP1 and increased p53 expression could be detected. In melanocytic lesions, none of the nevi showed pronounced huCOP1/p53 positivity compared to normal epidermis. In malignant melanomas, huCOP1/p53 expression intensity and number of positive cells gradually increased from lentigo maligna melanomas to superficial and nodular melanomas. Our expression data suggest that huCOP1 expression is lesion-specific, displaying both synergistic and antagonistic changes with p53, suggesting lesion specific alternative pathways of UVB-induced skin carcinogenesis.

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Subcutaneous Panniculitis-Like T-Cell Lymphoma Shows A Molecular Signature Characteristic For Autoimmune Diseases

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Subcutaneous panniculitis-like T-cell lymphomas (SPTL) with an α/β T-cell phenotype represent a group of cutaneous T-cell lymphomas (CTCL) difficult to diagnose and affecting mainly young people. The prognosis of SPTL and response to systemic steroids are usually favourable. Overlapping features with lupus erythematosus (LE) profundus may occur and SPTL patients often have a concomitant diagnosed autoimmune disease or at least a laboratory parameter suggestive of an autoimmune phenomenon.To further investigate the molecular pathomechanism of this malignancy, we analysed altogether 12 SPTL patients with gene expression microarray or immunohistochemistry. We compared the expression profiles of SPTL skin lesions to normal subcutaneous tissue and inflammatory panniculitis (erythema nodosum). Immunohistochemistry was used to show the cellular origin of the deregulated gene product. In addition, we analysed the same samples after successful treatment.The genes most significantly overexpressed in the SPTL lesions were several genes (e.g. CXCL9, CXCL10) affecting the CXCR3 pathway, which is involved in the development of autoimmune diseases. Their inducer, IFN gamma was also upregulated, as has previously been shown in cutaneous LE subtypes. Following successful treatment with systemic steroids (and methotrexate in some cases), the expression of the aforementioned genes lowered. The expression profiles of CTCL and SPTL were surprisingly different. In conclusion, these results suggest that an autoimmune type inflammation underlies the development of subcutaneous panniculitis like T-cell lymphoma. This is in line with the favourable response to steroids as well as favourable prognosis. Our results provide a novel link between autoimmunity and cancer.

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Gene expression microarray analysis of microdissected epidermis and dermis in mycosis fungoides and adult T-cell leukaemia/lymphoma

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Characteristic histopathological feature is epidermotropism in mycosis fungoides (MF) and adult T-cell leukaemia/lymphoma (ATLL). In this study, to reveal the mechanism for epidermotropism, total RNAs were obtained from the epidermis and dermis of skin biopsies of MF and ATLL patients (5 epidermal samples each from MF and ATLL and 3 dermal samples each from MF and ATLL, and 2 epidermal samples from dermatitis patients used as controls) by laser capture microdissection, and were served for subsequent cDNA microarray experiments. Expression levels of CCR4 and CLA, a well-known skin-homing receptor, were higher in the dermis of MF and ATLL, if compared to controls. CCR4 was also strongly expressed in the epidermis of ATLL. High expression of CCR10 was observed in the dermis of MF and ATLL, being higher in MF than ATLL. CCL27 expression in the epidermis was higher in MF and ATLL than controls. CCR7 and CCL21 play a role in homing of T cells to lymph nodes. Expression of CCR7 in the dermis was very high in both MF and ATLL. Correlation between CCR7 and CCL21 was observed in MF. Subsequently, we confirmed the expression of these chemokine receptors and the ligands by immunohistochemistry. These results strongly indicate that CCL27-CCR10 interaction, CCR4 and CLA play an important role in the homing of the tumor cells in both MF and ATLL, while high expression of CCL21 in the dermis of MF retains lymphoma cells in the skin and interrupt them to migrate to lymph nodes.

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Detection of Merkel Cell Polyomavirus in Kaposi Sarcoma

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We performed a systematic research of Merkel cell polyomavirus (MCPyV), a human virus discovered in Merkel cell carcinoma, in a series of patients with KS. DNA was extracted from lesional and nonlesional skin samples of patients with KS by fresh biopsies and superficial cutaneous swabs. MCPyV DNA was detected by PCR and quantified by real-time PCR assay in samples positive for MCPyV DNA detection. We included 10 patients with KS (8 M, 2 F, median age 69), 8 with the classic form of KS (68-81 yo) and 2 HIV-related KS (41-46 yo). Overall, 80% of our patients had MCPyV DNA in both healthy and affected skin, on biopsies and cutaneous swabs. MCPyV DNA was detected in 44% (4/9) of the biopsies, viral DNA being found both in affected and unaffected lesions (concordance rate 100%). MCPyV DNA was detected in all cutaneous swabs (face, trunk, upper and lower limbs) from 6 subjects. Mucosal swabs were negative (0/4). Prevalence is higher than previously reported. Main limitation is the small size of our sample. Non invasive cutaneous swabs are sensitive enough to detect MCPyV DNA in skin of patients and healthy controls. Viral analysis performed on FFPE samples is less sensitive than fresh biopsies. KS lesions may harbor MCPyV DNA. A true link with KS pathogenesis remains speculative. Indeed, MCPyV seroloprevalence is higher in elderly patients. Our results may simply reflect this tendancy. MCPyV may act as a co-factor in KS pathogenesis in a subgroup of patients, but this needs to be confirmed by larger studies.

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Whole transcriptome analysis of cutaneous SCC cells and epidermal keratinocytes reveals new differentially expressed genes

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Cutaneous squamous cell carcinomas (SCCs) are common skin cancers associated with a substantial risk to develop metastases. However, there is no good molecular marker for the early identification of the malignant SCCs. We have performed a whole transcriptome profiling of normal keratinocytes and cutaneous SCC cell lines using next generation sequencing. The analysis was carried out in four cultured normal human epithelial keratinocytes (NHEKs) and eight cutaneous SCC cell lines and differentially expressed transcripts were detected between NHEKs and SCCs using SOLiD next generation sequencing system. Thresholds for significance were based on false discovery rate ($p<0.05$) and fold-change (2-fold up- or down-regulation). Statistical analyses revealed 284 transcripts that were significantly and differentially expressed in SCC cells compared with NHEKs. 184 of them were significantly up-regulated and 100 were significantly down-regulated in SCC cell lines. The DNA microarray was performed also by the Affymetrix gene chip technology. Comparison of the differentially expressed features included 107 genes which were detected on both platforms and 177 transcripts which were detected only by SOLiD. Analysis of differentially expressed pathways between NHEKs and SCCs showed significant changes in expression levels of the cell cycle and inflammation related genes. Furthermore, expression levels of the genes involved in the VEGF and Erb signaling pathways showed significant changes between NHEKs and SCCs. These results reveal the complete gene expression profile of cutaneous SCC cells. Further functional studies of the differentially expressed genes are required to understand the significance of these genes in the carcinogenesis of cutaneous SCCs.

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Expression of tumour necrosis factor-like weak inducer of apoptosis in normal human skin, inflammatory dermatoses and non-melanoma skin cancer

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Tumour necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) is a member of the TNF superfamily of cytokines that possesses a variety of biological effects including stimulation of cell growth and angiogenesis, tissue regeneration, induction of proinflammatory cytokines and stimulation of apoptosis. To investigate the role of TWEAK in skin biology, we performed immunofluorescent analyses of TWEAK expression in normal human skin, psoriasis, lichen planus, actinic keratosis (AK), squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). In normal epidermis, strong positive cytoplasmic staining for TWEAK was observed in all keratinocyte layers except the basal cell layer which was entirely unstained. Similar pattern of staining was found in psoriasis with clearly observable difference between unstained basal and occasional suprabasal cells and strongly stained upper layers of epidermis. This "clear cut" difference in the staining pattern was not observed in lesions of lichen planus, where the overall staining intensity was decreased but occasional positively stained nuclei were also detected. Decreased staining was additionally observed in AK, particularly in the areas of cellular atypia. In SCC, strong signal was seen in the well-differentiated keratinocytes around squamous pearls whereas poorly differentiated cells showed weak or absent signal. In BCC, TWEAK was almost completely absent, with only a few individual cells in a limited number of BCCs positively stained. In conclusion, these data suggest that TWEAK is associated with keratinocyte differentiation in normal skin, inflammatory dermatoses and non-melanoma skin cancer and that it is possibly involved in the apoptotic keratinocyte death in lichen planus.

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DNA damage response in psoriasis - expression of 53BP1 and gammaH2AX

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DNA replication stress which occurs in hyperplastic and precancerous lesions is associated with the activation of DNA damage response (DDR). Since psoriatic epidermis is also characterized by hyperproliferation of keratinocytes, we aimed to investigate the expression of two DDR markers, p53-binding protein 1 (53BP1) foci and phosphorylated histone H2AX (gammaH2AX), in psoriatic lesions. Immunohistochemical and immunofluorescent staining was performed on paraffin-embedded tissue of lesional skin of patients with psoriasis and skin of healthy volunteers. The percentage of keratinocytes positive for Ki-67, 53BP1 and gammaH2AX and the number of foci per nucleus were determined. The number of cells positive for proliferation marker Ki-67, as well as the number of cells positive for gammaH2AX and 53BP1 foci, was significantly higher in psoriasis when compared to normal skin. In addition, the mean number of 53BP1 foci per nucleus, in cells expressing foci, was higher in psoriatic than in normal epidermis. Positive cells were predominantly localized in the basal and suprabasal epidermal layers. These results indicate that psoriasis, similarly to preneoplastic lesions, is characterized by high proliferative index and increased expression of DDR markers. It is possible that potent activation of DDR in psoriasis overcomes the effects of DNA replication stress and represents a part of the barrier to malignant transformation.

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Expression and physiopathologic function of T-plastin in cutaneous T-cell lymphoma

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A molecular feature of Sezary syndrome (SZ) is the abnormal expression of T-plastin by tumoral T-lymphocytes. T-plastin is a conserved actin-bundling protein that is normally not expressed in hematopoietic cells and its function in SZ T-cells is totally unknown. Herein, we studied T-plastin expression in a large cohort of SZ patients, compared with a wide range of T-cell lymphoproliferative disorders, and investigated T-plastin role in apoptosis resistance of tumor T-cells. Peripheral blood lymphocytes (PBL) of patients with SZ (n=84), MF (n=41), irrelevant lymphoproliferative disorders (n=18), healthy donors (n=72), SZ cell line HUT-78 and control Jurkat cells were studied for T-plastin expression by quantitative polymerase chain reaction assay and immunoblotting. Our results showed that T-plastin mRNA was expressed in 70% of SZ patients, while any expression was detected in PBL from patients with other diseases or healthy donors. T-plastin expression was induced by calcium entry and downregulated by calcineurin inhibitors (20-98%) in T-plastin negative PBL from SZ patients as well as in healthy PBL.To determine T-plastin function in T-cells, Jurkat T-cell line was stably transfected by a T-plastin expression vector. Our data show that specific expression of T-plastin in stable Jurkat clones allowed apoptosis resistance in response to antineoplastic agents and this is partially reversed by T-plastin siRNA. Pretreatment of T-plastin positive tumor lymphocytes by calcineurin inhibitors downregulates T-plastin and potentiates apoptosis induced by antineoplastic agents. Our results confirm that T-plastin is a specific marker of SZ tumor cells that is involved in apoptosis resistance of SZ tumor T cells.

327 [Oral 045]

Ustekinumab prevents development of psoriatic alterations in a humanized mouse model of psoriasis

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We used Ustekinumab a human monoclonal antibody directed against the p40 subunit of interleukin 12 and interleukin 23, as a model biological in tests aimed at further validating a humanized mouse psoriasis model. BNX mice were transplanted with 5 mm diameter full-thickness skin biopsies from non-involved skin from psoriasis patients. After 3 weeks PBMCs, isolated from each donors blood, were activated by incubating for 2 days with IL-2 and SEB and injected into the corresponding transplants to initiate a psoriatic lesion. 1 day before injecting the cells the mice were treated with the test compounds. Treatment continued for 3 weeks after which the mice were sacrificed and the biopsies were analyzed for epidermal ridge thickness, keratinocyte proliferation (Ki67) and epidermal differentiation (cytokeratin 16). The cell incubation media were analyzed for IL-23. Treatments with betamethasone (± 15mg topical, 2xday) and Ustekinumab (10mg/kg i.p, 2x week) resulted in a reduction in epidermal ridge thickness, expression of CK16 and number of Ki67 positive keratinocytes compared to the control group. There was no correlation between the concentration of IL-23 in the cell culture medium and the effect of cells on development of psoriasis characteristics. Human IL-23 was only detected in mouse serum when activated cells were injected into the transplants. We conclude that the psoriasis transplant model, using 5 mm diameter full thickness skin biopsies and BNX mice, is suitable for testing compounds that modulate T-cell activity by inhibiting interleukin function.

328 [Oral 046]

Efficacy of PUVA therapy compared with biologics in moderate to severe chronic plaque psoriasis

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Not much data are available comparing the clinical efficacy of biologics to conventional treatments such as PUVA in moderate to severe chronic plaque psoriasis. We therefore analyzed available data in patients treated over a 5-year period with biologics compared to (previous) PUVA treatment at the Department of Dermatology, Medical University of Graz, Austria. The data were extracted from the Graz Psoriasis Registry (http://www.psoriasis-therapieregister.at). In total, 162 patients with chronic moderate to severe plaque psoriasis had received 118 treatment courses of oral PUVA (8-MOP, n=86; 5-MOP, n=32) and/or 130 treatment courses with a biological agent (adalimumab, n=18; alefacept, n=32; efalizumab, n=17; etanercept, n=38; infliximab, n=7; ustekinumab, n=18). The primary efficacy assessment was psoriasis-area-and-severity-index (PASI) improvement at wk12 for biologics and at treatment completion for PUVA (median time, 10.3 and 9.2 wks, for 8-MOP and 5-MOP, respectively). Fifty-eight percent of PUVA-treated patients achieved at least PASI90% improvement, compared with 22% for adalimumab (P=0.0050), 3% for alefacept (P<0.0001), 6% for efalizumab (P<0.0001), 29% for etanercept (P=0.0025), 71% for infliximab (ns), and 39% for ustekinumab (ns) (P-value determined by Fisher-exact-test, comparing each biologic vs. PUVA). Sixty-nine percent of PUVA-treated patients achieved at least PASI75% improvement, compared with 56% for adalimumab (ns), 25% for alefacept (P<0.0001), 59% for efalizumab (ns), 39% for etanercept (P<0.0018), 100% for infliximab (ns), and 67% for ustekinumab (ns). This implies that PUVA had significantly superior efficacy in psoriasis than certain biologics and similar efficacy than infliximab or ustekinumab. Clearly, prospective studies are desirable to confirm these observations.

329 [Oral 047]

Aprepitant as an antipruritic agent: results of a first case series

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Chronic pruritus is one of the most frequent symptoms associated with uncurable conditions such as renal, liver and skin diseases with a high negative impact on the quality of life. The symptom often does not respond to conventional treatment. Therefore, antipruritic therapies which target physiological mechanisms of pruritus need to be developed. Substance P (SP) is one major mediator of pruritus which binds to the neurokinin receptor 1 (NKR1). In this study we evaluated if an NKR1 antagonist would significantly decrease chronic pruritus. We applied the NKR1 antagonist aprepitant 80 mg in a case series of 20 patients with chronic pruritus (12 females, 8 males; mean age, 66.7 years) for one week. This treatment achieved significant reduction in pruritus (p<0.001, CI 1.913-5.187) in 16/20 patients (80%), as assessed by the visual analog scale (VAS). The mean VAS value was reduced from 8.4 points (SD +/- 1.7) to 4.9 points (SD +/- 3.2). Interestingly, younger patients (n=6) responded significantly better (mean pruritus reduction, 66.7±24.2%) than elderly patients aged over 65 years (n=14; 29.3±29.5%; p=0.012). Side effects were mild (nausea, vertigo and drowsiness) and only occurred in three patients. In sum, therapy with aprepitant leads to pruritus reduction mainly in dermatological diseases such as atopic diathesis and prurigo nodularis. The high response rate suggests that an NKR1 antagonist is a promising treatment strategy based on the pathophysiology of chronic pruritus. Future controlled trials will have to confirm the efficacy and safety of this novel therapeutic strategy.

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Clinical and biological analysis of IL-12, IL-23, TNF-α and IL-17 specific targeting in mouse model of psoriasis

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Psoriasis is characterized by epidermal proliferation, inflammatory skin infiltrate and vascular proliferation. Mouse models of psoriasis reflect various aspects of human psoriasis. InvEE transgenic mice exhibit hyperproliferative and inflammatory skin lesions induced by constitutive Mitogen Activated Proteins (MAP) kinase activation in keratinocytes. Tie2 mice present conditional overexpression of Tie2 (Tek tyrosine kinase) which leads to recruitment of lymphocytes and polynuclear leukocytes as well as epidermic hyperplasia. These mice have obvious clinical as well as histological signs of psoriasis. In this study, we investigated effects of the following four anti-mouse antibodies in InvEE and Tie2 mice: anti-p40 (IL-12/23), anti-p19 (IL-23), anti-IL-17A and anti-p75 (TNF). After treatment with anti-p19, anti-p40, or anti-IL-17A, but not anti-TNF, InvEE mice exhibited decreased clinical signs as well as a reduction of the extent and depth of acanthosis. In contrast, clinical signs are less important in Tie2 mice and clinical differences were not observed. Tie2 and InvEE mice treated with anti-p19 and/or anti-p40 presented a down-regulation of circulating cytokines such as IL-12, IL-22 and IFN-γ and an up-regulation of IL-23. Furthermore, these mice presented a regulation of various cutaneous cytokines implicated in angiogenesis, chemotactism and glucose metabolism. In conclusion, in the InvEE mouse model, anti-p19, anti-p40 and anti-IL-17A, but not anti-TNF, antibodies improved the clinical and histological signs of disease. In the Tie2 mouse model, treatment did not improve some signs of disease, but the two models presented similar regulation of cytokines.

331 [Oral 049]

Etanercept in the treatment of toxic epidermal necrolysis

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Toxic Epidermal Necrolysis(TEN) is a rare life-threatening dermatological disease characterized by estensive destruction of the epidermis and mucosal epithelia, caused by the apoptosis of the epithelial cells. A large body of evidence suggest that TEN is an adverse drug reaction: over 100 different medications are implicate as a trigger for the development of this disease. The pathogenesis of TEN is not entirely clarified, but is appears to be mediated by a metabolic and immune mechanisms. Among the players in TEN pathogenesis TNF alfa has a preminent role. In the early phase of TEN keratinocytes are the main source of this cytokines. TNF alfa acting in autocrine/paracrine manner leads to the apoptosis of keratinocytes and the production of NO and other cytokines and chemochines that recruit and activate inflammatory cells. Up till now doesn't exist an evidence-based therapy for TEN: corticosteroids, immunoglobulins , cyclosporine, plasmapheresis, cyclophposphamide and others drugs showed doubtful therapeutical responses and not ameliorate the mortality rate. Because we retained TNF alfa is the chief responsible in the pathogenesis of TEN, we treated six TEN's patients (SCORTEN ranging from 3 to 6) with a single injection of etanercept 50 mg. An immediate block of the disease progression and a complete recovery within 8-10 days was obtained without any complications or said-effects. On the basis of these results we retain etanercept the drug of choice in the treatment of TEN, also because is antagonize as well the lymphotoxin alfa, a forgotten but important cytokine.

332 [Oral 050]

Notch 1 as a potential therapeutic target in cutaneous T-cell lymphoma

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Deregulation of Notch signaling has been linked to the development of T-cell leukemias and several solid malignancies. Yet, it is unknown whether Notch signalling is involved in the pathogenesis of mycosis fungoides and Sezary syndrome, the most common subtypes of cutaneous T cell lymphoma. By immunohistochemistry of 40 biopsies taken from skin lesions of mycosis fungoides and Sezary syndrome we demonstrated prominent expression of Notch1 on tumor cells, especially in the more advanced stages. The γ-secretase inhibitor I blocked Notch signaling and potently induced apoptosis in purified Sezary cells and in cell lines derived from mycosis fungoides (MyLa) and Sezary syndrome (SeAx, HuT-78). Specific downregulation of Notch1 (but not Notch2 and Notch3) by siRNA induced apoptosis in SeAx. The mechanism of apoptosis involved the inhibition of NF-κB, which is the most important prosurvival pathway in cutaneous T cell lymphoma. Our data show that Notch is present in cutaneous T cell lymphoma and that its inhibition may provide a new way to treat cutaneous T cell lymphoma.

333 [Oral 051]

MiR-122 regulates apoptosis in malignant T-cells derived from mycosis fungoides

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MicroRNAs (miRs), the non-coding RNAs regulating gene expression, are involved in the control of malignant transformation, cell proliferation and apoptosis. We have recently discovered that inhibition of Notch-1 by γ-secretase inhibitors (GSI) induces apoptosis in T-cell lines derived from cutaneous T-cell lymphomas (Kamstrup et al. Blood 2010, in press). Assuming that Notch-1-dependent apoptosis is regulated by miRs, we compared miR expression between GSI-treated and control cells by microarrays and RT-qPCR. We found an upregulation of miR-122 in apoptotic cells treated with GSI. Surprisingly, miR-122 silencing increased the sensitivity to GSI-induced apoptosis whereas miR-122 over-expression increased cell proliferation and protected against programmed cell death. Screening for possible signalling pathways involved in the observed miR-122 effect revealed the role of NF-κB. miR-122 is not expressed in quiescent T-cells, but is readily detectable in lesional skin biopsies from patients with mycosis fungoides and in purified Sezary cells. Taken together, our data suggest tha miR-122 is expressed *in vivo* in cutaneous T-cell lymphoma and may be involved in the protective, pro-survival circuit in malignant T-cells.

334 [Oral 052]

Th2 cytokines enhance tissue kallikrein 7 expression and the serine protease activity of keratinocytes in atopic dermatitis

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The expression of kallikrein 7 (KLK7), a chymotrypsin-like serine protease, is upregulated in atopic dermatitis (AD) lesion and it has been considered to be involved in AD pathogenesis. We hypothesized that Th2 cytokines such as IL-4 and IL-13 induce KLK7 expression in AD keratinocytes. First, we confirmed that KLK7 protein expression was increased in AD lesion by immunohistochemistry (n=6), and that the serum level of KLK7 correlated with that of IL-4 in AD patient samples (n=37, r=0.772, p<0.01). Cultured normal human epidermal keratinocytes (NHEK) treated with IL-4 (50 ng/ml) or IL-13 (50 ng/ml) increased KLK7 mRNA (2.73x, p<0.0001, or 2.55x, p=0.0007) and protein (8.16x, p=0.0015, or 8.96x, p=0.0010) in a time-dependent manner, while IFN-gamma (100 U/ml), a Th1 cytokine, and IL-17A (50 ng/ml), a Th17 cytokine, did not. On the other hand, Th2 cytokines did not induce the expression of kallikrein 5, a trypsin-like serine protease, in NHEK. Furthermore, the protease assay with the specific substrate showed that IL-4 or IL-13 enhanced chymotrypsin-like serine protease activity in NHEK (3.23x, p=0.0005, or 2.29x, p=0.0172, respectively). Trypsin-like serine protease activity also increased with IL-4 or IL-13 significantly but slightly (1.11x, p=0.0007, 1.12x, p=0.0008). These findings suggest that Th2 cytokines affect skin barrier function through increased KLK7 expression in AD.

335 [Oral 081]

Diabetes impairs adipose-derived stem cell pro-healing function

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Adipose-derived mesenchymal stem cells (ASCs) are gaining a great consideration in the therapeutic application for tissue repair. Besides showing the potential to differentiate into cell types of different lineages, these cells are easily accessible and abundant. The therapeutic administration of ASCs in impaired wound healing has recently been reported. With the aim of assessing the therapeutic potential of autologous versus heterologous ASCs in the treatment of diabetic ulcers, we have functionally characterized diabetic ASCs and investigated their pro-healing potential. ASCs were isolated from the inguinal fat of streptozotocin-induced diabetic mice and analysed either freshly isolated as stromal vascular fraction, or following a single passage of culture to preserve at most the diabetic phenotype. Diabetic ASCs showed decreased proliferative potential when compared to non diabetic ones. The pattern of expression of surface markers was also altered in diabetic ASCs, with a constant reduction in the levels of stem cell-specific markers (Sca-1, CD49e, CD54, CD73 and CD90). Importantly, Bioplex and ELISA analyses revealed that ASCs from diabetic mice released significantly lower amounts of different growth factors and cytokines (b-FGF, VEGF, PlGF, M-CSF, LIF, IL-15, MIG). Accordingly, the supernatant of cultured diabetic ASCs manifested reduced capacity to promote keratinocyte and fibroblast proliferation and migration, assessed by BrdU labelling and scratch assay, respectively. Our data indicate that the diabetic condition impairs ASC pro-healing activity and that this effect is due to decreased paracrine function. Experiments are ongoing to evaluate the *in vivo* effect of diabetic versus healthy ASC administration in diabetic wounds.

336 [Oral 082]

Acrosyringium is a target of vesicle/pustule formation in palmoplantar pustulosis (pustulosis Palmaris et plantaris)

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Pustulosis palmaris et plantaris (PPP) is a chronic recurrent dermatosis characterized by intraepidermal vesicles filled with neutrophils, which is recalcitrant to treatment. Despite the studies to dissect the nature of PPP, its pathomechanism remains unknown. Sweat participates in skin innate immunity by influencing temperature and hydration. In addition, sweat contains various antimicrobial peptides such as cathelicidins (hCAP-18/LL-37) and dermcidin protecting against certain bacteria, fungi, and viruses. These antimicrobial peptides were used as eccrine sweat gland markers to investigate whether PPP vesicle/pustules relate to the sweat gland. In order to evaluate the sweat secreting function in PPP, the palmar lesional sweat production was measured by a portable sweat meter. Skin biopsies of PPP were examined by immunohistochemistry with hCAP-18/LL-37, dermcidin, GCDFP-15, and EMA. Vesicular fluids were collected, and Western blotting for hCAP-18/LL-37 and dot blotting for dermcidin were performed. Polymorphonuclear leukocytes (PMN) from pustules and peripheral blood were collected for RT-PCR and real time PCR analyses for hCAP-18/LL-37. GCDFP-15 and EMA immunohistochemistry was positive for the lining cells of the vesicles, suggesting the acrosyringieal nature of the vesicles. Both hCAP-18/LL-37 and dermcidin expression in the fluid were also detected, but no over-expressions in the keratinocytes surrounding the vesicles was observed. There was no significant difference in hCAP-18/LL-37 mRNA levels between peripheral blood PMNs and lesional PMNs. These results indicate that hCAP-18/LL-37 in the fluid most-likely originates from the sweat gland and not from PMN or keratinocytes around the vesicles, suggesting that the acrosyringium is the primary location of PPP vesicle/pustule formation.

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Evaluation of Immature CD19$^+$CD21$^-$ B-lymphocytes as a potential biomarker for predicting and measuring success of steroid refractory/intolerant chronic Graft versus Host Disease therapy

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To broaden the observation that CD19$^+$CD21$^-$ B-lymphocytes, as a possible biomarker, predict response to photopheresis (ECP) , B cell subpopulations in peripheral blood (PB) of 74 patients (median age 40 years, range 20-59 years) given cyclosporine A (CSA n=24), tacrolimus (n=13), sirolimus (n=18) and ECP (n=19) for moderate (n=29) or severe (n=45) cGVHD were analyzed by multiparameter flow cytometry after staining for CD19, CD27, CD21 and surface Ig. cGvHD activity and response to therapy were assessed prior to start of immunosuppressives and every 3 months. Prior to immunosuppressives non-responders had significantly (p=0.03) higher proportions of immature CD19$^+$CD21$^-$ B-lymphocytes with a mean of 19.4% (range, 4.17-45) compared with a mean of 13.3% (range, 1.3-54) in responders to 6 months of therapy. Significantly higher proportions of immature CD19$^+$CD21$^-$ B-lymphocytes in non-responders compared to responders were observed. No significant difference in memory CD27$^+$ B-lymphocytes was observed prior to therapy . After 6 months all responding patients had a significant (p=0.01) decrease of percentages of immature CD19$^+$CD21$^-$ B-lymphocytes from a mean of 13.3% (range, 1.3-54) prior to therapy to a mean of 8.2% (range, 1.1-30) 6 months later. In complete responders, the immature CD19$^+$CD21$^-$ B-lymphocytes significantly (p=0.04) decreased from a mean of 14.06% (range, 1.3-54.6) to a mean of 7% (range, 1.1-36) after 6 months. In all non-responders percentages of immature CD19$^+$CD21$^-$ B-lymphocytes either increased or remained unchanged after 6 months of therapy. Relative amounts of immature CD19$^+$CD21$^-$ B-lymphocytes assessed prior to start of cGVHD therapy may predict response to immunosuppressive therapy.

338 [Oral 084]

Cytotoxic T lymphocyte stimulation with artificial antigen-presenting cells activates anti-melanoma natural responding cells

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Adoptive immunotherapy based on in vitro activation and expansion of tumor antigen-specific cytotoxic T lymphocytes (CTLs) is a very promising strategy that has been tried for many years. Our team used artificial antigen-prensenting cells (AAPCs; Latouche and Sadelain, Nat Biotechnol, 2000) to activate CTLs against MART-1, an auto-antigen which is overexpressed in melanoma.One major question with CTL stimulation by AAPCs is about either it activates lymphocytes that react in natural anti-tumoral immune response or it activates minor responding cells. We studied the proliferation and purification of MART-1-specific CTLs from peripheral blood of two healthy donors and two patients, using AAPCs stimulation and anti-MART-1 phycoerythrin-coupled pentamer staining and magnetic sorting with anti-PE beads. We obtained more than 95% of specific CTLs which were able to lyse specifically MART-1-prensenting cells in cytotoxic chromium assays. Flow cytometry staining revealed a high potency to produce mainly TNF-α, IFN-γ and Granzyme B. Among the major and expanding Vβ family for each donor after stimulation with AAPCs, we sequenced the highly variable CDR3 region of the β-chain of TCR and we found that there is at least few or one major clone among a few one which compose the population of a Vβ family. These particular sequences that differ between donors could be linked to the already described TCR sequences that have been found in natural anti-tumoral immune response in melanoma patients. In conclusion, our results indicate that stimulation of CTL with AAPCs activates anti-tumoral CTLs that respond in the restricted natural immune response to tumor.

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Application of Light Emitting Diode and Celecoxib (Cox-2 inhibitor) for the Management of Wounds

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Management of chronic wounds have always been a challenge for clinicians. Many factors come into play in order for wounds to heal properly and timely. These factors include control of infection, inflammation, stimulation of keratinocytes and fibroblast growth as well as adequacy of wound vascular bed. Of late, LEDs has been shown to upregulate keratinocyte proliferation in cell culture studies through upregulation of growth factors. Our study aims to evaluate the efficacy of Light-Emitting Diodes (LEDs) combined with topical COX-2 inhibitor in the management of wounds.We used a pig model of partial thickness burn injury and studied efficacy of various treatment modalities by a) wound contraction, b) Laser Doppler imaging, c) histology, and d) immunohistochemistry for Ki-67, proliferating cell nuclear antigen, and laminin. The various treatment modalities studied include LED monotherapy, Celecoxib monotherapy, LED + Celecoxib, Silverlon burn dressing and control group.Our findings show that LED + Celecoxib combined treatment is very effective and achieved the best wound healing profile among all treatment groups. The improvement of wound healing is significantly better when compared to control as well as with the other treatment groups. Even when used alone, LED treatment of burn wounds showed good healing when compared to control**.** Our results suggest that treatment of burn wounds with LED in combination with topical celecoxib significantly improves wound healing. This combination showed potential to be used as a novel therapeutic intervention for the management of wounds, thus possibly opening up new avenues for the management of difficult wounds.

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Adult Alopecia Areata-a Trichoscopic patterns study

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Dermoscopy of hair-Trichoscopy allows to explore the hair at 10 to 800 magnifications and to observe precisely the types of hair, follicular openings,the peripilar signs and to follow up the evolution of the disease or the treatment efficacy prior to naked eye clinical observation. We wanted to find wich are the most common trichoscopic patterns and how frequent they are in adult alopecia areata. We studied 84 adults with 143 plaques of alopecia areata by trichoscopy before and after 3 months of treatment. 71,3% of plaques had regulary distributed yellow dots-coresponding to hyperkeratotic plugs in hair follicle ; 51,7% had excalmation mark hair; 46,1% had distrofic –broken hair; 27,9% had cadaverised hairs-black dots in the hair follicles; 8,8 % had short pseudo regroing hairs-they are apparently regroing but they are atrophic hairs and they are a sign of activity of alopecia areata.They mostly disappear at 3 months trichoscopic follow up; 13,2 % had corkscrew hairs; 4,8 % had circle hairs; 4,1 %had vellus hairs-0,03 mm or less in thickness; 3,5 % had white dots-feature of fibrosis;they have extensive and three years persistent alopecia areata; We did not find any pseudomoniletrix hairs. The most frequent pattern is the presence of regular yellow dots (71,3 %),the second ithe presence of exclamation mark hairs (51,7 %) and the third is the presence of distrofic-broken hairs (46,1 %).The presence of pseudoregroing hairs is a sign of the activity of alopecia areata.They are thin hairs that differs from normal thick real regrowing hairs-sign of the treatment efficacy.

341 [Oral 048]

Cognitive performance and neuroimaging in patients with chronic plaque psoriasis

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Chronic plaque psoriasis is frequently associated to cardio-metabolic comorbidities, depression and unhealthy life behaviors (i.e. heavy smoking and drinking) which are risk factors for cognitive impairment. *We* investigated cognitive functions in patients with chronic plaque psoriasis. Cross sectional study on patients with moderate to severe psoriasis and controls. Cognitive functions were assessed through the Gainotti and Caltagirone Battery of Mental Deterioration. Neuro-imaging was studied by high-field magnetic resonance imaging (MRI) with four canal-brain spool. Moreover, in pre-contrast phase, 7 patients were submitted to Diffusion Tensor Imaging (DTI) and to fiber tractography. Mild cognitive impairment was found in 37 out 41 (90%) of patients with psoriasis compared to 26 out 37 (70%) of controls (p=0.02). In particular, patients with psoriasis obtained lower scores in the delayed recall of the Rey auditory verbal learning test (p=0.04); backwards digit span test (p=0.002), Weigl's sorting test (p=0.01) and Trail making test B (p=0.008). There were no degenerative alterations or signs of infra-supratentorial atrophy in patient with psoriasis investigated by the MRI. In all patients submitted to DTI a statistically significant reduction in the brain thickness in parahippocampal gyrus, superior temporal gyrus and superior frontal gyrus of left hemisphere was found. Patients with psoriasis may have a precocious impairment of long-term verbal memory, executive functions and attention. A reduction in the brain thickness in the parahippocampal gyrus, superior temporal gyrus and superior frontal gyrus of left hemisphere possibly justifying the cognitive impairment was found.

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Bone Mineral Density And Pro-Inflammatory Cytokines In Psoriatic Arthritis Patients

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Psoriatic arthritis affects 5-40 % of the patients with psoriasis. The aim: to investigate bone mineral density correlated with the blood and synovial fluid pro-inflammatory cytokines, in order to better understanding the pathogenesis mechanism of psoriatic arthritis (PsA) for biologic therapy application and monitoring the disease's evolution under treatment. Bone mineral density in lumbar spine (L2-L4) was determined by dual energy X-ray absorbtiometry (DEXA) method in 27 PsA patients. The blood samples and knees synovial fluid were collected from PsA patients and from 20 healthy subjects. As pro-inflammatory cytokines were determined IL-1, IL-6, TNF alfa, levels, by ELISA method (Enzyme Linked Immunosorbent Assay) using a specific kits for every interleukin. Osteoporosis and osteopenia in lumbar vertebral bone were present in 33 %, respectively 41 % of PsA patients, correlated with disease' stage. Serum pro-inflammatory cytokines IL-1 (4.6 ± 2.45 pg/ml), IL-6 (11.25 ± 4.75 pg/ml) and TNF alfa (3.5 ± 1.45 pg/ml) were higher in PsA patients than in control group (IL-1 = 2.32 ± 0.95 pg/ml, IL-6 = 6.55 ± 3.85 pg/ml, respectively TNF alfa = 1.02 ± 0.45 pg/ml), the variation was statistically significant ($p < 0.001$). In PsA patients, synovial IL-6 and TNF alfa were significant higher than serum values. Both, anti- TNF alfa antibodies and soluble TNF receptors can be equally effective in PsA patients, by reducing synovial tissue inflammation.The results showed that the inflammation in joint tissue can be produced especially by pro-inflammatory cytokines and was correlated with decreased bone mineral density.

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Lack of association between cigarette smoking and response to hydroxychloroquine in 200 patients with discoid lupus erythematosus

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Hydroxychloroquine is the oral therapy of choice for discoid lupus erythematousus (DLE).Previous studies demonstrate that cigarette moking is common amongst patients with DLE and suggest it may predict response to hydroxychloroquine.However, this association has not been tested in a large cohort of patients.We performed a multi-centre cross-sectional observational study in patients with DLE.Patients satisfied pre-defined clinical and histological criteria for DLE and also had received hydroxychloroquine.Response to hydroxychloroquine within the first 6 months of use was retrospectively designated following casenote review.All patients were recruited and interviewed by one investigator.A detailed smoking history was taken from each patient, making it possible to determine whether they had been smoking during the first 6 months of hydroxychloroquine use. 200 patients with DLE were recruited.Response to hydroxychloroquine within the first 6 months of use was observed in 120 patients(60%);70 patients(35%) were non-responders;8 patients(4%) had therapy withdrawn early because of side effects before response could be judged and in 2 patients(1%) response was equivocal.During the first 6 months of hydroxychloroquine use,75 patients(38%) were non-smokers,of whom 43(57%) responded and 29(39%) did not.125 patients(63%) were smokers, of whom 77(62%) responded and 41(33%) did not.There was no significant difference for achievement of response between non-smokers compared to smokers(OR 0.78; 95% CI 0.43-1.45; p=0.27).This is the largest study to investigate response to hydroxychloroquine in DLE.We confirmed the observation that smoking is common amongst patients with DLE. However, no significant difference in response rates were noted between smokers and non-smokers,casting doubt on the notion that smoking *per se* inhibits response to antimalarials in DLE.

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Immunologic characteristics of wart patients and cytokine changes after immunotherapy with squaric acid dibutylester

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Wart is a proliferative lesion on the skin caused by human papilloma virus. Contact immunotherapy is one of the many therapeutic options attempted to treat warts. However, its effectiveness differs from a patient to a patient. This study aims to evaluate immunologic characteristics of wart patients and cytokine changes after immunotherapy with squaric acid dibutylester (SADBE). Twenty two patients with five or more warts and nine healthy individuals were enrolled in the study. With respect to the patients whose warts have reduced by 90% or more, we reclassified them as a complete response group. The patients who showed 50% or less of improvement were reclassified as a treatment failure group. We measured the expression of cytokine by the flow cytometry, using the mononuclear cells in peripheral blood. The wart patients displayed a lower level of IL-4, Fas ligand and TNF-α and a higher level of IL-12 and IFN-γ/IL-4 ratio than the control group. The levels of IL-4 and Fas ligand and IL-10/IL-12 ratio of the complete response group, which was lower before the treatment, returned to the normal level, and IFN-γ/IL-4 ratio also recovered the normal level after the treatment. None of these changes were observed in the "treatment failure group". The fact that cytokine abnormalities detected amongst the complete response group before the treatment disappeared after the treatment with contact immunotherapy proves that SADBE normalized the immune responses and cured the patients who responded well to the treatment.

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Acne severity does not correlate with impairment of acne related quality of life

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Acne is a common disease especially among teenagers. It has a considerable psychological impact on affected individuals. The disease itself ranges from very mild affection to the most severe form. The aim of this study was to assess if the effect of acne on acne related quality of life is correlated to acne clinical severity. 102 university female students attending the university medical clinics with acne complaint were examined. Cardiff Acne Disability Index (CADI) was used to assess acne related quality of life and global acne grading system (GAGS) was used to assess clinical severity of acne. There was no correlation between acne severity (GAGS scoring system) and quality of life impairment as assessed by CADI score (r=0.145, p=0.127). Additionally CADI score did not correlate with disease duration or age of patients. Therefore, acne clinical severity alone does not affect acne related quality of life changes. Many other factors might play a role. Each patient should be treated individually taking into consideration mild disease does not mean little effect on quality of life.

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Dermal Exposure to Organophosphates Interacts with the Behavioral Effects of Opioids in the Mouse

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Azinphos methyl (AM) and malathion (MT) are organophosphates frequently used in agriculture and veterinary practice. They may come in contact with the skin of some individuals, causing adverse effects like neurotoxicity and behavioral impairment. Narcotics are among drugs of abuse globally with tremendous behavioral effects. The aim was to evaluate the possible interaction of dermal organophosphate exposure with these effects in an animal model. Chronic dermal effect of MT 1% and AM 1% solutions (and tap water as control) in mice was studied. For this purpose, tails of animals where dipped into the solutions once daily (10 sec) for 28 successive days. Morphine (1 mg/kg), tramadol (40 mg/kg) or saline solution (control) was injected to animals (SC, 3 times daily for 5 days on days 24-28). On day 29, the following behaviors were investigated: pain sensation, anxiety and learning. Dermal exposure to MT and AM caused anxiety and decreased exploring behavior and learning. MT and AM increased the anxiety in tramadol-treated animals by almost 30% and 55%, respectively ($P<0.05$). Learning was also impaired by MT and AM in the morphine- and tramadol-treated animals. No significant effect was seen on pain sensation after organophosphate exposure. Based on extrapolation from findings of the present study in an animal model, the behavioral abnormalities caused by opioids may be exaggerate in people with history of dermal exposure to long-term, low concentrations of organophosphates. Using tramadol instead of classic opioids seems to be less problematic.

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Withdrawn

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Carcinoid tumor arising on the abdominal wall: Calcitonin gene-related peptide as a candidate marker of primary cutaneous carcinoid tumor

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Carcinoid tumor, which is characterized by secretion of several neuropeptides and/or hormones, usually arises on stomach, intestine, and bronchi. The tumor often metastasizes to lymph nodes, liver and skin. However, primary cutaneous carcinoid tumor is extremely rare. Here, we report a case of carcinoid tumor arising on the abdominal skin of a Japanese man with lymph node metastasis. Tumor cells were positive for synaptophysin, chromogranin, cytokeratin (CK) -7, but negative for CK-20. Electron microscopy revealed 150-200 nm electron-dense granules, consistent with neurosecretery granules. No clinical or immunohistochemical evidence of visceral involvement was detected. Calcitonin gene-related peptide (CGRP) is one of the neuroendocrine peptides expressed on normal eccrine sweat gland. Immunohistological analysis and reverse transcription-polymerase chain reaction revealed that the tumor cells of skin and regional lymph node express CGRP. CGRP-expression was not detected in other carcinoid tumors originated from gastrointestinal tract or bronchi, suggesting that CGRP might serve as a useful marker for the skin-originated carcinoid tumor.

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An appraisal of oral retinoids in the treatment of pachyonychia congenita

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Pachyonychia congenita (PC), a rare autosomal-dominant keratin disorder caused by mutations in keratin (KRT) genes *KRT6A/B*, *KRT16* or *KRT17*, is characterised by painful plantar keratoderma and hypertrophic nail dystrophy. Available studies assessing oral retinoid treatment for PC are limited to a few case reports and case series. In a questionnaire-based retrospective cross-sectional survey of 30 genotyped PC-patients on oral retinoids at doses between 10 to 50 mg/day for 0,5 to 240 months, we here determined clinical score, visual analog pain scale (VAS) and side effects. 50% of patients reported that treatment was effective and plantar hyperkeratoses had improved. Only 14% observed an amelioration of their pachyonychia. While 33% experienced decreased plantar pain, 27% reported increased pain with oral retinoid treatment. All patients experienced adverse effects, most commonly dry lips, eyes and skin. A risk/benefit analysis favoured lower retinoid doses of ≤25 mg/d (significant mean overall satisfaction score) for more than 5 months, compared to higher doses of >25 mg/d for a shorter time. Acitretin was slightly more effective than isotretinoin in terms of overall change in calluses. In summary, therapy with oral retinoids at doses of ≤25 mg/d for greater than 5 months is superior to higher doses, shorter treatment duration or no therapy in PC. Further prospective studies are needed to confirm these findings.

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Treatment of refractory melasma with combination of topical 5% magnesium ascorbyl phosphate and fluorescent pulsed light in Asian patients

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The conventional therapies for melasma are often unsatisfactory because of inadequate response and recurrence following cessation of treatment.This study was designed to determine the effectiveness of treating melasma with combination of topical 5% Magnesium Ascorbyl Phosphate(MAP) and Fluorescent Pulsed Light(FPL). Patients with refractory epidermal and mixed melasma were treated for 12 weeks with topical application of 5% MAP at night, and 3 sessions of FPL(570-950nm) at 3, 6, and 9 weeks (fluence 12-14J/cm^2, pulse width 12-15msec, pulse repetition rate 2/3Hz, spot size 3cm^2). Post-treatment follow up was continued for another 12 weeks. Sunscreen (SPF-60) was prescribed throughout period of observation. Determination of treatment efficacy was based on the Melasma Area and Severity Index (MASI), calculated at beginning and then at weeks 6, 12, and 24. The subjective assessment was done by comparing pre and post-treatment photographs by an independent observer and self-assessment by patients at 12th week; using a 4-point scoring scale (1-poor, 2-fair, and 3-good, 4-excellent). Sixty five patients completed the study. The baseline mean MASI score of 14.80 decreased to 4.53 at 12th week (end of treatment), and 6.35 at 24th week (end of follow up). The overall regression of MASI at these end-points was 69.3% and 57% (p<.001). The pre and post treatment photographic evaluation by independent observer and patients' self-assessment at 12th week showed good to excellent response (scores 3 & 4) in 52.3% and 44.6% cases respectively. The combination of 5% MAP and Fluorescent Pulsed Light could be an effective modality in treating refractory melasma in Asian patients.

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A novel fibronectin derived peptide (P12) limits skin necrosis when delivered iv within 2 hours after burn in swine

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Skin burn injury progresses over several days secondary to continuing tissue cell necrosis and apoptosis. Fibronectin, a 500 kDa glycoprotein, is produced on-demand by most tissue cells during embryogenesis, morphogenesis and wound healing to become a critical component of the provisional extracellular matrix and is deficient in chronic wounds and burn patients. Last year we reported that a 14mer, growth factor-binding peptide (P12) derived from the first fibronectin type III repeat enhanced adult human dermal fibroblast (AHDF) growth and protected AHDF from necrosis and apoptosis secondary to oxidative and cytokine stress. We also showed that P12 reduced burn progression in a rat comb burn model. Now we demonstrate that P12 also limits progressive skin necrosis in a porcine hot comb model. Burns were created on the backs of outbred, 20-25kg Yorkshire swine using a brass comb preheated in boiling water and applied for 30 seconds resulting in four full thickness burns separated by three unburned interspaces. 20 burns with 60 unburned interspaces were created on each animal. Lactated Ringers (control) or 1, 3 or 10mg/kg P12 were infused intravenously at 1 h (one dose), or 1 h and 24 hrs (two doses) after burn over 30 min using a peristalsis pump. By macroscopic and microscopic analysis, necrosis progressed in 90% of interspaces in controls compared to 40 and 50% of interspaces in animals treated with 1 and 3 mg/kg P12, respectively (P<0.001). One dose was as good as two. These data suggest clinical trials with P12 are warranted.

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Determining the extent to which clinically effective treatment, ustekinumab or etanercept, reverses the molecular disease profile of psoriatic skin: Comparisons of lesional, non-lesional and normal skin

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ACCEPT, a randomized, active-controlled study, compared the efficacy of etanercept and ustekinumab in 903 patients with moderate-to-severe plaque psoriasis through wk12. Skin biopsies were performed in a subset of patients at baseline, wks1 and 12. Microarray analyses (Affymetrix U133+2 array) comparing non-lesional skin (n=85) to lesional skin (n=85) at baseline showed several thousand probe sets differentially expressed (>2-fold change FDR, p<0.05) in lesional skin. An additional 25 healthy skin biopsies were also analyzed. Comparison of nonlesional skin to healthy normal skin showed a series of lesional genes also dysregulated in non-lesional skin (DEFB4, S100A7A, CCL18, SERPINB3). Analyses to understand the impact of p40 cytokine (IL-12/IL-23) or TNF-alpha blockade on resident and inflammatory cells and on the expression of gene circuits that may drive chronic immune activation and inflammation in the skin were completed. In addition analyses to understand the residual molecular profile or „molecular scar" following 12 weeks of treatment were completed. Patients responding to each agent (>=PASI75, n=21 for etanercept, n=19 ustekinumab) had significant changes in ~4000 transcripts compared to untreated lesions, indicating significant resolution of pathological gene circuits back to nonlesional levels. However, genes such as DEFB4, S100A7, CCL18 and SERPINB3 though reduced to levels similar to non-lesional skin were not reduced to that of healthy normal skin by either treatment unlike ADAM10 and HSD3B1. Elucidation of the molecular pathways which remain dysregulated following effective treatment may provide insight into pathological mechanisms that remain active despite appearance of clinical and histologic resolution.

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Augmentation of Lipogenesis and Steroidogenesis-related Enzyme Expression by Gefitinib in Sebaceous Gland Cells *in vitro*

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A small-molecule tyrosine kinase inhibitor, gefitinib, which targets the ligand-binding domain of epidermal growth factor (EGF) receptors, exhibits anti-tumorigenic activity in patients with advanced non-small cell lung cancer. Many patients treated with gefitinib develop an acne-like rash on the face and upper body, most likely related to keratinocyte alterations, and hair follicle proliferation and maturation. Although acne is characterized as a functional disorder in sebaceous glands and pilosebaceous units, e.g., excess sebum production, accumulation, and secretion, there have been no reports to date that gefitinib may modulate sebum production in sebaceous glands. In the present study, therefore, we examined whether or not gefitinib directly influenced EGF-mediated cell proliferation, sebum production, and steroidogenesis in hamster and human sebocytes *in vitro*. Western blot analysis showed that EGF receptors were constitutively detectable in hamster sebocytes. In addition, gefitinib was found to dose-dependently inhibit the EGF-mediated sebocyte proliferation. Furthermore, nile-red staining revealed that intracellular lipid-droplet formation was augmented by gefitinib in both hamster and human sebocytes. Moreover, real-time PCR indicated that gefitinib increased the gene expression of cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc) and 5α-reductase in both sebocytes. These results provide novel evidence that gefitinib directly facilitates sebum production along with the augmented gene expression of P450scc and 5α-reductase in sebocytes. These findings are likely to increase the clinical understanding of the side effects of gefitinib.

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Quantitative and objective area extraction of tinea unguium

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Quantitative and objective evaluation of tinea unguium would be helpful for drug efficacy assessments. The aims of this study are to provide a quantitative assessment of the tinea unguium determined by dermatologists and to develop a computer-based quantification method of lesions. We examined a total of 38 clinical images of tinea unguium taken by a digital camera with polarized filters. The six images were clinically easy to identify the lesion areas while the others were rather difficult. The lesions were determined based on color information with the "location mask", which was generated based on a priori knowledge: nail is located near the border of finger and has an ellipse shape. Since the deviation in lesion area determined among dermatologists was large, we needed to define a gold standard for each image for evaluation of the proposed method. Five dermatologists manually drew the border of each lesion with a tablet computer. After an assessment of the extraction by dermatologists, the gold standard was defined as the area that was selected by two or more dermatologists, since the standard deviation of dermatologists' extraction were quite large (28.4%). Our method achieved a precision and recall score of 84.3% and 74.7%, respectively for the 6 clear cases and similarly, 63.8% and 58.3%, respectively for the total 38 cases. Our preliminary method determined the lesions almost accurately for relatively easy cases. Although our preliminary method needs further investigation, we confirmed a computer-based method has the capability to quantize the area of tinea unguium.

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Zileuton prevents the activation of the leukotriene pathway and reduces sebaceous lipogenesis

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Arachidonic acid (AA) activates the 5-lipoxygenase, induces leukotriene-B4 (LTB4) synthesis, enhances interleukin-6 (IL-6) release and increases intracellular neutral lipids in human sebocytes. Moreover, the enzymes of LTB4 biosynthesis are activated in acne-involved sebaceous glands. Zileuton, a 5-lipoxygenase inhibitor, reduces the number of inflammatory acne lesions and lipogenesis in patients with acne. In this study, we investigated the activity of zileuton on LTB4 generation, lipid content and IL-6 and -8 release from human SZ95 sebocytes *in vitro*. Pretreatment with zileuton partially prevented the AA-induced LTB4 and IL-6 release and increased neutral lipid content. IL-6 release and neutral lipid content were also reduced under long-term zileuton treatment. In conclusion, zileuton prevents the activation of the leukotriene pathway and enhancement of lipogenesis by AA in human sebocytes *in vitro*.

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Biomarkers for Adamantiades-Behçet's disease

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Purpose of the stidy was to detect serological predictive course-parameters in Adamantiades-Behçet's disease (ABD). Serum/blood of 122 ABD patients in active/inactive stages and of 75 controls was screened for IL1α, IL1β, IL8, TNFα, sICAM1, bFGF, CRP, ESR. Serum IL18, IL8, MIP1α and CRP and ESR were investigated prospectively in another 18 ABD patients (11 with disease exacerbations in two years) and 16 controls. Increased IL8 serum levels, but not IL1α, IL1β, IL6, TNFα, sICAM1, bFGF or ESR were found in active compared with inactive ABD patients and controls. Patients with oral aphthae and neurological features presented higher IL8 levels. An association of IL8 levels with the number of active clinical signs and the presence of severe oral aphthae was detected. Patients having systemic symptoms along with oral aphthae had significantly higher CRP and VEGF levels during the symptomatic period. In the longitudinal prospective evaluation**,** IL18 was increased in active and decreased in inactive periods in the individual patient's course. IL18 was elevated even in the presence of few oral aphthae or mild uveitis. Comparably, CRP was a useful course-indicator, while MIP1α and ESR showed no reliable correlation with disease activity. IL18 is the most sensitive, single sign-parameter correlating with disease activity in ABD, followed by IL8, which increases with the number of clinical signs. CRP and VEGF can also be used as biomarkers to identify systemic but not mucocutaneous disease. The combination of these four serological parameters can be useful in the follow-up of ABD patients, especially in therapeutic trials.

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Proteomics identified biomarker candidates to predict the effects of histone decetylase inhibitors on lymphoid neplasm cell lines

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Histone deacetylase inhibitors (HDACi) are novel agents for the treatment of the patients with lymphoid neoplasms. Although the effective treatments by HDACi have been reported in the patients with malignant lymphoid neoplasms, the sensitivities to the HDACi are variable among the patients, and the molecular backgrounds of the different sensitivities have long been explored to optimize the therapeutic strategies. To clarify the proteomic differences associated with anticancer effects of HDACi, we examined 33 lymphoid nelplasm cell lines. The 50% inhibitory concentrations (IC50) value for the valproic acid (VPA) was varied between 0.2 and 6.0 mM (average 1.8 mM) in these 33 lmphoid cell lines. The proteomic profiles of the cells were created by two-dimensional difference gel electrophoresis (2D-DIGE). We found that the intensity of 31 protein spots was positively correlated with the IC50 value ($rs > 0.4$). Among them, the intensity of nine protein spots was 1.5 times higher in the VPA sensitive cell lines (IC50 < 1.0 mM) than the resistant countarpart (IC50 > 2.0mM). The proteins corresponding to these protein spots were determined by mass spectrometry. These proteins were involved in stress response, T cell activation, cell proliferation, glucose metabolism, glutathione metabolism, histone modification and regulation of ARF protein signal transduction. The utilities of these proteins as a predictive biomarker for HDACi treatments will be considered in clinical specimens.

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Image analysis for senile lentigo and evaluation of therapeutic effects by hydroquinone

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As efficacy of hydroquinone for senile lentigo is often limited, evaluations of effectiveness would be difficult and unreliable.The aim of the study is to evaluate efficacy of hydroquinone objectively by image analysis and subjectively by QOL questionnaires.Forty patients with senile lentigo were enrolled. They were instructed to apply hydroquinone twice daily: group A; hydroquinone (HQ) alone, B; HQ plus chemical peeling once a week, C; HQ plus vitamin C application and D; HQ plus iontophoresis with vitamin C. The patients were assessed by spectrophotometer and image analysis of dermoscopy at 4, 8 and 12 weeks. Skin darkness was defined as Z score using the principal component analysis: $Z = 150 - \{(R - 120) \times 0.523 + (G - 73.8) \times 0.578 + (B - 60.5) \times 0.626\}$. The patients were further evaluated with DLQI and Skindex16.Significant improvements of melanin index were demonstrated by spectrophotometer in group B at 8 weeks and group D at 12 weeks, but with no statistical differences among the groups. Image analysis of dermoscopy exhibited substantial decrease of Z score after the treatments in combination therapy groups, but with no statistical differences. DLQI scores for daily life and leisure especially improved in combination groups. Skindex16 scores for emotions were also confirmed to be improved after the therapy by Wilcoxon t-test in combination groups. Combination therapies were shown to be more effective than HQ alone by image analysis and QOL assessments. Objective image analyses would be useful for assessments of subtle therapeutic effects.

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Narrow-band UVB exposures increase serum D-vitamin levels in chronic kidney disease patients requiring dialysis

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Narrow-band UVB (NB-UVB) treatment heals psoriasis and atopic dermatitis, and also increases effectively serum 25-hydroxyvitamin D (25(OH)D) concentration in these patients. Chronic kidney disease (CKD) patients requiring dialysis are especially prone to vitamin D insufficiency and deficiency and therefore, we studied whether NB-UVB exposures would improve their D-vitamin balance. Fifteen CKD patients (10 men, 5 women, mean age 48.3 years) received three times a week a total of 9 NB-UVB exposures with Medisun 700 UVB-311 apparatus. The NB-UVB exposures were given on the face, hands and chest, and the cumulative amount was 15 standard erythema doses (SED). Twelve healthy subjects (2 men, 10 women, mean age 43.6 years) received a similar course of NB-UVB.Before NB-UVB mean serum 25(OH)D was 32.5 ± 10.2 nmol/L in the CKD patients and 60.2 ± 18.0 nmol/L in the healthy subjects ($p< 0.001$). After nine NB-UVB exposures serum 25(OH)D concentration increased significantly ($p< 0.001$), i.e. 13.8 nmol/L in the CKD patients and 9.0 nmol/L in the controls. One and two months after NB-UVB exposures mean serum 25(OH)D levels had decreased in the CKD patients but were still 10 % higher than initially. The present study shows that a short course of NB-UVB exposures increases significantly serum 25(OH)D concentration in the CKD patients on dialysis. The effect is, however, short lasting suggesting that the patients need cyclic NB-UVB exposures to maintain their improved vitamin D concentration.

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Safety, tolerability, and tissue compatibility of Novabel®

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This study evaluated the short and long-term safety of Novabel® (Merz Pharmaceuticals, Germany). Novabel is a dermal shaper based on a new Geleon® technology derived from marine algae for temporarily aesthetic skin corrections. 34 adult healthy subjects received a 1 ml Novabel® injection (deep dermal to superficial subcutis of the inner left arm), at week 6 subjects received a 1 ml Novabel into the inner right arm (re-exposition/re-challenge). 4mm punch biopsies were obtained at week 6 week (right arm), at week 52 (left arm), and week 72 (right arm). Biopsies were analyzed microscopically. Skin irritation and sensitization were assessed using a 5-point scale or a 6-point scale, respectively. Palpability of implants was assessed at every visit. Adverse event monitoring was performed during the complete trial. Novabel® did not show a relevant locally irritant potential. No irritation or sensitization was observed after rechallenge. In biopsies obtained six weeks after rechallenge and 52 weeks after initial injection, Novabel® was still detectable in well-defined deposits. Light microscopy analysis did not reveal any pronounced tissue response. Around the deposits, there was a low-grade localized physiological reaction, typically for biomaterial implants. There were no signs of necrosis, clinically relevant inflammatory infiltrates or calcifications. Degradation with a volume reduction was recorded after 52 weeks and even more pronounced after 72 weeks. This study demonstrates a high degree of local tolerability, tissue compatibility and application safety of Novabel®. These safety and tolerability data support the excellent tolerability which Novabel® showed in a pivotal clinical trial for the correction of nasolabial folds.

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Adherence To Treatment In Patients With Psoriasis

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To evaluate patient adherence to different types of treatment in psoriasis including biologic therapy and factors which affects their adherence. Patients attending Dermatology Department for psoriasis completed a standard questionnaire. Self-assessed Psoriasis Area and Severity Index (SAPASI), Dermatology Quality of Life Index (DQLI) and patients' medication-taking behaviour were recorded. Patient's confidence, satisfaction and reasons for missing medications were also recorded for each treatment modality. A total of 106 patients on various treatment modalities were included in the study; 98 patients were on topical treatments, 43 on oral systemic therapies, 39 on phototherapy and 29 patients on biologic therapies completed the study. The overall rate of treatment adherence was found to be 85.8%. Rate of adherence was significantly higher in patients on biologic therapies and patients taking oral therapies ($p<0.001$). Patients taking biologic therapies reported 100% adherence to treatment while those on topical therapies had an average score of 77.32%. Patients on combination treatment also showed poor compliance with the topical therapies. Side effects, damage to other vital organs and skin malignancies from phototherapy were patients' main concerns. 56.8% of patients reported that messiness of treatment prevented them from adhering completely to their medications. There is a significant relationship between the types of treatment (topical, oral systemic, phototherapy, biologic therapy) and the number of combination of treatments and treatment adherence scores. It is very important to understand factors which contribute to treatment adherence and improve treatment adherence in patients with psoriasis to provide effective treatment for patients.

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Withdrawn

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Comparison of drug survival rates for adalimumab, etanercept and infliximab in patients with psoriasis vulgaris

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Adherence to treatment is a indicator of treatment success. Long-term data on adherence to biologic treatment in psoriasis are lacking. Here we compared the TNF-α inhibitors regarding drug survival rate and safety in patients with psoriasis, based on data from the Danish nationwide clinical database DERMBIO. All patients, who received anti-TNF-α in academic referral centers were included. In total, 882 treatment series with etanercept (n=311), adalimumab (n=427) or infliximab (n=129) were administrated to 747 patients. Significant positive predictors of drug survival were: male sex (hazard ratio [HR] 1.8, 95% confidence intervals [CI]=1.4-2.4), the anti-TNFa agent and the previous response to the anti-TNFa agent (HR 5.34, CI=3.8-7.4). In the group of the anti-TNFa naïve patients the longest drug survival was observed for infliximab, followed by adalimumab (HR vs infliximab 3.7, CI=2.0-6.9) and etanercept (HR vs infliximab 3.2, CI=1.7-5.9). The overall 4-year drug survival is in the range of 40% for etanercept or adalimumab vs 80% for infliximab. There was no difference in number of adverse events. In conclusion, the overall efficacy of anti-TNFa drugs diminish with time, as envisaged by the progressive loss of patient adherence to treatment. The major reasons for stopping treatment were loss of efficacy, followed by adverse events. Infliximab had the best patient retention ability with 70% patients being still on drug after 4 years of treatment.

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Clinical case of chronic chlamydial salpingo-oophoritis

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Incidence of genitourinary chlamydiosis among young European women ranges from 4.1 to 25%, among men - 1.2-12%. Approximately 8-19% of Ukrainian population is infected with Chlamydia. Patient A., complained of periodic pain in the lower abdomen. Anamnesis of the disease: the patient was referred by the obstetrician-gynecologist with a diagnosis genitourinary chlamydiosis. Objective and laboratory study: changes in the internal organs, cardiovascular system and locomotor apparatus were not detected; in general and biochemical blood analyses pathological changes were not found. Gynecological examination: signs of left-sided salpingo-oophoritis, endocervicitis. Data of additional examinations: ultrasound examination of the uterus and adnexa; dilatation of the left fallopian tube with exudates, ovary enlarged, hyperechoic inclusions in ovary parenchyma. PCR and ELISA- results were positive. In colposcopy - signs of endo- and exocervicitis were seen. Evident lymphoid follicles were observed in the area of the throat. Clinical diagnosis: exacerbation of chlamydial salpingo-oophoritis. On the 1st day of treatment immunomodulator manaxx was administered. From the 3rd to the 13th day of treatment patients took azithromycin orally 1.0 g on the 3rd day of treatment and 0.5 g every other day from the 5th to the 13th days of treatment. Final examination: changes in blood analysis, urethra and endocervix were not found. Etiologic diagnostics of chlamydiosis: PCR, ELISA - results were negative, obvious decrease in amount of antibodies was marked. Iimmunogram: all indicators were normal. Ultrasound examination of the uterus and adnexa: pathology was not detected. Colposcopy examination: cervical mucosa without any signs of inflammation. Clinical recovery.

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Monitoring of NO and selenium levels in pregnant women with sexually transmitted diseases

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Informative for clinical observations are tests for detection of NO and selenium levels in cervical canal secretion in daily urine, which characterize general immunologic mechanisms.30 women, were examined during different trimesters of pregnancy. Presence of inflammatory focus is known to be accompanied by an increase in nitrous oxide level by means of iNOS activation under the influence of cytokines, angiotensin II, E2 prostaglandin, interferon, and endotoxins of bacteria. In the 1st trimester the level of NO in cervical contents increased in 1.2 times in comparison with control group, in 1.3 - in the 2nd trimester, and in 1.4 - in the 3rd trimester. These data show that an obvious imbalance of local immunity in the cervical canal exists due to the presence of urogenital infection. Premature flow of amniotic fluid was marked in case of twice increased selenium level in daily urine of pregnant women. A complicated obstetric anamnesis (threatened abortions, congenital defects of fetal development, premature flow of amniotic fluid) was observed in case of decreased selenium level almost in 20 times in parturient women. Inflammatory diseases of genitourinary organs, caused by STD, are accompanied by increased production of NO, which intensifies intracellular oxidant stress and promotes adhesion of neutrophils, leucocytes to the endothelium that is important in inflammatory impairment of the vascular wall due to infection. Monitoring of selenium level in daily urine enables to conclude that a lack of selenium during pregnancy is accompanied by threatened abortions, spontaneous abortions, and congenital defects of fetal development.

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Cytokines, chemokines and growth factors "in vitro" and wound healing

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"Minced micrografts" is a simple, economic and effective technique recently introduced in our department consisting in the spreading on the bed of ulcers of a small skin specimen finely minced. We supposed that this procedure of cutaneous fragmentation facilitate the interaction between keratinocytes and fibroblasts and their release of mediators. So we decided to investigate the production of IL-6, G-CSF, TNF-α, MCP-1, PDGF, bFGF and VEGF (using Bio-Plex system) by 2 small specimens (diameter 4mm) excised from healthy patient. The first piece was minced and all its fragments were cultured in Keratinocytes Growth Medium (KGM), the second one was cultered in KGM without fragmentation. Samples of supernatants were taken every 24 hours for ten days.The "minced" specimens produced larger amounts of PDGF and bFGF; the other pieces released higher amounts of IL-6, MCP-1 and VEGF. We did not find significantly differences in TNF-α and G-CSF release. Our results indicate that healthy skin fragments, that could behave as "minced micrografts", produced expecially growth factors that play a role in the proliferation phase of wound healing; they are normally reduced in chronic ulcers. Non-minced healthy skin released large amounts of proinflammatory cytokines that usually are important mediators of inflammatory phase, but they are increased in chronic wounds delaying repair. In conclusion our data seem to confirm that the success of "minced micrografts" on chronic and inert wound could be related to the action of PDGF and bFGF and to the reduction of proinflammatory mediators on the bed of ulcers.

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Assessment of corticoid-therapy induced skin atrophy using the DermaInspect *in vivo* multiphoton microscope

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Multiphoton microscopy (MMP) is a non invasive optical imaging technique that allows the investigation of the 3D structure of human skin with sub-cellular resolution. It provides complemental modalities, mainly two-photon excited fluorescence (2PEF) and Second Harmonic Generation (SHG). 2PEF signals in skin are emitted by endogenous chromophores such as NADP(H), flavins, keratins, melanin or elastin, whereas SHG signals are specific for dense and ordered macromolecular structures such as fibrillar collagen. The aim of this study was to determine if MMP allows detecting the early signs of the corticoid-therapy induced skin atrophy. Four healthy volunteers (23-43 years) were topically treated with clobetasol propionate for 3 weeks, under occlusion during the night, on a small region of the dorsal side of the left arm. The treated region was investigated using MMP at J0 (before the treatment), J7, J15, J22 (end of the treatment) and J60 (38 days after the end of the treatment). At each time point, two stacks of images were acquired in two different zones of the treated region. Our results show that multiphoton microscopy allows detection of skin modifications following corticoid-therapy: J7 - thinning of the stratum corneum compactum; J7 to J22 - modification of keratinocytes morphology in the upper layers of the epidermis; J22 - complete de-pigmentation of the epidermis. These modifications disappear at J60. The evaluation of the skin pigmentation was firstly performed using qualitative scores, then using a quantification method based on the fraction of the volume occupied by melanin. Both methods showed similar results.

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Circulating follicular helper T cells are increased in psoriasis

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Follicular helper T cells (Tfh) are involved in regulating autoantibody production, producing abundant interleukin (IL)-21. IL-21 generates Th17 from central memory T cells (Tcm) and suppresses regulatory T cell (Treg) induction. In psoriasis, Treg are dysfunctional and circulating $CCR6^+$Th17 are increased. The Treg and Th17 imbalance is presumably an important factor for psoriasis activity. We previously reported that serum concentrations of IL-17 and IL-22 are significantly increased in psoriasis patients compared to healthy volunteers. Serum IL-22 concentrations in psoriasis patients correlated with the Psoriasis Area and Severity Index (PASI). IL-21 is not known to be involved in the pathogenesis of psoriasis. Therefore, we analyzed the Tfh population in psoriasis patients and healthy controls. Peripheral blood was obtained from 47 psoriasis patients and 10 healthy volunteers. $CD4^+$ cells were isolated from peripheral blood monocytes. Tfh were measured by fluorescence-activated cell sorting analysis. The proportion of $ICOS^+$T cells among $CD4^+$ T cells was significantly increased in psoriasis patients (3.91 ± 2.52%) compared with healthy volunteers (2.32 ± 1.28%). Circulating Tfh ($ICOS^+CXCR5^+CD4^+$T) were significantly increased in psoriasis patients compared with controls (0.397% versus 0.161%, p=0.040). The Tfh population was not correlated with other related factors, such as PASI score, Th17, and Treg. Some patients with guttate psoriasis had a higher Tfh population, suggesting that focal infection might have induced this increase. Moreover, Th17 were readily induced from psoriasis patient Tcm compared with healthy volunteers, suggesting that Tcm in psoriasis patients was already primed with IL-21.

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A case of short anagen syndrome: successfully controlled with topical minoxidil and systemic cyclosporine A combination therapy

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Short anagen syndrome is a recently recognized disease and it is poorly understood. This disease has normal features for the hair structure, strength and hair growth rate and it has no relationship with total hair loss, but the clinical aspects are fine, short hair with mildly decreased overall density. Because the disease is relatively uncommon, there are not many reports regarding its prevalence, pathogenesis and treatment. We experienced one case of short anagen syndrome that was successfully controlled with topical minoxidil and systemic cyclosporine A combination therapy. We report here on this interesting case and we review the relevant literature.

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Expressional change of inflammatory cytokines and nociceptive mediators in patients with female pattern hair loss complaining about trichodynia

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Many women worrying about hair loss by themselves usually complain about trichodynia. We want to evaluate the relationship between hair loss and trichodynia through checking out the expressional changes of several inflammatory and nociceptive mediators. Fifteen female pattern hair loss (FPHL) Korean patients (28-45 years) with M0F1 BASP type were enrolled. Tissue specimens were obtained by 4 mm punch skin biopsy from the suspected hair loss area. mRNA expressions of IL-1 beta, IL-2, IL-4, IL-6, TNF alpha, INF gamma, NGF, substance P and neurokinin-1(NK-1) receptor were determined by RT-PCR. These mediators were also checked in peripheral blood leukocytes. Eight scalp skin specimens were obtained from normal haired subjects during cosmetic surgery (22-53 years) as controls. The mRNA expression of substance P, NGF and NK-1 was significantly increased ($p<0.1$). Inflammatory cytokine TNF alpha and IL-6 mRNA levels were significantly higher as compared to controls ($p<0.1$). Although IL-2 mRNA was upregulated, it was no reached statistically significance. There were no mRNA expressional correlations between tissue and peripheral blood leukocytes. Trichodynia induced expressional alteration of some inflammatory cytokines and nociceptive mediators in FPHL lesional skin. Its alteration not influences the peripheral blood gene expressions. Albeit trichodynia associated hair loss is still controversial, our study propose the possibility of its involvement into hair loss through nociceptive mediators.

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Increased serum levels of secreted protein acidic and rich in cysteine in patients with localized scleroderma

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Scleroderma is a connective tissue disorder characterized by cutaneous sclerotic changes. It is classified into systemic sclerosis with internal involvement, including lung fibrosis and esophageal dysfunction and localized scleroderma, which is limited to the cutaneous tissue without systemic involvement. Secreted protein acidic and rich in cysteine (SPARC) is a multifunctional secreted glycoprotein found in the extracellular matrix. SPARC is expressed only at sites of tissue remodeling and wound repair. SPARC gene overexpression by cultured dermal fibroblasts from patients with systemic sclerosis was shown in a cDNA micro-array study. To determine the serum levels of SPARC in patients with scleroderma and investigate their clinical significance in this disease, serum samples from 33 patients with systemic sclerosis, 15 patients with localized scleroderma, 12 patients with dermatomyositis and 15 healthy volunteers were examined by a specific enzymelinked immunosorbent assay. Serum levels of SPARC were significantly higher in patients with localized scleroderma than those in other groups or healthy individuals. And the patients with elevated serum SPARC levels had significantly larger number of sclerotic lesions and significantly higher serum levels of anti-single strand DNA antibodies than those without them. These results suggested that the serum levels of SPARC might be a serological marker for the disease activity and the extent of skin involvement in localized scleroderma.

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Effect of anticellulite treatment on skin condition - instrumental and subjective analysis

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The presence of cellulite is an aestetically unacceptable cosmetic problem for many post-adolescent women worldwide. Therefore, appropriate research to investigate treatment options and objective methods measuring its efficacy are warranted.Recent studies using new diagnostic techniques such as ultrasound imaging can very well define cellulite-reducing efficacy of cosmetics. This study aimed to evaluate the efficacy of anticellulite treatment (topicaly applied cream and oral supplementation) on volunteers' skin condition using non-invasive investigation techniques.The efficacy of anti-cellulite treatment was assessed in a double blind placebo controlled trial. The study involved healthy female volunteers aged 25 between 55 presenting a cellulite of the thighs. They took dietary supplement or placebo 2 times daily and applied anticellulite or placebo cream once daily during 4 weeks. The instrumental analysis was performed using 13MHz ultrasound (Esoate Technos), as well as Corneometer probe and Visioscan camera (Courage-Khazaka Electronic GmbH). Each volunteer also completed a survey concerning their own evaluation of the treatment. Instrumental analysis demonstrated that anticellulite treatment comprising oral supplementation and topical application of anticellulite cream improved skin moisturization and smoothness. Using the 13MHz ultrasound we also demonstrated that the thickness of the subcutaneous tissue was decreased whereas dermis echogenicity was increased. The anticellulite treatment was very well evaluated by the volunteers. Although cellulite is not considered as a disease, complaints of women are real and their demand for care is very high. We conclude that ultrasound imaging and skin condition analysis can very well define cellulite-reducing efficacy from cosmetic point of view.

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Molecular markers associated with clinical response to bexarotene therapy in CTCL

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A new rexinoid, bexaroten (Targretin®), has been registered for the treatment of cutaneous T-cell lymphoma (CTCL) since 2002, and has reportedly induced 45% overall response. Mostly, the responses are partial or generate a stable, skin-restricted disease. We explored the usefulness of a novel cancer associated gene, the *NAV3* and corresponding chromosome 12 copy numbers as possible biomarkers to monitor therapeutic response to bexarotene in 21 Finnish CTCL patients. It was a prerequisite that the patient had early-stage CTCL refractory to skin-directed therapy or had received at least one standard systemic treatment modality with no benefit to the disease. Six patients (29%) reached complete remission (CR), 12 (57%) a partial response (PR, with one stable disease) and three were non-responders. The duration of bexarotene therapy to reach CR ranged from 1 month to 24 months. In the target skin lesions, an allelic *NAV3* deletion was observed at low frequency in five (24%) patients, four of whom showed an initial partial response of short duration but then progressed or died of disease. The fifth patient has remained with stable disease on bexarotene maintenance therapy for 2 years. Chromosome 12 tetraploidy was found in the lesions of two of three patients with CR who remained in remission, and polyploidy in one patient with PR. While such tetraploidy is a feature of proliferating normal T cells, this observation may reflect a favourable anti-tumor immune response among the skin-infiltrating lymphocytes.

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Transcriptional adaptation caused by vorinostat/bexarotene combination therapy in advanced cutaneous T-cell lymphoma

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Despite the broad spectrum of therapeutic interventions, curative treatment for CTCL is challenging. In this study we evaluated transcriptional adaptation during the Phase I study of vorinostat/bexarotene combination therapy in patients with advanced CTCL (ClinicalTrials.gov ID: NCT00127101).Transcriptional profiling was performed on skin biopsies from 15 patients: Pre- and On-treatment. In several cases both, tumor and plaque lesions were available for analysis. Human Exon 1.0 ST microarrays by Affymetrix were used and transcriptional modifications were identified by Partek and R-Bioconductor, enrichment analysis (EA) was performed using Genego Metacore.Analysis of transcriptional adaptation showed that plaque and tumor lesions were equally affected by the systemic treatment. Interindividual patient's variability was identified as the major factor influencing the molecular response. EA of differentially expressed genes revealed that progressive patients upregulate NOTCH and ER stress-responsive cascades and downregulate JAK-STAT signaling. In benefiting patients hormonal response network was the most represented with the emphasis on cell survival and inflammatory response (PPRG, RXR, MIF, ELK1, HDAC1, SOX5). Angiogenesis regulation through IL-8 and apoptosis were downregulated. Evaluation of alternative splicing after 14 days of the therapy showed that genes involved in cytoskeleton and matrix remodeling (MMP2, COL1A2, E-cadherin), cell proliferation (JAK2, ERK1/2, STAT5), and other processes tend to be modified. Vorinostat/bexarotene treatment caused tumor transcriptome to adjust, and progressive patients seem to escape through NOTCH signaling. Responsive patients characterized by apoptosis induction upon treatment. Results may help to initiate the development of personalized therapeutic strategies targeting responsive CTCL patients.

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Switching azathioprine to mycophenolate mofetil reduces UVA photosensitivity of the skin and DNA 6-thioguanine in PBMC of kidney transplant recipients

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Exposure to sunlight and immunosuppressive drugs are the most important risk factors for squamous cell carcinoma of the skin (SCC) in kidney transplant recipients (KTR). Azathioprine (AZA) causes incorporation of 6-thioguanine bases into DNA and when exposed to UVA allows direct mutagenesis. We measured photosensitivity and molecular changes (common deletion of mitochondrial DNA, p53 protein expression, IL1beta mRNA in skin biopsies, 6-TG in PBMC) in long-term KTR on AZA (n=23) and 3 months after switching to mycophenolate mofetil (MMF). 3 months after switching AZA to MMF, immediate and late photosensitivity decreased compared to baseline values on AZA. Greater reduction in 6-thioguanine content in PBMC DNA correlated to greater decreases in photosensitivity. Skin biopsies from UVA MED fields showed higher amounts of common deletion on AZA by PCR, increased p53 expression by immunohistochemistry and increased IL1beta mRNA expression by qRTPCR in the same patients.The switch from AZA to MMF normalizes photosensitivity in KTR. This is accompanied by changes on the molecular level indicating not only a quantitative improvement, but also a qualitative improvement of photosensitivity which together should result in reduced SCC formation.

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Statins effects on the skin

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Hypolipemiant drugs are widely-used for prevention of cardiovascular events.Statins and are by far the most widely used class of lipid-lowering drugs are the drugs class of choice for LDL-C reduction.Their cutaneous side effects reported in medical literature until present, can be classified into three types : autoimmune reactions, reactions due to dryness of the skin and severe reactions like –DRESS, but, with their expanding use, new side effects will inevitably continue to appear. Latest studies have proved that statins have immunomodularory activities also and thus they can be used in treatment of several immunological conditions which are characterized by a Th1 immune response, like psoriasis, alopecia areata, vitiligo, lichen planus. We examine patients receiving statins as their treatment for dyslipidemia and we observe the frequency of cutaneous side effects and the type of the reactions. We also try to identify the benefits of using statins in treatment of some skin disease like psoriasis.

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Effect of Intense Pulsed Light Treatment on Transforming Growth Factor Beta Expression in Acne Vulgaris

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Inflammatory processes play a pivotal role in acne pathogenesis and alterations in cytokine expression may be necessary to initiate and maintain acne development. Significant upregulation of inflammatory cytokines such as interleukin (IL)-8 has been observed in inflammatory acne. Thus, targeting these inflammatory mediators maybe a viable therapeutic option. Recently, much interest has been generated in the use of intense pulsed light (IPL) sources in acne treatment. However, its underlying mechanism of action has not been elucidated. In animal and *in vitro* models, IPL has been demonstrated to enhance transforming growth factor beta (TGF-β) expression. TGF-β inhibits inflammatory cytokine production via a Smad3-mediated signalling pathway. These findings suggest that TGF-β may have a potential role in acne resolution in response to IPL. Therefore we investigated the *in vivo* effects of IPL used for acne treatment, on the expression of TGF-β and down-stream effects of TGF-β signalling. Biopsies obtained from 13 patients with acne at baseline, 48 hours after the first IPL treatment and 7 days after the fourth treatment were immunohistochemically analysed to determine whether IPL alters the expression of TGF-β, Smads, and IL-8. Immunohistology revealed upregulation in the expression of TGF-β1 and Smad3 and down regulation of IL-8 in the post-IPL samples compared to baseline. These findings suggest that the Smad3-mediated negative regulation by TGF-β may be important in promoting resolution of inflammation in acne. The results of this study provide a framework for a potential mechanism of action of IPL in the treatment of acne.

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Expression of Glucocorticoid Receptor Alpha and Beta in Inflammatory Dermatoses

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Glucocorticoid receptor alpha (GRalpha) mediates the effect of systemic and topical glucocorticoids that are used to treat inflammatory dermatoses, e.g. atopic eczema. Alternative splicing of the glucocorticoid receptor (GR) pre-mRNA generates GRalpha, but also GRbeta, which does not bind glucocorticoids but antagonises the activity of GRalpha. Thus increased expression of GRbeta could account for glucocorticoid insensitivity. We investigated GRalpha and GRbeta expression in lymphocytes of patients with severe atopic dermatitis (AD) before and after systemic corticosteroid treatment. Peripheral venous blood was collected from 9 patients with severe AD. mRNA was isolated from peripheral blood mononuclear cells. Expression of GRalpha and GRbeta was determined by RT-PCR and quantitated by real time PCR. The expression of GRalpha and GRbeta was detected in all patients with variable response of the mRNA levels to systemic glucocorticoid treatment. 4 of 9 patients showed increase in the expression of GRbeta mRNA during treatment with systemic glucocorticoid with a concomitant decrease in the expression of GRalpha. This correlated positively with clinical glucocorticoid resistance in this group of patients. In addition, we studied immunohistochemical localization of GRalpha and GRbeta in skin specimens from patients with different inflammatory dermatoses. GRbeta signal was mainly found in lymphocytes and neutrophils. In conclusion, our results are consistent with GRbeta expression being an important mediator of glucocorticoid resistance.

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Selective activation of naturally occurring regulatory T cells (Tregs) by the monoclonal antibody BT-061 as a novel therapeutic opportunity in psoriasis: Early clinical results after single doses

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Tregs down-regulate excessive immune responses. In patients with autoimmune diseases, reduced numbers or functional impairment of Tregs is observed. The humanized agonistic monoclonal antibody BT061 binds to a unique epitope of CD4, thereby selectively activating Tregs but not helper T-cells. In the first placebo-controlled, double-blind clinical trial of BT-061, 55 patients with moderate to severe chronic plaque psoriasis received a single dose of BT-061 or placebo with doses ranging from 0.5 mg to 20 mg i.v. and 10 mg to 25 mg s.c.. BT-061 was well tolerated and the majority of adverse events were mild or moderate without increasing in frequency and intensity with increasing doses. There was no evidence of an increased risk of infections, no significant increase in cytokines and no depletion of CD4 T-cells. Efficacy parameters showed a long-lasting effect in some of the patients with an improvement in PASI-score persisting for up to 90 days after single dose administration. 15 of 41 patients (37%) receiving BT-061 exceeded a PASI 50 response including two patients with more than 75% improvement. Responses were also seen after placebo (29%), but none reached a PASI 75 response. The strongest improvements in PASI were observed in 2.5 mg i.v. and 25 mg s.c. dose groups. Based on these promising results, phase II clinical trials of BT-061 in patients with rheumatoid arthritis and chronic plaque psoriasis are ongoing.

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Subjective efficacy in everyday use of topical medications for scalp psoriasis

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Topical medications for scalp psoriasis are effective under strictly controlled study conditions. However, in everyday use they can be demanding for both patient and physician. Here we retrospectively analysed the efficacy and pitfalls of common topicals for scalp psoriasis, namely calcipotriol plus betamethasone dipropionate scalp formulation (Xamiol), betamethasone dipropionate lotion with salicylic acid (Diprosalic), clobetasol-17-propionate shampoo (Clobex) and 10% salicylic acid in PEG (Carbowax).The data was gathered by direct or telephone interviews of scalp psoriasis patients at our clinic with a standardized questionnaire. We investigated 81 cases of scalp psoriasis that had been treated with at least one of said products. All agents performed modestly to poorly when rated by patients on sustainability of effects. Xamiol and Diprosalic showed significant improvements in overall assessment of disease activity (total sign score TSS) and Xamiol improved pruritus significantly. Patients complied to the recommended duration of use in 62,5% for Xamiol and 63% for Diprosalic. Only 45% (Xamiol) and 44.4% (Diprosalic) of patients kept to the recommended frequency of application, while frequency and duration of use of Clobex differed widely from recommendations. Xamiol and Diprosalic had the best patient compliance to recommendations, led the sustainability ranking and obtained significant clinical score improvements. Taken together, topical treatment of scalp psoriasis remains a therapeutic challenge and patients' everyday experiences do not necessarily match phase III study results.

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Long-term results in patients with recalcitrant dermatoses of palms and soles after bath PUVA therapy

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Numerous studies confirmed the short-term effectiveness of 8-methoxypsoralen bath PUVA therapy in patients with recalcitrant dermatoses of palms and soles (RDPS), however, little is known on long-term results.In this retrospective study we examined the long-term results in 79 patients (mean age, 48 years) with RDPS treated with bath PUVA three times a week in an 8-year period. A good clinical response (more than 50% reduction of skin lesions) was observed after a mean of 23 treatments and a mean cumulative UVA dose of 39J/cm2 in 51 patients (65%). Best results were present in patients with hyperkeratotic eczema (17/22; 77% good clinical response) followed by patients with palmoplantar psoriasis (26/41; 63%), and patients with dyshidrotic eczema (8/16; 50%), respectively. In 2007 a questionnaire was sent to all 51 patients with good response to bath PUVA therapy to determine long-term outcome. Among the 34 patients (67%) who answered the questionnaire 10 (29%) reported continuous complete clearance and 12 (35%) reported an improved course of disease after PUVA therapy with reduced frequency and/or intensity of skin rash. Seventy nine percent of patients reported a long-term reduction in the use of topical steroids in the follow-up (mean, 4.3 years). In addition, 67% of patients reported a durable improvement of quality of life. These data show that bath PUVA has a beneficial influence on the course of disease in patients with recalcitrant RDPS and may lead a long-lasting clearance of skin lesions.

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Calcitonin infusions in systemic sclerosis -a retrospective study of clinical effects

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Vasculopathy apart from fibrosis and immunological features characterizes systemic sclerosis (SSs) and will result in the characteristic Raynaud phenomenon. n vitro studies indicate an important role of the calcitonin gene related peptide as mediator of sensory nerve innervation as well as in circulation. In fact, deficiency of this neuropeptide has been demonstrated in SSc. Though its therapeutic application has been advocated, it has only rarely been applied clinically. In our hospital this treatment has been used for a decade and results were now evaluated in a retrospective study by monitoring the disease activity and organ involvement as well as serological parameters. 49 patients with limited (n=32), diffuse (n=10) or undifferentiated SSc (n=7) have been followed-up for a median of 12,2 +/- 10,3 cycles of intravenous calcitonin (100U per day for 10 days every cycle), each one with an interval of 3-6 months. Whereas creatinine clearance improved slightly during continuing treatment, it deteriorated again after stop of treatment. Cardiologic functions remained stable in 65% of patients, whereas lung function (total lung capacity, CO-diffusion) improved in 14% of patients, remained stable in 46 % of patients and deteriorated in 37%. Serological parameters (ANA, BSR, CRP) were unchanged or without statistical significance. In a written questionnaire about their evaluation of clinical responses, 58 % of patients gave the overall judgement of „good", 17% modest, 29% neutral, 4% „bad". With regard to the limitations of a retrospective study, prospective studies are necessary to examine the positive clinical effects of calcitonin treatment indicated in this study.

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Impaired hyaluronate production is restored by defined size hyaluronate fragments in corticosteroid-induced skin atrophy

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Hyaluronate and its cell receptor CD44 contribute to skin homeostasis, as selective suppression of CD44 expression in mouse epidermis leads to skin atrophy. Topical application of intermediate size hyaluronate fragments (HAFi) results in an epidermal hyperplasia accompanied by an increase in CD44 expression and hyaluronate synthesis in mouse skin. In is study we explored the effect of corticosteroid and HAFi treatment on hyaluronate synthesis *in vivo* and *in vitro*. Clobetasol propionate impaired significantly the hyaluronate production and the expression of hyaluronate synthase 3 (HAS3) in cultured human keratinocytes and HAFi could restore this effect. Topically applied clobetasol propionate (0.05%) decreased epidermal and dermal hyaluronate staining and epidermal HAS2 and HAS3 expression in SKH1 hairless mice. Simultaneous topical application of HAFi with clobetasol propionate restored the hyaluronate staining and HAS2 and HAS3 expression. Topically applied betamethasone dipropionate (0.05%) resulted in a decreased expression of HAS3 in human skin which was reversed by HAFi. Our results suggest that hyaluronate metabolism plays an important role in corticosteroid-induced skin atrophy and that HAFi may be a therapeutic option for reducing atrophic effects of topical corticosteroids.

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Expression of steroidogenic protein mRNAs in human skin and hair follicles and *in vitro* steroid effects on dermal fibroblasts and keratinocytes

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In humans, compared to testosterone (TST) and estradiol (E2), significantly higher levels of adrenal steroids dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) circulate. Since human skin and hair follicles (HFs) are important target tissues, we have analyzed mRNA expression of key steroidogenic proteins cytochromes P450scc, P450c17, P450arom, steroid sulfatase (STS), OATP2B1 and 5α-reductase 1 and 2 in female abdominal skin biopsies (n=4), epidermal keratinocytes (n=3) and HF (n=5 donors). The whole transcriptome of keratinocytes after 24h incubation with DHEA-S (10μM), TST (50nM) and E2 (1nM) was assessed by Gene Expression Array. Migration of a keratinocyte cell line (NCTC) and scalp dermal fibroblasts (n=3) in response to steroids was assessed with a scratch wound assay. Skin, keratinocytes and HFs expressed 5α-reductase 1, with the highest expression in HFs. Only HFs expressed 5α-reductase 2. All three expressed similar levels of STS, OATP2B1, P450scc and P450c17. Low levels of P450arom were found in skin and HFs, but not detected in keratinocytes. Gene expression array demonstrated modulation of inflammation, proliferation and differentiation genes in response to steroids. All steroids stimulated NCTC migration; DHEA-S was reversed by an STS inhibitor (STX64). All steroids stimulated fibroblast migration; although both DHEA and TST were blocked by an aromatase inhibitor (Arimidex) and DHEA-S by the STS inhibitor. Human skin and HFs appear to be a potential synthetic site of sex steroids which can locally affect a multifunctional set of genes, while stimulating a migratory cell response. These mechanisms may have implications for hair growth, wound healing, skin aging and cancer.

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An original topical probiotic related ingrédient for dry skin: Efficacy evaluated in a clinical trial with the help of bioinstrumental measurements and proteomic tools

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The aim of this study was to evaluate the effect of a novel probiotic related extract on some symptoms of dry skin. For this purpose, a topical cream containing a *Bifidobacterium* lysate was tested in a randomized double-blind placebo-controlled trial, with sixty six female volunteers with dry skin. The volunteers applied twice a day the cream to the face, arms and legs for two months. Skin barrier repair ability, leg dryness, facial roughness and natural moisturing factors amounts in the *stratum corneum* (sc) were assessed at day 1, d29 and d57. Moreover, we used sc samples to perform a differential iTRAQ Peptide isobaric labeling-MS analysis. The results showed that the treatment led to significantly increase skin resistance compared to group who applied control cream (stripping number to obtain barrier function disruption was significantly increased (p=0.0044) at d57). Clinical and self-assessment revealed a significant decrease in skin dryness (p=0.028). Skin urea level for the active extract treated group increased while this natural moisturizing factor decreased in the control group in the same period of time. Proteomic evaluation showed that the levels of some proteins associated with the desquamation process increased while the quantity of some other proteins related to the SC immaturity decreased.The results of this study demonstrate that this specific bacterial extract have a beneficial effect on skin hydration and epidermal barrier homeostasis and thus may protect against environmental potentially irritating agents, external aggressions or stressed psychological conditions. This bacterial extract then prevents and treats skin dryness.

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The fatty acid profile of the skin surface lipid layer in patients with papulopustular rosacea

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Patients with papulopustular rosacea (PPR) frequently complain of dry, sensitive skin. We have previously demonstrated that PPR patients have reduced skin surface hydration levels, but normal sebum casual levels. This suggests that it may be the quality and not the quantity of sebum that plays a role in PPR. We therefore examined the skin surface fatty acid composition of 25 PPR patients and compared the results with 25 age and sex matched controls. Lipids were collected by placing a sebutape on the degreased skin of the central nose for 1 hour. Lipids were removed from the sebutape by a solvent procedure, and analysed by gas chromatography-mass spectrometry. 58 fatty acids were identified in the skin surface lipids. Of these, it was possible to quantify 28 and semi-quantify 27. The fatty acid with the highest concentration was palmitic acid (C16:0), followed by palmitoleic acid (C16:1 6+ 9), C17_1**, oleic acid (C18:1 9) and myristic acid (C14:0). There were statistically significant lower levels of the saturated fatty acids undecanoic acid (C11:0), behenic acid (C22:0), tricosanoic acid (C23:0) and lignoceric acid (C24:0), and the monounsaturated fatty acid cis-11-eicosenoic acid (C20:1) in the PPR group. There is increasing evidence that sebaceous fatty acids play a role in the maintenance of skin barrier integrity. We propose that the imbalance between skin surface saturated and unsaturated fatty acids may contribute to the cutaneous symptoms of PPR. These new findings may have therapeutic implications for the development of sebum-modifying non-antibiotic treatments for PPR patients.

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Primary cutaneous marginal zone lymphoma: Studies on Borrelia infection

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Primary cutaneous marginal zone lymphoma is a B-cell lymphoma with favorable outcome and a 5-year survival of 100 %. Nodal or systemic dissemination is uncommon and the treatment is successful in almost every cases. Borrelia infection has been suggested to be associated with disease development. Clinicopathological features and results of Borrelia studies in 11 patients with primary cutaneous marginal zone lymphoma are presented. A Borrelia burgdorferi ELISA in sensu lato revealed IgG seropositivity in one and IgM seropositivity in 2 other patients. In the former case the IgG positivity was not confirmed by Western blot and no Borrelia burgdorferi DNA was detected in the cutaneous lymphoma by polymerase chain reaction. These 3 patients have been treated with antibiotics, 2 patients responded well without recurrence, one patient relapsed. Two of the remaining 8 cases regressed spontaneously, 6 patients responded completely to the surgery or radiotherapy. During the 2 to 5 years follow-up 3 Borrelia negative patients relapsed with treatment responsive cutaneous symptoms. Extracutaneous manifestations were not observed. In conclusion favorable course, spontaneous regression or treatment responsive cutaneous symptoms were observed without systemic involvement in all of our patients. The etiological role of Borrelia subspecies could not be confirmed in this patients' population.

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Morphine Loaded Solid Lipid Nanoparticles (SLN) for the Local Treatment of Skin Wounds

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For the treatment of severe pain caused by skin wounds opioids are administered orally which is often related to systemic side effects like respiratory depression or obstipation. Topically applied opioids appear to induce local analgesic effects as well as to improve wound healing, while systemic side effects are reduced. Recently, we documented that both opioids and the drug delivery system solid lipid nanoparticles (SLN) accelerate the wound healing process in 2D/3D wound healing models by stimulating the keratinocyte migration. Now, we aimed for a formulation of morphine loaded SLN to combine the positive effects of both - opioid and carrier - and to obtain sustained release for several days fitting best with clinical needs in wound management. Therefore, morphine 0,125-0,5 mg/mL was loaded onto SLN composed of Compritol® ATO 888 and Poloxamer 188. Stability, particle size, the efficiency of drug-loading and drug release were determined according to standard procedures. The SLN with an average size of 195 nm are stable for at least 30 days. Morphine loading efficiency was about 90 % depending on the morphine concentration. Morphine release from SLN was prolonged compared to morphine solution. Total morphine release was reached within 6 hours, whereas the release from solution was already finished after 4 hours. For a further improvement, in the next step we will incorporate the SLN in a hydrophilic gel in order to extend the release of the formulation.

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Towards the Pathogenesis of Cutaneous Side-Effects Induced by Inhibitors of the Epidermal Gowths Factor Receptor

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Recently, inhibitors of the EGFR (EGFRI), like the small molecule tyrosine kinase inhibitors erlotinib (Tarceva®) and gefitinib (Iressa®), or the monoclonal antibody cetuximab (Erbitux®) have been established successfully as so called targeted cancer drugs in the clinical practice. Most frequent and most severe side-effects of this class of drugs are cutaneous toxicities occurring in more than 50 percent of the patients. Patients treated with EGFRI develop a characteristical inflammatory papulopustular rash (acneiform rash), xerosis cutis, as well as hair and periungual alterations. In the course, superinfections of the rash with Staphylococcus aureus occur frequently. These clinical observations assume, that the EGFR critically regulates cutaneous inflammation and infection. Nevertheless, the pathogenesis of EGFRI induced cutaneous side-effects has yet to be elucidated. This study analyses the *in vitro* expression of chemokines and antimicrobial peptides in primary human keratinocytes treated with inhibitors of the EGFR (EGFRI) using quantitative realtime PCR. Our results demonstrate that the EGFR in fact controls the cutaneous expression of chemokines and antimicrobial peptides. In particular, we show that the blockade of the EGFR induces an overexpression of proinflammatory chemokines as well as a suppression of antimicrobial peptides in human keratinocytes. In line with these findings, cell supernatants of EGFRI treated keratinocytes demonstrated a reduction of the cytotoxic activity against Staphylococcus aureus as compared to medium controls. Our results present interesting new insights into the pathogenesis of cutaneous side-effects associated with the use of EGFRI.

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Detection of melanoma-derived cancer-testis antigen CT16 in patient sera by a novel immunoassay

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Cancer-testis antigens (CTAs) are expressed mainly in various cancer tissues. In normal tissues they are only expressed in testis and placenta. Because of their restricted expression pattern, the CTAs can be potentially utilized for vaccine development and diagnostic applications. Cancer-testis antigen CT16 has been found to be expressed in lung and renal cancers as well as in melanomas. Detection of CT16 protein directly from melanoma patient serum could facilitate monitoring of tumor growth and response to therapy in CT16-positive patients. A highly sensitive time-resolved fluorescence-based immunoassay measuring cancer-testis antigen CT16 in serum was developed. CT16 level was detectable in 14 out of 23 metastatic patients (61%) whereas none of the healthy controls had measurable CT16 level. The Wilcoxon-Mann-Whitney exact test showed statistically significant difference (P=0.006) between the groups of healthy individuals and patients with metastatic melanoma. Four melanoma patients had exceptionally high serum CT16 level. The level of S100B, a recognized marker of progressing melanoma, did not correlate with CT16. CT16 levels of follow-up samples of three patients with metastatic melanoma were found to follow disease progression independent on S100B. As a conclusion, our results show that CT16 protein can be measured directly from patient serum, and the developed assay has a potential for clinical use.

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Innovative Therapies - Discovery and Development of Mapracorat, a Topical SEGRA for the Treatment of Inflammatory Diseases

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Concerted efforts in research have been focused in recent years on innovative New Chemical Entities with improved therapeutic indices for the treatment of inflammatory diseases. Mapracorat is a novel low-molecular-weight compound which belongs to the proprietary class of SELECTIVE GLUCOCORTICOID RECEPTOR AGONISTS (SEGRAs) that are structurally unrelated to steroids. Pharmacological properties of Mapracorat were studied in terms of the potential anti-inflammatory and side effects in functional bioassays. This novel potent compound binds with high affinity and selectivity to the GR yet is more selective than traditional glucocorticoids. The anti-inflammatory activity of Mapracorat was demonstrated in *in vitro* assays for inhibition of cytokine secretion and T cell proliferation. *In vivo*, in irritant contact dermatitis and T-cell-mediated contact allergy models in rodents, Mapracorat showed anti-inflammatory efficacy after topical application similar to traditional GCs, mometasone furoate and methylprednisolone aceponate. Mapracorat, however, exhibits a better safety profile after single and repeated treatment of animals. Likewise, Mapracorat was extensively studied in both *in vitro* and *in vivo* models of ocular disease. Mapracorat exhibits potent anti-inflammatory effects in cell-based systems comparable to traditional steroids, as well as potent efficacy in animal models of ocular inflammation, dry eye, and allergy. However, Mapracorat demonstrates a superior nonclinical ocular safety profile with respect to the potential for elevated intraocular pressure. Mapracorat is a potent anti-inflammatory compound with a lower potential for side effects, compared with traditional glucocorticoids. It represents a promising drug candidate and is currently in clinical development for application in inflammatory skin diseases and for ophthalmologic indications.

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What causes the pseudoxanthoma elasticum (PXE) skin lesions to look yellowish? : measurement and analysis of their optical properties

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Pseudoxanthoma elasticum (PXE) is a genetic disorder characterised by calcification and degeneration of elastic fibers. The term pseudoxanthoma refers to the yellowish papules found on flexural skin. However, mechanisms that cause the yellowish color are unknown. In this study, the optical properties of PXE skin lesions were examined to answer this question. First, a reflectance spectrophotometer was used to measure the skin color of lesional and non-lesional skin of 5 PXE patients. Reflection values in the longer-wavelength ranges, as well as *b** values (yellowness) in CIE *L*a*b** space, were higher in PXE lesions, indicating that they were objectively more yellowish. As degeneration of elastic fibers and calcium deposition occur mainly in the deeper dermis, we then performed a Monte Carlo simulation using a nine-layered skin model to test whether skin can be yellowish with increased light scattering from the dermis. By increasing the scattering coefficient of the reticular dermis, simulated spectral pattern assimilated that of PXE lesions. *b** values (yellowness) also increased. No differences were found regarding the content of chromophores such as melanin or blood vessels.Finally, to investigate if the masses of degenerated elastic fibers actually increase scattering, deparaffinized sections of biopsied specimens were examined under a dark-field stereomicroscope. Strong scattered light was observed in the areas corresponding to the masses of degenerated elastic fibers. Decalcification by EDTA abolished this increase. These results together suggested that increased scattered light from the deposited calcium in the reticular dermis might be the cause of yellowishness of PXE skin lesions.

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The chemokine CCL20 directs human sperm to the oocyte

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The interaction of sperm with the oocyte is pivotal during the process of mammalian fertilization. The limited numbers of sperm that reach the fallopian tube as well as anatomic restrictions suggest that human sperm-oocyte encounter is not a matter of chance but a directed process. Chemotaxis is the proposed mechanism for re-orientating sperm towards the source of a chemoattractant, and hence to the oocyte. Chemokines represent a superfamily of small (8-11 kDa), cytokine-like proteins that have been shown to mediate chemotaxis and tissue-specific homing of leukocytes. Here we show that human spermatozoa express chemokine receptors in a non-random manner and identify CCR6 as the most abundantly expressed receptor. Conversely, granulosa cells of the oocyte-surrounding cumulus complex as well as human oocytes represent an abundant source of the CCR6-specific ligand CCL20. In human ovaries, CCL20 shows a cycle-dependent expression pattern with peak expression in the preovulatory phase, and CCL20 protein induces chemotactic responses of human sperm. Neutralization of CCL20 in ovarian follicular fluid significantly impairs sperm migratory responses. Taken together, our study indicates that the chemokine CCL20 is a critical factor in the oocyte microenvironment for attracting human sperm.

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Expression of Cutaneous Lymphocyte Associated Antigen During TNF-alfa Inhibitor Biologic Therapy in Psoriatic Patients

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Th-1 mediated inflammatory answer has a major role in the pathogenesis of psoriasis. Homing receptor of T-cells, the cutaneous lymphocyte associated antigen (CLA) is responsible for the specificity of the inflammation processes developing in the skin. Authors examined CLA expression of 17 psoriatic patients recieving TNF-alfa inhibitor biologic therapy. Total lymphocyte CLA expression and CD4+, CD8+ subpopulations of CLA+ lymphocytes were measured by flow cytometry before treatment, two and six weeks after initiation of therapy. Correlation of CLA expression with the clinical severity measured by PASI during tratment was examined and evaluated in two groups: 13 responder and 4 relapsing patients. Results were statistically analized. Significant difference was detected between the initial total CLA expression of the responder and the relapsing group, and CD4+ CLA expression between responder and control group. An inverse tendency in CLA expression was detected in the two groups reacting differently to the treatment. These results indicate that the level and dynamics of CLA expression might predict the efficacy of the treatment.

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Profiling of sebaceous lipids: discovery of novel sebum alterations in juvenile acne

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Acne is a multifactorial disease that afflicts the majority of adolescents in western countries. To define the ultimate significance of sebum in the pathogenesis of juvenile acne, detailed compositional information is required. We have achieved comprehensive and simultaneous detection of intact lipids enriching normal sebum, which presented a broad range of chemical structures and concentrations. Compound-mining and structural characterization were undertaken with HPLC/TOF-MS and MS/MS experiments, respectively. These approaches enabled the identification of 95 and 29 families of triacylglycerols (TAG) and diacylglycerols (DAG), respectively. Among wax esters (WE), 28 species contained the C16:1 fatty acyl moiety. Detection of squalene (SQ), its oxidation products, and 9 cholesterol esters (CE) was accomplished in the same run. Additionally, more than 48 free fatty acids (FFA) were found. To address lipidomics of acne sebum, we applied the gathered compositional and structural information for the within-class profiling of individual sebaceous lipids. Sebum was collected from uninvolved forehead zones of 20 young acne patients (14.00 ± 1.78 years, F/M=1) and from the same area of 20 age matched controls (14.33 ± 2.20 years), with analogous female to male ratio. Significantly different distributions of intact components were detected among TAG, DAG, WE and FFA lipid classes, whereas no differences were found in the CE profiles. Moreover, levels of SQ epoxide were significantly higher in acne subjects. This is the first evidence of different distributional profiles of sebaceous lipids in acne. The structural information of the individual sebum components provided clues on the enzymatic pathways possibly affected in acne.

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Cervical spine compression leading to brachioradial pruritus: results of a prospective magnet resonance tomography study

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Brachioradial pruritus (BRP) describes a rare form of chronic localized itching occurring at the dorsolateral part of the forearms. Recent case reports suggest that BRP may be attributed to cervical lesions or spine neoplasms. To determine the incidence of cervical spine changes in BRP and to correlate the localization of spinal lesions with the dermatomal presence of pruritus, 40 patients (27 female, 13 male, 60.2+10.3 years) with BRP were investigated by magnetic resonance tomography (MRT) of the cervical spinal cord, chest x-ray, and skin biopsy. Patients filled in an itch questionnaire („NeuroDerm Questionnaire") including a dermatome chart and the Northwick Park Neck Pain Questionnaire. All patients showed MRT changes. In 80% of the patients, stenosis of intervertebral foramen or protrusions of cervical disc led to nerve compression (NC). The localization of the NC lesions significantly correlated with the dermatomal localization of the pruritus (Spearman's correlation coefficient: 0.938). No spinal neoplasm was observed. 20% had degenerative changes (DC) such as retrospondylolisthesis without significant correlation to the dermatomal localization of pruritus. BRP as a form of neuropathic pruritus may originate in cervical NC but improbably by DC. Our findings suggest that even slight cervical MRT changes may alter itch afferences leading to BRP. Spinal neoplasms as cause of BRP are rare but should be ruled out by cervical spine MRT.

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An *in vitro* synergetic effect of adapalene and benzoyl peroxide (BPO) on inflammatory acne lesions

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Acne is a chronic inflammatory disease of the pilosebaceous follicle characterized by increased sebum production, keratinocytes hyperproliferation and presence of *p. acnes*. Currently, association of retinoïds and BPO is recommended by the international guidelines for the treatment of acne. The purpose of this study was to investigate *ex vivo* modulator effect of Adapalene associated with BPO on keratinocytes proliferation/differentiation and innate immunity. An immunohistochemical study was carried out from acne papules and non involved acne skin biopsies of the back from seven patients with moderate acne. Keratinocyte proliferation was measured by the Ki67 expression, differentiation by the integrin α2, α3, α6, fillagrin and transglutaminase expressions and innate immunity by the TLR-2 and IL-8 expressions. It was demonstrated that, except for filaggrin, all the markers were overexpressed in inflammatory acne skin compared to non involved acne skin. Adapalene and BPO association produced significantly greater decrease of the proliferation marker (Ki67), differentiation markers (integrin α2 and α6, transglutaminase) and innate immunity markers (TLR-2, IL-8) in inflammatory acne skin than adapalene or BPO alone. Interestingly, the expression of the different markers was down regulated by adapalene and BPO association to a level close to the control skin. In conclusion, for the first time, we have demonstrated here a synergistic *in vitro* effect of adapalene and BPO association on keratinocyte proliferation/differentiation and on skin inflammation of acne lesional skin.

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Toll-Like Receptor-8 engagement on Artificial Antigen-Presenting Cell-expanded CD8+ T lymphocytes

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Adoptive transfer of *in vitro* activated and expanded tumor antigen-specific cytotoxic T lymphocytes (CTLs) is a promising approach to cure cancer but the main problem encoutered is to obtain highly cytotoxic cells that could home to the tumor. We studied the impact of Toll-Like Receptor-8 engagement on peripheral CD8+ T lymphocytes expanded by co-culture with artificial antigen-presenting cells (AAPCs; Latouche and Sadelain, Nat biotechnol, 2000). We defined the optimal conditions for obtaining MART-1-specific CTLs from 6 A2.1+ healthy donors, using AAPCs and anti-MART-1 phycoerythrin (PE)-coupled pentamer. After a 2 to 3 week co-culture, we obtained, in a donor-dependent manner, 3 to 25% of specific CTLs. We found that CTLs expressed TLR8 at the cell surface and in intracellular compartments. MART-1 specific CTLs activated by TLR8 agonist were able to induce an increase of cytotoxic activity against MART-1-pulsed target cells, between 10 and 20% for four donors. However, TLR8 engagement did not change significantly the production of cytokines implicated in cytotoxicity (TNF-α, IFN-γ and Granzyme B). We found, using a cytotoxic assay with T2-cells pulsed with a gradient of MART-1 peptides, that TLR8 engagement increased by ten fold the avidity of MART-1 specific CTLs to their targeted cells. In conclusion, we found that TLR8 expression levels as well as the increase of cytotoxity observed after TLR8 engagement were highly dependent on the donor. We found that TLR8 engagement increased the avidity of CTLs for their target cells but this mechanism is not correlated with an increase of cytokine production.

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Illness perception, psychological distress and quality of life in patients with cicatricial alopecias

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Cicatricial (or scarring) alopecias are a diverse group of inflammatory disorders that result in permanent hair loss. Very little is known about the psychological impact of these conditions on patient wellbeing and quality of life. The aim of this study was to evaluate illness perception, psychological distress and quality of life in patients with cicatricial alopecias.Patients with cicatricial alopecia were recruited from a specialist hair research clinic. The Dermatology Life Quality Index (DLQI; n = 88), Hospital Anxiety and Depression Scale (HADS; n = 77) and Revised Illness Perception Questionnaire (IPQ-R; n = 75) were used as validated measures of quality of life, psychological distress and illness perception, respectively. Patients displayed high levels of psychological distress (mean HADS score = 11.6) and impaired quality of life (mean DLQI score = 6.4). Participants reported significant emotional impact from the alopecia (mean IPQ-R score = 3.49), as well as a low sense of personal control (mean IPQ-R score = 2.27) and treatment control (mean IPQ-R score = 2.57). Stress, immune dysfunction and aging were the most commonly cited causal attributions for the alopecia. Psychological distress was associated with a strong illness identity ($r=0.412$; $p<0.001$) and beliefs that alopecia has serious consequences ($r=0.578$; $p<0.001$), and strong emotional representations ($r=0.653$; $p<0.001$). There was no significant difference in psychological distress or quality of life between the different cicatricial alopecia disease entities examined. The strong relationships between psychological distress and illness perceptions should be recognised when managing patients with cicatricial alopecia.

400 [Oral 053]

Improvement of epidermal barrier function and erythema by *cis*-urocanic acid emulsion cream in adult patients with mild to moderate atopic dermatitis

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The efficacy of repeated and multiple topical dosing of cis-urocanic acid (*cis*-UCA) was evaluated in adult patients with chronic mild to moderate atopic dermatitis (AD) up to 28 days. Thirteen female or male AD patients were treated with 5% *cis*-UCA (0.7 mg/kg daily) and placebo (Aqualan®) emulsion cream on symmetric volar arm skin areas twice daily for 10 days. Twelve of the patients continued treatment with *cis*-UCA (0.35 mg/kg daily) and placebo on study days 14-31. *cis*-UCA treatment reduced transepidermal water loss (TEWL) vs. placebo over the whole treatment period ($p=0.024$; RMANCOVA) and vs. baseline ($p=0.043$). Excluding days 14 and 35 with no ongoing treatments, the decrease from the baseline was highly significant for cis-UCA ($p=0.003$) but not for placebo ($p=0.26$). Erythema was reduced by *cis*-UCA only ($p=0.012$; excluding days 14 and 35, $p=0.012$). In a cohort of the AD patients with their baseline erythema above the mean + 1 SD of healthy individuals, *cis*-UCA reduced erythema vs. placebo on days 5 and 21 and vs. baseline on days 14-28 ($p< 0.05$). In the same cohort of high-erythema patients, *cis*-UCA reduced the initial TEWL vs. placebo on days 10 ($p=0.019$), 21 ($p=0.005$), and 28 ($p=0.003$). Significant improvements were observed in eczema area and severity index (EASI) and the physician's global assessment (PGA) for both treatments. The 5% *cis*-UCA emulsion cream improved skin barrier function (assessed by TEWL), erythema, and disease condition in adult patients with AD. The effect of *cis*-UCA may be the most beneficial in subjects with active AD.

401 [Oral 026]

First double-blind randomized clinical trial of intradermal allogeneic fibroblast therapy for severe generalized recessive dystrophic epidermolysis bullosa randomized against placebo injections resulted in similar wound healing that is independent of collagen VII expression

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Based on the observation that intradermal injection of cultured fibroblasts increased collagen VII expression in unblistered skin of RDEB-GS patients at three months, we initiated a single institution, double-blinded, intra-patient, placebo-controlled RCT to determine the clinical and molecular responses of fibroblast injections in RDEB-GS chronic erosions. Five patients each with up to 6 pairs of symmetrical wounds were injected with GMP-cultured allogeneic fibroblasts in transport solution (Transalyte with 2% albumex) or with transport solution alone. Wounds were biopsied and randomly injected at baseline and monitored at two weeks, three, six and twelve months with the Visitrak technique. Skin biopsies were performed at each time point to determine collagen VII protein and mRNA expression, anchoring fibril numbers and morphology, and inflammatory markers. All patients had rapid improvements in wound healing of both paired wounds treated with cultured fibroblasts and the placebo solution, an effect that lasted up to six months, was similar in therapeutic degree, and was unobserved in untreated wounds. Collagen VII expression and anchoring fibrils increased to a similar degree in both study arms for up to six months in three of the patients' wounds, however not all patients required increased collagen VII expression for wound healing. In conclusion, the injection of both allogeneic fibroblasts and transport solution alone encouraged wound healing in chronic non-healing RDEB-GS wounds, and the molecular results suggest increased collagen VII expression is not a requirement for this phenomenon. Thus, the injections provide a feasible new therapy for wound healing in patients with RDEB.

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Vitamin D serum levels are differentially influenced by UVB311 and UVA1 phototherapy

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UV phototherapy has been shown to increase 25(OH)D. Almost all studies were performed with UVBbb. 2 recent studies investigated the influence of UVBnb on vitamin D. Only 2 studies investigated the 25(OH)D under PUVA-therapy, with contradictory results. We could not find any studies on the effect of UVA1 on vitamin D in the medical literature. We investigated the serum elevation of 25(OH)D under UVBnb and UVA1 therapy in patients undergoing phototherapy, in order to determine the effect of these nowadays frequently used wavelengths for phototherapy on vitamin D plasma levels, and particularly to test the dogma, that UVA has no effect on vitamin D. In the contrary to UVA, UVA1 has almost no overlap with the UVB spectrum. 25(OH)D was determined before the start of light therapy, as well as 1 week after the start of therapy and after completion of therapy at 12 weeks. The first preliminary results show that, as expected, serum vitamin D increases under UVBnb therapy. Under UVA/UVB we also have an increase in vitamin D, however, less clear than with UVBnb. Under UVA therapy, no increase in vitamin D was measured. In conclusion, as UVA exposure does not increase vitamin D synthesis, and the UVBnb-induced increase in vitamin D synthesis is linked to a higher risk of skin cancer, the optimum wavelength for production of previtamin D3 corresponding to maximal DNA damage, intentional UV exposition is not an appropriate way to correct a vitamin D deficiency.

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Succesful Treatment Of 114 Patients With Alopecia Universalis (AU) With A Novel Oral Vaccine Based On Oral Tolerance Induction

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To reconcile the immune system with an unknown antigenic moiety of hair follicles, we used an oral preparation to treat refractory alopecia universalis. Oral tolerance induction is still theoretical. We used alopecia as the best model for it because of abundance of the antigen, relatively harmless disease without affecting vital organs and possibility of histological evaluation during the disease course.Forty seven patients received 800mg of autologous or allogeneic dark hair (partially hydrolyzed in some) in gelatin capsules TID under a strict preparation protocol as sole treatment and 67 in addition to other medications. Thirty of 47 patients responded although it was complete in about 10%. Sixty five of the 67 patients who received both the vaccine and other medications showed a reduction of their need to other drugs including steroids. They relapsed very late or did not relapse till the release of this paper. Later control of the disease was extremely easier and only 22 patients relapsed in the form of total hair loss (all because of arbitrary discontinuation of treatment). The relapse in all others were in the areata or multiple patchy form and easy to treat. As the patients were on many medications exact conclusion about drug/vaccine interaction is difficult to draw but some synergy between Vitamin B6 and the oral vaccine and relative antagonism between concomitant corticosteroids and oral vaccine were encountered. We conclude that oral tolerance induction is an antigen specific option in many auto immune diseases and alopecia is the best model for it.

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Efficacy and safety of etanercept for the treatment of moderate-to-severe psoriasis when used with adjunctive topical therapy as needed: the PRISTINE Trial

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The PRISTINE trial (NCT00663052) assessed efficacy and safety of 2 different dose regimens of etanercept (ETN) for treatment of moderate-to-severe plaque psoriasis. Subjects were randomised to either ETN 50 mg OW (n=137) or 50 mg BIW (n=136) for 12 weeks double-blind followed by ETN 50 mg OW open-label for all subjects. Mild topical steroids only were permitted on scalp, axillae, and groin for first 12 weeks; all potencies of topical steroids and/or vitamin D were allowed as needed for weeks 12-24. Primary endpoint was 75% improvement in Psoriasis Activity and Severity Index compared with baseline (PASI75) at week 24, evaluated for all randomised subjects who received at least 1 dose of ETN (n=137 for 50 mg OW/OW; n=133 for BIW/OW). Safety was assessed for all subjects. Subjects were adults with stable plaque psoriasis involving body surface area ≥10% or PASI≥10, and deemed not candidates for ≥1 of the following: MTX, ciclosporine or PUVA. 251 subjects (92%) completed 24 weeks. Mean baseline PASI was 21. At week 24, 60% in the OW/OW group and 78% in BIW/OW achieved PASI75 (P=0.0015); only 15 subjects (10.9%) in OW/OW and 9 (6.6%) in BIW/OW were electively using potent topical steroid preparations at week 24. No new safety signals were reported; 7 subjects (2.6%) experienced serious adverse events. Nine subjects (3.3%) discontinued due to adverse events. Etanercept at either dose regimen was efficacious in moderate-to-severe psoriasis, with few subjects electing to use concomitant potent topical medications. BIW/OW regimen provided better relief of skin symptoms.

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PK/PD analysis for the prediction of the effective dosage and administration of SUN13834 in Phase 2a study of human atopic dermatitis

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SUN13834, a novel chymase inhibitor, improved skin condition in animal models of atopic dermatitis (AD). A PK/PD analysis to predict the effective dosage and administration in human AD patients was performed.The inhibition of late-phase reaction in biphasic skin reaction was considered important for the improvement of AD because immunosuppressants and corticosteroids, but not anti-histamines, inhibited the late-phase reaction. SUN13834 inhibited late-phase reaction at the dose of 2 mg/kg (PO) or 0.4 mg/kg (IP). The plasma concentration of SUN13834 at the elicitation, which meant the minimum effective plasma concentration in mice, was calculated to be 0.13-0.2 ng/mL. Oral administration of SUN13834 improved skin condition in NC/Nga mice, which develop human AD-like skin lesions under conventional condition, at the dose of 15 mg/kg (BID) and 30 mg/kg (QD), but not 60 mg/kg (EOD). The duration time of 15 mg/kg (BID), 30 mg/kg (QD) and 60 mg/kg (EOD) over 0.13-0.2 ng/mL were 20.3, 12.2 and 10.1 hours, respectively, suggesting that it is important in NC/Nga mice to maintain the minimum effective concentration for more than 12 hours. Since the IC_{50} values of SUN13834 for human chymase was 2-fold higher than that for mouse chymase, the expected minimum effective concentration in human plasma concentration would be 0.26-0.4 ng/mL. In order to maintain these SUN13834 concentrations in human subjects for a similar duration as the corresponding plasma levels in the NC/Nga mice, we determined the dosage and administration in Phase 2a study as 50 mg TID based on Phase 1 PK data.

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Safety and efficacy of the new dermal shaper (Novabel®) for the correction of nasolabial folds: 18 months interim-results of a prospective, ongoing, multi-center study

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Novabel® (Merz Pharmaceuticals, Germany) is a new dermal shaper based on a new Geleon® technology. The objective of this prospective, multi-center, open-label trial was to evaluate safety and efficacy after bilateral injection for the correction of nasolabial folds in a long-term follow-up. Subjects (> 18 years) with moderate to severe nasolabial folds (NLF) using a 5-point severity rating scale (Merz Scales; SRS) were included. Novabel® was administered bilaterally into the deep dermis and upper subcutis with an optional touch-up within 2 weeks. The improvement was assessed by 3 blinded, independent raters after 18 months (SRS score of the NLF). Adverse events were assessed by the physician at each visit as well as by patient diaries. 154 subjects have been injected with the new dermal shaper initially. The overall mean improvement in the SRS score 18 months after the injection of Novabel® was 0.8 ± 0.59 as assessed by the photo rating of 3 independent blinded raters. None of the subjects dropped out of the study due to non-allowed follow-up treatment of the NLF. The adverse events were mostly injection related events such as bruising (1.9%), redness (21.4%), swelling (5.8%), and pain (1.3%) within the first 14 days after the injection (moderate to mild intensity). Only 3 patients dropped out due to an adverse event which were unrelated to the Novabel® according to the investigator. The study demonstrated a clinically improvement in NLF 18 months after the injection of Novabel®. Novabel® was well tolerated leading to a significant better patient comfort.

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Belotero® Basic has a better tolerability vs Restylane®: A prospective, rater-blind, randomized, comparative trial

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Belotero® Basic is the only monophasic HA with Cohesive Polydensified Matrix (CPM®) technology, whereas Restylane® is a biphasic, non-animal stabilized HA (NASHA) filler. The aim of this prospective, rater-blind, randomized, intra-individual study was to compare after a single injection the effectiveness and tolerability of these two HA for correction of moderate to severe nasolabial folds (NLF) and to demonstrate the advantage of monophasic versus biphasic HA. After a screening phase subjects were injected randomly with either Boletero® or Restylane® in one NLF in a split-face design. Rating was conducted at week 4. The rater and the subject were not aware of the randomization. Besides other endpoints pain experienced directly after injection using a 10-cm visual analog scale, feeling of implant, and recommendation of the study treatments were evaluated (descriptively). 20 Patients were treated with a mean of 1.4 mL of Belotero® Basic or Restylane®. Mean pain assessment scores on the VAS were lower for Belotero® Basic than for Restylane® with an intra-individual difference of -4.5 mm [95% CI: -14.0; 5.1]) in favour of Belotero. Moreover, less sujects reported the feeling of Belotero® Basic ompared to Restylane® (65% vs 75%). The majority of patients would recommend the use of both products, however, 25% would only recommend Belotero® Basic only (vs. 10% Restylane® only). 85% of subjects in the Belotero® Basic group and 80% of subjects in the group rated the tolerability as 'good' to 'very good'.Belotero® Basic demonstrated a numerical superiority regarding tolerability vs.Restylane® in this study.

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Baseline cardiometabolic abnormalities in subjects with moderate-to-severe plaque psoriasis in the PRISTINE trial

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The PRISTINE trial ((NCT00663052) assessed efficacy and safety of 2 different regimens of etanercept (ETN) for treatment of moderate-to-severe plaque psoriasis in subjects from 13 countries in Europe, Asia, and Latin America. Cardiometabolic parameters were also assessed to further characterise this population. Descriptive baseline statistics (mean values) are presented herein for 273 randomised subjects. Subjects were ≥18 yrs old with stable plaque psoriasis involving ≥10% body surface area or PASI≥10. Subjects were 70% male, 64% Caucasian, 33% smokers; mean age 44 yrs, BMI 28.3 kg/m^2 for males and 29.6 kg/m^2 for females, PASI 21, mean baseline hsCRP 7.37 mg/L (n=265; normal value <3.1 mg/L) and psoriasis duration 17 yrs. Thirty-one percent (31%) gave a history of psoriatic arthritis (duration 8 yrs). At baseline, 36% of subjects had hypertension; 39% met the definition of metabolic syndrome (71% large waist circumference, 36% elevated blood pressure by history, 27% low high-density lipoproteins, 37% high triglycerides, and 35% high fasting glucose by history or fasting glucose ≥100 mg/dL). Mean Framingham 10-yr risk score was 5.63 (n=260), and mean Reynolds Risk Score was 4.00 (n=257). According to the 2010 American Diabetes Association Guidelines, 14% (35/250) had diabetes mellitus (10% by history) and 34% (86/250) had prediabetes. In addition, 31% of diabetics (11/35) and 17% of prediabetics (14/84) had evidence of chronic kidney disease (based on glomerular filtration rate 15-59 mL/m/1.73m^2 or urinary albumin/creatinine ratio ≥30 mg/g). In this multinational study of moderate-to-severe plaque psoriasis, a high prevalence of cardiometabolic abnormalities was identified.

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A Pharmacometric Model of SUN13834 Efficacy in Adult Subjects with Atopic Dermatitis

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SUN13834 is a novel chymase inhibitor under development for the treatment of Atopic Dermatitis (AD). Subjects, 18 to 65 years old, diagnosed with AD (baseline total Eczema Area and Severity Index (EASI) scores of ≥5) were randomized in a Phase 2 study of SUN13834: 120 to placebo and 120 to 50 mg tid administered orally for 28 days with EASI scores assessed weekly. A two-compartment, first-order absorption and elimination model adequately characterized the SUN13834 pharmacokinetic profile with steady-state achieved within one week. Population pharmacokinetic estimates of clearance (CL/F) and volume of distribution (V2/F) averaged 113 L/h and 106 L, respectively (NONMEM© 6.1). Between-subject variability was 42.8% for CL/F and 62.1% for V2/F. Residual intra-subject variability was 52.9%. Individual empirical Bayesian estimates of SUN13834 steady-state area under the 24-hour concentration-time curve (AUC) were obtained for subsequent EASI score modeling. EASI score was adequately modeled as declining mono-exponentially over the course of treatment. Baseline EASI scores and first-order score improvement rate constants were log-normally distributed among subjects. Baseline EASI scores tended to be higher in atopy probable subjects (IgE >100 IU/mL) and improvement rates tended to be slower in these subjects, particularly in the placebo group. Placebo-adjusted improvement was greater in atopy probable than in non-atopy probable subjects treated with SUN13834.

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SUN13834 in the Treatment of Subjects with Atopic Dermatitis

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Atopic Dermatitis (AD) is a highly prevalent and symptomatic disease with limited treatment options. Mast cells containing chymase predominate in the skin, and degranulate upon stimulation with IgE. Chymase has been implicated in pruritus and biphasic allergic skin reactions consistent with AD. SUN13834 is a potent, selective orally administered chymase inhibitor that has demonstrated efficacy in animal AD models. SUN13834 was evaluated in a randomized, placebo-controlled, double-blind study for 28 days in 270 adults with AD of all severities (mean IGA = 3). Assessments included EASI, IGA, pruritus and insomnia. Mean improvement in EASI was 47% vs. 38% for placebo. Based on a comparison of LS-means, this difference was statistically significant; $P = 0.006$. Similar improvements were observed in IGA. Consistent improvement in EASI was observed in all disease severities, and all EASI disease components, including lichenification. Significantly more subjects on SUN13834 had little or no pruritus and sleep disturbance than placebo. A subset of atopy probable patients (plasma IgE > 100 IU/mL, N=120) showed enhanced response, with a mean improvement in EASI of 52% vs 28% for placebo ($P = 0.001$). 63.1% vs 30.9% of placebo subjects had a ≥50% improvement in EASI ($P = 0.002$). There were significantly more responders to pruritus at the first time point measured (Day 8). AEs with SUN13834 were similar to placebo except for diarrhea (5% vs 2% for placebo). SUN13834 was efficacious and safe in the treatment of AD subjects studied in this trial.

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Sèzary Syndrome Treated With Ecp And Interferon- Alpha

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Sèzary syndrome (SS) is a leukemic variant of primary cutaneous T cell lymphoma. The characteristic features include pruritic erythroderma, lymphadenopathy and the presence of circulating malignant T lymphocytes (Sèzary cells). In differentiating SS from other erythrodermic conditions, we use the following markers: monoclonality of the T lymphocytes (TCR analysis), the presence of at least 1000 circulating Sèzary cells/mm3, an expanded CD4+ population in the peripheral blood and loss of any T cell antigen (CD2, CD3, CD4, CD5). The prognosis of SS is poor with no curative therapy. The current therapies include extracorporeal photopheresis (ECP), psoralen and UV-A (PUVA), chlorambusil combined with prednisolone, electron beam therapy, bexarotene and interferon-α.We present a 68 year-old woman, who developed an erythrodermic itchy skin eruption starting from the palms in 2004. Repeated biopsies showed eczema and psoriformic histology. Laboratory tests and CT- scans were normal. To control erythema and itching cyclosporine was introduced in 2007. Some of the symptoms alleviated, but after 6 months of cyclosporine therapy, a follow-up biopsy revealed mycosis fungoides and over 40% of blood lymphocytes had a cerebriform nucleolus. TCR from skin biopsies showed clonal rearrengement and axillary lymph nodes were enlarged. Patient was diagnosed with SS and clorambusil/prednisolon treatment started for 3 months. The Sèzary cells were reduced to 35%. In 2009 ECP with prednisolone was started on 2 successive days every 4 weeks. After 2 cycles patient was concomitantly treated with interferon-α. The skin symptoms were clinically alleviated

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Pimecrolimus Cream Treatment Normalizes Clinical Symptoms, Skin Barrier Function and Antimicrobial Protein Expression in Seborrheic Eczema

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Seborrheic dermatitis is a chronic inflammatory skin disease, etiology is only partially known. Since *Malassezia* species are often found in the lesions, topical application of antimycotic creams, most commonly ketoconazole, is a standard therapy. The calcineurin inhibitor pimecrolimus is widely used for the topical treatment of atopic dermatitis because of its anti-inflammatory effects. To examine whether pimecrolimus improves clinical symptoms and barrier function in seborrheic dermatitis, 32 patients were treated either with 1% pimecrolimus cream or 2% ketoconazole cream twice daily for 4 weeks in a randomized, double-blinded fashion. In addition, in 25 patients skin washings were used for antimicrobial proteins monitoring. In a subgroup, skin biopsies were taken before and after treatment from the chest region. Both treatments improved clinical symptoms, but the pimecrolimus group reached significance more quickly than the ketoconazole group. In both groups enhanced transepidermal water loss values decreased towards normal levels. Both treatments enhanced hydration which was reduced in lesional skin. Epidermal hyperproliferation and thickness were significantly reduced only in the pimecrolimus group. A reduction of *Malassezia* spp. was seen after both treatments, but more pronounced using ketoconazole cream. Ketoconazole treatment revealed a slight reduction and pimecrolimus treatment a significant reduction of hBD-2 expression, which is related to inflammation and dedifferentiation (p=0.0367). This demonstrates that hBD-2 expression, epidermal proliferation and differentiation as well as clinical scoring were more favourable influenced towards normalization by using pimecrolimus compared to ketoconazole.

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Antipruritic potency of the triterpene betulin: results of an open-labelled trial in patients with chronic pruritus

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To date, experimental studies suggested that natural occuring triterpenes like betulin induce cutaneous anti-inflammatory, anti-nociceptive effects and wound-healing. An open-labelled trial aimed to investigate the antipruritic effects in 64 patients with chronic pruritus on inflammatory skin (group1: n=20, 10 f, 10 m; mean age 55.5 years), pruritus on unchanged skin (group 2: n=23, 10 f, 13 m; mean age 57.4 years) and with chronic scratch lesions (group 3: n=21, 13 f, 8 m, mean age 62.0 years). A Betulin containing cream was applied for a period of two weeks twice daily on the affected areas. Before and after therapy, patients received a detailed clinical investigation with documentation of present scratch lesions assessed by the prurigo-score. For daily documentation of pruritus intensity patients used the visual analogue scale (VAS) from 0 to 10. Statistical analysis was done by intention-to-treat analysis. An antipruritic effect was documented in 50% of group 1 (10/20), 39.1% of group 2 (9/23) and 57.1% of group 3 (12/21). The mean reduction of pruritus intensity (in percent) was 49.3% in group 1, 66.9% in group 2 and 65.3% in group 3. Patients of group 3 also showed a significant regression of scratch lesions within two weeks of cream application (mean prurigo score before/after therapy: 2.73/2.04; p=0.012). Most of the patients (90.6%) tolerated the therapy well. The present results suggest that the topical use of betulin is an effective antipruritic treatment option with good compatibility in patients with chronic pruritus, especially in patients with chronic scratch lesions.

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Expansion of Tumor Infiltrating Lymphocytes in a close system: interests for GMP production and adoptive immunotherapy

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Adoptive cell therapy (ACT) consists in the isolation, *ex vivo* expansion and reinjection of tumor reactive lymphocytes. ACT has emerged as an efficient treatment for patients with metastatic melanoma. However, large scale *ex vivo* cell amplification has several logistical concerns for widespread availability of ACT. One critical step is the initial stimulation of Tumor infiltrated lymphocytes (TIL) with feeder cells that is performed in multiple plates which have many limitations in terms of safety, reproducibility and handling. To address these problems we developed a specific compartmentalized bag that allows close contact between TIL and feeder cells, facilitating stimulation and amplification in an easy handling, close system. We performed a comparative study of TIL proliferation after stimulation with feeder cells in the compartmentalized bags versus standard protocol in plates. We also examined the production of specific tumor reactive CD8+ T cells. Firstly, the level of proliferation and cell viability was as good with the bags as with the standard protocol. Secondly, interestingly, by majoring IFNγ production by TIL in presence of the autologous melanoma cell line, we found that cells stimulated in bags were enriched in reactive CD8+ T cells compared to cells stimulated in plates. In conclusion, we demonstrate that stimulation of TIL with feeder cells can be amplified in a close system which is a crucial point for GMP. Moreover, the higher amplification rate of reactive CD8+ T cells could have significant impact for ACT.

415 [Oral 085]

Isotretinoin and Inflammatory Bowel Disease: Population-Based Study

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Case reports and small observational studies suggest that isotretinoin, a popular medication for nodulocystic acne, may cause Inflammatory Bowel Disease (IBD). To explore this association, we conducted a retrospective cohort study in the province of British Columbia (BC), Canada from January 1st, 1997 to December 31st, 2008. All provincial residents were categorized into one of three groups according to use of isotretinoin, topical acne products, or none of these medications. the main outcome measure was the adjusted rate ratio for the risk of IBD with untreated patients as the reference group. A secondary analysis examined patients with a history of IBD prior to exposure. Among 1,764,017 BC residents, 47,189 were prescribed isotretinoin. After adjusting for potential confounders, we observed an increased risk of IBD among young adults exposed to isotretinoin [rate ratio (RR) 1.51; 95% confidence interval (CI) 1.12, 2.04). However, an increased risk was also seen with topical acne products (RR 1.18; 95% CI 1.00, 1.39). In the secondary analysis, exposure to isotretinoin among IBD patients did not increase the risk of IBD flare up (RR 0.72; 95% CI 0.48, 1.23). In fact, isotretinoin exposure was associated with decreased risk compared to topical acne products (RR 0.52; 95% CI 0.29, 0.92). In conclusion, we found increased risk of IBD after isotretinoin exposure. Given that the risk was also increased in the topical acne products group, drugs classes unlikely to cause IBD, the increased risk is likely related to acne vulgaris rather than acne interventions. We also found a decreased risk for IBD flare up following isotretinoin exposure.

416 [Oral 086]

Obesity and atopic diseases: evidence from a population-based cross-sectional study in Germany

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The purpose of this study is to analyse the assocation of obesity with hayfever, eczema and asthma in German children and adolescents. Data was drawn from the public use files of the German Interview and Examination Survey for Children and Adolescents (KIGGS), a nationwide cross-sectional representative survey conducted between 2003 and 2006. 14362 participants with no missing values on hayfever, eczema, asthma and obesity were eligible for analysis. The association of hayfever, eczema and asthma with obesity was analysed by means of multivariable logistic regression, using proc surveylogistic in SAS. Obesity was defined using German reference values (>97th age and sex-specific percentile) according to Kromeyer-Hauschild et al. In crude analyses (N=13022), the lifetime prevalences of ever-physician diagnosed hayfever and asthma were significantly higher in obese compared to normal weight children (15,5 % vs 11,9%, p=0.01; 9,5% vs 5,0%, p<0.0001). In contrast, the lifetime prevalence of ever-physician diagnosed eczema was significantly lower in obese compared to normal weight children (10,9% vs 14,1%, p=0.02). In multivariable analyses (N=9671), neither hayfever (OR 1.22, 95% CI 0.91-1.63) nor eczema (OR=0.92, 95% CI 0.71-1.19) were significantly associated with obesity. Asthma, however, was significantly associated with obesity (OR 1.85, 95% 1.37 - 2.49). Asthma, but not hayfever or eczema, is significantly associated with obesity in German children and adolescents. This finding is consistent with existing studies and calls for research on the mechanisms linking asthma, but not hayfever or ezcema, to obesity.

417 [Oral 087]

Determinants of atopic and non-atopic eczema: results from a population-based cross-sectional study in Germany

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The purpose of this study is to investigate the prevalence of atopic and non-atopic eczema in German children and adolescents and to examine associated factors. Data was drawn from the public use files of the German Interview and Examination Survey for Children and Adolescents (KIGGS). 12747 children and adolescents, aged 3-17 years, were eligible for analysis. Allergic sensitization was defined as sensitization (specific IgE >= 0.35 kU/l) to at least one aero- or food allergen. Weighted prevalences of potential determinants were compared in atopic and non-atopic eczema. The weighted prevalences of atopic and non-atopic eczema were 8,2% (95% CI 5,3%-6,3%) and 5,8% (95% CI 5,3%-6,3%), respectively. The percentage of atopic eczema in all eczema cases increased significantly with age group. The proportion of girls was significantly higher in non-atopic eczema (p< 0.0001). Family history of eczema was strongly related to atopic as well as non-atopic eczema in the offspring (p< 0.0001). Family history of respiratory atopy was significantly more prevalent in children with atopic eczema. Migration background was associated with a significantly decreased prevalence of both atopic and non-atopic eczema (p< 0.0001). Lifestyle and environmental factors (social position, living area, mould, pets, breastfeeding, smoking) were associated with both atopic and non-atopic eczema to the same degree. Depending on age group, up to 54% of German children/adolescents with eczema do not show concomitant allergic sensitization. Both atopic and non-atopic eczema are strongly influenced by a family history of eczema, but asthma and hayfever do not play a role in non-atopic eczema.

418 [Oral 088]

Suicidal Ideation, Mental Health Problems and Social Impairment are increased in Acne: A Population-Based Study in Adolescents

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Psychosocial problems in acne are scarcely explored in large adolescent populations, and the relation to suicide ideation and depression is of great interest because of the controversy regarding possible psychological side-effects of isotretinoin. In a cross-sectional, questionnaire-based study, a total of 4744 adolescents aged 18-19 years were invited and 3775 (80%) participated. Fourteen percent (n=493) of respondents reported having a lot and very much (substantial) acne. Suicidal ideation was more than twice as frequently reported among those with very much acne compared to the whole sample (24.1% versus 10.9%). Suicidal ideation remained significantly associated with substantial acne (OR 1.80, 95% CI 1.30-2.50) in a multivariate model including adjustments of ethnicity, family income and symptoms of depression. Mental health problems, as assessed by Strengths and Difficulties Questionnaire (OR: 2.25), low attachment to friends (OR; 1.52), not thriving at school (OR: 1.41), never had romantic relationship (OR: 1.35), and never had sexual intercourse (OR:1.51) were all statistically significantly associated with substantial acne in a multivariate model. Information from the National Prescription Database shows that the maximum number of isotretinoin-users in the sample was 27. Substantial acne is frequent in late adolescents and is associated with psychosocial problems in this population with low use of isotretinoin. Our findings complement results from quality of life studies in acne patients. Adverse events including suicide, suicidal ideation and depression that have been attributed to isotretinoin may therefore reflect the burden of substantial acne rather than the effects of the medication.

419 [Oral 089]

Low winter levels of 25-hydroxyvitamin D, not summer levels, are associated with increased risk of internal cancer in organ transplant recipients

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High serum levels of 25 hydroxyvitamin D (25(OH)-vitD) have been related to reduced risk of non-cutaneous ('internal') cancers. Adequate summer levels and corresponding sun exposure are considered primary determinants of reduced risk. The impact of recurrent low winter levels at mid latitudes (40 - 60°) remains obscure. We have investigated this potential risk in organ transplant recipients (OTRs). A total of 6609 measurements of 25(OH)-vitD were registered from 1991 through 2007 of 1455 OTRs, 81 of whom developed internal cancer with 304 prior measurements of 25(OH)vitD levels, and 78 developed cutaneous squamous cell carcinomas (SCC) with 341 prior measurements. Cox proportional hazard analyses were used to estimate the risk of cancer. In contrast to summer levels, winter levels of 25(OH)-vitD were significantly lower among people who contracted internal cancers (34 vs. 41 nmol/l, p = 0.002). Hazard ratios showed a significant increase with lower winter levels of 25(OH)-vitD (< 12 vs. > 50 nmol/L gave 5.6 fold increase, p = 0.024). No trend in risk was observed with summer levels of 25(OH)-vitD. Higher summer levels corresponded with significant increases in SCC risk (p = 0.026). Only winter time levels of 25(OH)-vitD were associated with the risk of internal cancers, which casts doubt on the efficacy of risk reduction by increasing summer levels through increased sun exposure. SCC risk was related to higher levels of 25(OH)-vitD which may be attributable to increased sun exposure. Adequate vitamin D supplementation from October through March would appear advisable.

420 [Oral 022]

Filaggrin loss-of-function mutations are associated with early onset eczema, eczema severity, and transepidermal water loss at three months of age

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Filaggrin (FLG) mutations are associated with eczema and skin barrier impairment, but it is unclear whether skin barrier impairment precedes phenotypic eczema in FLG mutation carriers, or whether it is primarily an epiphenomenon of disease activity. 88 infants were examined for eczema. Disease severity was determined by SCORAD score. Transepidermal water loss (TEWL) was measured on unaffected forearm skin, and venous blood samples were screened for the four commonest FLG mutations in the UK white population (R501X, 2282del4, R2447X, and S3247X). 31.8% (28/88) of children had eczema. Median SCORAD was 10.6 (range 0-31). TEWL was higher in children with eczema compared to unaffected infants (median TEWL [g/m²*h] 14.24 vs 11.24, p<0.001). Higher TEWL was associated with more severe disease (median TEWL SCORAD <15 13.76 vs SCORAD ≥15 29.60, p=0.029). Clinically dry skin was associated with higher TEWL, even in the absence of eczema (median TEWL 17.55 vs 11.08, p=0.008). 17.0% (15/88) of children carried at least one FLG mutation. FLG mutation carriers were more likely to have eczema by three months of age (OR=4.26 (1.34-13.57), p=0.014), and FLG mutations were associated with higher median TEWL (FLG 'yes' 21.59 vs FLG 'no' 11.24, p<0.001), even without clinical eczema (FLG 'yes' 15.99 vs FLG 'no' 10.82, p=0.01). Already at three months, FLG mutations are associated with eczema phenotype and increased transepidermal water loss. The observation that TEWL is elevated in unaffected FLG mutation carriers suggests that skin barrier impairment precedes clinical eczema, but longitudinal follow up of this cohort is required.

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Quality of Life and Self-Concept in Patients with Psoriasis

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Numerous studies have documented that psoriasis can exert a profound detrimental effect on quality of life in the afflicted individuals. It is unclear, however, whether the chronic, disease-related decrease in quality of life can affect the structural components of personality, for instance, modify self-concept. The aim of this study was to investigate the relationships between the levels of health-related quality of life and different dimensions of self-concept in patients with psoriasis. Fifty patients with psoriasis completed the questionnaires measuring quality of life (Skindex-29) and self-concept (Adjective Check List - ACL). Lower levels of quality of life were found to be associated with a lower number of positive and a higher number of negative adjectives selected for self-description. Decreased quality of life was also related to lowered interpersonal needs, including nurturance (the need to engage in behaviors that provide material or emotional benefits to others) and affiliation (the need to seek and maintain personal friendships). Lowered quality of life was also associated with compromised self-control, lower personal adjustment, decreased spontaneity and higher attitudes of evaluation, severity, skepticism, and dissatisfaction with oneself. The findings suggest that chronically lowered quality of life due to psoriasis co-occurs with negative changes in self-concept, particularly with different facets of dissatisfaction with oneself (lowered self-esteem) and with compromised interpersonal needs.

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Development of a new biological instrument applied to cutaneous tissues in order to anticipate the evolution of radiological burns

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Radiological accidents of past ten years are caracterized by localized dose of up to 2000 Gy. The diagnosis and prognosis of radiological burn evolution are partly based on the evaluation of the dose locally received by skin. The physical reconstruction of the dose helps to get this information, but requires specific knowledge of the accident geometry and irradiations conditions. However, these data are rarely available in case of radiological accidents. The aim of this project is to develop a new biological dosimeter that could be applied to cutaneous tissue. First, in order to avoid high-dose culture of irradiated cells, we work directly on human skin explants as an *in vitro* human model of radiological burns. Secondly, the Fluorescence In Situ Hybridation (FISH) is applied to skin fibroblasts directly isolated from skin biopsies in order to count extra chromosome segments. Human skin biospies are irradiated from 0 to 50 Gy. Twenty fours hours after irradiation, FISH painting are performed on skin fibroblasts. The number of extra chromosome per cell is mesured. At the same time, each explant is characterized (metabolic activity, histological features and KI67 immunostaining). During the conference, we will present whether there is a relationship between the number of extra chromosome segments per cell and the dose. We will then conclude of the effectiveness of your tool as a biological dosimeter applicable to skin or as an indicator of exposure.

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Sleep lines: facial locations and risk factors in French middle-aged Caucasian women

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The aim of the study was to investigate possible risk factors for sleep lines in Caucasian women. Sleep lines are different from expression wrinkles since they are not located on tension lines and are not related to Langer's lines but they are caused by individual's sleeping positions. This study involved a sample of 542 French middle-aged women (44 to 70 years old). Three standardised facial photographs (face and profiles) were examined independently by two dermatologists allowing for the identification of sleep lines and the evaluation of the severity of several features. Possible impacts of genes polymorphisms (MC1R, elastin, MMP1 and MMP9) were tested using logistic regression models. Sixty women (11%) had facial sleep lines and showed generally more than one sleep line. The sleep lines were often located on the forehead, along the nose, on the cheeks and under the eyes, and more rarely on the chin. As expected, the sleep lines were associated with age, and the women with sleep lines showed also more severe signs of skin ageing. After adjustment on possible confounders, the presence of two major diminished function variants of the MC1R gene was identified as a strong risk factor for sleep lines (AOR [95% CI]: 8.25 [2.6225.97]). No such association was found with the other genes tested. Our results suggest that genetic variations of MC1R are important determinants of the development of sleep lines.

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Risk Factors of Solar Lentigines in French middle-aged Caucasian Women

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The aim of this research was to investigate risk factors of solar lentigines in French adult Caucasian women. Beforehand, a first sample of 320 women completed a questionnaire specifically designed to explore sun exposure and sun protection behaviour. These data were used to build a typology: different types of behaviour have been defined. Then, to be able to assign any new individual into a given type, a decision tree was built based on the three most important items. Independently, a second study was conducted on 542 adult women. Phenotypic data and items related to sun exposure and protection were collected. Moreover, MC1R polymorphisms was determined, as well as the presence of solar lentigines on the face. After having assigned each woman in the sun behaviour typology, possible risk factors of solar lentigines were examined using logistic regression models. As expected, the risk of solar lentigines was found significantly linked with age (AOR [95% CI]: 1.08 [1.051.11]), presence of freckles (3.00 [1.994.52]), dark hair colour (1.77 [1.092.86]) and dark skin colour (1.47 [0.922.36]). Moreover, significant links were found with the presence of solar lentigines and the practice of nautical and mountain sports (1.71 [1.152.55]), the most risky behaviours regarding sun exposure (3.27 [1.467.31], 2.26 [1.074.76], 1.88 [0.893.95]), and a trend with the presence of at least one major diminished function variants of MC1R gene (1.42 [0.852.35]). Our findings highlight complex relationships between individuals' genotypic background and its phenotypic expression, sun exposure behaviour and sun protection habits.

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Chromosome 11q13.5 variant: No association with atopic eczema in Japanese population

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A single nucleotide polymorphism (SNP) on chromosome 11q13.5 [rs7927894] has been reported to show highly significant association of a common variant of rs7927894 with atopic eczema (AE) in the German and Irish population. We evaluated the association between rs7927894 and AE in our cohort of 194 Japanese AE patients and 113 unrelated Japanese control individuals. 22.7% and 1.5% of the patients in our Japanese AE case series are heterozygous and homozygous for rs7927894[T], respectively. rs7927894[T] is also heterozygously and homozygously carried by 23.0% and 0% of the Japanese control individuals. Thus, there is no statistically significant association between the rs7927894[T] and Japanese AE. After stratification for Japanese specific *FLG* mutations that we previously identified, 26.0% and 4.0% of our Japanese AE case series with *FLG* mutations are heterozygous and homozygous for rs7927894[T], respectively. 21.5% and 0.7% of the Japanese AE patients without *FLG* mutations are heterozygous and homozygous for rs7927894[T], respectively. There is no statistically significant association between the rs7927894[T] and AE without *FLG* mutations or rs7927894[T] and AE with *FLG* mutations. Our case-control study in the Japanese population did not confirm that rs7927894 is at increased risk for AE. The present data suggest that the association of rs7927894 with AE established in the European populations is not seen in the Japanese population.

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A Preference Based Approach to Assess Quality of Life of Family Members of Patients with Inflammatory Skin Diseases

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The aim was to assess the family impact of skin diseases using two preference based measures: willingness-to-pay (WTP) and time trade-off (TTO). Family members accompanying patients with inflammatory skin diseases were recruited from the out-patient dermatology department of two secondary referral centres. Participants were asked to complete the Family Dermatology Life Quality Index (FDLQI), a WTP and a TTO questionnaire. These two questionnaires asked how much of their monthly household income and daily time, family members would be willing to spend if they were offered a hypothetical treatment that would completely suppress symptoms of patients' skin disease. A total of 104 subjects (mean age=45.9 years) were recruited who were family members to patients with 13 different inflammatory skin diseases. The mean FDLQI score of family members was 7.9 (SD=6.4; normal range=0-30). Participants were willing to pay a mean of 17% (SD=21) of their monthly income and trade off a mean of 185 minutes (3.1 hours) per day (SD=228) for the hypothetical treatment. There was a highly significant correlation between the values of two preference-based measures (r_s=0.51, p<0.001) and between the FDLQI and the two preference-based measures (WTP to FDLQI: r_s=0.34, p=0.001; TTO to FDLQI: r_s =0.35, p=0.001). Self-assessed disease severity was found to be the strongest predictor of family members' QoL scores (β=.57, p<0.001). This is the first study to assess the family QoL of dermatology patients using a preference-based approach that has highlighted the substantial secondary impact that many skin diseases can have on patients' family members.

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Post exposure prophylaxis (PEP) to HIV - epidemiological profile of patients from the Medical University of Vienna (MUV) general hospital in years 2008-2009

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PEP to HIV is a course of antiretroviral drugs administered within 72 hrs after events with high risk of exposure to HIV aiming to reduce the odds of established infection.We evaluated the putative HIV exposed individuals referred to the MUV and indicated for PEP in years 2008-2009. Our so far analyzed data of 180 individuals (research in progress) demonstrated that:

- 44.1 % are females,
- indication type: unprotected homosexual contact [28.5%, from which 45% of source patients (SPs) were HIV positive], needlestick injuries (22.8%, 37.5% HIV positive SPs), unprotected heterosexual contact (21.4%, 20% HIV positive SPs), occupational exposure (12.8%, 100% HIV positive SPs), rape (11.4%) and needle exchange by IVDUs (2.8%) where HIV status of SPs were unknown,
- PEP regimens were Kaletra®/Truvada® (79.4%) or Combivir®/Truvada® (20.5%),
- 58.8% of individuals tolerated the PEP without any adverse events, 35.3% had minor adverse events (nausea, fatigue, diarrhea, abdominal discomfort or slight elevation of pancreatic enzymes) and in 5.8% PEP was modified or discontinued (severe adverse events: strong diarrhea, abdominal pain and vomiting or significant elevation of liver function parameters),
- 77.1% of patients missed at least one of their follow-up visits planned at 1, 3 and 6 months after PEP start, and
- no case of seroconversion was observed.

In conclusion, approximately equal numbers of sexes seek counseling service for PEP. Most prevalent types of exposure include high risk sexual contact and needlestick injuries. Kaletra®/Truvada® combination seems to be a well tolerated and effective therapy.

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Validation and refinement of the Millennium Criteria for atopic dermatitis

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There is no gold standard for a definite diagnosis of atopic dermatitis (AD). The Millennium Criteria (MC) have been proposed to diagnose AD and to differentiate it from atopiform dermatitis (AFD). Our objective is to further refine the MC into a manageable set that can differentiate between AD, AFD and other entities. The hereby renewed MC will be compared with the UK criteria and the Hanifin & Rajka criteria. New consecutive patients in whom the diagnosis of AD was considered in the differential diagnosis were screened for eligibility. Clinical diagnosis was performed by a panel of dermatologists and patients were assessed according to the various criteria lists by study investigators. To refine the MC, a forwards logistic regression model was used. The refined MC were then compared for validity with the UK and Hanifin & Rajka criteria. Data of 210 included patients were used. After logistic regression of the individual criteria of the diagnostic lists, a set of 5 criteria were identified as best discriminators: typical morphology, early age of onset, Dennie-Morgan fold, historical and actual flexural involvement. The refined MC were constituted from these criteria. When comparing the different list for validity in diagnosing AD, the refined MC showed a relative value of 0.81, the UK criteria 0.71 and the Hanifin & Rajka criteria 0.51.This refinement and validity study shows that the refined MC are a valid tool to diagnose AD and AFD in a hospital based setting and therefore could be incorporated in clinical practice and trials.

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Dermofit: a novel software that improves novices' diagnostic accuracy to a level above that of trained medical students

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The cornerstone of dermatological diagnosis is attaching semantics to images. Dermatologists use non-analytical reasoning to achieve this, but non-experts are not afforded the clinical exposure required to develop such skills. To address this we have developed a prototype content based image retrieval system ("Dermofit") to improve non-experts' diagnostic accuracy. 12 test images were randomly selected from the Department of Dermatology's image library. All students (n=60) attending their 10 day undergraduate dermatology attachment between November 2009 and January 2010 were enrolled. The 60 students were randomly split into two groups; the Dermofit group (n=31) used our Dermofit software to achieve a diagnostic match, and the non-Dermofit group (n=29) provided a written diagnosis. On Day 1 the non-Dermofit group diagnosed a median of 1 image (mean=16%) correctly and the Dermofit group identified a median of 12 images (mean=99%) ($p<0.0001$). On Day 10 the non-Dermofit group correctly diagnosed a median of 6 images (mean=51%) correctly and the Dermofit group matched 12 images (mean=99%) ($p<0.0001$). We have demonstrated that student diagnostic scores are increased significantly by using a structured image database coupled with matching of index and referent images. We have replicated similar results in lay novices. Our Dermofit software allows users to achieve a high degree of diagnostic accuracy without explicit definitions of likeness or rule-based strategies, instead capitalising on the users' intrinsic image recognition abilities. If scalable this could set a new paradigm for acquiring dermatological expertise.

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Living Knowledge of the Healing Plants: Results from a Cross-Sectional Study in Habiganj District of Bangladesh

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Dermatology has been prevalent and even endemic in various parts of the world since ancient times. In recent years, attention has focused on these diseases because of the emergence of drug-resistant varieties of these diseases. As a result, it has become imperative to discover novel compounds to treat such diseases. Since plants form one of the best sources for obtaining pharmacologically active constituents, which can be used as remedy for diseases like cosmetic problems of the skin, scalp, hair, and nails; a study of traditional medicinal practitioners in Habiganj district of Bangladesh; to obtain information on plants used by them as remedy for the above-ailments. It is noteworthy in this regard that all the above-mentioned ailments are prevalent in Bangladesh, and the primarily rural population of the country relies on plants or plant parts prescribed by the traditional medicinal practitioners to treat the above-ailments. Interviews were conducted of traditional medicinal practitioners with the help of a semi-structured questionnaire and plant specimens were photographed and identified at the Bangladesh National Herbarium. The collected information showed that the following plants were used to treat dermatology: *Linum usitatissimum* (L.), *Nigella sativa* (L.), *Aconitum napellus* (L.), *Agaricus albolutescens* Zeller, *Olea europaea* (L.), *Brassica napus* (L.), *Ricinus communis* (L.), and *Polygonum persicaria* (L.). Since the rural patients appeared to be generally satisfied with the treatment offered through these plants, it is important to conduct proper scientific studies towards discovery of compounds of interest in these plants, which can be used as safe and effective medicines.

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Role of aryl hydrocarbon receptors (AhR) in tobacco smoke extract induced-matrix metalloproteinase (MMP)-1 expression

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Epidemiologic studies suggest a link between smoking and extrinsic skin aging, and we previously reported that matrix metalloproteinases (MMPs) mediate connective tissue damage in skin exposed to tobacco smoke extracts. Tobacco smoke contains more than 3800 constituents, including numerous water insoluble polycyclic aromatic hydrocarbons that trigger the aryl hydrocarbon receptor (AhR; also called the dioxin receptor) signaling pathway. To analyze the molecular mechanisms involved in tobacco smoke–induced skin aging, we exposed primary human fibroblasts and keratinocytes to tobacco smoke extracts. Hexane- and water-soluble tobacco extracts significantly induced MMP-1 mRNA in both human cultured fibroblasts and keratinocytes in a dose-dependent manner. To clarify the involvement of the AhR pathway, we used stable knockdown cell lines for AhR in HaCaT cells. AhR knockdown abolished the increase in transcription of the AhR-dependent gene CYP1A1/CYP1B1 and MMP-1 upon treatment with either tobacco smoke extract. Furthermore, the tobacco smoke extracts induced 7-ethoxyresorufin-O-deethylase activity, which was almost completely abolished by AhR knockdown. Likewise, treating fibroblasts with AhR pathway inhibitors, i.e., the flavonoids 3-methoxy-4-nitroflavone and a-naphthoflavone, blocked the induction of CYP1B1 and MMP-1. These findings suggest that the tobacco smoke extracts induced MMP-1 expression in human fibroblasts and keratinocytes via activation of the AhR pathway. Thus, the AhR pathway may be pathogenetically involved in extrinsic skin aging.

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Clinical and epidemiological characteristics of pemphigoid in Podlaskie voivodship, 1998-2009.

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The purpose of the study was the analysis of clinical and epidemiological aspects of pemphigoid cases in 1998-2009 in Podlaskie voivodship (about 700,000 inhibitants). Seventy-eight cases, based on clinical picture confirmed with direct or indirect immunofluorescence studies were diagnosed: 35 males-M and 43 females -F. The mean incidence was 5,9 per million (the highest-10,82 in 2009) and increased since 2001. The majority of patients (51,28%) were inhibitants of rural areas and the mean incidence in this group was 7,41 per million (the highest - 14,29 in 1999). The mean patients' age was 72.12+/-13,12 years (76,37+/-10,60 in M and 68,65+/-14,36 in F, $p<0,05$) and was lower in patients from urban than rural areas (66,70+/-14,95 and 76,50+/-8,37, $p<0,05$, respectively). The mucosal involvement (mostly oral) was seen in 29,39% of patients. Fourteen patients (17,95%) had not any concomitant disorders before diagnosis of pemphigoid. Majority (62,82%) had cardiological diseases. Almost half of patients were treated with drugs that are known to induce pemphigoid. Malignances were found in 6 patients before and 6 - after diagnosis of pemphigoid (15,38%). The incidence of co-morbidities rose significantly during the treatment of the disease. The mortality rate was 26,92%, 16, 88% of patients died during the first year after diagnosis of pemphigoid. The study results indicate that pemphigoid remains an important multidisciplinary disorder in ageing society, adversely affecting life style in the elderly. These patients require careful and systematic follow-up to diagnose internal organ diseases, including these, that might be induced by therapy of pemphigoid.

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Co- morbidities in Patients with Psoriasis: A Pilot Study on 100 Czech Patients

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The aim of the study is to confirm the hypothesis of the increased prevalence of metabolic syndrom, atherosclerosis and autoimmune inflammatory gastrointestinal diseases is higher in patients with chronic plaque psoriasis. 35 patients with plaque psoriasis of PASI 0-20 (av.5,0) and BSA from 0%-80% (av.13,1)and 65 controls were included between January and May 2010. Questionnaires, physical investigation inc. blood pressure, waist perimeter, BMI index and laboratory testing (serological tests of markers of IBD and coeliac disease, blood count, iron level, total protein and glucose level, lipid spectra, CRP) were performed.Results will be presented and the hypothesis that patients with chronic plaque psoriasis are suitable for selective screening and the well-timed intervention will be recommended.

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Evidence that adequate vitamin D levels can be maintained in healthy adults in the absence of environmental ultraviolet radiation exposure

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Exposure to sunshine increases vitamin D in humans and many groups advocate regular exposure to sunlight to maintain vitamin D levels. However, ultraviolet radiation (UVR) exposure is associated with increased risk of skin cancer, and the wavelengths that are most efficient at vitamin D production are also carcinogenic. Many publications in the literature state that UVR exposure is the main source of vitamin D in man, yet it is unclear whether regular exposure to sunshine is necessary for maintenance of adequate levels. In order to establish whether UVR is the major source of vitamin D, and whether sun exposure is required to maintain adequate vitamin D concentrations, we conducted a systematic review of studies looking at UVR exposure and serum vitamin D levels. Multiple databases (n=21) were searched using synonyms of vitamin D, UVR, diet and supplementation; 29,095 articles were screened by two independent reviewers. Two-hundred and forty-seven studies reporting the effect of UVR on serum levels of vitamin D in healthy adults were assessed; these included interventional and observational studies on different ethnicities and skin types. Seventeen studies provided 25 sets of participants from populations that received negligible UVR for prolonged periods. In 15/25 data sets, the mean serum 25OHD level of the participants remained adequate (>50nmol/L) despite absent UVR. This suggests that sun exposure is not the dominant source of vitamin D in these populations and that sufficient levels of vitamin D can be achieved solely through diet and or supplementation in the absence of regular UVR exposure.

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Children and adolescents' health related quality of life in relation to eczema, asthma and hay fever: results from a population-based cross-sectional study

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We attempted to quantify the impact of eczema, asthma and hay fever on health-related quality of life in a large community-based sample of children and adolescents (n = 6518, 11-17 years) by means of univariate and multivariate analyses (adjusting for sociodemographics, psychological functioning and atopic co-morbidity) and to identify the areas most affected by each condition. We analysed data from the public use files of the German Health Interview and Examination Survey for Children and Adolescents by general linear model analyses for complex samples. The grouping variable was presence of the allergic condition (in the past four weeks and 12 months). Total health-related quality of life was significantly reduced when eczema or hay-fever was present but not when asthma was present within the past four weeks and 12 months (univariate analysis). Multivariate analyses revealed an impact of hay fever but not of eczema or asthma within the last 12 months on total health-related quality of life. Total health-related quality of life was significantly affected when eczema and hay fever but not asthma was present within the last four weeks even after controlling for psychological functioning. The pattern of association with the subscales varied among conditions. While 6 – 20% of variance in total and subscales was accounted for, most of this variance appeared to be due to variation in SDQ scores. All conditions affect health-related quality of life (total and/or subscales) even after controlling for psychological functioning. The association is stronger when presence within the last four weeks is considered.

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Epidemiology of chronic pruritus: Population-based cross-sectional study on prevalence, correlates and characteristics

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We aimed at providing prevalence estimates (point, 12-months, lifetime) of chronic pruritus (> 6 weeks) in the general population, assessing its association with sociodemographic variables and describing its characteristics. 4500 individuals received a validated postal questionnaire. A reminder was sent after 2 months to all non-responders. The remaining non-responders were contacted by telephone if their number was listed in the telephone directory; if the number could not be obtained a third reminder including a shortened version of the questionnaire was sent. Logistic regression modelling was performed with current, 12-months and lifetime chronic pruritus as the dependent variables, and the following independent variables: sex, age, occupational status, schooling, ethnic origin and place of residence (urban vs. rural). The response rate was 57.8%. The point prevalence of chronic pruritus was 13.5% (95% CI 12.2%-14.9%), the 12-months prevalence 16.4% (95% CI 15.0%-17.9%) and the lifetime prevalence 22.0% (95% CI 20.4%-23.7%). Multivariate analyses found only ethnic origin to independently be associated with chronic pruritus. Ethnic origin other than German increased the odds for current and 12-months chronic pruritus. Chronic pruritus is endured for many years (46.8% for more than 3 years). The impact of chronic pruritus on quality of life and affect appears to depend on severity rather than the presence of the symptom alone. To the best of our knowledge this is the first study providing estimates of the point-, 12-months and lifetime prevalence of chronic pruritus in the general population. It suggests a high burden of the symptom in the general population.

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Differential effects of a tertiary individual prevention programme for patients with occupational skin disease depending on diagnosis

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The purpose of this investigation was to evaluate whether the effects of a 3 week inpatient tertiary individual prevention programme on socio-cognitive determinants (attitudes, control beliefs, perceived social norms and intention) of skin protection behaviour vary between patients with work related atopic dermatitis and other work related skin diseases. A total of 101 patients (14 inpatients with work related atopic dermatitis and 87 inpatients with other work related skin diseases) completed measures on socio-cognitive determinants of skin protection behaviour before and after a 3-week inpatient tertiary prevention programme. Mixed model analyses, using maximum-likelihood estimation tested whether there were differential effects of the intervention on socio-cognitive determinants of skin protection behaviour. Although patients with atopic dermatitis reported more favourable cognitions towards skin protection behaviour than patients with other skin diseases at admission, these cognitions deteriorated or remained on the same level. Patients with other forms of work related skin disease on the other hand developed more favourable cognitions during the intervention. Professionals working in the field of work related skin disease should not cease to assist atopic dermatitis patients in achieving optimal skin protection behaviour. Tertiary individual prevention measures may need to pay more attention to the needs of individuals with an occupationally relevant atopic dermatitis. This may contribute to their being able to remain active in the workforce. The alternative would entail regular sick leave, poorer quality of life and economic hardship for the atopic dermatitis patient.

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Discrepancy in patient and physician global assessments of dermatologic diseases.

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To investigate discrepancy in the perception of dermatologic diseases (DD) severity between patients and physicians.A descriptive study was performed: 2459 patients with DD rated their level of disease severity on a five level scale: very mild, mild, moderate, severe, very severe (PtGA) . Physician global assessment (PhGA) was performed on the same scale. 53 physicians were involved in an out-patient setting for three weeks (March 2010) in a dermatologic research hospital, Rome, Italy. Patients were predominantly females (59%), with an high education and the majority were employed; mean age was 45.9 ±18.5 for females and 44.5 ±18 for males. No discrepancy between PhGA and PtGA was observed in 37% of cases; PtGA under-rated compared to the physician in 35%; and PtGA over-rated relative to the physicians in 28%.Statistically significant differences were observed between PtGA and PhGA in each of the five levels of judgement ($p<0.001$). Higher percentages of patients, in respect to physicians, reported very mild, severe and very severe evaluations. Physicians tended to overestimate for mild and moderate levels. Differences were observed between male and female physicians in the severity judgement, reaching a statistically significant difference for the very mild level ($p<0.001$) where females were more represented. The perceived severity disease in DD was different between patients and physicians and it was different in patients in respect to sex. Only for very mild DD there was a difference in PhGA between males and females, with males underestimating the severity.

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Withdrawn

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Assessment tools to measure pruritus intensity: study on validity of visual analogue scale (VAS), numeric rating scale (NRS) and verbal rating scale (VRS)

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The most commonly used tool for self-report of itch intensity is the visual analogue scale (VAS). Similar measurement tools are the numeric rating scale (NRS) and verbal rating scale (VRS). We herein present data of the first study on reliability and concurrent validity of VAS, NRS and VRS. 200 randomly selected chronic pruritus patients of our out-patient department (88 m, 112 f, mean 62.06 years) recorded their pruritus by VAS (10 cm line), NRS (0-10) and a four-point VRS scale. Mean point difference of VAS to NRS was -0.26 to 0.70. Pearson's correlation coefficient for VAS with NRS was 0.939; p<0.01. On the VRS, 26 patients stated to have no itch («0») which was scored on average as 0.11 (VAS) and 0.10 (NRS); 96 patients to have low («1») pruritus (mean VAS/mean NRS: 1.36/1.90), 54 patients to have moderate («2») pruritus (mean VAS/mean NRS: 4.28/4.83) and 16 patients to have severe («3») pruritus (mean VAS/mean NRS: 8.79/8.56). 9.5% did not record their pruritus intensity by VAS, 2.5% not by NRS and 4.0% not by VRS. Spearman's correlation coefficient for VAS with VRS was 0.788 and for NRS with VRS 0.859; p<0.01. In sum, all scales especially NRS and VAS showed a high correlation and concurrent validity with a low point difference of mean 0.39, SD 0.96. Thus, all scales are applicable in clinical trials to assess valid data of itch intensity. However, the discrimination of itch intensity on the VRS is not as sensitive as on VAS and NRS.

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Melanoma screening in the nuclear power plant in Hungary

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Many exogenous, occupational reasons may play role in the formation of melanoma and non-melanoma skin cancer. Attempts have been made to reveal the relationship between ionizing radiation and melanoma formation. Some of these studies suggested increased incidence of melanoma. Hungary's single nuclear power plant is the Paks Nuclear Power Plant, the nominal total power of the blocks increased to 500MW in 2009. The question was raised, weather the melanoma incidence is higher or not among the workers of the power plant. Between 2008 and 2009 October we screened 556 nuclear workers, 281 men and 275 women for melanoma and non-melanoma skin cancer. We investigated also the number of sunburns, the skin type, the number of nevi and the nuclear risk of work. In both genders most of the examined workers were in the age group between 50-54 years (22% of the men, 25% of the women). 28% of the men and 25% of the women worked 20 to 24 years in the plant.We confirmed in one patient in situ melanoma, and in two patients basal cell carcinoma histologically. In 2006 the melanoma incidence was 17/100.000 in Hungary. If the 1/556 incidence is extrapolated to 100.000 persons, that would mean 180 melanoma cases out of 100.000 persons. In our study the size of the screened population is too small, so we can not confirm any enhanced incidence of melanoma.

442 [Oral 090]

Targeting of human interleukin-12B by small hairpin RNAs in xenografted psoriatic skin

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Psoriasis is a chronic inflammatory skin disorder driven by a dysregulation of the cytokine production; monoclonal antibodies targeting the proinflammatory cytokines tumor necrosis factor alpha (TNFα), interleukin-12 (IL-12), and IL-23 have already been approved for clinical use. Previously, we documented a therapeutic applicability of targeting TNFα mRNA by anti-TNFα small hairpin RNAs (shRNAs) delivered by lentiviral vectors to xenografted psoriatic skin. The present study aimed at targeting mRNA encoding the shared p40 subunit (IL-12B) of IL-12 and IL-23 by cellular transduction with lentiviral vectors encoding anti-IL12B shRNAs. Effective anti-IL12B shRNAs were identified and shRNA-expressing lentiviral vectors were intradermally injected in xenografted psoriatic skin. Treatment effects were evaluated by clinical psoriasis scoring and by measuring epidermal thickness and IL-12B mRNA levels. The efficiency and persistency of lentiviral gene delivery were investigated by bioluminescence analysis of skin treated with lentiviral vectors encoding the luciferase gene. Intradermal injection of lentiviral vectors in xenografted human skin demonstrated potent and persistent transgene expression. Stable IL-12B mRNA knockdown and reduced epidermal thickness were achieved after three weeks treatment. These findings mimic the results obtained with anti-TNFα shRNAs but, in contrast, anti-IL12B shRNAs do not ameliorate the psoriatic phenotype as evaluated by semi-quantitative clinical scoring throughout the course of the experiment. Our studies consolidate the properties of lentiviral vectors as a tool for potent gene delivery and for evaluation of mRNA targets for anti-inflammatory therapy. However, in contrast to local anti-TNFα treatment, the therapeutic potential of targeting IL-12B at the RNA level in psoriasis is questioned.

443 [Oral 091]

Zebrafish Type XVII Collagen: Gene Structures, Expression Profiles, and Morpholino "Knock-Down" Phenotypes

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The human *COL17A1* gene encodes type XVII collagen (also known as the 180-kDa bullous pemphigoid antigen), an integral component of hemidesmosomes, attachment complexes providing integrity to the dermal-epidermal junction. Zebrafish, a facile model system to study skin development, displays fully developed hemidesmosomes at approximately 5 days post-fertilization (dpf). We have identified two *COL17A1* orthologues in the zebrafish genome, *col17a1a* and *col17a1b*, which are expressed in the skin and the neural system, respectively, as determined by *in situ* hybridization. These genes have 26% identity at the amino acid level, and have 27 and 51% identity with the corresponding human protein. The proteins coded by these genes have structural module organizations homologous to the human type XVII collagen, including collagenous segments within the ectodomain, a transmembrane segment, and a relatively small intracellular peptide segment with putative binding affinities to other intracellular components of the hemidesmosomal protein complex. "Knock-down" of the expression of *col17a1a* with a specific morpholino targeting the 5′ UTR of the gene resulted in blister formation and manifested with perturbations in the basement membrane zone. "Knock-down" of *col17a1b* expression had no gross morphologic changes in the skin but resulted in reduction of hair cells in the neuromast in the lateral line. Thus, zebrafish has two *COL17A1* orthologues which may have evolved into tissue-specific functions during vertebrate development. Collectively, zebrafish provides a model system to study the molecular aspects of skin development and offers insights to the corresponding human diseases.

444 [Oral 092]

Transcriptional profiling after lipid raft disruption in keratinocytes identifies new actors in atopic dermatitis

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Cholesterol is particularly important in cellular physiology of proliferating and differentiating keratinocytes. Lipid rafts are cholesterol-rich cell signaling platforms and their physiological role can be explored by cholesterol depletion. To characterize transcriptional changes ongoing after lipid raft disruption in epidermal keratinocytes, we performed whole-genome expression profiling. Microarray results show that over 3000 genes are differentially regulated. Particularly, interleukin-8, urokinase-like plasminogen activator receptor (PLAUR) and metalloproteinases are highly upregulated after cholesterol extraction. qRT-PCR validation and protein release measurements demonstrate the physiological relevance of microarray data. Major enriched terms and functions, determined by IPA analysis, identify cholesterol biosynthesis as a major function, but also the inflammatory disorder atopic dermatitis is the skin disease that associates most closely with the profile of lipid raft-disrupted keratinocytes. This interesting finding is confirmed in skin of atopic dermatitis patients where transcript levels of major lipid raft target genes were analysed. Gene expression in atopic lesional skin was compared to surrounding non-lesional skin, as well as to healthy skin. Our study demonstrates typical downregulations in atopic skin of filaggrin and loricrin, two critical epidermal barrier proteins. Moreover, we show that HB-EGF, transglutaminase-1, IL-8 and PLAUR are specifically upregulated in lesional skin. Possibly the gene expression profile of these genes could constitute a new expression signature of atopic dermatitis. These results suggest that lipid raft organization and signaling may likely be perturbed in keratinocytes of atopic lesions.

445 [Oral 093]

COL17A1 splicing mutations: lessons for design of molecular therapies

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Recent pilot studies on causal therapies for genetic skin diseases have revealed that relatively small biological changes, e.g. moderately increased levels of a missing protein in the skin, can have substantial clinical effects. Epidermolysis bullosa (EB), a group of hereditary skin fragility disorders caused by mutations in the genes encoding components of the epidermal adhesion complex, has served as a prototype for such investigations. The junctional EB (JEB) subgroup is mostly associated with mutations in the genes for laminin 332 or collagen XVII (*COL17A1)* and characterized by trauma-induced tissue separation within the lamina lucida. The phenotypes associated with *COL17A1* mutations range from mild to severe, and features like dystrophy or loss of nails, mucosal involvement, enamel defects and alopecia occur to a differing extent. Here, the systematic analysis of 34 different *COL17A1* mutations in 43 patients with JEB-other revealed new insights into the phenotypic variability. We focused on the effects of splice-site mutations, i.e. the nature and amounts of transcripts and polypeptides synthesized and their association with the phenotypic outcome. Careful molecular genetic and protein biochemical analysis revealed that even small amounts of collagen XVII have a remarkable effect on the phenotype. In contrast to complete null phenotypes, patients with only about 4 % of collagen XVII of the control levels had clearly milder cutaneous involvement and a long life span. These data have significant implications for design of molecular therapies for JEB, since they suggest that a low degree of collagen XVII restoration may deliver substantial skin stability.

446 [Oral 094]

Germline melanocortin-1-receptor variants are associated with severity of phenotype in individuals with congenital melanocytic naevi

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During development neural crest-derived melanoblasts populate the epidermis and hair follicles of human skin. In children with congenital melanocytic naevi (CMNs), collections of poorly-differentiated melanocytes also reside in the epidermis and dermis. The genetic basis of CMNs has not been elucidated. We noticed a high prevalence of red hair/freckling in families of children with CMNs, thus we hypothesised a role for melanocortin-1-receptor (*MC1R*) genotype in CMN development. *MC1R* sequencing was conducted in a cohort of 100 children with CMNs and their parents. Effect of *MC1R* status on phenotypic variables relating to the CMN was modelled using multiple logistic regression analysis; phenotypic variables included size of the largest CMN and total number of naevi, clinical features linked to risk of neurological and malignant complications in this condition. The prevalence of at least one non-synonymous *MC1R* variant in individuals with CMNs was 72%, with V92M the most common variant (allele frequency 0.105). Carrying either V92M or an R allele (R151C, D294H, D84E, R160W) was significantly associated with size of the principal CMN (projected adult size >60cm odds ratio 2.606, 95%CI 1.121-6.060, p=0.026) and with the number of naevi in this population (>50 naevi OR 2.386, 95%CI 1.041-5.471, p=0.040). The numbers of cutaneous melanocytes are comparable across ethnic groups and *Mc1r*-null mice have similar melanocyte numbers as wild-type mice. However, our data suggest that *MC1R* genotype can influence the size and number of CMNs, and that *MC1R* variants play a role in the development of CMNs, increasing melanocyte numbers within these lesions.

447 [Oral 095]

3′ *trans*-splicing repair of *COL7A1* mutations in recessive dystrophic epidermolysis bullosa patients

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The severe autosomal recessive types of dystrophic epidermolysis bullosa (RDEB) are caused by premature termination codons on the *COL7A1* gene. As a result, type VII collagen is absent at the dermal-epidermal junction (DEJ) of the skin, leading to skin blistering. Using RNA *trans*-splicing as a gene therapy tool we were able to restore type VII collagen expression in RDEB keratinocytes. *Trans*-splicing exchanges parts of the coding sequence of the endogenous target transcript by the wildtype coding sequence, which is exogenously delivered by a repair molecule called pre-*trans*-splicing molecule (PTM). Retroviral transduction of primary RDEB keratinocytes with a 3′ PTM encoding a 3,300-bp portion of the wildtype *COL7A1* transcript resulted in correction of full-length type VII collagen expression in cultured keratinocytes and at the basement membrane zone in skin equivalents *in vitro*. To attest to the full phenotypic and functional reversion of *trans*-splicing corrected RDEB keratinocytes *in vivo*, patches of skin equivalents cultured from these keratinocytes were grafted onto immunodeficient mice. Histological and immunohistological analysis of five-week old specimens of grafted tissue showed no blistering and strong labeling of human type VII collagen between dermis and epidermis. Localization of type VII collagen was restricted to the basement membrane, with no expression in the suprabasal cell layers. In this work we demonstrated that 3′ *trans*-splicing within the endogenous *COL7A1* gene generates tissue-specific stable expression of human type VII collagen *in vitro* and *in vivo*. Thus, 3′ *trans*-splicing may be suitable for an *ex vivo* gene therapy approach for treatment of DEB.

448 [Oral 009]

***Ex-vivo* gene therapy restores LEKTI activity and corrects the architecture of Netherton syndrome derived skin grafts**

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Netherton syndrome is a debilitating congenital skin disorder caused by mutations in the *SPINK5* gene which encodes the Lympho-epithelial Kazal-type-related inhibitor (LEKTI). The condition is characterised by defective keratinisation, recurrent infections and hypernatraemic dehydration and is associated with a mortality rate of about 10% in the first year of life. Currently, there are no curative treatments for NS. We have developed a HIV-1 based, self-inactivating lentiviral vector to express *SPINK5* in keratinocytes as part of an *ex-vivo* gene therapy strategy for this disease. High transduction efficiency was achieved in primary Netherton syndrome keratinocytes and reconstitution of LEKTI expression was confirmed in previously deficient cells. These genetically corrected keratinocytes were further tested in a three dimensional *in vitro* organotypic culture system and also *in vivo* using a mouse model of human skin engraftment. Results showed correction of epidermal architecture in both organotypic cultures and regenerated skin grafts. Importantly, the results from corrected skin grafts indicated that even where detectable LEKTI expression was restored to a limited numbers of cells, a wider bystander benefit occurred around these small populations. As LEKTI is a secreted protein, the genetically modified graft may provide not only an immediate local protective barrier, but could also act as a source of secreted LEKTI which would then provide generalized benefit following *ex vivo* gene therapy.

449 [Oral 011]

Loss of corneodesmosin due to early truncating autosomal recessive nonsense mutations leads to peeling skin disease

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Generalized peeling skin disease (PSD) [MIM 270300] with pruritus and atopic diseases, referred to as peeling skin syndrome type B, is an unusual autosomal recessive congenital ichthyosiform erythroderma characterized by lifelong patchy peeling of the entire skin. It shares features with Netherton syndrome (NS) - clinically and at the ultrastructural level, e.g. enhanced detachment of corneocytes, and has to be differentiated from acral peeling skin syndrome (APSS). We characterized a large consanguineous family with PSD and carried out a whole-genome linkage analysis using chip-based SNP analysis, which identified a candidate region on chromosome 6p, and for the first time identified recessive early N-terminal nonsense mutations in CDSN, the gene encoding corneodesmosin, an important adhesive protein of the extracellular part of the corneodesmosomes. As could be demonstrated by immunoblot and immunofluorescence analysis skin of the patients shows a complete loss of corneodesmosin expression. Components of the desmosomes (Dsc1 and Dsg1) showed regular expression; LEKTI, missing in NS, presented a broadened signal; transglutaminase 5, affected in APSS, was normal; further late epidermal differentiation markers showed enhanced expression.We conclude that PSD is due to recessive CDSN nonsense mutations, which lay prior or within the first functionally important glycine loop of the protein. Hence, the pathophysiologic basis as much as clinical expression of PSD is distinct from that of hypotrichosis simplex (HTSS), which is associated with particular dominant C-terminal CDSN mutations. Corneodesmosin is vastly important for the epidermal barrier integrity, and its absence may give rise to the strong predisposition to atopic manifestations.

450 [Oral 014]

Skin grafts recruit bone marrow-derived mesenchymal stem cells through SDF-1α/CXCR4 interaction to enhance tissue regeneration

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Use of avascular skin grafts is common in surgical practice but how the vasculature is restored to maintain the engrafted skin is not known. In this study, we evaluated how bone marrow-derived cells contribute to the skin graft repair process. We evaluated mouse skin that had been grafted onto the back of a mouse that had received green fluorescent protein (GFP) transgenic bone marrow cell-transplantation following lethal dose irradiation. On day 3 after engraftment, significant numbers of microvasculature endothelial cells were noted to be GFP-positive in the skin graft, indicating a contribution of bone marrow-derived cells in vasculogenesis and thereby ensuring restoration of blood flow to the graft. On day 4, further GFP-positive cells were noted in the skin. We detected mesenchymal fibroblastic cells in the dermis and keratinocytes in the hair follicles that were GFP-positive. These data clearly demonstrate that engrafted skin can recruit endothelial, mesenchymal, and epithelial progenitor cells from bone marrow. We then analyzed the molecular mechanism that recruits bone marrow-derived mesenchymal stem cells (MSCs) to the grafted skin. We found that the skin graft could release a soluble factor or factors that resulted in MSCs highly expressing chemokine receptor CXCR4, which is a receptor for stromal derived factor-1α (SDF-1α) that is expressed in endothelial cells of hypoxic damaged tissues such as a skin graft. Our data suggest that the interaction between SDF-1α and CXCR4 could be a key mechanism to regenerate engrafted skin through the recruitment of bone marrow-derived circulating MSCs to the graft.

451 [Oral 015]

Identification of novel psoriasis susceptibility loci and genetic interaction between *HLA-C* and *ERAP1* provides evidence for an integrated pathogenic pathway

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To identify new susceptibility loci for psoriasis, we undertook a genome-wide association study (GWAS) powered through the analysis of 594,224 SNPs in 2,622 unrelated psoriatic individuals from the British Isles and 5,667 control subjects. In addition to a strong signal at the Major Histocompatibility Complex (MHC; $P < 10^{-200}$), we confirmed association for all eight previously reported non-MHC psoriasis susceptibility loci. We discovered new associations for a further eight independent regions of the genome, of which seven contain genes with recognised immune functions (*IL28RA*, *REL*, *IFIH1*, *ERAP1*, *TRAF31P2*, *NFKBIA* and *TYK2*). We found evidence for allelic heterogeneity at one of these loci, with inclusion of both risk classes substantially increasing estimates of genetic risk for the locus. These findings were confirmed through the analysis of seven replication panels ascertained from different regions of Europe (3,174 psoriasis cases and 5,464 controls). All of the loci generated a combined $P < 5 \times 10^{-8}$. We also report strong evidence for an interaction between the *HLA-C* and *ERAP1* loci (interaction $P < 10^{-4}$ in the discovery dataset and 0.03 in the replication set), with the *ERAP1* disease associated variant only affecting psoriasis susceptibility in individuals carrying the *HLA-C* risk allele. This is one of the first compelling examples of interaction between GWAS loci. Taken together, these findings provide robust genetic evidence to implicate specific pathways that integrate epidermal barrier dysfunction with innate and adaptive immune dysregulation in the pathogenesis of psoriasis.

452 [Oral 018]

Long term genetic correction of xeroderma pigmentosum epidermal stem cells : a preclinical model of *ex vivo* and *in vivo* of cutaneous gene therapy

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A major challenge of corrective gene therapy *ex vivo* is a to safely target and select sufficient numbers of somatic stem cells destined to correct the diseased organ they originate from. Here we aimed at permanent correction of epidermal stem cells derived from patients suffering from Xeroderma pigmentosum (XP), a rare, cancer prone photo genodermatosis due to inneficient nucleotide excision repair (NER) of UV-induced DNA lesions. XP cells fall into seven groups of genetic complementation (XP-A to XP-G). XP-C patients are usually exempt from neurological problems and constitute priviledged candidate for resurfacing severly altered skin areas using genetically corrected stem keratinocytes. To fully correct XP-C primary epidermal keratinocytes, a CD24-XPC cassette was retrovirally transduced, allowing CD24 immuno-affinity selection together with nuclear XPC re-expression. Corrected mass population was serially propragated for 100 population doublings (PDs). 32 clones were derived form the initial mass populations and candidate holoclones were propagated until 100 PDs. The *XPC* gene was still present and expressed in high passage holoclones with some decrease from 50 to 65 PDs. However, both mass populations and individual clones at 100 PDs exhibited normal levels of UV-cell survival. Mass population also retained a full capacity of skin regeneration both *ex vivo* and *in vivo* over the long term (22 weeks). Regenerated corrected skin exhibited normal levels of DNA repair UV resistance. Our results indicate for the first time successful transduction and safe selection of epidermal stem cells in a preclical model of *ex vivo* cutaneous gene therapy of XP-C.

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Dysregulated tropomyosin receptor kinase (Trk) signalling in *CYLD* mutant tumours

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Patients with germline mutations in the tumour suppressor gene *CYLD* develop disfiguring cutaneous appendageal tumours, of which the defining tumour is the cylindroma. The key pathways abrogated in human cylindroma tissue, however, are not fully understood and represent an important step in developing therapeutic agents. We adopted a genome wide approach, characterising the genomic and transcriptomic changes in 31 rare microdissected tumours from our pedigrees, and discovered dysregulated tropomyosin kinase signaling (Trk) in the tumour tissue. CYLD, an ubiquitin hydrolase, has been previously shown to regulate receptor internalisation and signal transduction of TrkA, by cleaving lysine 63 linked ubiquitin chains. TrkB and TrkC homeostasis may also be perturbed in the absence of functional CYLD as they have recently been shown to bind to p62, a component of the scaffold that facilitates TrkA regulation. Immunohistochemical validation at the protein level demonstrated increased membranous expression of TrkB and TrkC, compared to perilesional epidermis. To investigate the therapeutic utility of drugs that inhibit Trk signalling, we developed an *in vitro* cylindroma cell culture model. We demonstrated that stimulation of TrkB and TrkC with their cognate ligands resulted in upregulation of phospho ERK and BCL2, consistent with active Trk signalling in our model. Growth of these *in vitro* cylindroma models in the presence of the pan Trk inhibitors K252a, AG879, and lestaurtinib, demonstrated reduced cell viability at micromolar concentrations. Trk inhibition therefore represents a novel therapeutic target for *CYLD* mutation carriers that warrants further investigation as a non-surgical treatment alternative.

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Connecting base excision repair (BER) and nucleotide excision repair (NER) within mitochondria: Cockayne syndrome A and B interact in complexes with mitochondrial (mt)DNA repair associated proteins 8-oxo-guanosine glycosylase (hOGG)-1 as well as mt single strand binding protein (SSBP)-1

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DNA damage induced by ultraviolet (UV)-B radiation predominantly gets repaired by the nucleotide excision repair (NER) mechanism, while UV-A induced oxidative DNA damage is preferentially repaired by base excision repair (BER). Defective NER either causes skin cancer prone Xeroderma pigmentosum (XP) or premature aging in Cockayne syndrome (CS). While mitochondrial BER is well characterized, mitochondria are thought to be free of NER. Mutations of mitochondrial (mt)DNA have been linked to aging. We previously showed localization of CSA and CSB proteins in mitochondria, their enrichment upon oxidative stress, premature induction of oxidatively induced mtDNA mutations in CSA and CSB cells as well as binding of CSA and CSB proteins to mtDNA. However, it has been unclear if CSA or CSB interact with other mitochondrial proteins. We provide evidence that CSA and CSB are associated to complexes of the DNA repair associated mitochondrial single strand binding protein (mtSSBP)-1 and the BER-associated 8-oxo-guanosine glycosylase (hOGG)-1 in oxidatively stressed mitochondria by Coimmunoprecipitation. These data present a link between mtCSA/CSB proteins and protection from large scale deletions of the mtDNA via a previously unreported interaction of two hitherto separate repair mechanisms NER and BER.

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Distribution of mitochondrial DNA deletions in *Csb$^{m/m}$ and Csa$^{-/-}$* mice of different ages

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Defects in the repair mechanism nucleotide excision repair either lead to skin tumors in Xeroderma pigmentosum or premature aging and neurodegeneration in Cockayne syndrome (CS). Aging-associated reduction of subcutaneous fat is a hallmark of physiological aging and premature aging of CS patients. Accumulated mutations of mitochondrial mtDNA are also linked to chronological aging, premature skin aging and neurodegeneration. Less is known about the age dependent distribution of mtDNA large scale deletions in multiple organs of prematurely aging mice. We investigated spleen, liver, skin, quadriceps, heart and brain of mice of different age and different genotypes (*Csb$^{m/m}$*, *Xpa$^{-/-}$* and wildtype) for the relative amount of large mitochondrial deletions (mtD17 and mtD1) and found an age dependent increase of mtD17 in many organs in all mice especially in the skin of aged *Csb$^{m/m}$* . For further investigation we microdissected subcutaneous fat of *Csa$^{-/-}$* and *Csb$^{m/m}$*, *Xpa$^{-/-}$* and wildtype mice of different age and analyzed the relative amount of mtD17 and the mtD1 deletion. Aged *Csa$^{-/-}$* and *Csb$^{m/m}$* mice show a strong increase of mtD17 and mtD1 in age-dependently reduced subcutaneous fat tissue which was not observed in other DNA repair deficient mice without prematurely reduced subcutaneous fat. MtDNA deletions were increased in the epidermis and dermis of these animals but at lower levels compared to subcutaneous fat. These data show that mtDNA mutations age-dependently increase in many tissues of progeroid animals with the highest levels in subcutaneous fat, indicating that these mutations may be involved in age dependent loss of subcutaneous tissue.

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Increased risk of developing squamous cell carcinoma in adult junctional epidermolysis bullosa patients

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In the literature the risk of developing squamous cell carcinoma (SCC) among patients suffering from the inherited trauma-induced blistering disease junctional epidermolysis bullosa (JEB) is unclear. In our centre we noticed an unexpected number of SCCs among adult JEB patients. The purpose of this study was to review all documented JEB patients who developed an SCC. A search in our EB-registry and a systematic literature search were performed. In our EB-registry we found seven cases complicated by SCC. The frequency of developing an SCC among adult JEB patients (n=28) was 25%. The incidence rate of a primary SCC in JEB patients is 4.6 per 1000 person-years; in the general population this is estimated at 0.08-1.36 per 1000 person-years. In the literature we found seven case reports of JEB complicated by SCC, bringing the total number to 14 documented cases. All cases were adults classified as JEB, type non Herlitz. The first SCC developed at an average age of 50 years (median 52 y, range 28-70 y). In 64% of the cases multiple primary SCCs occurred. The localization of SCCs is most often seen on the lower extremities. Three (21%) patients developed metastases and died an average of 8.9 years after diagnosis of the initial SCC. In summary our results show that adult JEB patients have an increased risk (one out of four patients) of developing SCC. The SCCs have an aggressive behaviour with a tendency to metastize. Therefore we recommend annually checking all JEB patients for SCC from age 25.

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A novel enhancer in the first intron is involved in transcriptional regulation of peptidylarginine deiminase type I gene by chromatin looping in human keratinocytes

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Peptidylarginine deiminase (PAD), which has five isoforms (PAD1-4,6), catalyzes the conversion of protein-bound arginine residues to citrulline residues in a calcium-dependent manner. In human epidermis, three PADs (PAD1, 2 and 3) are detected with specific patterns of expression depending on the keratinocyte differentiation state. Their main target is filaggrin, a major protein for the epidermal barrier function. It is unclear the mechanism controlling the transcriptions of *PADI*1-3 in the human epidermis. Previous characterizations of the PAD-encoding gene promoters have shown that the proximal regulation alone is not sufficient to explain this specificity of expression. In present work, we describe an evolutionarily highly conserved nucleotide segment located in the first intron of the PAD1 gene (*PADI*1). Luciferase reporter assays showed that it enhances the activity of the *PADI*1 promoter, in a calcium- and orientation-independent manner. Mutation of a putative NF-κB *cis*-element markedly reduced its enhancer activity, which also confirmed its potential regulatory function. Chromatin immunoprecipitation assays evidenced the binding of both p65 and p50 NF-κB subunits to the *cis*-element, and RNA interference inhibition assays confirmed that NF-κB contributes to the *PADI*1 transcriptional control. Furthermore, the intronic enhancer and promoter of *PADI*1 potentially interact through chromatin-looping, as indicated by chromosome conformation capture assays. Eukaryotic gene transcription is controlled not only by promoters but also by intragenic *cis*-elements. In this study, Our findings provide evidence that an intronic NF-κB-mediated signaling pathway is involved in *PADI*1 regulation in human epidermal keratinocytes.

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Revertant mosaicism due to second-site mutation in *COL7A1* in patient with recessive dystrophic epidermolysis bullosa

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Revertant mosaicism due to *in vivo* reversion of an inherited mutation has been described in the genetic skin disease epidermolysis bullosa (EB) for the genes *COL17A1*, *KRT14* and *LAMB3*. Several mechanisms can underlie this reversion process, such as gene conversion, intragenic crossing-over, true back mutation, and second-site mutation. Despite of the high incidence of revertant mosaicism in patients with EB due to correcting mutations in the genes *COL17A1* and *LAMB3*, i.e. 35%, revertant mosaicism has not been described for *COL7A1*. Mutations in *COL7A1* are responsible for the most devastating form of EB in adults who will develop cocooned 'mitten' deformities of the hands. Our study shows *in vivo* reversion of an inherited *COL7A1* mutation in a patient with recessive dystrophic epidermolysis bullosa (RDEB), who was homozygous for the frame-shift mutation *COL7A1*:c.6527insC,p.2176FsX337. The female patient displayed a patch of clinically healthy revertant skin on her left forearm. The second-site mutation c.6528delT, only present in revertant keratinocytes, resulted in correction of the reading frame. As the new CCC codon codes for the same amino acid proline as the wild-type codon CCT, the revertant cells expressed wild-type type VII collagen polypeptide leading to restoration of skin function. We hypothesize that revertant mosaicism might be more common in patients with type VII collagen-deficient EB, if one looks carefully for it. Further, the revertant keratinocytes offer the possibility to explore cell-based therapeutic strategies, by culturing *in vitro* and grafting as part of bioengineered dermo-epidermal substitutes to affected skin.

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A case of novel G8569T mutation in the type VII collagen gene in dystrophic epidermolysis bullosa

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Dystrophic epidermolysis bullosa (DEB) is a hereditary mechanobullous disorder characterized by fragility of the skin and mucous membranes caused by abnormal anchoring fibrils. Both dominant and recessive DEB are caused by mutations in COL7A1, the gene encoding type VII collagen, the major component of anchoring fibrils. We performed mutation analysis of COL7A1 in one patient with recessive DEB. The diagnosis of DEB was based on the characteristic clinical features, such as mitten-like deformity, and confirmed by histopathologically and EM image. The mutation detection strategy consisted of PCR amplification of genomic DNA, followed by heteroduplex analysis and nucleotide sequencing of the PCR products demonstrating altered mobility. All 118 exons and flanking intron boundaries of COL7A1 were amplified. One novel mutation (G8569T) was detected in Exon 116. This mutation resulted in substitution Gly to Tyr. Further studies will be required whether the clinically unaffected parents are heterozygous carriers of this mutation or this mutation arose as a de novo occurrence of autosomal dominant DEB. Importantly, using this mutational analysis and genetic pedigree, we will provide a precise genetic counseling of individuals at risk for recurrence of DEB in subsequent offspring or future generations.

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A sporadic case of hereditary angioedema with a heterozygous large deletion including *C1-INH* gene

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Hereditary angioedema (HAE; OMIN 106100) is an autosomal dominant disease with recurrent episodes of subcutaneous or submucosal edema typically involving the face, extrimities, hands, feet, tongue, bowels, or upper airways. We investigated the molecular basis of a sporadic Japanese patient 33-year-old woman with repeated episodes of edematous swelling especially on the face and arms. The blood examination showed a low level of C1 inhibitor (C1-INH) protein with a remarkably reduced C1-INH functional activity through the term of remissions and attacks of angioedema. The level of D-dimer was high during the angioedema attack, especially during severer attack. Her parents are healthy and showed normal levels of the CI-INH activity. Direct sequencing of genomic DNA revealed no point mutation in the *C1-INH* gene. Gene copy number analysis of genomic DNA by using quantitative real time PCR identified that the copy number of *C1-INH* gene in the patient was half that of the healthy control. This result indicated a heterozygous large deletion including *C1-INH* gene. Further analysis identified the deleted genomic length was about 650 kbp. We could not find any reported evidence showing that any gene locating in the deleted region can link to the clinical phenotypes of angioedema. This study has identified the heterozygous large deletion including *C1-INH* gene in a sporadic case of HAE. The level of D-dimer has reflected the activity of HAE in this patient.

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Intracellular cholesterol trafficking proteins, STARD4 and D5, regulate cholesterol homeostasis and differentiation status in HaCaT keratinocytes

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This study explored the hypothesis that cytosolic cholesterol trafficking/sensing proteins, STARD4 and STARD5, members of the START family of lipid trafficking proteins, impact on keratinocyte cholesterol homeostasis and differentiation status, providing a novel strategy to target defects in lipid metabolism causative of a range of skin disorders.Human immortalised keratinocytes (HaCaT) were transiently transfected (48h) with *pCMV.6*, *pCMV.6_STARD4* or *pCMV.6_STARD5*, incorporation of [^{14}C] acetic acid used to measure *de novo* lipid biosynthesis, and quantitative polymerase chain reaction determined steady state mRNA levels of transcription factors, enzymes, receptors and transporters implicated in cholesterol homeostasis, and markers of keratinocyte differentiation. Over-expression of *STARD4* (≥ 75 fold; p< 0.05) or *STARD5* (≥ 57 fold; p< 0.001) reduced levels of keratin 1 mRNA by 88.2% (p<0.001) and 63.3% (p<0.05), respectively, while only STARD4 induced Loricrin (54%; p<0.05) gene expression. Both cholesterol trafficking proteins repressed cholesterol and cholesteryl ester biosynthesis, maximally by around 40% (p<0.01); these changes associated with distinct gene expression patterns. STARD4 stimulated *SREBPF2* gene expression (4.6 fold; p<0.01), while *STARD5* increased the expression of *PPARD* (26 fold; p<0.001), *PPARG* (1.7 fold; p<0.001) and repressed *SREBPF2* (43%; p<0.05). Downstream, these changes were reflected in reciprocal regulation of *ABCA1* (D4: 98.2%; p<0.05; D5 12.5 fold increase; p<0.05) and *ABCG4* (D4: 4.2 fold increase; p<0.05), while STARD5 additionally repressed expression of *LDLR* (82.8%; p<0.01). Thus, cytosolic cholesterol transporters, STARD4 and STARD5, regulate keratinocyte cholesterol homeostasis and differentiation status, and may provide novel therapeutics for treatment of lipid-related clinical skin disorders.

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Lamellar ichthyosis in Ibadan, Nigeria - a case of two sisters

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Lamellar ichthyosis, an autosomal recessive inheritable disorder of keratinization with estimated worldwide incidence of 1 per 300,000 live births is rarely reported in Nigeria despite being the most populous country in Africa.Two sisters, aged 18 and 15 years presented to the Dermatology Clinic of the, University College Hospital Ibadan for evaluation of their skin conditions. They are from a monogamous family with a set of twins. The 18-year old girl ranks third while the younger sister is one of non identical twins and fourth in the family. They were born as full-term collodion babies and have had a lifelong history of dryness and excessive desquamation of the skin without notable waxing and waning period. They had episodes of heat intolerance and chronic infections. Both had typical features of lamellar ichthyosis: large, brown, polygonal scales diffusely present over the entire cutaneous surface with flexural accentuation, no apparent underlying erythema. They had malodorous body odor, hyperkeratotic/hyperlinear palms and bilateral ectropion. Their nails, hair and extracutaneous systems were essentially normal. There is no history of similar skin conditions in the other siblings, or consanguinity in the family; their parents hail from Ibadan in Oyo State, Nigeria. Case studies of lamellar icthyosis will encourage attempts to better refine the classification of this highly heterogeneous skin disorder. In Nigeria, the complications and social stigmatization associated with lamellar ichthyosis is unfavourable, thus an effective mode of management needs to be developed.

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Hyper-activation of kallikrein 5 does not up-regulate interleukin 8 expression in Netherton syndrome

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Netherton syndrome (NS) is a congenital skin disease caused by mutations in SPNK5, resulting in loss of function of its encoding protein LEKTI. LEKTI regulates kallikrein 5 (KLK5) which activates protease activated receptor2 (PAR2). PAR2 up-regulates pro-inflammatory factors including interleukin8 (IL-8). Previous studies have shown up-regulation of KLK5 activity and PAR2 expression in NS. However, whether the PAR2 activity is up-regulated in NS is obscure. To further understand the patho-mechanism of NS, KLK5 and IL8 were examined in skin biopsies from NS patients (n=3) and normal donors (n=3). SPINK5 mutations in patients were confirmed by DNA sequencing. LEKTI, KLK5 and IL-8 protein levels in the epidermis, detected by immunohistochemistry, were quantified by optical intensity of staining using a computerised programme. Heterozygous mutations were found in two NS patients but only one mutation was found in the third patient. Of the five mutated alleles, one was a novel mutation (A1478G) in intron 16, near the intro-exon boundary to exon 17. All NS patients lacked LEKTI expression in the epidermis but had intensive KLK5 staining pattern throughout the entire epidermis. KLK5 expression was increased significantly compared to normal skin ($p<0.01$). By contrast, the level of IL-8 remained unchanged ($p>0.05$). In conclusion, we have identified a novel mutation in SPINK5. Our results also suggest that PAR2 activity may not be up-regulated in NS, as levels of the PAR2 targeted chemokine IL-8 are unchanged; possibly because long-term sustained hyper-activation of KLK5 desensitises the PAR2 receptor, leading to relatively low activity of PAR2.

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A recurrent COL7A1 silent mutation in an exonic splicing enhancer sequence underlies dominant dystrophic epidermolysis bullosa pruriginosa.

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Intense itching and blistering tendency characterize dystrophic epidermolysis bullosa pruriginosa (DEB-Pr), a rare DEB subtype inherited either as dominant (DDEB-Pr) or recessive trait. At present, about 40 different COL7A1 mutations were documented as causing DEB-Pr, but no clear genotype-phenotype correlation has been found. Here, we report two unrelated Danish pedigrees presenting DDEB-Pr phenotypes and intrafamilial clinical variability. COL7A1 genotyping in patients from both families identified a common haplotype distinguished by three novel heterozygous point variations with no obvious pathogenic role. One of these sequence variations, c.6846G>C in exon 87 (sited 15-bp from the acceptor splice site) was translationally silent. However, computer prediction with all available matrices revealed that nucleotide 6846G is embedded within overlapping exonic splicing enhancer (ESE) sequences recognized by serine/arginine-rich (SR) splicing factors SF2/ASF and SRp40 and that the mutation either creates a new juxtaposed binding site for SC35 SR protein or abolishes an ESE motif. Reverse transcription (RT)-PCR analysis of the mRNA transcribed from either the endogenous gene in patients' fibroblasts or a recombinant mutant COL7A1 minigene transfected in primate cells allowed to demonstrate that the c.6846G>C prevents exon 87 usage leading to in-frame skipping. These findings demonstrate that exon 87 definition is strongly dependent on ESE. In addition to the identification of a recurrent allele in DDEB-Pr families of Danish ancestry, our study substantiates for the first time the involvement of an exonic splicing regulatory sequence in the pathogenesis of DEB.

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ABCA12 regulates other ABCA subfamily expression in epidermis through PPARδ

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Mutations in the lipid transporter ABCA12 have been linked to Harlequin Ichthyosis (HI), one of the most severe genodermatoses. ABCA12 belongs to the ABCA subfamily of lipid transporters, whose members play an important role in lipid transportation and homeostasis throughout the body. The expression of other ABCA subfamily members has been poorly explored in the epidermis. We screened the expression of all ABCA sub-family members and looked at the effect of loss of ABCA12 of ABCA subfamily member expression in the epidermis of both mouse and human. We used wild type (WT) and *Abca12*$^{-/-}$ mice and normal (NHEK) and harlequin Ichthyosis keratinocytes for this study. PCR revealed the expression of ABCA1, 3 and 7 in both mouse and human keratinocytes. ABCA 1, 3 and 7 were significantly up-regulated in HI patient keratinocytes, but there was a significant reduction in ABCA1 and 7 expression in *Abca12*$^{-/-}$ mouse keratinocytes. At the protein level, there was a striking relocation of ABCA3 and 7 in HI patient epidermis. A loss of ABCA1 fluorescence was observed in mouse skin. These discrepancies were reflected in the changes in PPARδ, a known regulator of ABCA expression. In HI patient keratinocytes there was a significant up regulation in expression, but little change in PPARδ was observed in *Abca12*$^{-/-}$ mouse keratinocytes compared to WT. Thus, ABCA12 regulates ABCA subfamily expression in the epidermis, perhaps through PPARδ.

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Molecular markers in forecasting of the efficiency of treatment with Infliximab in patients with psoriasis

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Infliximab selectively blocks the action of TNF-α which plays the important role in inflammation in derma of psoriatic plaques. However not in all patients with psoriasis Infliximab causes the expressed therapeutic effect. For the purpose of forecasting of the therapeutic efficiency of Infliximab molecular markers biosamples (skin tissue samples taken from psoriatic plaques and blood serum) from 22 patients with moderate-to-severe psoriasis aged 19-57 treated with Infliximab were analyzed. The molecular structure of genes TNF-α, TNF-R-I and TNF-R-II, contents of cytokine TNF-α and soluble receptors sTNF-RI and sTNF-RII and proteome composition were analyzed in skin tissue samples; levels of cytokines TNF-α, IL-2, IL-4, IL-6, IL-8 and IL-10 were analyzed in the blood serum. The homozygous TT genotype of TNF-R-II gene at the 676 locus of 6 exon and high levels of IL-10 in the blood serum (>2.7 pg/ml) were associated with the high intensity of the response on treatment with Infliximab in patients with psoriasis; the homozygous GG genotype of TNF-R-II gene at the 676 locus of 6 exon and low levels of IL-10 in the blood serum (<1.0 pg/ml) were associated with a poor response or no response on treatment with Infliximab. These results were the basis for developing a method of forecasting the efficiency of treatment with a genetically engineered biological drug as Infliximab in patients with psoriasis. Pilot results of this investigation indicate a possible mutual relationship between the composition of the skin proteome and the therapeutic response on treatment with Infliximab in patients with psoriasis.

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Novel and recurrent filaggrin gene mutations in Taiwanese ichthyosis vulgaris families

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Filaggrin is a key structural protein that facilitates terminal differentiation of the keratinocytes and formation of the skin barrier. Filaggrin gene (*FLG*) mutations have been identified as the cause of ichthyosis vulgaris (IV) and have been shown to be a major predisposing factor for atopic eczema (AE). *FLG* mutations were initially identified in European populations, and subsequently in Japanese, Singaporean Chinese, Taiwanese, Korean, and mainland Chinese populations. Notably, there is a clear difference in filaggrin genetics between the European and Asian populations. Our previous study showed that Taiwanese population, as an East Asian group, share *FLG* mutations with both the Japanese and the Singaporean Chinese population. The purpose of this current study was to identify novel and recurrent filaggrin gene mutations in Taiwanese IV patients. Totally, six individuals from unrelated Taiwanese IV families were examined for *FLG* mutations by comprehensive sequencing of the entire *FLG* coding region using an overlapping PCR strategy. We identified three recurrent mutations and four novel mutations in Taiwanese IV patients. Among the identified recurrent mutations, c.3321delA is prevalent among Asian populations; p.Lys4022X and p.Gln2417X have been previously identified in Japanese and Singaporean Chinese IV patients, respectively. The four novel mutations are located at *FLG* repeat 1, 5, 10 in exon 3. Our findings further support the notion that *FLG* mutation spectra of the white European and the Asian ancestral groups are different, and facilitate the establishment of global population genetic maps for IV and AE patient *FLG* mutations.

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The Inversa Type of Recessive Dystrophic Epidermolysis Bullosa is Caused by Specific Arginine and Glycine Substitutions in Type VII Collagen

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The inversa type of recessive dystrophic epidermolysis bullosa (RDEB-I) is a rare variant of dystrophic epidermolysis bullosa, characterized by blistering in the body flexures, trunk, and mucosae. The cause of this specific distribution is unknown. So far, only 12 *COL7A1* genotypes have been described in RDEB-I and genotype-phenotype correlations have not been studied. The aim of the study was to gain more insight into the intriguing pathophysiology of RDEB-I. We identified 20 Dutch and British RDEB-I patients, with genotypes in 18, and conducted the first genotype-phenotype correlation study in RDEB-I. All had generalized blistering at birth and during early infancy. The age of transition from generalized to inversa distribution ranged from 1 to 18 years, but in most before the age of 4. Most patients had severe involvement of the oral and esophageal mucosae, and genital mucosae in half of the female patients. We noted a spectrum of disease severity ranging from the mildest 'mucosal only' phenotype to the severest phenotype with limited acral involvement. The 21 complete genotypes of our RDEB-I patients and those reported in the literature revealed that RDEB-I is associated with specific recessive arginine and glycine substitutions in the triple-helix domain of type VII collagen. Why these substitutions cause the inversa distribution remains unknown. However, we hypothesize that the higher skin temperature in the affected areas plays an important role in the pathophysiology of RDEB-I.

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Zebrafish Model to Study Heritable Skin Diseases - Expression of the Structural Genes and "Knock-Down" Phenotypes

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Zebrafish (*Danio rerio*) has a well characterized genome, comparable to mammals, which can be easily manipulated using morpholino-based antisense oligonucleotides to down-regulate the corresponding gene expression. The larvae develop rapidly, with all major organs, including the skin, having developed by 5-6 days post-fertilization (dpf). During the early stages of zebrafish skin development (<15 dpf) the epidermis consists of two layers, separated from the dermal matrix by a basement membrane zone, with fully developed hemidesmosomes at ~5.5 dpf. In this study, we have systematically surveyed the gene expression profile of structural genes in 1 and 6 dpf larvae, and in adult zebrafish by RT-PCR. Expression of epidermal genes, keratins 1 and 5, was readily detected at 6 dpf and in adult, similar to a number of basement membrane zone genes, including *bpag1*, *itga6* and *itgb4*, as well as *lama5* and *plectin*. A repertoire of collagen genes was also expressed, including those encoding type I, IV, V, VI, VII, XII, XIV, XV, XVI, XVII, XVIII and XIX collagen subunit polypeptides. Morpholino-mediated down-regulation of several genes have resulted in cutaneous phenotype, including blistering in col17a1 "knock-down" animals, mimicking junction epidermolysis bullosa. The expression of zebrafish genes also expressed in human skin suggest that zebrafish provides a facile model system to study molecular aspects of skin development, and pathogenesis and treatment of heritable skin diseases.

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Large-scale analysis of *COL29A1* gene variation for eczema and related traits

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Eczema (atopic dermatitis) is one of the most common skin disorders influenced by both genetic and environmental factors. Based on a recent positional cloning approach it was suggested that *COL29A1*, which encodes a novel epidermal collagen, represents a major eczema disease gene. We investigated reported *COL29A1* variants for association with eczema, subtypes of eczema, and related traits in 5 independent and large study populations: a set of 1687 eczema cases and 2387 population controls, a collection of 274 eczema families, a cross-sectional population of German children (n = 3099), the Swedish population-based birth cohort BAMSE (n=2033), and the European cross-sectional PARSIFAL cohort (n=3113). An additional set of 19 *COL29A1* coding SNPs was analyzed in BAMSE and PARSIFAL. We found no evidence for a relationship between *COL29A1* SNPs or haplotypes and eczema or any other atopic trait. Furthermore, we were unable to confirm an excess of maternal transmissions to eczema-affected offspring. The equivalence test rejected the hypothesis of association with eczema, even excluding small contributions (OR=0.80-1.25) to disease development. In situ hybridization did not show any differences in the cellular distribution pattern of COL29A1 expression between lesional and non-lesional eczema skin and skin from healthy controls. Our results suggest that *COL29A1* is unlikely to contain genetic variants that have a major impact on eczema or atopy susceptibility.

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The Mouse *Samd9L* Gene: Transcriptional Regulation and Developmental Tissue-Specific Expression

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Normophosphatemic familial tumoral calcinosis (NFTC) is characterized by progressive dermal calcification, despite normal plasma calcium and phosphate levels, and caused by mutations in the sterile alpha motif domain 9 gene (*SAMD9*). *SAMD9* is deleted in mouse and its function is postulated to be substituted by its paralogous gene, *Samd9L*. We investigated the transcriptional regulation and the expression pattern of mouse *Samd9L*. A 1.5-kb mouse *Samd9L* promoter fragment was cloned, and a series of 5′ truncated constructs were linked to a luciferase reporter gene. All constructs displayed significant activity in transfected cultured cells of diverse origins, suggesting the presence of regulatory *cis*-elements as close as 87 bp upstream of the transcription start site. Ras-responsive element binding protein (RREB-1) was identified in this region by protein-DNA binding array. The expression of *Samd9L* mRNA was up-regulated in the cells cultured with calcitonin, preceded by significant increase in expression of *Rreb-1* mRNA. qRT-PCR analysis of *Samd9L* revealed near-ubiquitous expression, with the highest level in the kidney. The expression of *Samd9L* in the mouse embryos was first noted at 12.5 pc, and the expression in the kidney progressively increased with age. Tissue-specific expression was also confirmed both by *in situ* X-gal staining and quantitative enzymatic activity assay in a transgenic *Samd9L*$^{-/-}$ mouse model in which the LacZ gene replaced exon 2 in the *Samd9L* gene. These findings assist in understanding the function of *Samd9L* and its paralogous genes, and in elucidation of the mechanisms responsible for extraosseous calcification in NFTC.

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New insight into genotype/phenotype correlation in *ABCA12* mutations in harlequin ichthyosis

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Harlequin ichthyosis (HI) is one of the most severe genodermatoses, caused by loss-of-function mutations in *ABCA12* encoding a keratinocyte lipid transporter. To date, various *ABCA12* mutations have been reported in HI families, although the genotype/phenotype correlation in *ABCA12* mutations has been little elucidated.We found two HI patients from two independent Japanese families, who were compound heterozygotes for *ABCA12* mutations. Both patients had the same paternal nonsense mutation p.Arg1515X which leads to truncation in the first ATP-binding cassette of ABCA12 probably resulting in ABCA12 loss of function.In the other allele, the first patient had a recurrent splice acceptor site mutation c.3295-2A>G. This splice site mutation was reported to result in production of mainly ABCA12 peptide lacking three amino acids (1099_1101delYMK) in the first transmembrane domain.The second patient carried missense mutation p.Gly1179Arg in the other allele. The glycine 1179 is a highly conserved amino acid residue located in the first transmembrane domain of ABCA12, and this mutation substitutes an uncharged polar glycine residue for a positively charged arginine residue.As for the phenotype expression, clinical symptoms of the first patient have been remarkably improved during infancy. In contrast, the symptoms of the second patient did not improve apparently, and she died at the age of 5 months.The marked difference in clinical severity of the two patients indicated that the p.Gly1179Arg has far bigger negative functional effects than that of p.1099_1101del, and shows new insight into genotype/phenotype correlation in *ABCA12* mutations in harlequin ichthyosis.

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Allogeneic fibroblast therapy in RDEB may induce mutant type VII collagen synthesis by keratinocytes through up-regulation of HBEGF.

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When allogeneic fibroblasts are injected intradermally into individuals with recessive dystrophic epidermolysis bullosa (RDEB) they survive for only a few days but are able to increase type VII collagen (C7) expression at the dermal-epidermal junction (DEJ) for several weeks or months. However, the precise mechanism and duration of the response are not known. To investigate this further, we injected 5 x 10^6/cm^2 allogeneic neonatal fibroblasts (Vavelta™, Intercytex plc) into a 9 cm^2 area of non-blistered skin in one RDEB individual (*COL7A1* mutations: p.Arg682X and IVS87+4A>G) and examined *COL7A1* gene and C7 protein expression, and undertook microarray profiling on skin biopsy RNA (Sentrix Human-6 Whole Genome Microarray Gene Expression Analysis; Illumina Inc.) at serial time points up to one year. Immunofluorescence microscopy of skin sections showed increased C7 labelling for up to 6 months (LH7.2 antibody) and RT-PCR (including primers specific for the splice site mutation) revealed increased *COL7A1* expression for up to 3 months. The increased *COL7A1* expression was paralleled by increased expression of *HBEGF* (encoding heparin-binding EGF-like growth factor). Treatment of control and patient cultured keratinocytes and fibroblasts with recombinant HBEGF led to increased *COL7A1* gene expression, in association with increased AP-1 transcription factor levels; the responses were greatest in keratinocytes. Our data indicate that HBEGF maybe a key cytokine in the response of RDEB keratinocytes to allogeneic fibroblast injections in increasing mutant but partially functional C7 at the DEJ, and that a single injection of cells can alter a RDEB patient's intrinsic skin defect for approximately 6 months.

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RNA *trans*-splicing technology facilitates targeted delivery of a suicide gene to cancer cells

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Spliceosome mediated RNA *trans*-splicing constitutes a technology which has the ability to reprogram genetic information on the mRNA level, thus *trans*-splicing has already been successfully used to repair the mutated part of disease-causing genes *in vitro* and *in vivo*. This is accomplished by a specifically designed pre *trans*-splicing molecule (PTM) that binds to the endogenous target RNA and induces a *trans*-splicing reaction between the target and the PTM. We are investigating the ability of a PTM to be used as a therapeutic molecule in a suicide gene therapy approach for cancer treatment in recessive dystrophic epidermolysis bullosa (RDEB). In this experimental setup a tumor associated marker gene is reprogrammed in order to convert it into a new gene product coding for a cell death inducing peptide/toxin. Previously we have designed a PTM, targeting the pre- mRNA of matrix-metalloproteinase-9 via an antisense binding domain (BD) and have shown that the PTM is able to elicit cytotoxicity through the introduction of streptolysin O in an RDEB-associated squamous cell carcinoma (SCC) cell line. We further investigated the specificity of the PTM to selectively target cancer cells expressing the marker gene. Notably, only RDEB SCC cells but not RDEB keratinocytes showed significant reduction in cell viability upon transfection with the PTM which occured by a target-specific RNA replacement. Therefore *trans*-splicing offers great potential to be used in suicide gene therapy approaches because tumor restricted expression of the toxin represents one of most crucial aspects in this area.

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Screening for the best 5´pre-*trans*-splicing molecules in COL7A1 in order to develop a 5´*trans*-splicing gene therapy approach for dystrophic Epidermolysis bullosa

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Mutations in COL7A1, the gene coding for type VII collagen, are the cause of dystrophic Epidermolysis bullosa, a heritable mechanobullous skin disease. Because of its size COL7A1 exceeds the size capability of most viral vectors commonly used for delivery in gene therapy. Furthermore endogenous regulation of expression is crucial in tissues with a complex differentiation program. Therefore we chose a mRNA based gene therapy to repair defects in COL7A1. We repair mutated genes by *trans*-splicing a wild type copy mRNA of the mutated gene with the target gene. The advantages in comparison to a standard gene therapy are maintenance of endogenous transcription control, the ability to target dominant negative mutations and the size reduction of the delivery vector. To select the best molecules to correct mutations in the 5´part of COL7A1 we used a fluorescence-based screening procedure to evaluate a library of randomly cloned pre-*trans*-splicing-molecules. Thereby we identified molecules with a repair-rate of up to 96% in our test system in Hek293FT cells. These constructs shall now be tested in keratinocytes on their endogenous *trans*-splicing potential. Endogenously functional test molecules will be adapted to exchange the 5´part of COL7A1 and therefore be able to correct mutations situated in the first 64 exons of COL7A1 and recover type VII collagen expression in patient cells. The long term goal of our approach is an *ex vivo* gene therapy, in which skin grafts taken from patients are transfected with specific pre-*trans*-splicing molecules and are then transplanted to the patients again.

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Mutation and SNP analysis of TMC6 and TMC8 in patients with epidermodysplasia verruciformis

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Epidermodysplasia verruciformis (EV) is a rare autosomal recessive dermatosis, characterized by a combination of gene mutation and HPV infection. Patients with EV suffer from the development of planar warts, which may end in squamous cell cancer (SCC). In 2002 Ramoz et al. discovered that patients with EV reveal mutations in the genes *TMC6* and *TMC8* (both on chromosome 17q25.3). TMC6 and TMC8 are members of the transmembrane channel-like proteins which are localized in the endoplasmic reticulum. The function of TMCs is widely unknown and the linkage to development of EV is not well understood. Both genes contain a numerous SNPs with unknown influence to EV. Mutations in *TMC6* or *TMC8* could be found in 75% of all patients with EV. Further responsible genes are supposed on chromosome 2p and a X-chromosomal inheritance is also described. We examined patients presenting the typical signs of EV and analyzed *TMC6* and *TMC8* by direct sequencing on an ABI prism 3100. In this connection a high number of SNPs could be detected. We will analyze their occurrence and distribution more detailed, compare them to the general population, and discuss a possible connection of single SNPs to development of EV.

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The H Syndrome – A New Autosomal Recessive Genodermatosis with Systemic Manifestations

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The H syndrome is a recently described autosomal recessive disorder, characterized by cutaneous hyperpigmentation, hypertrichosis, hepatosplenomegaly, heart anomalies, hearing loss, hypogonadism, short stature (low height), hyperglycemia/diabetes mellitus, hallux valgus, camptodactyly or fixed flexion contractures of the toe joints and the hands' proximal interphalangeal joints. Histological findings include a dermal and subcutaneous infiltrate composed of monocyte-derived cells (CD68 histiocytes and CD34 and FXIIIa dendrocytes), admixed with mast cells and plasma cells. Later, this infiltrate is replaced by fibrosis with thickened, fragmented and partially calcified elastic fibers and psammoma bodies. Following homozygosity mapping and further sequencing we found a total of seven mutations in the SLC29A3 gene. Three different mutation have been detected in patients from Israel (p.G427S, p.G437R and c.1045delC), all in exon 6 of the gene. Four additional mutations have been detected in patients from Japan, India, Spain and Morocco (p.S184R, p.R133C p.R363Q and p.R363W). The SLC29A3 gene encodes the ubiquitously expressed equilibrative nucleoside transporter; hENT3. This transporter localizes to the mitochondria and assists mitochondrial homeostasis including mitochondrial DNA synthesis/repair. An increasing number of patients are being identified around the world, some of them had been erroneously given other diagnoses prior to the recognition of the disorder, thus implying that the H syndrome is more common than previously thought. According to our knowledge this is the first description of a disease caused by mutations in nucleoside transporters, and the prominent cutaneous findings suggest a critical role for hENT3 in maintaining skin, pigmentation and hair homeostasis.

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A novel CYLD mutation identified in Brooke-Spiegler syndrome causes decreased NEMO activity and thus increased NF-κB signaling

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Brooke-Spiegler syndrome (BSS; OMIM 605041) is an autosomal dominant disease characterized by the development of multiple skin appendage tumors, which are the consequences of mutations in the cylindromatosis (*CYLD)* gene. The encoded CYLD protein acts as a negative regulator for NF-kappaB-dependent inflammation *in vivo*. We have recently identified a Hungarian pedigree with two members (father and his daugther) affected by BSS . Using direct sequencing, a novel missense mutation of *CYLD* (c.2613C>G; p.His871Gln) was detected located in exon 19 and affecting the ubiquitin-specific protease domain of CYLD protein. To reveal the pathogenic role of the newly identified mutation functional studies were performed on CD4+ T lymphocytes of the two patients since CYLD is highest expressed in this cell type. CD4+ cells of BSS patients were stimulated by TNFα and the activation of the NF-κB pathway was monitored by the expression of NEMO transcription factor. Our data demonstrated a significant reduction in the protein level of NEMO in the samples of the BSS patients compared to healthy controls. Additionally, we characterized peripheral blood lymphocytes by flow cytometry and found minor alterations, the most significant was an increase in CD3+/CD4+/CD26- cell numbers in the CYLD patient with more advanced symptoms. We hypothesize that this novel mutation leads to increased NF-κB signaling via the impairment of the deubiquitinating activity of CYLD protein.

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H syndrome: a recurrent Arg363Gln mutation in *SLC29A3* gene and ultrastructural skin analysis in three patients.

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The *SLC29A3* gene, located on 10q22.1, encodes the hENT3 equilibrative nucleoside transporter and has been shown to be mutated in H syndrome. This autosomal recessive disease described in 2008 is characterized by hyperpigmented and hypertrichotic skin lesions, hearing loss, low height, hepatosplenomegaly, heart abnormalities, hypogonadism and hyperglycemia. We here report on molecular and ultrastructural analyses performed on 3 men from 2 consanguineous families with typical H syndrome. Cutaneous ultrastructural analysis showed numerous melanosomes in the keratinocytes of basal and spinous layers, laminated basement membranes surrounding the capillaries and perivascular infiltrates of histiocytes and eosinophils. The 6 coding exons and the intron/exon boundaries of *SLC29A3* were sequenced. In the three patients, a homozygous G-to-A transition in exon 6 at position 1088 was observed. This 1088 G>A transition substituted arginine for glutamine at position 363 and was not found in 384 normal controls. This Arg363Gln mutation reported once before and located between the 8th and 9th transmembranous domains probably is deleterious since i) it changed a positively-charged amino acid for a neutral amino acid, ii) conversion of arginine 363 to glutamine would be expected to affect the conformation of hENT3 protein since *in silico* analysis predicted a structural change of threonine 363 from sheet to coil, iii) arginine 363 is highly conserved among all species suggesting a functionally important role of this amino acid. In conclusion we detected the recurrent Arg363Gln mutation in Algerian and Tunisian families evoking a founder effect and described ultrastructural cutaneous changes in the newly defined H syndrome.

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Induction of phenotype modifying cytokines by FERMT1 mutations

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Kindler syndrome (KS) is a skin disorder caused by mutations in the *FERMT1* gene encoding kindlin-1, an epithelial-specific phosphoprotein involved in b1 integrin activation. Early in life, KS manifests as a mechanobullous disease reflecting diminished cell adhesion, but many aspects of its later phenotypic features, including progressive poikiloderma and fibrosis of the skin and mucosa, remain unclear. Against this background, we addressed the pathogenesis of dermal abnormalities in KS by exploring cytokine profiles of KS keratinocytes and by characterizing KS skin fibroblasts *in vitro*, and by validating the findings *in vivo* in the skin of nine KS patients. We show that kindlin-1 negative keratinocytes upregulate the expression of IL-24, IL-20, transforming growth factor-b2 (TGF-b2), IL1F5, platelet derived growth factor B (PDGFB) and connective tissue growth factor (CTGF), and that KS fibroblasts exhibit an activated phenotype. These findings correlate with the presence of macrophages and of mediators of fibrosis, like a-smooth muscle actin, TGF-b1, IL-6 and CTGF, in KS skin. Based on these data we predict that mutations in *FERMT1* gene cause epithelial cell stress and, as a stress response, secretion of cytokines that mediate local inflammation and fibrosis. The repeated cycles of epidermal cell stress, cytokine secretion, dermal inflammation and fibrotic processes underlie the phenotypic changes in different tissue compartments in the skin. Our data uncover cytokine-mediated paracrine cell communication processes as novel phenotype modulators in KS and thereby yield a new starting point for development of therapeutic strategies.

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COL7A1 mutation spectrum in Polish Dystrophic Epidermolysis Bullosa patients.

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The aim of the study was to investigate the spectrum of mutations in Polish Dystrophic Epidermolysis Bullosa (DEB) patients and to implement population specific scheme for molecular analysis of COL7A1 in Poland, as it was already done in other countries of Central and Western Europe i.e. Germany, Hungary and England. 33 DEB patients (29 recessive DEB- RDEB, 4 dominant DEB-DDEB diagnosed on the basis of clinical symptoms and immnofluorescence mapping were enrolled to the study. DNA was isolated from leukocytes and analyzed by direct sequencing of COL7A1 coding region and intronic flanking sequences. Full genotype was identified in 23 probands. In further 10 cases molecular analysis is still proceeding due to incomplete genotypes (8 cases with one identified mutation, 2/10 with no identified mutations) Our preliminary data shows that c.425G>A is the most frequent mutation in our group of patients (62% of RDEB patients; 40% of RDEB alleles), followed by equally distributed: c.682+1G>A and, unreported before, c.7154delC (14% of RDEB patients; 7% RDEB of alleles). In other cases we found also rare mutations, including 7 variants unreported before. In conclusion, we present the first molecular study performed in Polish DEB patients. Our results indicate that mutations spectrum and frequency in Polish DEB patients differs from other population tested and support the general idea of developing population specific molecular diagnostic scheme.

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Reconstructed human epidermis demonstrates a severe epidermal barrier defect caused by deficiency of corneodesmosin

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Generalized peeling skin disease (PSD) is an autosomal recessive ichthyosiform erythroderma characterized by lifelong patchy peeling of the skin associated with pruritus and atopic disease. Whole-genome linkage analysis identified a homozygous nonsense mutation in *CDSN* encoding corneodesmosin in four affected children, resulting in a premature termination codon, p.Lys59X. Western blot analysis demonstrated complete absence of corneodesmosin in the patients, whereas differentiated keratinocytes of healthy controls showed a strong signal. We generated a three-dimensional model using primary keratinocytes and a collagen matrix populated with fibroblasts, which precisely mimicked the epidermal skin, with regular formation of the basement membrane zone and expression of late differentiation markers such as involucrin and filaggrin. The epidermis equivalents showed normal epidermal barrier activity and enabled us to further analyse its integrity. Test substances were the OECD validated standard compounds for cutaneous absorption studies, caffeine and testosterone. The P_{app} values with caffeine and testosterone were 15.7 x 10^6cm/s and 8.58 x 10^6cm/s in patient models compared to 10.9 x 10^6cm/s and 5.30 x 10^6cm/s, respectively, in control models ($p<0.01$). To further characterize the importance of corneodesmosin, we knocked down *CDSN* in normal keratinocytes used for model preparation. We observed ablation of corneodesmosin throughout the cultivation of the model, which replicated the patient epidermis with an increased granular layer and strong hyperkeratosis. Thus we demonstrate that lack of corneodesmosin accounts for the predisposition to atopy and propose PSD as a new model for atopic disorders induced by a disturbed and ineffective epidermal barrier.

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A new *LAMA3* mutation in 2 patients with junctional epidermolysis bullosa

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The *LAMA3* gene encodes the α3 chain of laminin 5 and is mutated in 9% of junctional epidermolysis bullosa (JEB). Here we report 2 patients with non-Herlitz JEB and a novel *LAMA3* mutation. Two Pakistani brothers with consanguineous parents presented with skin fragility and bullae starting after birth and regressing completely at adolescence except for a pretibial plaque. Enamel hypoplasia and nail dystrophy of hands and feet were also noted. Skin histology showed a subepidermal bulla, and immunomapping revealed a substantial reduction of laminin 5. Electron microscopy showed a cleavage in the lamina lucida and focally in basal keratinocytes, with abnormal and scarce hemidesmosomes. Sequencing of *LAMA3* gene revealed in both patients a homozygotous mutation in exon 19 (c.2444 T>C) leading to the substitution of phenylalanine 815 by a serine. This variation has not been found in 100 other individuals, ruling out that it could be a polymorphism. It is probably deleterious because a small hydrophilic amino acid replaces a large hydrophobic amino acid and because Phe 815 is highly conserved among species. This novel mutation is located in the C-terminal globular subdomain LG1 and could be pathogenic by disrupting the secretion of laminin 5.

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Association of the chromosome 11q13.5 variant with atopic eczema in Austrian patients

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Recently, the two single nucleotide polymorphisms rs7927894 on chromosome 11q13.5 and rs877776 within the region of the hornerin gene were identified as novel susceptibility variants for atopic eczema in the first genome wide association study in atopic eczema. The aim of our study was to evaluate the influence of these two genetic variants on atopic eczema and disease-related phenotypes in the Austrian population. 275 atopic eczema patients and 228 controls were genotyped for the two variants rs7927894 and rs877776 by using Taqman based allelic discrimination assays. When comparing patients with controls we found a significant association of the rs7927894 variant on chromosome 11q13.5 with atopic eczema (p= 0.005; OR: 1.81; CI 1.19-2.75). Subgroup analysis revealed no significant association of rs7927894 with age of onset, concomitant asthma and allergic rhinoconjunctivitis, total serum IgE levels and family history of atopy. The analysis of the rs877776 variant showed neither a relevant difference in the allelic distribution between patients and controls nor a statistical significant association with any of the analyzed atopic eczema phenotypes. In summary our data show a statistical significant association of the rs7927894 variant on chromosome 11q13.5 with atopic eczema but not with other disease-related phenotypes. Therefore, we assume that the rs7927894 single nucleotide polymorphism selectively influences eczema development. More investigations in distinct study populations are needed to assess the role of this interesting polymorphism in atopic eczema.

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Exon 87 skipping of the *COL7A1* gene in dominant dystrophic epidermolysis bullosa

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Dystrophic epidermolysis bullosa (DEB) is a rare inherited blistering disorder resulting from mutations in the *COL7A1* gene, which encodes type VII collagen, the major component of anchoring fibrils at the dermal-epidermal junction. We here report a Japanese girl with dominant DEB (DDEB) with a recurrent splice-site mutation in *COL7A1*. The patient initially presented with scattered blistering and erosions healed with pruritic scars on the extremities, although these features markedly decreased by 18 years of age. The clinical manifestations of the present case were very mild. Direct nucleotide sequencing of genomic DNA from the patient disclosed a heterozygous G to A transition at nucleotide c.6900 that does not lead to an amino acid change in p.Gln2300 residue. RT-PCR, using RNA extracted from skin biopsy, showed, in addition to a normal transcript, a short in-frame mutant transcript with exon 87 skipping and other minor mutant transcripts. This may account for the mild phenotype. Five different *COL7A1* mutations leading to exon 87 skipping have been previously reported in rare forms of DEB: four DDEB pruriginosa and one pretibial DDEB. Therefore, exon 87 skipping in *COL7A1* seems to cause phenotype of DDEB pruriginosa. However, the present case was clinically quite different from DDEB pruriginosa. Modified genes and/or environmental factors might influence the phenotypic variations in DEB. Genetic counseling in such cases is fraught with difficulty. We should be aware of the clinical diversity in DEB and offer appropriate genetic counseling.

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Localization of psoriasis candidate gene product CCHCR1 at centrosomes

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CCHCR1 (coiled-coil alpha-helical rod protein 1) is a plausible PSORS1 candidate gene whose role as a psoriasis effector gene is unsettled although it resides in the region of strongest associations even in GWA studies. CCHCR1 has different expression pattern in psoriatic vs. healthy skin and is suggested to have a role in cell proliferation. We have recently cloned a novel variant of CCHCR1 (isoform 1) in which the N-terminal domain is 89aa longer than in the previously studied form (isoform 3). The SNP (rs3130453) that controls formation of the alternative isoforms has now been genotyped using samples from 435 Finnish and Swedish psoriasis families. The allele for the variant 3 shows preferential transmission from heterozygous parents to affected offspring ($p<10^{-5}$). Here we show by immunofluorescent staining that both CCHCR1 isoforms have a centrosomal localization. They co-localize with a centrosome marker g-tubulin but not with microtubule proteins α- and β-tubulin. However, the localization of CCHCR1 is partially dependent of microtubules indicated by Nocodazole treatment that disrupts the microtubules. After the treatment CCHCR1 can still be observed in association with centrosomes but also as granules in the cytoplasm. Stable over-expression of CCHCR1 in HEK293 cells results in nuclear aberrations. As the centrosomes have a role in organization of the microtubules, cell cycle and division, the novel localization of CCHCR1 provides a connection to the abnormal proliferation and offers a link to the cellular pathways that are altered in psoriasis.

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Identification and characterization of regulatory networks contributing to psoriasis susceptibility

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The non-lesional skin of psoriatic patients possess some inherent characteristics that are already present in the otherwise healthy-looking skin, and make them prone to develop the typical psoriatic symptoms in response to various stimuli. Our major aim was to identify and characterize genes and proteins that are differentially expressed in uninvolved psoriatic and normal epidermis, and to discover regulatory networks that are responsible for these differences. To this end, a cDNA microarray experiment was performed where we compared the gene expression profile of 4 healthy and 4 psoriatic uninvolved epidermis samples upon T cell lymphokine induction in organotypic cultures. The cDNA microarray experiment identified 57 annotated genes with known functions, and another 11 expressed transcripts with unknown functions that were differentially regulated in psoriatic uninvolved and healthy epidermis following T cell lymphokine induction. 11 of the annotated genes have already been implicated in the pathogenesis of psoriasis. Pathway analysis using various software packages and public databases suggests that many of the identified genes play an important role in cellular processes; regulation of cell morphology, development and cell death, and also in the metabolism of small molecules and lipids. Our results can help to uncover basic mechanisms of this complex disease and lead to novel therapeutic approaches to prevent disease development.

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The role of the 8.1 ancestral haplotype in the pathogenesis of acne vulgaris

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Acne is a common inflammatory disease of the pilosebaceous follicles. As genetic predisposition clearly plays a role in its pathogenesis, earlier we investigated the function of single nucleotide polymorphisms (SNP) of primary cytokine genes (IL1A, TNFA). Functional polymorphisms of both genes resulting altered mRNA and protein functions were identified as genetic predisposing or protective factors. Among these the TNFA 308G>A SNP was found to be a predisposing factor, and showed interesting gender differences in our study population. In order to investigate if this association is truly depending on the function of the SNP itself, or a result of other closely linked polymorphisms on the short arm of chromosome 6, we decided to extend our studies, and analyze the role of the 8.1 ancestral haplotype (AH) in acne. For that the distribution of known marker polymorphisms of the 8.1 AH (TNFA -308G>A, LTA +252A>G, HSP70-2 +1267A>G and AGER -429 T>C) are currently being investigated in case-control studies using the DNA collection of 126 controls and 226 acne patients by PCR-based methods.Preliminary results suggest that the carrier frequency of the 8.1 AH tends to be higher in patients, suggesting a role of this haplotype in the genetic predisposition of acne vulgaris.Carriage of the 8.1 AH has been shown to cause immune system dysfunctions, affecting especially the early stages of cellular activation, altering the balance of various cytokines. These results could help us to understand the genetic and molecular nature of individual differences in acne vulgaris.

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Cockayne syndrome mutations in TFIIH impair RNA polymerase I transcription

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Cockayne syndrome (CS) is a devastating childhood disease with signs of premature aging and early death. It can be caused by the recessive mutation in five different genes. The gene products are involved in DNA repair and basal transcription mechanisms. To define the critical function altered in the severe disease we are investigating the function of CS-genes in transcription of RNA polymerase I. Analysis of RNA polymerase I transcription in XPB/CS and XPD/CS cells reveal a severe reduction of ongoing transcription *in vivo*. Nuclear extracts from these cells show a distinct impairment of RNA polymerase I transcription *in vitro* that can be overcome by addition of affinity-purified TFIIH. "Immobilized-template" experiments demonstrate that TFIIH associates with the initiation complex of RNA polymerase I and leaves the promoter with the polymerase after start of transcription. Binding of TFIIH to the rDNA promoter is significantly reduced in nuclear extracts of TFIIH/CS cells. Density-gradient centrifugation analysis and co-immunoprecipitation experiments revealed that TFIIH associates with the enzyme after one round of transcription, indicating that the function of TFIIH in RNA polymerase I transcription differs from its role in RNA polymerase II transcription. Moreover, ChIP analysis revealed that TFIIH associates in addition to the rDNA promoter with gene-internal sequences of the rDNA, implying a role of TFIIH in transcription elongation of RNA polymerase I. TFIIH/CS cells have less ribosomes per cell, indicating that a possible reduction of the protein synthesis capacity of the cells could contribute to the premature aging of Cockayne syndrome patients.

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The first *COL7A1* locus specific online mutation database

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Dystrophic Epidermolysis Bullosa (DEB) is a genodermatose characterized by spontaneous or mechanically induced bullous formation. The *COL7A1* gene, which defects cause DEB, spans 32 kb and comprise 118 exons. There are about 520 published mutations in the gene, although the real number remains unknown due to lack of the possibility to exchange the data between specialists worldwide. In this abstract we present *COL7A1* mutation database, which is the first tool aimed at gathering, integrating and sharing the data concerning genotype and phenotype details. There about 700 locus specific mutation databases dedicated to other genes/diseases, which are extremely useful in molecular diagnostics and research. The database project is in agreement with HGVS guidelines. The system is free of charge. The data can be submitted by every logged in user after being revised by curator. Information deposed in db include among others: mutation name (traditional and HGVS's nomenclature), graphic view of mutations and SNPs (DNA, RNA and protein level), identification method, pattern of inheritance, and clinical summary in accordance with the Third International Consensus Meeting on Diagnosis and Classification of EB and statistic data. Majority of information gathered in the system is based on "yes/no" answers thus searching and statistic tools are expanded. *COL7A1* mutation database was designed in order to unify and simplify molecular diagnostics and to collect multicenter genotype-phenotype data, which will, as we hope, serve in the future to find correlation between them.

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New insight into genotype-phenotype correlation in recessive dystrophic epidermolysis bullosa with a range of *COL7A1* missense mutations.

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Most cases of recessive dystrophic epidermolysis bullosa (RDEB) are caused by compound heterozygous *COL7A1* mutations, therefore it is usually difficult to assess the clinical phenotype and function of each mutant type VII collagen (COL7) derived from different mutations; particularly missense mutations which produce a range of aberrant COL7. To clarify this question, genotype-phenotype correlation was analyzed in 12 RDEB patients specifically having common *COL7A1* mutations (c.5818delC or c.6573+1G>C) on one allele, which is known to lead to complete loss of COL7 expression through a premature termination codon (PTC). On the other allele, the patients have a range of other *COL7A1* mutations, including 5 missense mutations, 3 nonsense mutations, 2 deletion mutations, and 2 splice-site mutations. In patients having nonsense, deletion or splice-site mutation with PTC (c.5818delC or c.6573+1G>C), COL7 expression level detected by immunofluorescence was well-correlated with clinical severity. In contrast, there was no correlation between clinical severity and COL7 expression levels in patients having missense mutations with PTC (c.5818delC or c.6573+1G>C). COL7 immunostaining was slightly reduced (R2063W, G2316R, G1815R, G2623S or R1957Q), however, clinical severity was varied according to respective mutations, severe (R2063W or G2316R), moderate (G1815R or G2623S), and mild (R1957Q) RDEB phenotypes. These results can show the functionality of each mutant COL7 produced by a specific missense mutation in RDEB patients.

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Upregulation of HERV-H env gene in *psoriasis vulgaris*

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The influence of Human Endogenous Retroviruses (HERVs) on autoimmune disorders is a widely discussed matter. The pathomechanism of *psoriasis vulgaris* is apparently related with those remnants of ancient viral infections. Here we investigate changes in RNA levels of a HERV-H group member with an ORF coding for a 62 kDa potential translational product. The expression levels of this particular sequence seem to vary significantly between different autoimmune disorders including both increasing and decreasing trends. Total RNA was isolated from PBMCs of 26 patients suffering from *psoriasis vulgaris* and 20 healthy volunteers, following reverse transcription to cDNA with the use of random hexamer primers. The transcription levels of HERV-H envelope gene (101bp, HERV ID: 10816, 2q24.3) was assessed by means of Real-Time PCR and normalized to GAPDH. The data was interpreted basing on three types of analyses. Median, windsorised mean and the Two Independent Sample Wilcoxon-Mann-Whitney (U) test were calculated. The Two-Sided P-Value of the Two Independent Sample Wilcoxon-Mann-Whitney (U) test was 0.005, which supports the claim of the groups differing statistically. The windorised mean of cDNA copies was 6.70-fold higher for the investigated psoriatic patients than for the control group. Median value was 2.12-fold higher for the psoriasis group, which is similar to our previously reported upregulation of the same sequence in *pepmhigus vulgaris* by the factor of 2.87. The results indicate an apparent increase of the HERV-H envelope RNA level in *psoriasis vulgaris*. The reason for such variation of expression of this sequence in different autoimmune diseases remains unclear.

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Gene Mapping Study of Constitutive Skin Color in a Genetically Isolated Population

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To elucidate the responsible genes governing constitutive skin color, we measured the extent of skin pigmentation in buttock, the sun-unexposed area for the life time, and conducted a gene mapping study on skin color in genetically isolated population composed of 344 individuals from 59 families who live in Dashbalbar, Dornod Province, Mongolia. Through the linkage analysis of 1039 short tandem repeat (STR) microsatellite markers, we found novel genomic region regulating constitutive skin color on 11q24.2 with an LOD score of 3.39. Moreover, we found other five candidate regions controlling intrinsic skin color. To test further association in regions of linkage, we selected chromosome 11, 17, and 6 (maximum LOD score > 2.5), and in each chromosome, high linkage peak regions (LOD score >1.5) were analyzed. On linkage region of chromosome 11, we identified 19 significant SNPs ($p < 9.29 \times 10^{-6}$). Further we found two ($p = 9.74 \times 10^{-7}$) and one ($p = 2.00 \times 10^{-6}$) significant SNPs on linkage regions of chromosome 17 and chromosome 6, respectively. In that the strongest locus of linkage on 11q24.2 harbors and significant SNP is located adjacent to ST3GAL4, we suggest ST3GAL4 as the novel candidate gene responsible for controlling constitutive skin color. Taken together with our linkage analysis and association study, other candidate genes are DRD2, MPZL3, BRIP1 and ZBTB17.

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Immortalized keratinocytes from Epidermolytic Ichthyosis-patients reproduce the disease phenotype in vitro

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Epidermolytic Ichthyosis (EI) is a rare, autosomal, dominantly inherited skin fragility disease caused by mutations in keratin genes. The phenotypes of the disease are heterogeneous and characterized by frequent blistering, redness and scaling and hyperkeratosis of the stratum corneum (EI). While the etiology is known, model systems that display structural defects in EI are needed for studies of cellular effects of mutations and development of putative therapies. We established and characterized immortalized keratinocyte lines in serum-free medium from three EI-affected patients; EH11 (K1_p.Val176_Lys197del), EH21 (K10_p.156Arg>Gly), EH31 (K10_p.Leu161_Asp162del). Keratinocytes were cultured in the presence of low or high Ca^2+ content in the medium. Cells were either heat stressed, in the presence or absence of chemical chaperones. The cells were stained with an antibody recognizing K1 and the presence of keratin aggregates were assessed by immunofluorescence. Furthermore, organotypic epidermis established on cell culture inserts were also exposed to heat stress. Under normal culture conditions only cells from a severe EI-phenotype (EH31) showed keratin aggregates. Heat stress induced aggregates in all EI cell lines which were reduced by pre-treatment with chemical chaperones. Heat stress exposure of organotypic EI epidermis resulted in suprabasal cytolysis.The results indicate that *in vitro* cultured EI-keratinocytes can recapitulate some of the abnormalities observed *in vivo* and could help for the understanding of the molecular pathomechanisms of EI as well as gives new ideas about therapeutic options.

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Tumour microenvironment is implicated by dermal fibroblast expression profiling in the development of aggressive squamous cell carcinoma in patients with recessive dystrophic epidermolysis bullosa

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Patients with recessive dystrophic epidermolysis bullosa (RDEB) suffer from skin blistering caused by mutations in the gene encoding type VII collagen and are predisposed to develop squamous cell carcinoma (SCC) at an early age. The SCCs developed in these patients are aggressive - almost 80% of the patients will die from metastatic SCC within 5 years. As we have previously demonstrated very few genetic differences between RDEBSCC and non-RDEBSCC primary keratinocyte cultures, we proceeded to examine the contribution of the dermal fibroblast in the development of RDEBSCC. Global gene expression analysis was performed using the Agilent platform and the data collected analyzed in Genespring GX. Primary fibroblast cell cultures were grouped into normal-F (n=3), RDEB-F (derived from SCC-free RDEB patients n=4), SCC-F (derived from UV-induced SCC n=5) and RDEBSCC-F (n=4). Based on one-way ANOVA statistical testing, 1098 genes transcripts were significantly differentially expressed among the four groups. Hierarchical clustering analysis separated all three disease states from normal-F, with RDEBSCC-F clustering furthest away. Interestingly, this analysis was unable to separate SCC-F and RDEB-F, suggesting that RDEB and SCC dermal fibroblasts share a similar genetic profile in culture and that the RDEB dermal microenvironment may be conducive to tumourigenesis. Furthermore, identification of RDEBSCC-F as being distinct from RDEB-F and the most dysregulated among the three disease states indicates that the transformation from RDEB to RDEBSCC fibroblasts may be crucial in accounting for the aggressive nature of SCC observed in RDEB patients.

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Effect of synthetic retinoids on keratin aggregate formation in immortalized cells from Epidermolytic Ichthyosis patients

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Epidermolytic Ichthyosis (EI) is an autosomal dominant skin fragility disorder caused by mutations in genes coding for keratin 1 and 10. Despite the knowledge of its etiology, pharmacological treatments are limited to synthetic retinoids and calcipotriol. We have evaluated the effect of retinoic acid receptor (RAR)-selective retinoids in an in-vitro model for EI in hope of finding new therapies. Three immortalized cell lines from EI patients; EH11 (K1_p.Val176_Lys197del), EH21 (K10_p.156Arg>Gly), EH31 (K10_p.Leu161_Asp162del) with varying clinical severity (EH31>EH21>EH11), and NKc21 cells (wildtype) were used. The cells were grown as monolayer cultures, differentiated by exposure to PD153035 (an epidermal growth factor receptor inhibitor) and treated with 1μM of CD336 (RAR-α agonist), CD2314 (RAR-β agonist), CD437 (RAR-γ agonist) and CD367 (pan-RAR agonist), respectively. The cells were subjected to a short period of heat stress and stained with a K1 antibody and keratin aggregates were monitored by immunofluorescence microscopy. Cytoplasmic keratin aggregates after heat shock was observed in all cell lines except NKc21. Without retinoid treatment 12% of EH31 cells exhibited keratin aggregates, which was reduced to 3-7% when pre-treated with retinoids. No retinoid were significantly more potent than the other. Heat stressed EH21 and EH11 cells showed less aggregates than EH31 (6% respectively), and the effect of retinoid treatments was almost similar as in EH31 cells. In conclusion, isoform-selective RAR-agonists reduced the formation of keratin aggregates in heat-shocked immortalized EI cells. This effect on keratin aggregation could in parts explain the beneficial effects of retinoid therapy *in vivo*.

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Gene expression profiles are differently affected by pimecrolimus and betamethasone in lesional skin in atopic dermatitis

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Topical corticosteroids and calcineurin inhibitors are effective in the treatment of atopic dermatitis (AD) but differ in their side effects. A study in AD patients has demonstrated that the repair of a disrupted skin barrier is impaired by betamethasone valerate (BM) but not by pimecrolimus. The present study elucidates the mode of action of topical BM and pimecrolimus cream in AD. These two components were subject for gene expression profile analysis in lesional AD skin after topical treatment. BM resulted in a significant reduction of mRNA levels of genes encoding for markers for dendritic cells, T cells, cytokines, chemokines and serine proteases, whereas pimecrolimus exerted minor effects only. This corroborates the clinical finding that BM reduces inflammation more effectively than pimecrolimus. Genes encoding molecules important for skin barrier function were differently affected; both BM and pimecrolimus normalized the expression of filaggrin and loricrin. BM, but not pimecrolimus, significantly reduced the expression of rate-limiting enzymes for lipid synthesis and the expression of involucrin and small proline-rich proteins, which covalently bind ceramides. This may be the reason for the observed lack of restoration of functional stratum corneum layers after BM treatment. The gene expression profiles are consistent with previous findings that corticosteroids may exert a more potent anti-inflammatory effect but may impair the restoration of the skin barrier. Hence, this study confirms on a molecular level the concept that corticosteroids are a suitable treatment for acutely exacerbated AD, but that pimecrolimus is preferable for long-term treatment and stabilization.

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Comparative quantitation of proteome alterations induced by loss-of-function of kindlin-1 in Kindler syndrome

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Kindler syndrome is an autosomal recessive genetic condition involving severe disruption of the dermal epidermal junction characterized by skin fragility, skin atrophy, photosensitivity, and carcinogenesis. It results from loss-of-function of kindlin-1, an epithelial-specific phosphoprotein. As an intrinsic component of the focal adhesion complex and an interactor of integrins, kindlin-1 specifically regulates cell attachment to the extracellular matrix and influences cell shape and migration by controlling lamellipodia formation via Rho-GTPase signaling. Uncharacterized phosphorylation sites of kindlin-1 indicate its involvement also in other signaling pathway. Here, we used a comparative proteomic approach to address molecular interactions of kindlin-1 and to uncover hitherto unknown functions. We utilized stable isotope labeling in cell culture (SILAC) to reveal quantitative proteomic differences of Kindler Syndrome cells, as compared to primary normal human keratinocytes (pNHKs). To study skin diseases on a proteomic level in relevant model systems we also evaluated proteome changes of freshly isolated cells during culture conditions, compared the proteome of the respective primary cells to a softly immortalized keratinocyte line and to HaCaT (Human adult low Calcium high Temperature) cells. We found minor proteomic alterations in pNHKs in culture and in softly immortalized NHKs indicating aging and increased proliferative capacity, respectively. HaCaT cells and Kindler Syndrome cells showed major proteomic alterations relating to the cytoskeleton, cell junctions and matrix adhesion.

499 [Oral 040]

ARNT controls the expression of epidermal differentiation genes through EGFR- and HDAC-dependent pathways

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Previously we have demonstrated a role for the aryl hydrocarbon receptor nuclear translocator (ARNT - a key factor in hypoxia and toxic responses) in regulating expression of differentiation-associated genes (EDC complex) and EGFR ligands in mouse epidermis *in vivo*. In order to examine the potential role of ARNT in epigenetic and EGFR-mediated control of differentiation in human keratinocytes, we used stable shRNA-mediated suppression and plasmid-mediated transient over-expression of ARNT in N-TERT and HaCaT cells. Expressional studies of ARNT-depleted keratinocytes show an upregulation of differentiation markers such as keratins 1 and 10, filaggrin and loricrin (up to 70 fold mRNA). Furthermore, ARNT-depleted keratinocytes show significantly reduced proliferation compared to control cells. ARNT deficiency also results in the downregulation of EGFR ligands such as TGFα, EGF and AREG and in consequent suppression of EGFR phosphorylation. ARNT-depleted cells also show an increase in total HDAC activity and in the protein levels of HDAC1 and HDAC3. Conversely, ARNT over-expressing cells are characterized by prominent depletion of HDAC3 level. Finally we demonstrate that the increased expression of differentiation markers mediated by ARNT deficiency can be abolished by TSA and AG1478 (HDAC and EGFR inhibitors respectively). These results further support a significant role for ARNT in the regulation of epidermal differentiation and demonstrate at least two different mechanisms implicated in this regulation - modulation of EGFR pathway and control of HDAC activity. Possible interactions between these two ARNT-dependent pathways require further investigation.

500 [Oral 041]

Activation of wnt5a in epidermis triggers massive hypersensitivity to double-stranded RNA

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Previously, we have shown that Wnt5a is expressed in the basal layer of the epidermis, that it is highly upregulated in psoriasis and that, functionally, Wnt5a causes hypersensitivity to type I interferon *in vitro*. This suggests that Wnt5a contributes to the upregulation of interferon responsive signalling in psoriasis. We have now established a transgenic mouse model to study the role of Wnt5a in adult skin *in vivo*. To avoid the pre-natal lethality associated with Wnt5a knock-out or overexpression, we developed a doxycycline inducible system, in which full length Wnt5a is controlled by a keratin 14 promoter. In the absence of doxycycline, transcription of the transgene is feedback-repressed by a KRAB repressor fused to a tet-transactivator protein encoded in a second cistron, resulting in tightly regulated transcription of the transgene. In transgenic, as well as in wild type mice, endogenous Wnt5a is constitutively expressed in hair follicles. However, only the additional induction of Wnt5a in the epidermis, enforced through doxycycline administration, causes a massive hypersensitivity toward the double-stranded RNA model compound polyIC, which triggers the release of endogenous interferon. This leads to rapid release of lethal concentrations of inflammatory cytokines IL-6 and TNF-α. Thus, it appears that the upregulation of Wnt5a seen in psoriasis is sufficient to trigger significant upregulation of interferon signalling characteristic of the disease.

501 [Oral 013]

SIRT1 counteracts STAT3 activity and STAT3-dependent gene expression induced by IL-22 in human keratinocytes

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IL-22 is a cytokine highly released by T helper 22 and 17 lymphocyte subsets having a pivotal pathogenetic role in psoriasis. In psoriatic skin, IL-22 is responsible for the altered proliferative and differentiative processes observed in the epidermis, and, induces antimicrobial peptides as well as chemokines. The majority of the IL-22-induced effects are mediated by signal transducer and activator of transcription (STAT)3, whose activity is proportional to acetylation in lys685 residue, which is, in turn, indispensable for the phosphorylation in tyr705 residue. Lys685 acetylation of STAT3 depends on acetylation and deacetylation processes executed by p300 acetylase and sirtuin (SIRT)1 deacetylase enzymes, respectively. Interestingly, SIRT1 has been described as important inducer of keratinocyte differentiation. The aim of this study was to investigate on the possible involvement of SIRT1 in regulating IL-22-induced effects on keratinocytes. We found that SIRT1 opposes STAT3 activation and, thus, the STAT3-dependent effects of IL-22 on proliferative, inflammatory and differentiative processes in keratinocytes, by deacetylating STAT3 and reducing STAT3 tyr705 phosphorylation. In addition, although SIRT1 levels are similar in both healthy and psoriatic cultured keratinocytes, they are reduced in psoriatic lesions, especially in areas close to the inflammatory $CD3^+$ infiltrate. Of note, IFN-γ inhibited SIRT1 expression and, concomitantly, enhanced STAT3 lys685 acetylation, and rendered keratinocytes more responsive to IL-22 effects, in terms of induction of STAT3 tyr705 phosphorylation. These data suggest that in psoriasis the STAT3-dependent IL-22 signalling and effects on keratinocytes can be amplified by IFN-γ through a direct inhibition of SIRT1 expression and function.

502 [Oral 020]

MicroRNA-21 mediates the effects of the BMP signalling pathway in keratinocytes

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Bone morphogenetic proteins (BMPs) play essential roles during skin development, postnatal tissue remodelling and tumorigenesis. BMP signaling inhibits the initiation phase of the hair follicle development, as well as operates as tumour suppressor in adult skin. To explore whether some of these inhibitory effects of the BMP signalling are mediated by micro-RNAs, *miRNAs were isolated from the* primary mouse epidermal keratinocytes treated with BMP4 *and processed for analysis of global miRNA expression using the microarray approach. Microarray and real-time PCR analysis revealed BMP4-dependent changes in the expression of distinct miRNAs including miR-21, which expression* was strongly decreased in the keratinocytes after BMP4 treatment. However, miR-21 expression was substantially higher in the skin of transgenic mice over-expressing BMP antagonist Noggin under K14 promoter. By in situ hybridisation, miR-21 expression was observed in the epidermis and hair follicle epithelium in mouse anagen VI skin. In K14-Noggin skin, miR-21 expression was seen in the peripheral portion of the tumours containing low differentiated, proliferative cells. Transfection of the keratinocytes with miR-21 mimic revealed existence of two groups of the BMP target genes, which are differentially regulated by miR-21. Some of the BMP target genes (i.e., PTEN) were down-regulated by miR-21, whilst the others of others (i.e., Msx2) were not affected by miR-21. Thus, our study establishes a novel mechanism involved in the realisation of the BMP effects in the skin and suggests micro-RNAs as important regulators modulating the effects of growth factor signalling pathways on skin development and tumorigenesis.

503 [Oral 002]

The survival of DNA damaged cancer cells is ensured by mTOR-dependent phosphorylation of Sirtuin 1

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Premature senescence (PS) is an irreversible stress condition in which damaged cells are unable to proliferate. PS induction in cancer cells by DNA-damaging chemotherapeutic drugs is potentially a novel approach for controlling tumor growth. However, because prematurely senescent cancer cells are viable and inherently less sensitive to DNA-damaging drugs, a major drawback with this approach is that PS tumor cells can re-enter the cell cycle and ultimately lead to tumor recurrence. Here, we identified a novel mechanism that regulates the survival of DNA damage-induced prematurely senescent squamous cell carcinoma (SCC) cells. Treatment of human SCC cells with a plant polyphenol resveratrol (RES) or anticancer agent such as anthracycline antibiotic doxorubicin (DOX) induces DNA damage-dependent PS induction. RES/DOX-induced PS involves the nuclear translocation of mTOR, followed by the mTOR-dependent inhibitory phosphorylation of SIRT1 at S47. This phosphorylation-dependent inhibition of SIRT1 deacetylase activity leads to the concomitant upregulation of an anti-apoptotic Bcl-2 family gene, *Bfl-1/A1*, via increased acetylation of p65/RelA NF-kB. SIRT1 S47 phosphorylation requires physical interaction between the mTOR complex and SIRT1. This novel SIRT1 phosphorylation occurs predominantly in human recurrent/metastatic SCCs, suggesting a role in tumor progression. RES/DOX-induced PS SCC cells eventually re-enter the cell cycle, and rapamycin-mediated mTOR inhibition can subsequently block the re-growth of PS SCC cells. Rapamycin treatment sensitizes UVB-induced DOX-resistant SCCs for apoptosis in mice. Our data indicate that the mTOR inhibition of SIRT1 fosters the survival of DNA damage-induced PS SCC cells, and that targeting mTOR/SIRT1 signaling may be effective in treating apoptosis-resistant cancer cells.

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Deletion of SOCS3 in the epidermis causes impaired skin wound healing in vivo

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STAT3 is a latent cytoplasmic protein that transduces signals to the nucleus upon stimulation with IL-6, EGF and many other growth factors. STAT3 plays critical roles in biological function such as cell proliferation, migration and so on. Suppressors of cytokine signaling (SOCS) regulate the strength of cytokine signals. SOCS3 is strongly induced by a variety of cytokines and other stimulators. The suppressive effect of SOCS3 has been shown to be relatively specific to STAT3. Therefore we investigated the role of SOCS3 in epidermal keratinocytes. Since germline targeting of the SOCS3 gene resulted in embryonic lethality, we generated keratinocyte-specific SOCS3-deficient mice (KS-SOCS3-/-mice) using Cre/loxP technology in combination with the keratin 5 promoter. KS-SOCS3-/-mice were viable and their skin appeared normal at birth. Previously we have reported KS-SOCS3-/-mice develop psoriasis-like skin phenotype. In this study we investigated whether wound healing was affected in KS-SOCS3-/-mice. A cutaneous wound was made on the backs of 20 week-old mice using a 6-mm punch biopsy and wound area was measured. Wound closure was markedly impaired in KS-SOCS3-/- mice compared to wild-type mice (25 and 55% on day 3, 64 and 86% on day 5, 82 and 92% on day 7 in KS-SOCS3-/- and wild-type mice, respectively). Histological analysis of skin lesions showed a hyperproliferative epidermis at leading edges and neutrophil infiltration. These results demonstrate an imbalance of SOCS3-STAT3 pathway results in delayed wound healing.

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Insulin resistance of keratinocytes contributes to the manifestation of the psoriatic phenotype

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Besides insulin's well known effects on classic metabolic tissues, such as liver, muscle, and adipose tissue, several reports suggest a role in non-metabolic tissues such as the skin. Psoriasis patients often display signs of systemic insulin resistance. Therefore we examined the role of insulin signaling in the skin and whether a disturbed insulin response contributes to the development of the psoriatic phenotype.Psoriatic patients show locally (dermal) and systemically elevated levels of inflammatory cytokines such as IL-1b, which is known to confer insulin resistance in metabolically active tissues. Using HaCaT cells we found that IL-1b transiently activates the Akt/PKB cascade. This effect is not only mediated via PI3-K but also via p38 MAPK, IKK and JNK. On the other hand we could show that chronic IL-1b treatment can indeed induce insulin resistance in keratinocytes that is mediated via p38 MAPK. We also found that insulin induces expression of cytokeratin 10, a marker of terminal differentiation, suggesting that insulin drives differentiation of healthy keratinocytes. This effect is blunted under chronic Il-1b treatment, resembling the inflammatory situation in psoriasis, where keratinocytes differentiation is abnormal and shifted towards hyperproliferation. Using immunohistology we found that in patients, components of the PI3-Kinase signaling pathway show hyperactivation compared to control skin. We therefore assume that IL-1b interferes at the intersection between insulin dependent differentiation and proliferation and that this mechanism contributes to the development of the disease's pathology. Thus, controlling correct insulin signaling in the skin might represent a novel anti-psoriatic strategy.

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Valrubicin activates PKCα and inhibits phosphorylation of p38 MAPK

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Valrubicin, a second-generation anthracycline, was recently shown to have a beneficial effect in animal models of psoriasis and skin cancer; disorders both characterised by hyper-proliferative keratinocytes and by increased inflammation.The aim of the present study was to investigate the effect of valrubicin on two separate signalling pathways: PKCα and p38 MAPK, respectively a proliferation and an inflammation pathway. Signalling through protein kinase C (PKC) is suggested as valrubicin structurally resembles diacylglycerol, a natural PKC ligand; moreover PKCα is located in the suprabasal layers of the epidermis and is important for keratinocyte growth. Signaling through p38 MAPK is investigated as it plays a pivotal role in inflammation. The effect of valrubicin on activation of PKC was evaluated by its translocation from the cytosol to the plasma membrane and of p38 MAPK by its level of phosphorylation. The study was carried out in HaCaT-cells and the effect of valrubicin visualized by western blotting. Valrubicin stimulation increased the levels of PKCα in the plasma membrane mirrored by a decrease in the cytosol indicative of translocation and thus activation of PKCα. Activation peaked after two minutes and returned to basal level after two hours; the rapid response suggests a direct effect of valrubicin binding. Additionally, valrubicin decreased the level of phosphorylated p38 MAPK indicative of decreased activation. In conclusion, the observed increase in PKCα activation and decrease in p38 MAPK activation may help explain valrubicin's mode of action in reducing both proliferation and inflammation observed when treating psoriasis and skin cancer.

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Overexpression of LEDGF/DFS70 induces IL-6 via p38 activation in HaCaT cells, which resembles psoriasis

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Lens epithelium-derived growth factor (LEDGF)/dense fine speckles 70 kDa protein (DFS70) is a transcription cofactor that enhances growth and is overexpressed in various cancers. In the epidermis, LEDGF/DFS70 localizes to the nucleus of keratinocytes (KCs) in the basal layers and to the cytoplasm of cells in the upper layers. However, the biological and pathological relevance of LEDGF/DFS70 in the epidermis is virtually unknown. Compared to a normal epidermis, we detected strong nuclear staining of LEDGF/DFS70 in the spinous layer as well as the basal layer of the epidermis of psoriatic skin. To investigate the roles of LEDGF/DFS70 in the epidermis of psoriatic skin, we generated HaCaT cells that constitutively express enhanced green fluorescence protein (EGFP)-LEDGF (EGFP-LEDGF-HaCaT) or EGFP alone (EGFP-HaCaT) as a control. EGFP-LEDGF-HaCaT cells had increased expression of interleukin-6 (IL-6), which was attenuated by LEDGF-specific RNA interference and the p38-specific inhibitors SB-239063 and SB-203580. Furthermore, EGFP-LEDGF-HaCaT cells had increased expression of S100A7 and S100A9 and decreased expression of filaggrin. These findings are compatible with the expression pattern in psoriatic tissues. Taken together, these results strongly suggest that ectopic expression of LEDGF/DFS70 in KCs could be involved in the pathology of psoriasis vulgaris.

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Interferon (IFN) γ inhibits tumor necrosis factor (TNF) α-induced cutaneous T cell attracting chemokine (CTACK)/ C chemokine ligand (CCL) 27 production from keratinocytes through signal transducers and activators of transcription (STAT) 1 and STAT3 signaling pathways

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CTACK is one of the chemokines indispensable in skin inflammation. Its expression is exclusively limited in epidermal keratinocytes, which characterizes the skin inflammation. TNFα is an inflammatory cytokine playing important roles in varieties of inflammatory diseases including psoriasis and atopic dermatitis, and is reported to induce CTACK production. IFNγ is also a major inducer of inflammation, and known to synergistically enhance the TNFα-induced production of various chemokines. In this study, we investigated the effect of IFNγ on TNFα-induced production of CTACK from keratinocytes and its signaling pathway using the cultured HaCaT keratinocytes and normal human keratinocytes (NHKs) *in vitro*. We stimulated keratinocytes by TNFα with or without IFNγ. TNFα induced, but IFNγ suppressed CTACK production at mRNA and protein levels. TNFα induced CTACK production through nuclear factor κ B (NFκB) as previously reported, but IFNγ did not inhibit NFκB activity. We investigated the effect of STAT1 or STAT3 by utilizing adenovirus vector of dominant negative or wild type STAT1 and 3. Introduction of dominant negative STAT1 partially reversed the inhibitory effect of IFNγ. IFNγ activated STAT3 in keratinocytes and transfection of wild type STAT3 inhibited TNFα-induced CTACK production. Inhibitor of Janus kinases (JAKs) reversed the suppression of CTACK by IFNγ, but dominant negative vector of suppressor of cytokine signalings (SOCSs) did not. From these results, we concluded that IFNγ suppressed CTACK production though activation of STAT1 and STAT3, a novel finding of the effect of IFNγ on keratinocytes.

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A pathological role for the IL-21 - STAT3 axis in Sézary Syndrome

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Sézary Syndrome (SS) is a rare cutaneous T cell lymphoma with malignant CD4+ T cells (SS cells) present in skin, lymph nodes and blood. We previously showed that STAT3 is constitutively activated in SS cells, and that STAT3 inhibition results in apoptosis. Upon *in vitro* culturing of SS cells, STAT3 phosphorylation is lost, indicating that the activation observed *in vivo* is the result of STAT3 activating factors present in the micro-milieu of the malignant cells. The aim of current study is to assess which factors are involved in STAT3 activation in Sézary Syndrome, and to analyze its downstream effects.Stimulation of SS cells with cytokines of the common-gamma chain family showed that STAT3 is strongly activated by IL-21, whereas IL-2, IL-7 and IL-15 mainly activate STAT5. SS cells freshly isolated from peripheral blood from SS patients displayed increased mRNA expression of IL-21 and its receptor, when compared to CD4+ cells from healthy controls and patients with benign erythroderma. In addition, IL-21-expressing neoplastic cells were detected in skin biopsies from SS patients. Stimulation of SS cells by IL-21 resulted in a STAT3 dependent increase in expression of the alpha subunit of the IL-2 receptor. Consequently, SS cells pre-incubated with IL-21 appeared more sensitive to stimulation with IL-2. Thus, our results suggest that autocrine activation of SS cells by IL-21 is involved in the maintenance of constitutive STAT3 activation. In addition, SS cells are sensitized to the T cell proliferation and activating cytokine IL-2 via the IL-21 - STAT3 pathway.

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Basic fibroblast growth factor impairs PPAR-gamma ligand-induced adipogenesis in rat nestin-positive cutaneous stem cells

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Neurosphere-forming nestin-positive skin progenitors (SKP) are easily reachable pluripotential stem cells with a promising potential in regenerative medicine, but a better understanding of the determination processes is required first. In order to know how SKP commit into mesenchymal or neural lineage, we exposed rat SKP to a Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) agonist and/or basic fibroblast growth factor (bFGF). PPAR-γ is a major regulator of adipogenesis in Mesenchymal Stem Cells (MSCs) but it enhances neural differentiation in embryonic midbrain Neural Stem Cells (NSCs). Conversely, bFGF alone inhibits mesodermal differentiation in NSCs while it is known to cooperate with PPAR-γ to induce adipogenesis in MSCs. SKP isolated from rat vibrissae were amplified for a month as described in the literature. For differentiation, cells were exposed to bFGF and/or rosiglitazone (RGZ). After 15 days, almost all cells exposed to RGZ were adipocyte-like cells and only a few were neural-like cells. Compared with bFGF alone, 3.3% (+/- 4.3) of the cell were neurofilament-positive and only few adipocytes were detected (< 1%). Surprisingly, with both bFGF and RGZ, adipogenesis was sharply inhibited since only one third of the cells presented lipid-droplets, while 6.5% (+/- 4.3) of the cells produced neurofilaments, similar to what observed with bFGF alone (Student t test, P-value=0.08). To conclude, these data demonstrate that PPAR-γ is a potent inducer of adipogenesis in SKP but this PPAR-γ pathway is inhibited by bFGF. This result contrasts with the data obtained in MSC and suggests that these two pathways interact differentially in SKP.

ABSTRACTS

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Divergent effect of EGFR- inhibitors on the expression of two functional distinct chemokines in keratinocytes

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Treatment with Epidermal Growth Factor Receptor Inhibitors (EGFRI) in various cancer types does not have the severe systemic side effects usually seen with cytotoxic drugs. Unfortunately, treatment is often associated with severe *cutaneous* side effects, such as a papulo-pustular rash, dry skin and hair abnormalities, resulting in profound impact on the quality of life for the patients.Using ELISA and PCR, we investigated the chemokine- profile of keratinocytes stimulated with EGFR-I (Panitumumab and Cetuximab), EGF (Epidermal Growth Factor), and TNFa with regard to CCL27 (CTACK), that primarily attracts skin prone T cells, and CXCL8 (IL-8) which receptor is readily expressed by T cells and neutrophils. Data are expressed as mean ± (SD).Cultured human keratinocytes treated with EGFR-I, in combination with TNFa, resulted in a 4.3 fold increase (Panitumumab), and a 6.8 times increase (Cetuximab) in CTACK mRNA level, compared with TNFa alone. In contrast the IL-8 expression was regulated down 7.5 fold (Panitumumab) and 6.6 fold (Cetuximab). Similarly, protein synthesis of CTACK increased to 353.4 ±(95.6) pg/ml for Panitumumab, and 236.7 ±(11.2) pg/ml for Cetuximab, compared with 104.1 ±(41.2) pg/ml in un-stimulated cells.Our data shows, that keratinocytes stimulated with EGFRI, induces a chemokine profile with high CTACK, thus making it possible for skin prone T cells to home to the skin. We also show that this is not an unspecific effect, because CXCL8 is decreased. We believe this could play an important role in our understanding of EGFRI´s ability to induce side effects primarily in the skin.

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Up-regulation of Jagged 1 (Notch Ligand) and Hes 1 (Notch Response Gene) correlates with Calcium-induced Differentiation in HaCaT Keratinocytes

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While, Notch-Delta-Jagged signalling plays a key role in cell fate determination and growth control during embryonic development, the role in adult tissues is not so clear. We studied expression (both protein and mRNA) of two receptors (Notch 1 [N1] and Notch 3 [N3]), three ligands (Jagged 1 [J1], Jagged 2 [J2] and Delta 1 [DLL1]) and four response genes (Hes-1, Hes-5, Hey-2 and HeyL) during calcium-induced differentiation of HaCaT keratinocytes. Terminal differentiation was defined using keratin expression (K1, K5, K10, K14) and Ki67 to indicate proliferation. HaCaT keratinocytes were grown in low calcium (0.06 mM) medium and shifted to high calcium (1.8 mM) when 70-80% confluent and cultured for another 3, 6 or 10 days. Protein expression was determined by immunofluorescence or immunoperoxidase microscopy, SDS-PAGE and western blotting using antibodies to K10, K14, Ki-67, N1, N3, J1, J2, DLL1 or Hes-1. Expression levels were estimated by Q-RT-PCR. As HaCaT cells proliferated, K14 mRNA levels increased but in high calcium medium, K14 levels reduced and K10 levels rose 10x by day 6 ($p<0.01$). N3 levels increased 4x by day 3 ($p>0.05$), J1 levels increased 5x by day 6 ($p<0.05$) and Hes-1 expression increased 12x by day 3 ($p>0.001$). Housekeeping gene (human ARP) levels did not significantly change. We conclude that notch signalling via N3, J1 and Hes-1 may be involved in the switch from a basal cell phenotype to a cell committed to terminal differentiation. Knock-down experiments are now in progress to test this hypothesis.

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Stress-induced PRINS gene expression in normal human cultured keratinocytes and HaCaT cells

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Psoriasis is a chronic inflammatory skin disease affecting approximately 2-4% of the population. Recently we described a novel non-coding RNA, PRINS (psoriasis susceptibility related RNA gene induced by stress), that was overexpressed in psoriatic uninvolved epidermis, and its expression was induced by various stress factors in HaCaT keratinocytes. In our recent study, we further investigated the role of PRINS in cellular stress response in HaCaT keratinocytes and primary cultured keratinocytes. We found that PRINS expression was increased in HaCaT cells but not in normal cultured keratinocytes when cells were exposed to cycloheximide and lipopolysaccharide (LPS). To determine whether altered nuclear factor-κB (NF-κB) signaling in HaCaT cells was responsible for the observed differences, we silenced PRINS expression by siRNA in HaCaT cells and normal cultured keratinocytes transfected with an NF-κB responsive element containing luciferase reporter construct. In the transfected cells, we monitored NF-κB signal transduction after LPS treatment. Silencing of PRINS had no effect on LPS-induced NF-κB activity either in HaCaT cells or in primary keratinocytes. This indicates that PRINS may signal independently of NF-κB. Other yet undiscovered cellular processes may lie behind the differences in LPS induced PRINS expression between the two cell types.

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IL-17C is Regulated by a p38 MAPK and NF-κB Dependent Mechanism in Cultured Human Keratinocytes

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We have previously demonstrated that the expression of IL-17C is increased in lesional compared with nonlesional psoriatic skin. However, the precise role of IL-17C in psoriasis remains to be investigated. Thus, the purpose of this study was to identify and characterize the mechanisms regulating the expression of IL-17C. By quantitative PCR (qPCR) we found that the increased expression of IL-17C in lesional psoriatic skin was significantly reduced already 4 days after start of adalimumab treatment, i.e. before clinical and histological improvement was detectable. Immunohistochemical analysis conducted on paraffin-embedded psoriatic skin biopsies demonstrated IL-17C to be localized primarily in the epidermis. In addition, IL-17C was found to be produced by cultured human keratinocytes after stimulation with TNFα, and this production was mediated by a NF-κB and p38 MAPK dependent mechanism as determined by both qPCR and ELISA. Moreover, we identified four NF-κB binding sites in the promoter region of IL-17C and by electrophoretic mobility shift assay we demonstrated an increased DNA binding activity to these sites in cultured human keratinocytes after stimulation with TNFα. Finally, preincubation of cultured human keratinocytes with decoy oligonucleotides containing the identified NF-κB binding sites significantly reduced the TNFα-induced IL-17C expression. We conclude that IL-17C is one of the early gene targets of adalimumab treatment and that IL-17C is produced by human keratinocytes by a p38 MAPK and NF-κB dependent mechanism.

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Investigation of pathogenic roles of karyopherin alpha2 in keratinocyte proliferative disorders

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Despite a number of studies on signal transduction in keratinocyte proliferation and differentiation, very little is known about precise molecular mechanism how those signals selectively move from the cytosole to the nucleus. we reported that karyopherin alpha 2 (KPNA2) plays important roles for the regulation of keratinocyte differentiation. Recently, KPNA2 has been reported to be over expressed in the tumor cells including breast carcinoma and malignant melanoma, although the roles of the elevated KPNA2 is still unclear. Here, we investigated the roles and mechanism of KPNA2 in the cutaneous proliferative diseases. Immunohistochemical anlaysis showed that KPNA2 is over expressed in basal layer keratinocytes of psoriatic skin, and in both basal and suprabasal keratinocytes of epidermal tumors such as Bowen's disease and squamous cell carcinoma (SCC). These observations evoked us a hypothesis that a loss of suppressive regulation in KPNA2 expression may lead the abnormal keratinocyte proliferation. To explore the molecular mechanism underlying KPNA2-dependent proliferative regulation of keratinocytes, KPNA2-binding proteins in HaCaT keratinocytes were purified by tandem affinity purification (TAP) method. Obtained KPNA2-binding protein library was analyzed by LC mass spectrometry. We explored that some unique transcriptional factors, several chromatin structural and regulatory proteins were shown to be transferred into the nucleus by KPNA2, that may contribute to the DNA replication or mitosis. Although further precise studies are necessary to conclude, these data suggest that over-driven activity of KPNA2 in keratinocytes may induce both transcriptional dysregulation, resulting in hyper proliferative conditions in the cutaneous diseases such as psoriasis and SCC.

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D type cyclins and Ki67 regulate each other and mitosis in HaCaT keratinocytes

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Results of our previous expression studies indicated that D type cyclins have differential functions in keratinocyte cell cycle regulation. In order to further examine the regulatory functions of D type cyclins in keratinocytes we silenced D1, D2 and D3 cyclins individually and together in HaCaT keratinocytes. The lack of one D type cyclin function had no effect on the cell cycle, but double and triple D type cyclin silencing resulted in G2/M arrest and the formation of multinucleated HaCaT cells. To elucidate the gene expression changes due to D type cyclin double and triple silencing, a real-time RT-PCR- based array method was applied. The results of this experiment showed a significant decrease in Ki67 mRNA expression, indicating a regulatory connection between D type cyclins and Ki67 in these cells. Silencing of Ki67 resulted in the appearance of multinucleated aberrant cells and a relevant decrease in the mRNA expressions of all D type cyclins. These data indicate that beside their well known function during the G0-G1/S phase, D type cyclins also regulate mitosis. This regulatory function of D type cyclins during mitosis may take place via influencing Ki67 expression. Although widely used as a proliferation marker, not much is known about Ki67 function. The formation of multinucleated cells in Ki67 silenced HaCaT keratinocytes indicates that Ki67 has a cell cycle regulatory function in mitosis. Furthermore our data suggest the existence of a regulatory cycle between D type cyclins and Ki67 and it is a key in mitosis regulation in keratinocytes.

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Effective AP1 dependant repression of TGFα mediated MMP9 upregulation by PPAR δ agonists in keratinocytes

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PPAR agonists have been shown to control inflammatory processes by inhibition of distinct proinflammatory genes. Several studies demonstrate that aberrant activation of the epidermal growth factor (EGF) and/or overexpression of its ligand transforming growth factor (TGF)-α are key features of both neoplastic and inflammatory hyperproliferative epithelia. Matrix metalloproteinase 9 (MMP9) belongs to the set of genes that are effectively induced by TGFα in keratinocytes. Induced MMP 9 expression has been linked to regenerative skin repair mechanisms and inflammatory skin diseases. We therefore explored whether the known anti-inflammatory effects of different PPARd-ligands are mediated in part through inhibition of TGFα-mediated MMP9 upregulation. PPARδ agonists are found to potently inhibit TGFα-induced MMP9 expression by HaCaT keratinocytes, This inhibition is demonstrated both at the level of protein and mRNA MMP9 expression. Zymographic assays of culture supernatants show that PPARδ ligands inhibit the catalytic activity of MMP9. As PPAR ligands do not interfere with expression and phosphorylation of the EGF receptor, we hypothesized that the inhibitory effects PPARδ agonists are mediated by suppressing the transcriptional activity of the MMP9 promoter. Transcriptional activation studies reveal that PPARd agonists mediate their inhibitory effects via an AP1 binding site. EMSA analysis demonstrated TGFa induced c-fos homodimer complex formation to this sequence is decreased by PPARd agonist treatment, indicating that MMP9 gene expression is inhibited by repressing c-fos site-dependent DNA binding and transactivation. Our data provide first evidence that TGFα-induced keratinocyte MMP9 expression is a valid target of PPARδ ligands, involving distinct mechanisms of AP1 dependant transcriptional repression.

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A Novel DNA Damage Pathway Responsible for Reduced Ribosomal Protein S6 Specific Translational Control Accelerates Aging in Connective Tissue Specific Manganese Superoxide Dismutase Deficient Mice

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Previously, the hypothesis was forwarded that DNA damage inhibits IGF-1 growth axis with substantial reallocation of energy from growth to DNA repair eventually leading to organ atrophy and aging. Here we have used connective tissue specific manganese superoxide dismutase deficient mice, which closely recapitulate intrinsic aging including skin atrophy, osteoporosis, kyphosis, and reduced lifespan to understand the underlying mechanism of DNA damage response pathway. The mutant fibroblasts evidenced a decrease in intramitochondrial H_2O_2, with an increase in $O_2^{\cdot-}$. Western blotting and immunohistochemistry suggested higher oxidized and nitrosylated proteins and increase DNA double strand breaks in mutants. In fact, reduced serum IGF-1 concentration and impaired IGF-1 signalling were found with reduced levels of phosphorylated (activated) AKT/PKB (Ser473) and p70S6 Kinase in the mutant mice. Notably, the ribosomal S6 protein, a downstream target of the p70S6 kinase which specifically controls the translation of mRNA transcripts containing an oligopyrimidine tract in their 5´untranslated region (5´TOP), was less phosphorylated (activated) in mutant skin. 5´TOP genes, like Cyclin D1, responsible for cell cycle progression and the major structural protein, collagen type-I, were significantly reduced on protein but not on mRNA level in mutant skin, while the cell cycle inhibitor p16 was highly induced. Collectively, these data for the first time show that pro-oxidant damage to the fibroblasts via double damage and attenuated IGF-1 signalling results in reduced translation of specific proteins responsible for the observed aging phenotype. These data unequivocally opens new avenues for the prevention and treatment of age-related diseases.

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KGF and KGFR expression and its changes induced by slight injury differ in normal appearing (uninvolved) skin of psoriatic patients compared to healthy skin

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Beside immune dysregulation, data suggest that abnormal skin homeostasis maybe crucial in psoriasis development; in fact it may drive the immune response. In the past few years we focused on investigating the uninvolved psoriatic skin in order to uncover differences between the psoriatic and the healthy skin. We applied tape stripping on non-lesional skin of psoriatic patients and on normal skin of healthy subjects and looked at changes in the epidermis and the dermis. Last year we reported that we found a striking difference in epidermal alpha5 integrin expression between psoriatic and healthy people, here we report that both KGF and its receptor KGFR is expressed differentially in healthy and psoriatic uninvolved skin, even before injury occur. KGF was expressed in the dermis and all layers of the epidermis in both normal and psoriatic uninvolved skin; staining intensity was much stronger in psoriatic tissue compared to normal. Twenty-four and 48 hours after tape stripping KGF expression slightly increased in healthy skin and no change was noted in the psoriatic uninvolved tissue. Six hours after tape stripping KGF mRNA increased 10 fold in normal dermis and only 5 fold in psoriatic. In healthy skin KGFR was expressed only in the basal, immediate suprabasal keratinocytes, but in the psoriatic uninvolved skin KGFR expression was detected in all layers of keratinocytes. Injury enhanced the expression intensity, but did not change the localization of KGFR expression in both normal and psoriatic epidermis. These data provide additional evidence of altered skin homeostasis in psoriasis.

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Nickel differentially regulates NFAT and NFκB activation in T cell signaling

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Nickel is a potent hapten inducing contact hypersensitivity in human skin. While nickel induces the maturation of dendritic cells via NFκB and p38 MAPK activation, nickel also has immunosuppressant effects on T cells with unknown mechanism. To elucidate molecular mechanisms of its effects on T cells, we examined the effects of $NiCl_2$ on mRNA expression in human CD3+ T cells activated with anti-CD3 and anti-CD28 antibodies. Using DNA microarray and Gene Ontology, we identified 70 up-regulated and 61 down-regulated immune responsive genes by $NiCl_2$. The former contained IL-1β, IL-6, and IL-8, while the latter did IL-2, IL-4, IL-10, and IFN-γ. Real-time PCR and BioPlex™ suspension protein array followed DNA array results. Suppression of IL-2 and IFN-γ genes transcription by $NiCl_2$ was also confirmed using Jurkat T cells transfected with IL-2 or IFN-γ luciferase reporter genes. To explorer $NiCl_2$ regulating signaling pathway, we examined the binding activity of the nuclear protein to NFAT, AP-1, and NFκB consensus sequences. $NiCl_2$ significantly and dose-dependently suppressed NFAT and AP-1, but augmented NFκB, binding activity. Moreover, $NiCl_2$ decreased nuclear NFAT expression in stimulated T cells. Using Jurkat T cells stimulated with PMA/ionomycin, we demonstrated $NiCl_2$ significantly suppressed stimulation-evoked cytosolic Ca2+ increase, suggesting $NiCl_2$ regulates NFAT signals by acting as a blocker of Ca2+ release-activated Ca2+ (CRAC) influx in T cells. These data showed that $NiCl_2$ decreases NFAT and increases NFκB signaling in T cells. These results would shed light on nickel effects of the molecular regulation in T cell signaling.

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***Propionibacterium acnes* activates the IGF-1/IGF-1R system in the epidermis and induces keratinocytes proliferation**

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Propionibacterium acnes plays a major role in the development of inflammatory acne. IGF-1 had been reported to stimulate the keratinocytes proliferation via an activation of IGF-1R and could play a central role in the potential link between diet and acne. Our first objective was to study the modulation of epidermal IGF-1 and IGF-1R expression by *P.acnes* extracts, and the second one was to determine their modulation with zinc gluconate which has proven both efficiency against inflammatory acne lesions and modulation of the IGF-1 system. *In vivo*, we analysed biopsies of acne lesions and healthy skin, and *in vitro* we used human skin explants incubated with two *P.acnes* extracts (membrane fraction (MF) and cytosolic proteins (CP)) with or without zinc. The expression of IGF-1 and IGF-1R in keratinocytes was evaluated using immunohistochemistry, and the production of IGF-1 in culture medium was measured by ELISA. *In vivo*, we observed that both IGF-1 and IGF-1R were overexpressed in acne lesions compared to healthy skin. *In vitro*, MF both increased IGF-1 and IGF-1R expression by skin explants keratinocytes and this modulation was associated with an overexpression of both Ki-67 and filaggrin. Zinc downregulated IGF-1 and IGF-1R levels. These results demonstrate for the first time that *P.acnes* can play a role in the formation of comedo via the modulation of the IGF-1/IGF-1R pathway in addition of the diet.

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Co-operation of Zinc Binding Protein 89 and Mitogen-activated Protein Kinase Pathways in the Enhancement of Matrix Metalloproteinase-1 and -3 Expression in Dermal Fibroblasts

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Matrix metalloproteinases (MMPs) control connective tissue remodeling in physiologic situations including tissue repair and angiogenesis, but also in pathological situations like tumor invasion and metastasis. Zinc binding protein 89 (ZBP-89) is a widely expressed transcription factor that binds to a variety of promoters involved in growth regulation, e.g. promoters for type I collagen and MMP-3. ZBP-89 may function both as a transcriptional activator and repressor. The up-regulation of MMP-1 (collagenase-1) and MMP-3 (stromelysin-1) expression is mediated by ERK1,2, JNK, and p38 mitogen-activated protein kinase (MAPK) pathways. We show here, that MAPKs and ZBP-89 function co-ordinately in the expression of MMP-1 and MMP-3. In normal dermal fibroblasts, enhancement of MMP-1 and MMP-3 expression and proMMP-1 and proMMP-3 protein production by tumor necrosis factor-α, but not by interleukin-1β, is enhanced by adenoviral expression of wild-type ZBP-89. Enhancement of MMP-1 and MMP-3 expression by the activation of p38 MAPK pathway by the adenoviral transduction of constitutively active MKK3b is further enhanced by adenoviral overexpression of wild-type ZBP-89. Enhancement of MMP-1 and MMP-3 expression by the specific activation of p38β, but not p38α, by the transduction of constitutively active MKK3b and wild-type p38β or wild-type p38α, is further enhanced by overexpressing wild-type ZBP-89. These results show that the expression of MMP-1 and MMP-3 is regulated by ZBP-89 transcription factor in dermal fibroblasts, which may have a role in proteolysis in, e.g., wound repair and tumor invasion.

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Keratinocytes Cultured From Psoriatic Patients Overexpress IGFBP2

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Psoriasis is one of the most common inflammatory skin disorders. In our search for a disease-specific signature, we performed oligonucleotide microarray analysis of the transcripts of keratinocytes cultured from the plaques of psoriatic patients (n = 6) and normal skin of healthy controls (n = 6), and found that one of the most dysregulated transcripts encoded for insulin-like growth factor binding protein 2 (IGFBP2). Specificity of IGFBP2 dysregulation was confirmed on independent keratinocyte sets, with massive overexpression in cells from 8 out of 10 psoriatic patients, but no dysregulation in cells from healthy donors (n = 6) or atopic patients (n = 6). Psoriatic keratinocytes cultured in serum-free conditions were enriched of senescent cells with high expression of $p16^{INK4a}$, a condition that further boosted IGFBP2 synthesis. Indeed, $p16^{INK4a}$ over-expression in normal keratinocytes led to enhanced IGFBP2, although $p16^{INK4a}$ was not indispensable for IGFBP2 expression. Conversely, IGFBP2 gene silencing led to down-regulation of the cyclin-dependent kinase inhibitors $p16^{INK4a}$ and $p21^{WAF1}$. In cells from healthy subjects and psoriatic patients, IGFBP2 could be slowly induced by IGF1 or down-regulated by TNF-alpha. When concentrated from the supernatant of unstimulated psoriatic keratinocytes, IGFBP2 inhibited IGF1 bioactivity, with abrogation of proliferation and CXCL8 expression induced by IGF1 in normal keratinocytes. In the epidermis of psoriatic lesions, IGFBP2 accumulated suprabasally, and colocalized with high $p16^{INK4a}$. Our study strongly suggests that IGFBP2 over-expression could be involved in the counterregulation of pathological mechanisms leading to hyperproliferation and in the establishment of a senescent phenotype in the psoriatic epidermis.

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Altered Redox Signaling in Senescent Human Dermal Fibroblasts

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The free radical theory of ageing postulating increased concentrations of reactive oxygen species (ROS) to drive ageing is still controversially discussed. We addressed the question whether alterations of redox balance may modulate signaling pathways *in vitro* and *in vivo* during ageing. Using proteomic approach with 2D fluorescence difference gel electrophoresis and mass spectrometry, we identified manganese superoxide dismutase (SOD2) to be 13-fold increased in senescent compared to young fibroblasts. Increased SOD2 expression and activity were confirmed using immunostaining/blot and activity assays. Most interestingly, SOD2 expression is also increased in the skin of old individuals (>70 years) compared to young individuals (≤ 25 years). Using adenoviral transduction of the H_2O_2 sensitive HyPer construct, we found increased mitochondrial H_2O_2 concentrations in senescent fibroblasts most likely due to enhanced SOD2 activity with enhanced superoxide dismutation to H_2O_2. Interestingly, senescent fibroblasts revealed a 3-fold overexpression and increase in the activity of interstitial collagenase/matrix-metalloproteinase-1 (MMP-1) which is responsible for collagen degradation during skin ageing. We have used AP-1 Transcription Factor ELISA and complementary lentiviral vectors containing AP-1 transcription response element (TRE) coupled with firefly luciferase to measure AP-1 activation responsible for collagenase expression. We found increased AP-1 transactivation by cJUN phosphorylation in senescent and H_2O_2-treated young fibroblasts. Collectively, we have found that enhanced SOD2 activity in senescent fibroblasts via unbalanced H_2O_2 overproduction and detoxification induces MMP-1 by transactivation of AP-1. Targeting strategies for fibroblasts may hold considerable promise to re-establish redox balance and prevent or treat connective tissue degradation, a hallmark in skin ageing.

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Novel neuroendocrine controls of amphibian and human skin reepithelialisation through thyrotropin-releasing hormone (TRH) and bombesin and its mammalian homologues

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Rapid reepithelialisation of chronic human skin ulcers is a largely unsolved medical problem for which new wound healing-promoters are urgently needed. However, there are not enough simple and physiologically relevant test systems for the preclinical screening of such candidate substances. Amphibian organisms possess a high capacity for tissue regeneration that has not yet been systematically exploited for human wound healing. Therefore, we have dveloped parallel wound healing assay systems in which whole skin „punch within a punch" samples from frog and human were cultivated in comparable serum-free growth media. In the human samples the newly formed epithelial tongues were analyzed by immunofluorescence using a new set of quantifiable markers for proliferation (Ki67), metabolic activity (cytochrome c oxidase subunit I MTCO), keratinocyte activation (K6) and early and late stages of differentiation (MSX2 and Involucrin, respectively). In the frog samples proliferation was measured by staining phosphorylated Histone 3 (pH3). Thyroid-stimulating hormone (TRH), which is highly expressed in frog and human skin, was tested first and found to significantly stimulate growth of epithelial tongues (compared to vehicle controls) in both frog and human samples. TRH also accelerated the onset and increased the intensity of K6, Ki67 and MTCO1 in human and of pH3 in frog samples, respectively. Bombesin, another very abundant peptide hormone in frog skin, and its mammalian homologues Neuromedin B (NMB) and Gastrin Releasing Peptide (GRP) also had stimulating effects on these parameters in human and frog samples. This demonstrates the effectiveness of our inter-species approach to wound healing research.

526 [Oral 096]

Progressive Decrease in Number and Change in Niche Preference of the ABCB5$^+$ Mesenchymal Stem Cell Subset in the Skin during Aging

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ABCB5, a P-glycoprotein of the ABC superfamily of transporter proteins, was expressed in a newly defined mesenchymal stem cell subpopulation in skin. Even though its decrease in number and/or function may result in impaired regenerative capacity and aging, *in vivo* data are not available. We studied cell surface expression pattern, number and localization of ABCB5-positive mesenchymal stem cells in young (0-20 years), middle aged (21-70 years) and old healthy individuals (>71 years). Dermal ABCB5$^+$ cells showed variability for expression of the hematopoietic progenitor cell antigen CD34 or CD133, indicating a distinct stem cell subset. In contrast, ABCB5$^+$ cells expressed mesenchymal stem cell markers CD29, CD90, CD59 and CD44. A significant decline in ABCB5$^+$ cell numbers was found in the skin of old individuals. In an attempt to understand the mechanisms underlying the ABCB5$^+$ stem cell decline in aging, we studied the expression of gH2AX, a phosphorylated histone protein detecting DNA double strand breaks and initiating a DNA damage response with the induction of cell cycle inhibitors like p16. We found that by contrast to age-dependent increase in gH2AX and p16 in dermal fibroblasts, no consistent increase in gH2AX and p16 was found in ABCB5$^+$ mesenchymal stem cells. Interestingly, ABCB5$^+$ mesenchymal stem cells were found in close association of CD31$^+$ vessels in younger individuals, while this perivascular localisation was lost in the old age group. Collectively, the decrease in ABCB5$^+$ mesenchymal stem cells with changes in niche preference may contribute to a reduced regenerative capacity in skin aging.

527 [Oral 097]

Functional importance and hormonal regulation of nestin expression in multipotent adult human skin progenitor cells *in situ* and *in vitro*

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Neurofilament nestin+ cells of adult human skin are one focus of cell-based regenerative medicine strategies, since they may serve as an easily accessible source of adult, autologous progenitor cells for the generation of different tissue lineages. While the cultivation of nestin+ progenitors from murine and human skin have been achieved by several laboratories, it remains unclear which specific functional role(s) the expression of nestin itself plays in these progenitors, e.g. in terms of cell growth, differentiation, cell fate and hormonal regulation. To address these questions, we organ-cultured nestin+ progenitor-rich human scalp skin and isolated sweat gland (SwG)-derived nestin+ cells in the presence or absence of the adipokin leptin, which we had previously shown to up-regulate intra-mesenchymal nestin expression in human skin *in situ*. Therefore, leptin was used as a tool to up-regulate nestin expression. In addition, nestin expression was knocked-down by RNAi in cultured SwG-derived nestin+ cells. Both experimental approaches revealed that strong nestin expression is associated with increased cell proliferation and nestin expression is essential for the glial cell, but not for the neuronal cell fate of nestin+ cells. After leptin stimulation, nestin+ progenitors managed to continue on a glial differentiation path *in vitro* even after (likely incomplete) nestin silencing. As nestin+ cells could be differentiated into glial and neuronal derivatives, we conclude that nestin expression alone is primarily responsible for the glial differentiation fate of nestin+ progenitors and that leptin administration may facilitate the isolation, extended culture, and glial differentiation of primary, adult human skin-derived nestin+ stem cells.

528 [Oral 012]

The Hedgehog response gene Gli1 marks multipotent stem cells in the telogen bulge

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Hair follicles cycle through phases of growth (anagen), regression (catagen), and quiescence (telogen). During anagen, stem cells in the bulge region regenerate the proximal follicle, whereas distinct stem cells maintain the interfollicular epidermis. The outer root sheath and matrix in the proliferative anagen follicle are known to express the Hedgehog (Hh) response gene *Gli1*. Moreover, blockade of Hh signaling with neutralizing antibodies prevents anagen regeneration. However, it is not clear if Hh signals directly to follicle stem cells, or if it simply drives proliferation of transient amplifying cells during anagen regrowth. We identify a novel population of Hh-responding cells in the telogen bulge marked by *Gli1* expression. These cells function as multipotent stem cells when observed in their native niche, with the ability to repeatedly regenerate the anagen follicle during homeostasis. Furthermore, fate mapping experiments suggest a role for Gli1-expressing follicle cells in the healing of epidermal wounds. Interestingly, follicle contributions to epidermal regeneration occur only during a limited period immediately after wounding. Finally, comparison of *Gli1* expression with other stem cell markers provides a molecular map of the progenitor cells in the quiescent telogen hair follicle.

529 [Oral 004]

Genetic Dissection of a New Biochemical Pathway in Epidermal Differentiation

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Cystatin M/E (*CST6*) is a non-redundant, epithelium-specific protease inhibitor with a presumed role in epidermal differentiation and tumor suppression. We have previously reported that cystatin M/E deficiency in *Cst6*$^{-/-}$ mice causes neonatal lethality due to excessive transepidermal water loss. Biochemical evidence suggests that cystatin M/E controls the activity of legumain, cathepsin L, cathepsin V and transglutaminase-3. Using a genetic approach we sought to define the role of cystatin M/E in epithelial biology by identification of its target proteases and their downstream functions. Ablation of cathepsin L in a *Cst6*$^{-/-}$ background (*Cst6*$^{-/-}$*Ctsl*$^{-/-}$ double knockout mice) restored viability and resulted in normalization of stratum corneum morphology. Ablation of legumain or transglutaminase-3 in *Cst6*$^{-/-}$ mice, however, did not rescue the lethal phenotype. Intriguingly, both *Cst6*$^{-/-}$*Ctsl*$^{+/-}$ and *Cst6*$^{-/-}$*Ctsl*$^{+/-}$ mice were viable, but the absence of cystatin M/E caused scarring alopecia in adult animals. In the cornea of *Cst6*$^{-/-}$*Ctsl*$^{+/-}$ mice, we observed keratitis, hyperplasia and transition to a cornified epithelium. Evidence is provided that activation of cathepsin D and transglutaminase-1 are downstream events, dependent of cathepsin L activity. We conclude that a tightly regulated balance between cathepsin L and cystatin M/E is essential for tissue integrity in epidermis, hair follicles and corneal epithelium.

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Dissociation and re-complementation of Alpha Keratin and Melanin from hair to build a novel biopolymer that can be used as different biocompatible prostheses at wide polymerization ratios tensile and impact strength

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We tried to dissociate and re-complement Alpha Keratin and Melanin from hair to build a novel biopolymer that can be used as biocompatible prostheses at wide polymerization ratios with a spectrum of tensile and impact strength. Keratin is a ubiquitous polymer of low tensile and impact strength. Melanin, the strongest natural UV absorbent is also a ubiquitous macromolecule whose function in brain and adrenals is not well understood. During treatment of alopecia, we encountered patients who regained only white hair upon treatment and never relapsed. Besides all patients show regrowth of white hair at beginning and the disease never attacks white hair. We postulated that interaction of Melanin and Keratin is not only a physical co-impaction as microscopic studies show but a chemical co-polymerization that gives rise to new antigenic determinants. In two different chemical environments, detergent treated black hair without fat was treated in a way that once Keratin and once Melanin could be extracted. After drying, the two were let to interact in a very delicate basic microenvironment that enabled growth of the crystals. Different non stochiometric proportions were used to evaluate chemical and physical properties. Co-polymerization of the two is a real event with birth of compounds distinct from the parents. The largest product is at present 30mmX20mmX7mm. Some are very resistant to tension and/or impact. As the condition of co-polymerization is not optimum shape and physical properties cannot be controlled yet but as they are biocompatible they open a new horizon for prostheses in plastic surgery.

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Spermidine as a stimulator of human hair follicle growth and regulator of stem cell function

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Polyamines are indispensable for cellular proliferation and for the growth of rapidly regenerating tissues. Given that the hair follicle (HF) is a highly proliferative organ, polyamines are also important for normal hair growth. Though inhibiting polyamine synthesis does inhibit human hair growth, the role of polyamines in human HF biology *in situ* remains largely unknown. To explore this, we have studied the effects of the prototypic polyamine spermidine (0.1-1 μM) on human scalp HFs in serum-free organ culture. Spermidine significantly promoted hair shaft elongation, prolonged the growth phase of the hair cycle (anagen), and modulated hair keratin gene expression *in situ* (e.g., downregulation of *KRT31*, *KRT32* and *KRT35*). In addition, spermidine was found to enhance the proliferation of cultured primary human keratinocytes. Microarray analysis suggested multiple differentially regulated spermidine-target genes. Spermidine upregulated expression of the epithelial stem cell-associated keratins K15 and K19, altered K15-promoter activity *in situ*, and dose-dependently affected colony forming capacities and K15 expression of isolated K15-GFP+ cells *in vitro*, thus suggesting a novel role for spermidine in regulating stem cell function. In contrast, the ornithine decarboxylase inhibitor eflornithine downregulated K15 expression. These physiologically and clinically relevant data provide the first direct evidence that spermidine is a potent stimulator of human hair growth and that this polyamine is an important modulator of human epithelial stem cell functions.

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Dermal perivascular niche of mesenchymal stem cells in human scalp skin

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In mammalian skin, epidermal and follicular bulge stem cells are well characterized by numerous studies. In contrast, existence of stem cells in dermis is still poorly understood. Previous studies have indicated that mesenchymal stem cells (MSCs) are situated as pericytes in various mammalian tissues. In adipose tissue, progenitor cells of adipocytes reside in the mural cell compartment of the vasculature as CD34 positive cells. We speculated that human adult dermis also contains MSCs-like cells positive for CD34 at the perivascular sites. To prove this hypothesis, fibroblastic cells isolated from adult scalp were examined their self-renewing and differentiating potency *in vitro*. Confocal microscopic observation was also performed to visualize the sites of MSCs-like cells in human scalp dermis. At first, fibroblastic cells from adult scalp skin tissues showed colony-forming ability, and differentiated into mesenchymal lineages (osteogenic, chondrogenic, and adipogenic). Three-dimensional analysis of scalp skin with a confocal microscope clearly demonstrated that perivascular cells were positive for not only NG2 but also CD34 immunoreactivity. Perivascular CD34 positive cells were abundant around follicular portions. Furthermore, CD34 positive cells collected with magnetic cell sorting from scalp dermis were indeed capable of differentiating into mesenchymal lineages. Consistent with these observations, cultured dermal sheath cells derived from lower hair follicles essentially displayed mesenchymal differentiation. This study suggests that dermal perivascular sites worked as a niche of mesenchymal stem cells in human scalp skin.

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The Development of the Cutaneous Neurofibromas

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Cutaneous neurofibromas are the hallmarks of neurofibromatosis type 1 (NF1). They are composed of multiple cell types, and are currently believed to arise from small nerve tributaries of the skin. A key finding in the context of this view has been that only subpopulations of tumor Schwann cells harbor bi-allelic inactivation of the *NF1* gene (*NF1*$^{-/-}$). Our aim was to further elucidate the pathogenesis of cutaneous neurofibromas. Cells expressing biomarkers associated with multipotency were detected in cutaneous neurofibromas. A method for isolating and expanding multipotent neurofibroma-derived precursor cells (NFPs) from dissociated human cutaneous neurofibromas was developed and employed to analyze their growth and differentiation potential. In analogy to solitary cells resident in neurofibromas, NFPs were found to express nestin, and had the potential to differentiate at least to Schwann cells, neurons, epithelial cells and adipocytes. Mutation analysis of the NFPs revealed that their genotype was *NF1*$^{+/-}$. The results lead us to speculate that the development of cutaneous neurofibromas includes the recruitment of multipotent *NF1*$^{+/-}$ precursor cells. These cells may be derived from the multipotent cells of the hair roots, which are often intimately associated with microscopic neurofibromas.

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Vitamin-D3 modulates human hair follicle epithelial progenitor cells *in situ* and *in vitro*

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Vitamin-D3 and its receptor (VDR) are key regulators of normal skin biology. For example, calcitriol (hormonally active form of vitamin D3) inhibits proliferation and stimulates differentiation of keratinocytes and VDR null-mice develop alopecia. This led us to hypothesize direct effects of calcitriol on human epithelial hair follicle stem cells (HFSCs), which we had recently shown to express VDR. Here, we have assessed the effects of calcitriol on HFSCs *in vitro* and *in situ* by using transfected cytokeratin-15 (K15)-promoter-driven GFP demarcated HFSCs or isolated HFSCs under serum- and calcium-free conditions. Within 3 days, low dose calcitriol, significantly up-regulated the recognized HFSC markers K15 and CD200 as well as VDR expression. After 6d, low dose calcitriol began to down-regulate *K15* mRNA, while a higher calcitriol dose selectively blocked K15 expression and induced a cytoplasmatic redistribution of VDR protein. In line with these findings, a low dose of calcitriol slightly enhanced proliferation and colony forming efficiency (CFE) whereas higher doses strongly induced apoptosis and impaired CFE. In addition, *deiodinase-2* (converts thyroxin to triiodothyronin) and *CYP27B1* (catalyzes the hydroxylation of calcidiol to calcitriol) were down-regulated by calcitriol. We conclude that HFSCs are sensitive to calcitriol/VDR-mediated controls, which may contribute to maintaining or depleting HFSCs in a dose-dependent manner. These newly identified endocrine controls of human HFSCs *in situ* and *in vitro* encourage one to examine, whether topical calcitriol application may be clinically exploited to protect (alopecia) or delete (hirsutism) HFSCs *in vivo*. Calcitriol administration may also become useful for human HFSC-based regenerative medicine strategies.

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Starburst Perifollicular Hyperplasia – A New Trichoscopy Finding In Patients With Folliculitis Decalvans

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Trichoscopy (videodermoscopy of hair and scalp) is gaining popularity as a valuable tool in differential diagnosis of hair loss. This noninvasive method allows viewing hair and scalp at high magnification. Characteristic trichoscopy findings of numerous hair and scalp diaseases have been described. These include alopecia areata, androgenic alopecia, frontal fibrosing alopecia and several other diseases. The aim of the study was to evaluate trichoscopy features of folliculitis decalvans. We investigated a total of 1223 patients, who visited our out-patients offices for hair loss and 60 normal controls. In all patients anamnesis, clinical evaluation, trichogram, trichoscopy and, if necessary for diagnosis, pathology evaluation of scalp biopsies were performed. Trichoscopy was performed with the use of FotoFinder 2 videodermoscope. Working magnifications were 20x and 70x. In all cases trichoscopy was performed in variants: dry and with the use of an alcohol solution as immersion fluid. Six patients (6/1223, 0,049%) were diagnosed with folliculitis decalvans. In all these patients trichoscopy showed focal hair loss, whitish areas of skin fibrosis, multiple pilosebaceous units with multiple (4-13) hairs and, in affected areas, perifillicular epidermal hyperplasia in a starburs pattern. This pattern was not observed in any other of the 1223 patients with hair loss nor in normal controls. This trichoscopy finding corresponded to perifollicular excess of fibrous tissue in the mid and deep dermis. In conclusion, perifollicular hyperplasia arranged in a starburst pattern is a specific finding in folliculitis decalvans.

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Development of a co-culture system using 3T3-L1 differentiated adipocytes and Reconstructed Human Epidermis (RHE) to test the efficacy of an active on the lipid content of fat tissue.

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Cellulite or lipodystrophy is an aesthetically problem that is claimed to occur in most postpubertal females. The localized accumulation of fat is responsible for the vertical extension of the superbasal fatty nodules and their distribution between the bundles of connective tissue which results in a topographic skin change. The slimming process consists on reducing the adipocytes diameter and for that, slimming formulations have to penetrate the epidermis and the dermis, to reach the adipose tissue. To assess the effect of slimming active on the fat cells, an *in vitro* system was developed in which well differentiated 3T3-L1 cells were kept in co-culture with Reconstructed Human Epidermis (RHE) Episkin® for three days, with 2 topical applications (t_{+24h}, t_{+48h}) of 26µg of the slimming product to test. Then, the cell viability of RHE is determined and the intracellular content of lipid in adipocytes is evaluated by Oil Red O staining. In parallel, human IL-1alpha and mouse IL-6 are quantified each day in culture medium. Adipocytes cultivate alone in co-culture die, but stay alive in presence of RHE, showing a very close interaction between keratinocytes and adipocytes. The use of green coffee oil in a formulation containing Biobase (Bioderma, excipients cream), water and ethanol lead to a decrease of lipid content. RHE in the presence of adipocytes releases less amount of IL1 alpha and stimulates the secretion of IL6 by adipocytes. These findings indicate that co-cultured keratinocytes and 3T3-L1 *in vitro* provides a potential new model to study inflammation interactions and slimming efficacy.

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The role of γ1 chain containing laminins for the formation and function of the dermo-epidermal basement membrane

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The main laminin isoforms in the dermo-epidermal basement membrane (BM) are laminin 332 containing the γ2 chain and laminin 511 and 311, both containing the γ1 chain. Mice lacking the laminin γ1 chain die around E5.5, thus not allowing analysis of skin development. Therefore, we have generated a mouse strain with keratinocyte-specific deletion of the laminin γ1 chain (*LAMC1*^{EKO} mice). These mice die postnatally showing severe hair and pigmentation defects. Histological analysis revealed that hair follicles (HFs) were malformed, irregularly orientated and hyperproliferative in the outer root sheath. In contrast, proliferation of hair matrix cells was decreased. HF morphogenesis was delayed and the number of HFs reduced in knockout animals. However, HF differentiation was normal. The epidermis showed thickening and altered differentiation caused by hyperproliferation of basal keratinocytes. Immunofluorescence revealed that laminin 511 was completely lost from the dermo-epidermal BM and replaced by ectopic deposition of laminin 211, whereas all other BM components were unchanged. Electron microscopy revealed local disruptions as well as thickening of the dermo-epidermal BM in the mutant skin. Interestingly, hemidesmosome formation seems to be unaffected. However, basal keratinocytes appear less polarized, rounded-up and desmosomal cell-cell interactions are partially lost. These data indicate that laminin 511 plays an important role not only in HF development, but also in pigmentation and homeostasis of the interfollicular epidermis. However, laminin 511 appears to be dispensable for the maintenance of epidermal-dermal cohesion. Our data also demonstrate that laminin 211 is not able to functionally replace laminin 511.

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Scanning Electron Microscopy and Atomic Force Microscopy in the study of damaged and weak hair treatment

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Healthy hair is highly important and desired. There are a lot of causes of bad condition and weakness of the hair, which lead to hair loss. That's why everyday hair care product and treatment must have high protecting properties. The aim of this study was to evaluate the *in vivo* efficacy of the hair care products using Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM).The SEM and AFM study were conducted on volunteers hair. The samples had been collected in three batches: before the first use of the test product, after 1 and 5 uses. The samples were examined using Zeiss Scanning Electron Microscope LEO 1430VP and AFM Bioscope II Veeco.The results showed visible improvement of hair condition noticed after first use of products. We observed smoothing of hair surface, which could be achieved through the restoration of the slate-like hair scale arrangement. The products left a thin protective film on the hair surface, filling all hollows and voids, and consequently restoring hair smoothness. This film also protects hair from the negative impact of environmental factors and prevents mechanical damage during daily hair care procedures. We also examinated the micro/nanoscale properties of human hair. Scanning Microscopy (SEM) and Atomic Force Microscopy (AFM) are very interesting and advanced technology which let us to observe all the morphological and changes in examined hair samples. Collected results help to estimate objective impact of treatment on a hair structure, which is nowadays especially important on dermatological and cosmetological area.

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Effect of aquaporin-7 modulation on glycerol release and fat droplet size in 3T3-L1 adipocytes

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Aquaporin-7 (AQP7) is an aquaglyceroporin abundantly expressed in adipose tissue and essential for glycerol release from adipocytes. In the current study, we were interested in modulating AQP7 expression and studying the incidence on glycerol release and fat droplet size in 3T3-L1 adipocyte cell line. For that purpose, immunofluorescence staining of AQP7 on 3T3-L1 cells was performed after treatment of cells with a compound targeting aquaporin expression. Our results showed that IV09.010, increased the expression of AQP7. This effect was similarly observed in presence of caffeine or isoproterenol. Quantification of glycerol indicated that IV09.010 increased glycerol release from adipocytes, when applied alone and in combination with isoproterenol. To further investigate the relation between AQP7 expression and the size of fat droplets, 3T3-L1 cells were transfected with AQP7 specific siRNA and stained by Oil Red. This transfection provoked the differentiation of these cells as indicated by the presence of fat droplets. IV09.010 allowed to partially reversing the effect of AQP7 siRNA. Furthermore, the effect of IV09.010 on the size of fat droplets was evaluated. Nile red staining showed a reduction of the size of fat droplets in cells treated with IV09.010, with or without caffeine or isoproterenol. These results indicate that inducing the expression of AQP7 in 3T3-L1 cells would be of great interest to modulate fat cell content, especially in combination with lipolysis inducers.

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Enhancing anti-oxidative protection in the hair follicle

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Hair scalp is constantly exposed to different environmental stresses including UV radiation, as well as oxidative and chemical stresses that can influence hair growth and also affect hair color. In this study, we investigated the effects of UV, oxidative stress induced by H2O2, and chemical stress induced by SDS on human scalp skin grafts maintained in culture. For UV irradiation, the biopsies were submitted to successive exposure to 5 and 0.2 J/cm2 of UVA and UVB, respectively. For oxidative and chemical stress, H2O2 at 5 mM and SDS at 1% were topically applied. Outcomes on hair follicle structure were studied by hematoxylin-eosine (HE) staining 24 hours after stress exposure. Damage was mainly observed in the outer root sheath, and included apoptotic cells, vacuoles, oedemas, and disorganization of the basal layer of the ORS. Using immunofluorescence staining, we observed an increase in catalase expression and a decrease in p63 staining, as a marker of hair regeneration. Moreover, application of an anti-oxidative stress compound (IV09.003) on human scalp skin grafts 24 hours before UV or H2O2 stress allowed to reduce the level of stress damage in the HF. This protective effect went along with a reduction of UV and H2O2 -induced increase in catalase expression, and maintenance of p63 expression. This study demonstrates the importance of hair follicle protection against environmental stress that can damage hair

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Modulation of circadian protein expression and hair growth

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Hair follicle is a complex organ that consists of mesenchymal and epithelial tissues, interacting together to ensure hair growth and regeneration. During the growth phase (anagen) of the hair cycle, the dermal papilla cells (DPC) interact with the hair matrix keratinocytes and activate them to proliferate and differentiate into several cell types.Hair follicle possesses a circadian clock cycle that correlates with the hair growth cycle. Circadian genes are up-regulated during the telogen-anagen transition and regulate the cell cycle progression. In this study, we investigated the effects of a new active ingredient IV09-019 (1%) on the circadian protein expression on DPC, then on growth and structure of the hair follicle. DPC and scalp skin biopsies were first treated with the active for 48h to study protein expression. Then, scalp skin biopsies were treated up to 17 days to evaluate hair growth. Our results indicated that DPC treated with the active showed an increase in expression of the circadian proteins Clock, B-mal1 and Period-1, as well as Ki67 protein. In scalp skin biopsies, an increase in Ki67 and keratin 15 expression was also observed. Moreover, the hair growth measured by hair shaft elongation was significantly increased after 7, 10, 14 and 17 days of treatment. This work shows the expression of circadian proteins in DPC, and demonstrates that their modulation could go along with an improvement of the hair follicle structure and hair growth.

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Androgen action inactivates canonical Wnt signalling in scalp dermal papilla cells impairing hair follicle stem cell differentiation in androgenetic alopecia

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Hair follicle (HF) formation begins when signals from the mesenchyme-derived dermal papilla cells (DPC) reach multipotent epidermal stem cells in the bulge region. In androgenetic alopecia (AGA) androgens action causes HF miniaturization and baldness through a mechanism which remain unclear. Circulating androgens act on DPC probably by altering the regulatory paracrine factors involved in the differentiation and proliferation of the HF multipotent cells. The aim of this work is to determine the role of androgens in the differentiation of HF stem cells and to identify the DPC signaling pathways or effector molecules involved in its action. The profile of DPC secreted proteins in response to androgens is modified as revealed by bidimensional gels and HPLC analysis. In a co-culture model with human DPC from patients suffering AGA and HF stem cells, androgens treatment abrogates hair differentiation. The expression of the keratin K6hf, hair differentiation marker, was significantly downregulated in HF stem cells co-cultured with androgen-treated DPC. Association of β-catenin to androgen receptor was observed, indicating that β-catenin is a cofactor of AR signalling in DPC. Androgens significantly upregulate β-catenin expression, both at mRNA and protein levels. Analysis of the cytoplasmic pool by Western blot showed that the ratio cytoplasmatic/total β-catenin is significantly lower in androgen-treated DPC showing that androgens are able to inhibit canonical Wnt signalling in DPC. These results suggest that androgens may modulate the secretion of paracrine factors involved in normal HF stem cell differentiation inactivating the canonical Wnt signaling pathway.

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Multiple miliary osteoma cutis: Three case reports and review of the literature

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The purpose of this study was to describe three patients with multiple miliary osteoma cutis (MMOC) and to study if these patients have features related to other forms of primary cutaneous ossification. MMOC is a rare nodular skin disease where tiny bony nodules are formed usually on the facial skin usually in middle age.The patients were examined clinically several times and a wide spectrum of laboratory tests was taken, including GNAS sequencing. Selected molecules taking part in bone formation were studied histologically. A review of literature was made. Histological analyses revealed intramembranous bone formation as in GNAS gene based osteoma cutis disorders. However, we could not find any correlation to germ-line GNAS gene inactivation or endocrinologic disorders, which were studied from peripheral blood. In histological staining BMP2 and BMP4 were positive in several skin cells but patient samples did not differ significantly from controls. FGF8, collagen XIII and estrogen receptor were negative. In the literature, we found 47 MMOC cases, both females (87%) and males (13%). 55 % had had pre-existing acne and 15 % had extra-facial osteomas. On the basis of literature review and our three cases we suggest that MMOC is a distinct disease entity of still unknown etiology. Tiny nodular osteomas show intramembranous superficial ossification and they increase slowly in number after appearing in middle age. We could not find any association with germ-line GNAS gene inactivation or the endocrinologic disorders. As a treatment, surgical extirpation is possible only in limited number of tumours.

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Trichohyalin-like 1, a novel human hair follicle-specific S100 fused-type protein

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Genes of the S100 fused-type protein (SFTP) family, clustered within the epidermal differentiation complex on human chromosome 1q21.3, encode essential components that maintain epidermal homeostasis and barrier functions. We have recently identified hornerin and filaggrin-2 as two members of this family which are important components of healthy epidermis. Here we report the identification of trichohyalin like-1 (TCHHL1), a novel member of the SFTP family, predominantly present in hair follicles. The *TCHHL1* gene encodes a glutamine- and lysine-rich protein of approximately 99 kDa, which shares common structural features with other SFTP members. *TCHHL1* transcripts were detected predominantly in the body sites where the skin is rich in hair follicles, such as in the chin and scalp. Immunohistochemical analysis revealed that unlike other SFTP members, TCHHL1 is specially localized in the inner root sheath cells. *In vitro* studies demonstrated that TCHHL1 could be cross-linked to itself by transglutaminase 1. To investigate whether TCHHL1 contributes to antimicrobial skin barrier as similar as other SFTP members, we recombinantly expressed two anionic peptide fragments of TCHHL1, $THHL1_{90\text{-}248}$ (17.5 kDa, pI 4.9) and $THHL1_{369\text{-}784}$ (45 kDa, pI 4.6). Using radial-diffusion antimicrobial assays, we found that only the 45 kDa-protein showed killing activity against *E. coli* at a concentration of 22 μmol/l. Our data suggest that TCHHL1 might play a critical role in the maintenance of hair follicle homeostasis and barrier function.

545 [Oral 107]

The *Malassezia* metabolites indirubin and malassezin are potent activators of the aryl hydrocarbon receptor in HaCaT cells

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The association of *Malassezia furfur* produced Aryl hydrocarbon receptor (AhR) indolic ligands, malassezin, indolo[3,2-*b*]carbazol and indirubin with seborrheic dermatitis has been reported previously. These substances are synthesized *in vitro* by the yeast when L-tryptophan is used as the single nitrogen source. The biomimetic synthesis of indirubin by one step oxidation of the *Malassezia* metabolite indol-3-carboxaldehyde was described as a putative biosynthetic pathway. This pathway may also lead to the simultaneous synthesis of the potent AhR ligand tryptanthrin. Our aims were: 1) to analyze *Malassezia* extracts for novel bioactive indoles, particularly tryptanthrin, as predicted by the biomimetic approach and 2) to quantify AhR agonistic activity of malassezin and indirubin. Analytical HPLC was employed in search for not yet identified AhR agonists in *Malassezia* extracts. For AhR activation profile, HaCaT cells in sub-confluency were treated with indirubin and malassezin for 24h at different concentrations (1nM to 50μM). mRNA expression of AhR related genes (AhR, AhRR, CYP1A1, CYP1B1, ALDH3A1, GST-P1, GST-T1) was assessed with quantitative RT-PCR. As expected tryptanthrin was identified in *M. furfur* extracts. Malassezin and indirubin had a dose dependent agonistic effect at the mRNA levels of CYP1A1, CYP1B1 and ALDH3A1. The induction was ~4 orders of magnitude higher and at lower concentrations with indirubin compared to malassezin (100nM vs 10μM). Both agents had insignificant effect on AhR, AhRR and GSTs' transcription. The description of novel potent AhR agonists (tryptanthrin) that have the potential to differentially activate the AhR pathway underscores the biologic significance of the *Malassezia* produced indoles.

546 [Oral 112]

Induction of Broadly Cross-neutralizing Antisera against Mucosal and Cutaneous Human Papillomaviruses (HPV) by HPV16L1-16L2 Virus-like Particles (RG1-VLP)

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Licensed HPV vaccines comprising major capsid protein L1-VLP induce persisting, high-titer, yet predominantly type-restricted antibody responses. In contrast, immunization with minor capsid protein L2 can induce low-titer cross-neutralizing antibodies. Particularly a highly conserved peptide motif HPV16L2 aa 17-36 (RG1) has been characterized as a broad-spectrum cross-neutralization epitope. We have previously developed chimeric HPV16L1-VLP that display RG1 within the DE-surface loop of L1 (RG1-VLP). Rabbit antisera raised against RG1-VLP plus human-applicable adjuvant Alum-MPL showed high-titer neutralizing antibodies to HPV16 and cross-neutralizing antibodies to mucosal high-risk HPV18/31/45/52/58, mucosal low-risk HPV6/11, and beta-skin HPV5. The objective was to more completely characterize the spectrum and robustness of RG1-VLP-based cross-neutralization. Rabbit antisera to RG1-VLP (50 microgram plus Alum-MPL i.m., weeks 0, 4, 6, 8) additionally cross-neutralized mucosal high-risk HPV33/68/76, alpha-skin HPV3 and Heck's disease type HPV32 (titers 50 to 1,000) in newly developed pseudovirion assays. Moreover neutralization of cutaneous alpha types HPV2 and HPV27 was demonstrated by RT-PCR infectivity assays, using native virions isolated from common warts. To evaluate the vaccine's potential for long-lasting protection, sera were drawn 10 months after the 4th immunization. L2-mediated cross-neutralizing antibodies were detectable with a similar decline (10- to 100-fold) to 16L1 antibody-titers. Importantly, an additional boost raised antibody titers to former levels at the minimum, indicating a functional B-cell memory response to cross-neutralization epitope RG1. Immunization with RG1-VLP in adjuvant applicable for human use can induce enduring and broadly cross-neutralizing antibodies to evolutionary divergent mucosal and cutaneous, high-risk and low-risk, alpha and beta HPV types.

547 [Oral 021]

HIV-1 activates Cdc42 and induces filopodia in immature dendritic cells to facilitate cell-to-cell virus propagation

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Dendritic cells (DC), due to their unique localization at mucosal surfaces, coupled with their known proficiency in capturing antigens, are among the first potential targets for HIV-1 during transmission. One of the limiting steps for HIV-1 propagation is the transfer of virus at an infectious synapse (IS) between DC and $CD4^+$ T cells. Bacterial pathogens can hijack the host actin cytoskeleton to facilitate invasion and propagation. We report here the first evidence that a virus, HIV-1, induces the formation of filopodia in DC through activation of the Rho GTPase Cdc42. We provide direct evidence that filopodia are obligate components of the HIV-1 induced DC-T cell infectious synapse and required for transfer of HIV-1 infection to target $CD4^+$ T cells. HIV-1 at the surface and near the tip of filopodia was observed by confocal microscopy, electron microscopy, live imaging and 3D-EM. Silencing of Cdc42 in dendritic cells dramatically reduced number of filopodia and decreased HIV-1 transfer via dendritic cell-T lymphocyte infectious synapses. Transfection of dominant-negative or constitutively active mutants of Cdc42 in dendritic cells decreased or increased HIV-1 transfer, respectively. Finally, we show that filopodia play an essential role in the transfer of virus when a low number of DC are co-cultured with T cells, a situation that mimics DC-T cell ratios in mucosal tissues or lymph nodes. In conclusion we identify a critical role for Cdc42-dependent filopodia induction by HIV-1 in the transfer of HIV-1 from DC to T cells thereby identifying a novel pathway for HIV-1 cell-to-cell propagation.

548 [Oral 007]

Concerted activation of TLR2 and IL-4R mediates Staphylococcus aureus dependent exaggeration of atopic dermatitis inflammation

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Atopic dermatitis (AD) is a chronic inflammatory skin disease with Th2 cells preferentially found in acute flares and Th1 cells dominating chronic lesions. Staphylococcus aureus is detected in most AD patients providing potent TLR2 ligands, however, the impact of TLR2 ligands on AD inflammation is unclear. Therefore, we established a model for acute AD inflammation by adoptively transferring and activating OVA-specific Th2 cells in the ear skin of naive mice, where ear swelling correlates with antigen-specific inflammation. While Th2 cells or OVA alone only lead to minor changes, Th2 cells plus OVA provoked inflammation and strong ear swelling after 24h. Importantly, addition of Staphylococcus aureus cell wall component lipoteichoic acid, a pathogen associated molecular pattern and TLR2 ligand, or of synthetic TLR2 ligand Pam2Cys provoked prolonged and increased dermatitis similar to OVA-specific dermatitis following Th1 cell transfer. Cross-over experiments with TLR2-deficient mice and Th cells revealed that TLR2 on accessory cells but not T cells is responsible for TLR2 mediated exacerbation of cutaneous inflammation. Hence, dendritic cells were activated with TLR2 ligands and produced Th1 inducing IL-12 as well as IL-10. However, IL-10 is completely downregulated by IL-4-costimulation. This shift was also observed *in vivo* and applying our AD model to IL10ko mice resulted in prolonged cutaneous inflammation compared to wildtype. Thus, we identified IL-10 as key regulator of TLR2 mediated inflammation. These data indicate that S. aureus derived TLR2 ligands shift Th2 cell dominated cutaneous inflammation towards chronic and persistent dermatitis through a concerted activation of TLR2 and IL-4R.

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Cure rate, duration required for complete cure and recurrence rate of onychomycosis according to clinical factors in Korean patients

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Despite great advances in the treatment of onychomycosis, there are still many problems with treatment, including a long duration required for a complete cure and a high rate of treatment failure. Many factors affect the cure rate (CR), duration for complete cure (DC) and the recurrence rate (RR). This study is to evaluate the CR, DC and RR in onychomycosis according to various clinical factors. We retrospectively reviewed medical records of the 637 Korean patients of onychomycosis between December 2000 and December 2006. We examined six clinical factors for effects on the CR, DC and RR: age, sex, clinical type, treatment pattern, presence of diabetes mellitus (DM), and the extent of nail involvement. On the view of the clinical nail appearance and potassium hydroxide (KOH) preparation, we designated the CR, DC and RR. In addition, we examined the differences in the CR, DC, and RR in terms of the above-mentioned clinical factors. A total of 207 eligible patients were finally analyzed. The CR as a whole was 78.3%, the DC was 31.7 ± 18.4 weeks, and the RR was 36.0%. There was significant relevance of the CR, DC and RR according to the extent of nail involvement. Age affects the CR and DC, and DM also affects the DC and RR. On the other hand, there were no significant differences according to gender, clinical types and treatment patterns. In conclusion, we hope that these results would be helpful in establishing therapeutic plans and predicting the prognosis of onychomycosis.

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Role of microbial populations in wound healing: new molecular methods

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This study analyzes the microbial population of wounds using robust new molecular tools. Chronic wounds contain persistent microbial populations that can function as impediments to wound healing. Recent genomic advances provide more accurate methods than standard culture to analyze the bacterial composition in wounds. Metagenomic analysis of the microbial population in wounds was compared to similar analyses of bacteria from normal skin. Increased proportions of anaerobes, gram negative rods (i.e. *Pseudomonas*, *Proteus*, *E. coli*, *Klebsiella*, etc.), *Staphylococcus* and *Streptococci* were found in chronic wounds. Furthermore, chronic wounds had significantly lower populations of *Propionibacterium* compared to normal skin. Using epifluorescence microscopy, a semi-quantitative biofilm grading scale was developed to define the morphology and bioburden of these bacterial communities. Samples showed that wound bacteria exist in a spectrum from highly organized thick continuous biofilms to individual bacterial cells in a planktonic state. Fluorescent in-situ hybridization provided further information by visualizing the location of specific bacteria in the wound. Quorum sensing molecules were measured by bioassay to understand signaling patterns among the bacterial colonies. All samples had greater than normal levels of autoinducer-2, a quorum sensing molecule implicated in biofilm formation and the regulation of virulence factors. These data suggest that wounds have a significantly different microbial biome from that of normal human skin and the presence of biofilms may impede wound healing. It is possible that certain bacteria play a greater role in the pathogenesis of chronic wounds, while large numbers of benign colonizers may improve healing rates.

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Impact of innate defense antimicrobial peptides in hidradenitis suppurativa

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Hidradenitis suppurativa (HS) is a chronic and debilitating inflammatory disorder of the apocrine gland bearing areas with frequent bacterial superinfection. We therefore aimed to investigate a potential deficient expression of innate defense antimicrobial peptides (AMPs). Skin biopsies (n=36), sweat (n=26) and suction blister fluid, gained 20 hours after blister formation to ensure neutrophilic infiltration (n=13), were collected from HS patients for analysis of dermcidin, human neutrophil peptides 1-3 (HNP1-3), and human β-defensin-3 (hBD-3). Severity of HS was graded according to the classification by Hurley in grade 1 (n=5), grade 2 (n=21), and grade 3 (n=10). 54 healthy individuals served as controls. Analysis of sweat samples and blister fluids from HS patients and controls revealed overall comparable levels of dermcidin and HNP1-3 by ELISA. However, if patients were distinguished with regard to HS severity, patients with higher severity (grade 3) had lower dermcidin and HNP1-3 levels than HS patients with less severe HS (grade 1 or 2). With regard to HBD-3 expression in lesional HS skin, there was a tendency to a lower expression of HBD-3 in more severe HS as assessed by RT-PCR (mean CT ranged from 7,9 for HS grade I to 11,2 for HS grade III). In conclusion, we found lower levels of innate defense AMPs dermcidin, HNP 1-3 and HBD-3 in patients with severe HS. Thus, deficient constitutive production of dermcidin and HNP1-3 and/or reduced HBD-3 induction possibly contribute to propensity of bacterial superinfection and thereby to HS severity.

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Removal of One or More Arsenic Related Infections by Using Medicinal Plants: Findings from a Rapid Assessment Study in Barguna District of Bangladesh

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One of the more perplexing ground water problems currently facing Bangladesh is the high concentration of arsenic in drinking water, which poses a relatively large risk to human health of this region. Traditional health practitioners (THPs) of Bangladesh primarily use medicinal plants for treatment of various ailments. The selection of medicinal plant is a closely guarded secret and is usually kept within the family. As a result, the use of medicinal plants varies widely between THPs of different areas within the country, and is based on both medicinal plant availability and the THP's unique knowledge derived from practice. The present study was to conduct a survey amongst the THPs to learn more about the medicinal plants used to treat one or more arsenic related infections in Barguna district of Bangladesh. This area is unique in its proximity to the Sunderbans forest region and contains quite different medicinal plants from other parts of the country because of high salinity in the soil and water. Semi-structured questionnaires were administered to twenty-one THPs to evaluate the THPs' perceptions and practice relating to causation and treatment of one or more arsenic related infections. The THPs described the signs, symptoms, and causes of one or more arsenic related infections. Information on twenty-four medicinal plants was obtained. Information on indigenous use of medicinal plants has led to discovery of many medicines in use today. Scientific studies conducted on the medicinal plants may lead to discovery of more effective drugs than in use at present.

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Expression and sequence diversity of the complement regulating outer surface protein E in *Borrelia afzelii* vs. *B. garinii* in patients with erythema migrans or neuroborreliosis

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Outer surface protein E (OspE) is a complement factor H-binding virulence factor of various borrelial subspecies. It is usually absent from *in vitro* grown *Borrelia garinii*, although *in vivo B. garinii,* the causative agent of neuroborreliosis (NB) appears to escape complement attack. We analyzed the presence and sequence spectrum of the *ospE* genes *in vivo* in *Borrelia* spirochetes. DNA samples from the skin, serum and cerebrospinal fluid (CSF) of patients with infections caused by *B. afzelii* or *B. garinii* were studied, and anti-OspE antibodies in the corresponding patient sera were detected by IgG ELISA using recombinant OspE as an antigen. *ospE* genes were found in 20 of 23 erythema migrans (EM) skin biopsies with *B. afzelii*, in 2 EM skin biopsies with unknown underlying subspecies, in 5 of 9 EM biopsies with *B. garinii*, and in 1 of 4 CSF samples of NB patients with *B. garinii* infection. All OspE sequences from *B. garinii* samples were identical. In contrast, OspE of *B. afzelii* origin showed more variation. Anti-OspE antibodies were found in 8/21 (38.0 %) sera from patients with *B. afzelii*-associated EM. In conclusion, our results indicate that all borrelial subspecies, but not necessarily all strains, causing human infections can carry *ospE* genes to protect themselves against complement attack *in vivo*.

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Reactive oxygen species and pro-inflammatory cytokines are produced in an in vitro S. pyogenes-stimulated keratinocyte model

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Group A Streptococcus pyogenes (S. pyogenes) is responsible of major suppurative and inflammatory infections of the skin like erysipelas. It has been shown that keratinocytes participate to the innate immune response through the production of reactive oxygen species (ROS) contributing to eliminate and/or enhance the inflammatory reaction. To date, no information are available in the production of ROS and pro-inflammatory molecules by keratinocytes stimulated by S. pyogenes. We stimulated HaCaT cells with S. pyogenes strains isolated from patients with erysipelas. Spectrofluorometric analysis was used to measure the ROS production. PCR array and RT-qPCR were used to screen the genes implicated in the inflammation reaction. HaCaT cells stimulated by S. pyogenes produced a large amount of $O_2^{\bullet-}$ in a dose and time-dependent manner while the production of H_2O_2 and NO appears to be steady. Superoxide anions are produced in the cytoplasm by the NADPH oxydase, detoxified into H_2O_2 by the superoxide dismutase, and may be responsible for the large amount of cell death contributing to the inflammatory reaction more than eliminating the bacteria. Cytokines and chemokines genes CXCL2, CXCL3, TNF-α, IL-8, IL-1α and IL-1β appears to be up-regulated from 2 to 100 time in our model suggesting a role of keratinocytes in the inflammation response. All together, the results showed that the keratinocytes contribute to the early innate immune response after being stimulated by S. pyogenes.

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Individualized therapy of patients with genitourinary chlamydiosis

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The incidence throughout the world, including Ukraine, is high, which is, in particular, associated both with the peculiarities of the course of the infection, ways of spreading of the agent in the body, variety of immune responses; and with certain difficulties in diagnostics and treatment of diseases, caused by Chlamydia trachomatis. According to contemporary conception of genitourinary chlamydiosis, its treatment is based on general principles of complex and individualized therapy in infectious diseases. Since chlamydiosis is regarded as systemic disease, etiotropic, pathogenic and symptomatic therapies should be used in the treatment of this ailment. In administration of individualized therapy, medications necessary for individual patient were used, depending on localization of inflammatory process, character of pathologic condition, which arose during course of the disease, and general condition of the body. The therapy also included correction of the immune system, physiotherapeutic treatment, fight against concomitant disorders and stases in pelvic organs, pharmacologic protection of the liver. Starting from the first day of treatment, patients took immunomodulator of plant origin. Depending on the severity of the course of the disease, patients took azithromycin orally in the dose of 1.0g in the third day of treatment and starting from the 5th day 0.5g every other day up to 13th-17th days of treatment. Following treatment 107 patients felt practically healthy. Two patients had slight periodic itching and burning sensations in the urethra and morning mucous discharge from the urethra. Individualized complex treatment of genitourinary chlamydiosis allowed achieving clinical and etiologic cure of 96.33% of patients.

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Problem of HIV/AIDS in Obstetrics and Neonatology

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A number of the HIV-infected among pregnant women has been rapidly increasing in recent decades. It is expedient to examine infants, born to HIV-infected mothers, for presence of perinatal infections. Infants, born to HIV-infected women, should be kept on artificial feeding.Examined pregnant women were placed into 2 clinical groups. The first clinical group included 13 HIV-infected pregnant women, who had undergone prevention of HIV transmission; the second group included 7 HIV-infected women, who had not received proper prevention. Average body weight of the newborns from the first group constituted 3200g, but 4 infants were born with the weight ≤ 3000g (31%), 2 infants (15%) with signs of delayed intrauterine development of the I stage. Manifestations of prenatal impairment of the CNS were detected in five (38%) infants from the first group: increased nervous-reflexive excitement syndrome was marked in 3 (23%) infants, CNS inhibition syndrome - in 1 infant (7.6%), syndrome of vegetative-visceral disorders - in 1 infant (7.6%). Average body weight of infants from the second group constituted 2700g: two (28.5%) newborns with signs of delayed intrauterine development of the I stage, and 3 (42.8%) infants with signs of delayed intrauterine development of the II stage. In the 2nd group increased nervous-reflexive excitement syndrome was marked in 5 (71%) infants, CNS inhibition syndrome - in 1 infant (14.2%), syndrome of vegetative-visceral disorders - in 1 infant (14.2%). AIDS virus penetrates the brain and the spinal cord, thus, infants should be supervised by the neurologist, and undergo monthly neurosonography examinations.

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Generation of cytoplasmic DNA of an RNA virus by human LINE-1

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Formation of DNA complementary to non-retroviral RNA virus genomes has been rarely reported. This process can be mediated by endogenous retroviruses or retrotransposons that have been implicated in autoimmune disease, such as SLE. Here we detected cDNA of vesicular stomatitis virus (VSV) following acute, cytopathic infection of human cells. We aimed to define the structure of the VSV DNA and to identify the cellular reverse transcriptase which is involved in the VSV DNA formation. We infected various human cell lines with VSV and the extracted DNA was subjected to PCR using VSV-specific primers and DNA sequencing. Inverse PCR was performed to determine the 5′ and 3′ sequence of the VSV DNA. Furthermore, human NP-2 cells producing little VSV DNA were transfected with a plasmid expressing long interspersed nuclear element 1 (LINE-1). The cells were infected with VSV and the amount of VSV DNA was compared by real-time PCR. Several reverse transcriptase inhibitors were tested whether they could inhibit the VSV DNA formation. VSV DNA complementary to VSV mRNA was present in cytoplasm and not integrated into the host genome. VSV DNA generation was cell dependent and over-expression of long interspersed nuclear element 1 (LINE-1) in cells restored this activity. The endogenous activity of VSV DNA generation and that induced by exogenous LINE-1 transfection showed similar sensitivity profile against reverse transcriptase inhibitors, indicative that LINE-1 is responsible for VSV DNA generation. Such DNA copies of non-retrovural RNA viruses generated by LINE-1 might contribute to autoimmune diseases via DNA sensors.

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A Spectrum of Tuberculids And The Value of Interferon-Gamma Release Assays In Their Diagnosis

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The diagnosis of tuberculids is challenging and requires correlation of clinical findings and diagnostic tests. Traditional tests such as tuberculin skin tests (TSTs) and detection of Mycobacterial DNA by polymerase chain reaction (PCR) are subject to interpreter variability, and limited in sensitivity and specificity. The objective of this study is to demonstrate the usefulness of interferon-gamma release assays (IGRAs) as additional diagnostic tools in tuberculids. We present a series of different forms of tuberculids where skin biopsy could not demonstrate mycobacteria by stain, culture or PCR, but IGRA results are positive. All three patients in our study responded to anti-mycobacterial therapy, lending support to the initial diagnosis of tuberculids based on clinical features and IGRA results. Methodological limitation inherent of a case series, however, does not allow us to draw conclusions on the sensitivity of IGRAs in diagnosing tuberculids. Dermatologists accustomed to depending on traditional tests such as TST or PCR in diagnosing tuberculids should consider adding IGRA in their diagnostic work-up.

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RNase 7 protects healthy skin from *Staphylococcus aureus* colonization

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Staphylococcus aureus (*SA*) is one of the most common pathogens related with human skin infections. Despite the high carrier rate in the normal population skin is usually not infected by *SA* which is explainable by the ability of healthy skin to keep the bacteria within a limited number. One mechanism to control the growth of *SA* may be the expression of antimicrobial proteins (AMP). The aim of our study was to investigate the physiological relevance of the AMP RNase 7 in cutaneous defense against *SA*.Therefore skin explants were exposed to *SA* for 2, 6 and 20 hours. ELISA revealed that the secretion of RNase 7 was induced after 2 hours, whereas 6 and 20 hours showed no significant change. To assess the functional relevance of RNase 7 in cutaneous defense against SA we investigated whether the killing activity of skin extracts was inhibited through blocking of the antimicrobial activity of RNase 7 by a RNase 7-specific antibody. As a result, the potent *SA* killing activity of the skin extract was reduced when RNase 7 was neutralized by the specific antibody. In further *ex vivo* experiments using skin explants and the RNase 7 neutralizing antibody we could confirm the functional role of RNase 7 in cutaneous defense against *SA*. These data demonstrate that skin infected with living *SA* responds with an increased secretion of RNase 7 which contributes to limit the growth of *SA* thus indicating the physiological relevance of RNase 7 to protect human skin from *SA* infection.

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A3 APO a proline-rich antibacterial peptide in burn infections

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The designer antibacterial peptide A3-APO is efficacious in mouse models of *Escherichia coli* and *Acinetobacter baumannii* systemic infections. Here we compare the efficacy of the peptide with that of imipenem and colistin in *A. baumannii* wound infections after burn injury. In three sets of independent experiments, mice were inflicted with burn wounds and different loads of *A. baumannii*, isolated from a Canadian veteran, were placed into the injury site. The antibiotics were added intramuscularly 1-5 times. The systemic toxicity of colistin and A3-APO was studied in healthy mice. While toxicity to colistin was observed at 25 mg/kg bolus drug administration, the lowest toxic dose of A3-APO was 75 mg/kg. In all three *A. baumannii* models 5 mg/kg A3-APO reduced the bacterial counts in the blood and in the wounds and improved wound appearance significantly better than any other antibiotic treatment. Peptide A3-APO with an intramuscular therapeutic index of 15 is more efficacious and less toxic than any existing burn injury therapy modality against multi-drug resistant Gram-negative pathogens.

561 [Oral 098]

SLC24A5/NCKX5 influences melanocyte cholesterol homeostasis and MC1R expression: a novel mechanism for natural human skin colour variation?

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Natural variation in human skin colour is determined by genetic variation in a very few genes. Variation of a non-synonymous single nucleotide polymorphism (pA111T, rs1426654) in one of these genes (SLC24A5) is associated with marked differences in constitutive skin colour in peoples from South Asia. NCKX5 is expressed in melanocytes and functions as a potassium-dependent sodium-calcium exchanger. Using NCKX5-specific antibodies alongside *trans*-Golgi-network (TGN) disrupting treatments we have confirmed that NCKX5 is a TGN resident protein. When heterologously expressed the 111Thr variant of NCKX5 confers significantly lower ion-exchange activity than the Ala111 variant, with reduced exchanger function proposed to mediate the lower melanogenic activity of lighter skinned individuals. Using genome wide expression microarrays we assessed the impact of siRNA-mediated knockdown of SLC24A5 on cultured normal human melanocytes. Surprisingly the expression levels of very few genes associated with melanogenesis were changed with the notable exception of MC1R. Furthermore alpha-MSH treatment increased both SLC24A5 transcript and NCKX5 protein expression, whilst MITF knockdown decreased their expression, suggesting that SLC24A5 interacts with this well characterized melanogenic signaling pathway. Unexpectedly the expression of a number of sterol and cholesterol homeostatic genes was altered after SLC24A5 knockdown and the total cholesterol content of NHM was increased. Cholesterol has previously been identified as a potential melanogenic regulator and is known to be essential for vesicular budding at the TGN. Our data imply that SLC24A5 affects natural variation in skin pigmentation through a TGN resident ion exchange mechanism and a novel mechanistic route for regulating melanocyte intracellular sterol/cholesterol levels.

562 [Oral 099]

Shot gun proteome analysis identified the PPARγ ligand 15d-PGJ2 (15-deoxy-D12,14-prostaglandin J2) as a novel drug interfering with melanoma-stroma interaction

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Peroxisome proliferator-activated receptors (PPARs), members of the nuclear hormone receptor superfamily, have been originally thought to be restricted to lipid and lipoprotein metabolism, glucose homeostasis and cellular differentiation. Recently, evidence is growing that PPARγ ligands have inhibitory effects on tumor growth by regulating cell proliferation and differentiation.To shed light on the potential therapeutic effects on melanoma we tested a panel of PPARγ agonists on a variety of melanoma cell lines *in vitro*. Whereas most of the PPARγ agonists showed only moderate effects on melanoma cell proliferation, 15d-PGJ2 displayed profound anti tumor activity on all cell lines tested. Additionally, 15d-PGJ2 inhibits proliferation of tumor associated fibroblasts and tube formation of endothelial cells. Furthermore, 15d-PGJ2 led to an induction of the tumor suppressor gene p21, a G/2M arrest and an inhibition of tumor cell migration in a matrigel assay in a panel of melanoma cell lines. Shot gun proteome analysis revealed that 15d-PGJ2 might exert its effects via downregulation of HSP-90 (heat shock protein 90) and other related chaperones. Applying the recently established CPL/MUW database with a panel of defined classification signatures we demonstrated an interference of 15d-PGJ2 in replication and protein synthesis including a significant regulation of mediators such as cell division cycle protein 27, angio-associated migratory cell protein, proliferating cell nuclear antigen, cell division protein kinase 2 or proliferation-associated protein 2G4. Our data revealed for the first time a profound effect of a single compound 15d-PGJ2 on melanoma cells in addition to the tumor microenvironment suggesting high therapeutic efficiency.

563 [Oral 100]

-catenin is activated by cAMP in normal human melanocytes and melanoma cells: implication in melanogenesis regulation

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Wnt/β-catenin signaling plays important roles in many developmental processes, including neural crest-derived melanocyte determination, proliferation and differentiation. Accumulating evidence suggests that the Wnt/β-catenin signaling plays an important role in melanogenesis regulation of adult cells. Here we investigated the crosstalk between cAMP/PKA signaling, the principal pathway involved in control of pigmentation, and canonical Wnt/β-catenin signaling in B16-F0 mouse melanoma cells and normal human melanocytes (NHM). Using a β-catenin-specific siRNA approach, we demonstrated that β-catenin is required for adenylyl cyclase signaling via PKA and its target transcription factor CREB-directed melanogenic gene expression. Chromatin immunoprecipitation assays demonstrated an increased association of β-catenin with the proximal promoter of microphthalmia-associated transcription factor (M-Mitf), the master regulator of pigmentation, as a consequence of cAMP level elevation. Activated PKA phosphorylated β-catenin at Ser675 stabilizing cytoplasmic and nuclear β-catenin. Consistent with nuclear β-catenin increase, several Wnt target genes (Axin2, Lef1, Mitf, Sox9 and Wisp1) were coordinately upregulated in B16 cells and the observed stimulation was significantly reduced in β-catenin-siRNA-treated cells. Surprisingly, transfection of exogenous wild type β-catenin or a constitutively active mutant form, that cannot be phosphorylated by CK1 and GSK3β, did not significantly up-regulated any of the genes studied indicating that in B16 cells, there might be some other limiting factors involved in the regulation of Wnt target genes, such as for example activating phosphorylation. It is of interest that in NHM increased cAMP was not able to stimulate Axin2 and Sox9 suggesting that β-catenin signaling is differently regulated in NHM and B16 melanoma cells.

564 [Oral 101]

Astaxanthin attenuates the stem cell factor-stimulated pigmentation of human epidermal equivalents through interruption of Raf-1 activity within melanocytes

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We previously demonstrated that MAPK signaling, including microphthalmia associated transcription factor (MITF) and CREB phosphorylation, is a major pathway involved in regulating melanogenesis within human melanocytes. Recently, a redox imbalance was shown to be linked to a variety of altered cellular responses in which the precise balance between levels of oxidizing and reducing equivalents that reflect the intracellular redox condition profoundly affects intracellular signaling pathways, especially the MAPK pathway. To elucidate the effects of redox balance regulation on epidermal pigmentation, we used the potent antioxidant astaxanthin (AX) to assess its effect on the stem cell factor (SCF)-stimulated pigmentation of a human epidermal equivalent (Melano-Model) and analyzed its biological mechanisms. The addition of AX elicited a marked depigmenting effect on the SCF-stimulated pigmentation over 14 days of treatment, which was accompanied by diminished melanin production and a significant decrease in eumelanin (PTCA) content. Real-time RT-PCR and western blotting revealed that the SCF-stimulated gene and protein expression of tyrosinase, tyrosinase-related proteins-1/2, Pmel17 and endothelin B receptor was significantly suppressed at days 7 and 10, respectively, by AX, which suggests an impairment in intracellular signaling upstream of gene expression. Western blotting to detect the phosphorylation of intracellular signaling molecules revealed that in AX-treated ALM melanoma cells, there is a marked deficiency in the SCF-stimulated phosphorylation of MEK, ERK, MITF and CREB but not of Raf-1 compared with the control cells at 15 min post-SCF incubation. These findings indicate that AX attenuates the SCF-stimulated pigmentation by interrupting Raf-1 activity within melanocytes.

565 [Oral 102]

Loss of E-cadherin increases metastasic potential of melanoma

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E-cadherin is a calcium-dependent cell-cell adhesion molecule expressed by melanocytes and responsible for their adhesion to the surrounding keratinocytes in the epidermis. Disruption of E-cadherin-mediated cell-cell adhesion has been implicated in tumour progression and metastasis. In order to investigate its role in melanomagenesis *in vivo*, we have generated a mouse model in which E-cadherin is specifically deleted in melanocytes (Tyr::Cre/°, E-cadherin$^{floxed/floxed}$ = Ecad mice). At the molecular level, E-cadherin is neither replaced by N- nor P-cadherin in defloxed melanocytes. Furthermore the amount of β-catenin, normally bound to E-cadherin, is less important at the membrane but accumulates in the cytoplasm and nucleus, which has been linked to 30% of human melanoma. However Ecad mice do not develop melanoma, suggesting a minor role of E-cadherin in the initiation steps of melanomagenesis including proliferation and immortalization. We crossed Ecad mice with Tyr::Nras^{Q61K} and Ink4a mice to induce proliferation and immortalization *in vivo*. As expected the latency period and the penetrance of melanoma production were not altered in the absence of E-cadherin. However, the lack of E-cadherin in melanocytes promotes dramatically lymph node invasion and lung macrometastasis.

566 [Oral 103]

a-MSH-mediated melanocyte differentiation involves PPAR-γ activation through a Phospholipase-C mediated pathway

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In melanocytes PPAR-γ is expressed at detectable levels and can promote differentiation when pharmacologically stimulated. We investigated, in primary cultures of human melanocytes, whether or not PPAR-γ could act as an effector component of differentiation, downstream a-MSH-dependent MC1R stimulation. We demonstrated that a-MSH up-regulated PPAR-γ expression and promoted its transcriptional activity. In order to define the mechanism responsible for a-MSH-mediated PPAR-γ activation, we focused on the cAMP/PKA pathway, for its main role in mediating melanogenesis, and surprisingly, Forskolin failed to promote PPAR-γ activation. Since PPAR-γ is induced by lipid mediators, and being MC1R a seven-transmembrane G-protein-coupled receptor, we have investigated whether or not stimulated MC1R could mediate PPAR-γ induction through the Phophoinositide-Phosphoplipase-C (PLC)-dependent pathway. Treatment with the PLC inhibitor, U-73122, abrogated PPAR-γ activation induced by a-MSH, and exposure to 3M3FBS, a PLC inductor, promoted PPAR-γ activation. We obtained a direct evidence of phosphoinositide-PLC-dependent pathway activation, in response to a-MSH, by assessing, by HPLC-MS-TOFF analysis, the generation of inosytol triphosphate and diacylglycerol as products of PLC activity. We also investigated the influence of PPAR-γ downstream a-MSH-dependent MC1R stimulation on melanogenesis. The silencing of PPARγ expression partially counteracted melanogenesis induced by a-MSH. This study delineates a new signalling axis from the a-MSH to its G-protein coupled receptor MC1R, through PLC, down to PPAR-γ. This pathway, which is independent from the well characterised cAMP/PKA pathway, is also involved in the control of melanocyte differentiation.

567 [Oral 104]

Inhibition of protein deacetylase SIRT1 suppresses in vitro and in vivo invasion of melanoma cells

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SIRT1 is a NAD-dependent protein deacetylase that deacetylates histones and transcription factors. It is a nucleocytoplasmic shuttling protein, but the role of SIRT1 in the cytoplasm is unknown. When SIRT1 is downregulated, mobilization of embryonic neural cells is significantly inhibited. We found that SIRT1 was predominantly expressed in the cytoplasm of melanoma cells and accelerated their migration. SIRT1 was highly expressed beneath the cellular membranes and protrusions and colocalized with filamentous actin. Administration of pharmacological inhibitors of SIRT1 prevented formation of protrusions and migration of B16F1 melanoma cells, whereas NAD and resveratrol, activators of SIRT1, enhanced cell migration. Among siRNAs for seven NAD-dependent protein deacetylases, only SIRT1-siRNA inhibited cell migration of B16F1 cells. Administration of nicotinamide, an inhibitor of SIRT1, suppressed abdominal invasion and lymph node metastasis of transplanted melanoma cells *in vivo*. These results suggested that SIRT1 might have some role in the progression of melanoma, and that suppression of SIRT1 by inhibitors of SIRT1 could inhibit metastasis of cutaneous melanomas.

568 [Oral 105]

Polo-like kinase 1 expression is indirectly regulated through MAPK signaling pathway and is a potential therapeutic target in human melanoma

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Disruption of the cell cycle regulation has been implicated in the development and progression of malignant melanoma. By using cDNA microarray technique and pathway enrichment analysis we could identify cell cycle pathway and its member polo-like kinase 1 (Plk-1, a mitotic serine/threonine kinase) to be significantly overexpressed in primary melanomas and melanoma metastases. This finding could be confirmed using real-time RT-PCR analysis on an independent set of specimens. In *in vitro* analysis of 8 human melanoma cell lines we observed the peak expression of the Plk-1 to be at the G2/M phase of the cell cycle. Transfection of human melanoma cell lines with two independent Plk-1 siRNAs led to reduction of Plk-1 mRNA/protein, significant decrease in cell proliferation, induction of mitotic catastrophe, and apoptotic cell death. Apoptosis was caspase 3/8-, Bid- and Bcl-2-dependent but TP53-independent. CGH and SNP arrays showed no genetic alteration in Plk-1 locus. MAPK signaling pathway was also significantly enriched in primary melanomas and melanoma metastases in our samples. Inhibition of this pathway using the 4 MAPK inhibitors resulted in decreased expression of Plk-1 (a result of G1 cell cycle arrest and not a direct regulation), cell death and apoptosis. This study shows that in human melanoma: Plk-1 expression is dynamically regulated during the cell cycle, knock down of Plk-1 can lead to inhibition of cell proliferation/survival and induction of apoptosis and, Plk-1 expression to be regulated indirectly through MAPK signaling pathway. We conclude that Plk-1 could be a potentially attractive target in melanoma therapy.

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A novel treatment for severe photodamage

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Octatrienoic acid is a new molecule, belong to psittacolfulvin family, sharing with carotenoids and retinoids some structural features .20 subjects with severe facial photoaging were enrolled in this double-blind, randomized, parallel group study. Subjects were divided in two different groups: 10 subjects in the active cream group, 10 subjects in eccipient cream group. All the subjects applied the topic (active or placebo) once a day for 45 days.. At basal time (T0), after 45 days (T1) and after 75 days (T2) from the beginning of therapy we evaluated: redness, dryness, scales, and texture of the face skin in all the subjects. At T0 and T2 we evaluated the photoaged skin (face, front area) by the reflectance confocal microscopy (RCM) to study the effect of octatrienoic acid on the skin. All the considered symptoms were significantly modified from T0 to T1 in the active group respect the placebo group (redness $p < 0.01$, dryness and scales $p < 0.05$).At T2 (1 month after the end of therapy) the same values were present. At the RCM the skin before the treatment showed an important hyperkeratosis, with scales, a significative activation of epidermal melanocytes expression. In the dermis we could see a significative enlargement of diameter of dermal capillaries (vasodilatation) and markers of inflammation. At T2 we noted and improvement of the epidermis physiological condition and a decrease of vasodilatation and inflammation in active group. The clinical evidence and the instrumental evaluation provide a strong evidence that octatrienoic acid can improve photoaged skin.

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Targeting XIAP to increase endoplasmic reticulum stress-induced apoptosis for melanoma therapy

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Malignant melanoma remains largely unresponsive to current chemotherapy due to the notorious resistance of such tumours to apoptosis. Recent evidence suggests increased expression of XIAP, a member of the inhibitor of apoptosis protein family, contributes to apoptosis resistance. We have recently demonstrated targeting endoplasmic reticulum stress with fenretinide (a synthetic retinoid) or bortezomib (a 26S proteosome inhibitor) represents a novel strategy by which to induce apoptosis of metastatic melanoma cells both *in vitro* and *in vivo*, although the efficacy of such treatment may be impaired by XIAP over-expression. The aim of the current study was to correlate XIAP expression with cancer stage and test the hypothesis that inhibition of XIAP signalling increases the efficacy of ER stress-induced cell death. Western blotting confirmed increased expression of XIAP in metastatic melanoma cell lines compared to normal melanocytes. Immunohistochemical studies of XIAP expression in primary melanoma tissue derived from a cohort of patients with differing stage disease also confirmed XIAP expression significantly increased with progressive cancer stage as defined by both Breslow thickness and the American Joint Committee on Cancer staging system ($P<0.001$). Retroviral transfection of metastatic melanoma cell lines (CHL-1, A375 and WM266-4) with a short hairpin RNA targeting XIAP resulted in significantly increased apoptosis in response to both fenretinide and bortezomib ($P<0.0001$) in a caspase-dependent manner. Collectively these data suggest targeting XIAP with a small molecule inhibitor (currently in clinical development) may increase the efficacy of ER stress-induced apoptosis as a more effective treatment for metastatic melanoma.

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Expression of CD10 predicts tumor progression and unfavorable prognosis in malignant melanoma

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CD10 expression in malignant melanoma (MM) has been reported to increase according to tumor progression and metastasis; however, its association with patient outcome has not been clarified.We examined the immunohistochemical expression of CD10 in MM to determine whether or not it could serve as a marker for tumor progression and prognosis.Sixty-four formalin-fixed, paraffin-embedded samples of primary MM from 64 patients were immunostained for CD10. Similarly, 35 samples of melanocytic nevus and 20 samples of metastatic MM were analyzed for comparison. The following clinicopathologic variables were evaluated: age, gender, histologic type, tumor site, Breslow thickness, Clark's level, and overall survival. Statistical analyses were performed to assess for associations. Several parameters were analyzed for overall survival using the Kaplan-Meier method and Cox proportional-hazards model. Immunohistochemical analysis revealed that 34 of 64 cases (53%) of primary MM expressed CD10, compared to 15 of 20 cases (75%) of metastatic MM and only 2 of 35 cases (6%) of nevus. There was a significant positive relationship between CD10 expression and Breslow thickness and Clark's level. Univariate analysis revealed three significant factors for shorter survival periods: CD10 expression, high Breslow thickness, and high Clark's level ($p < 0.01$ each). In multivariate analysis, only CD10 expression and Breslow thickness were statistically significant and independent prognostic factors.CD10 expression may serve as a progression marker and can predict unfavorable prognosis in melanoma patients.

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HSP70 inducers from Chinese herbs and their effect on melanin production

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Skin hyperpigmentation disorders due to abnormal melanin production induced by ultraviolet (UV) irradiation are both a clinical and a cosmetic problem. This melanin production is mediated by tyrosinase whose expression is positively regulated by microphthalmia-associated transcription factor (MITF). On the other hand, UV-induced modest melanin production plays an important role in protection against UV-dependent skin damage. Expression of heat shock proteins (HSPs), particularly HSP70, is induced by various stressors, including UV irradiation to provide cellular resistance against such stressors. We recently found that expression of HSP70 inhibits melanin production and proposed that HSP70 inducers could be beneficial as hypopigmenting cosmetics and medicines. In this study, we searched for HSP70 inducers from Chinese herbs and selected an ethanol extract of *Eupatorium lindleyanum* (*E. lindleyanum*) which induced expression of HSP70 in mouse melanoma cells (B16) at concentrations that did not significantly affect cell viability. Not only melanin production but also the activity and expression of tyrosinase were significantly suppressed in B16 cells treated with *E. lindleyanum* extract as well as in HSP70-overexpressing cells. The expression of MITF was clearly suppressed in B16 cells treated with *E. lindleyanum* extract but not in HSP70-overexpressing cells. These results suggest that *E. lindleyanum* extract suppresses the expression of tyrosinase and melanin production through both HSP70-dependent and HSP70-independent mechanisms. We propose that *E. lindleyanum* extract could be beneficial as a hypopigmenting cosmetic and medicine because it would not only suppress melanin production but also protect against UV-induced skin damage through its HSP70-inducing ability.

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TPA inhibits melanoma growth by dephosphorylation of Tyr705 on STAT3 through PKC-activated tyrosine phosphatase(s)

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The growth of most melanoma cells is inhibited by the tumor-promoting phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). In this study, the involvement of the signal transducer and activator of transcription 3 (STAT3) in the TPA-induced growth inhibition of melanoma cells was examined. The growth of five melanoma cell lines, whose Tyr705 on STAT3 was strongly phosphorylated (activated), was inhibited by TPA, whereas that of WM35 and WM39 cells, whose phosphorylation of Tyr705 on STAT3 was at negligible levels, was considerably slow and not affected by TPA. Blockade of STAT3 activity by siRNAs suppressed the growth of WM1205Lu cells containing constitutively activated STAT3. Treatment of WM1205Lu cells with TPA decreased both the phosphorylated STAT3 and the DNA-binding activity of STAT3. Pretreatment of WM1205Lu cells with either a protein-tyrosine phosphatase inhibitor or a protein kinase C (PKC) inhibitor prevented the inhibitory effects of TPA on the level of phosphorylated STAT3. The five melanoma cell lines containing phosphorylated STAT3 commonly expressed PKCα, PKCδ, and PKC . Introduction of the dominant negative mutant of one of these PKC isoforms into WM1205Lu cells inhibited the TPA-induced dephosphorylation of STAT3. A Src inhibitor attenuated the STAT3 phosphorylation in WM1205Lu cells. These results indicate that constitutively activated STAT3 is positively regulated by c-Src and negatively regulated by a PKC-activated tyrosine phosphatase(s) in melanoma cells. Because TPA did not affect c-Src activity, we conclude that the growth inhibitory effect of TPA on melanoma cells is mediated through dephosphorylation of Tyr705 on STAT3 by a PKC-activated tyrosine phosphatase(s).

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Association study between *IL-4* gene polymorphism and susceptibility to vitiligo in Korean populations

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Vitiligo is a depigmenting disorder characterised by the loss of melanocytes from the cutaneous epidermis. Although the exact cause of the condition remains to be established, an autoimmune etiology has been suggested, and several observations support this theory. In this study, we searched if the genetic polymorphism of *IL-4* intron3 could be demonstrated in Korean vitiligo patients. And we examined the differences in the distribution of the *IL-4* alleles and genotypes between each clinical subtype of vitiligo. Patients with vitiligo (n=358) and the healthy controls (n=744) were genotyped for *IL-4* variable number of tandem repeat(VNTR) polymorphism using polymerase chain reaction(PCR) or PCR restriction fragment length polymorphism. Genotype distributions and allelic frequencies were compared between patients and controls. No significant differences between patients and controls were found in allele and genotype frequencies of *IL-4* VNTR polymorphism. There were no significant differences between generalized type and localized type or between non-segmental type and segmental type. *IL-4* polymorphism was not associated with susceptibility to vitiligo patients.

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The role of Smac/DIABLO, p53, NF-kB, and MAPKs in Apoptosis of Keratinocytes from Perilesional Vitiligo Skin: Protective Effects of Curcumin and Capsaicin.

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Vitiligo is a chronic acquired hypomelanotic disorder affecting 0.5-2% of the world's population. Oxidative stress, has been suggested as one of the possible initial pathogenetic event in melanocyte degeneration in vitiligo, but also the possible responsible for the ultrastrucural alterations of keratinocytes observed in our previous study. We have taken biopsies from the perilesional skin of 12 patients suffering from nonsegmental vitiligo to better investigate the intracellular pathways involved in keratinocytes damage and apoptosis and the antioxidant protection of curcumin and capsaicin in these cells. High levels of activated p38, NF-kB p65 subunit, p53, and Smac/DIABLO proteins were observed in keratinocytes from perilesional vitiligo skin while low levels of ERK phosphorylation were present. For the first time, our study demonstrates the pivotal role of p38 MAP kinase as an upstream signal of perilesional keratinocyte damage, and the important contribution of p38 and NF-kB on p53 accumulation. Curcumin and capsaicin also increase ERK phosphorylation, thus inhibiting apoptosis. Moreover, pretreatment with such natural antioxidants inhibited caspase activation, increased total antioxidant capacity, repressed intracellular ROS generation and lipid peroxidation, and improved mitochondrial activity. These results suggest the possible protective role of antioxidants against vitiligo progression.

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The microRNA molecular signature of atypic and common acquired melanocytic nevi: Differential expression of miR-125b and let-7c

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MicroRNAs (miRNAs) are small, non-coding RNA species, which regulate gene expression through base pairing with the target messenger RNA. A proportion of miRNAs the so-called oncomiRs have been shown to be functionally involved in carcinogenesis. The presence of acquired melanocytic nevi showing clinical atypia (atypic nevi, AN) is an independent predictor of malignant melanoma (MM). We compared the miRNA molecular signature between AN and common acquired nevi (CN). We obtained 22 biopsies of AN and 19 biopsies of CN from patients with no priory history of melanoma. The miRNA expression profiles were obtained from total RNA. Data were validated with qRT-PCR. We show that AN can be differentiated from CN on the basis of the expression of 35 miRNAs (adjusted p-value <0.05). Cluster analysis showed a complex clustering pattern of AN into two major groups. The microarray data and qRT-PCR analysis identified miR-125b and Let-7c as the species the expression of which differed most between AN and CN. This is the first report showing that AN and CN differ on the molecular level. Interestingly, we have showing an independent study that miR-125b is downregulated in maligant melanoma producing sentinel node metastasis comparing to the non-metastasizing tumors. Deregulation of miR-125b and let-7c may constitue early steps in the malignant transformation of melanocytes.

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The Phenolic Compound, *N*-Propionyl-Cysteaminylphenol is Selectively Incorporated into B16F1 Melanoma Cells and Induces Apoptotic Cell death.

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Melanin synthesis uniquely occurs in pigmented cells and is highly elevated in malignant melanoma. Thus, therapy that targets to melanin synthesis can be a new molecular targeting therapy for malignant melanoma. We have previously reported that phenolic compounds are good substrates for tyrosinase as they are selectively incorporated into melanoma cells and induce a melanoma-specific antitumor effect. However, it has not been determined how they inhibit the growth of melanoma cells. In this study, we aimed to elucidate the mechanism of the cell death induced by *N*-propionyl-4-*S*- cysteaminylphenol (NPrCAP) and its antimelanoma effect. We first conducted MTT viability assay for assessment of the growth-inhibitory activity, followed by flow cytometric analysis and caspase assay to elucidate the mechanism of the cell death induced by NPrCAP *in vitro*. Our results showed that the cytotoxic effect of B16F1 mouse melanoma cells induced by NPrCAP was dose-dependent and an evident sub-G1 fraction with activation of caspase 3 was observed when cells were cultured in NPrCAP-containing medium. Furthermore, we examined whether NPrCAP could suppress B16F1 tumors transplanted via three or five intratumoral administrations of 24.4 mM NPrCAP every day or every other day. Our *in vivo* study showed that NPrCAP injection resulted in the inhibition of growth of melanoma tissue and an increase in the life span of melanoma-bearing mice. These results suggest that NPrCAP has an anti-melanoma growth effect by inducing apoptotic cell death and that it can be utilized as a chemotherapeutic agent specific to malignant melanoma.

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The intracellular trafficking of tyrosinase-related proteins-1/2, Pmel17 and MART-1 to melanosomes is interrupted independent of the trafficking of tyrosinase in amelanotic B-16 melanoma cells induced by the Golgi secretory inhibitor monensin: A model similar to the silver mutation

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We have previously demonstrated that the Golgi secretory inhibitor monensin (MS) disrupts the melanosome membrane, resulting in completely unpigmented B-16 melanoma cells despite the successful transfer of tyrosinase (TYR) to those vacuolated melanosomes. To characterize the trafficking of other melanosomal proteins (TYR-related proteins (TRP) 1/2, Pmel17, and MART-1) in the MS-induced amelanotic B-16 (MS-B-16) cells, we used subcellular fractionation, western blotting and confocal laser microscopy (CFLM) to examine differences in their intracellular distribution compared with melanotic non-MS-treated B-16 (Non-B-16) cells. Although the melanosome-rich large granule fraction contained similar levels of TYR protein/activity in the amelanotic MS-B-16 and the melanotic Non-B-16 cells, there were significantly decreased levels of TRP-1(80KDa)/2(100KDa) and Rab27A/B proteins or the complete loss of processed Pmel17 (HMB45) in the amelanotic MS-B-16 cells compared with the melanotic Non-B-16 cells. This suggests that the disrupted melanosomes in the amelanotic Mon-B-16 cells contain TYR but not the other melanosomal proteins (processed Pmel17 and Rab27A/B). Analysis of merged images obtained by CFLM revealed that whereas all melanosomal proteins studied (TYR, TRPs-1/2, Pmel17, and MART-1) sharply co-localized with each other in the vicinity of the nuclei in the melanotic Non-B-16 cells, those proteins, except for TYR, also co-localized with each other in the amelanotic MS-B-16 cells. These results suggest that the intracellular trafficking of TRPs-1/2, MART-1 and Pmel17 to melanosomes is interrupted independent of the trafficking of TYR in the amelanotic MS-B-16 cells. This provides a model similar to the silver mutation where melanosomes are structurally disrupted, leading to the loss of melanization.

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Glucosamine, an asparagine-linked carbohydrate core synthesis inhibitor, attenuates endothelin-1+stem cell factor-stimulated pigmentation in human epidermal equivalents by interrupting the endothelin-1-triggered intracellular signaling pathway within melanocytes.

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Although a carbohydrate core synthesis inhibitor, glucosamine (Glc) has been implicated in producing amelanotic B-16 melanoma cells, it remains unclear whether the inhibition is also effective in preventing the stimulation of pigmentation in human skin. In this study, we evaluated the effects of Glc on the stimulation of pigmentation in a human epidermal equivalent (Melano-Model) and analyzed its biological mechanism. Epidermal equivalents were cultured in DMEM medium supplemented with endothelin-1 (ET-1: 10 nM) and stem cell factor (SCF: 5 nM) and were treated with or without Glc (0.1%). The addition of Glc elicited a marked depigmenting effect on the stimulation of pigmentation in the epidermal equivalents after 14 days of culture. Real-time RT-PCR and western blotting revealed that increased gene and protein expression levels of melanogenic proteins (MITF, tyrosinase, tyrosinase-related proteins-1/2, Pmel17 and ETB Receptor) were significantly suppressed at days 7 and 10, respectively, which suggested an impairment in intracellular signaling upstream of their gene expression. Western blotting of intracellular signaling intermediates and the melanogenic proteins in human melanocytes or ALM human melanoma cells revealed that in Glc-treated cells, there is a marked deficiency in the ET-1+SCF-stimulated phosphorylation of CREB but not of ERK and MITF at 15 min post-stimulation and a significant decrease in the gene and protein expression of the melanogenic proteins at 12 or 24 h post-stimulation, respectively, compared with the control non Glc-treated cells. These findings indicate that Glc attenuates the ET-1+SCF-stimulated pigmentation in epidermal equivalents by interrupting the preferentially ET-1-triggered intracellular signaling pathway.

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The intracellular trafficking of tyrosinase to melanosomes is disrupted independent of the trafficking of tyrosinase-related proteins-1/2 and Pmel17 in reduced glutathione-induced amelanotic B-16 melanoma cells: A model for oculocutaneous albinism type 2.

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We have previously reported that reduced glutathione (GSH) abolishes the intracellular trafficking of tyrosinase (TYR) to melanosomes, which results in completely unpigmented B-16 melanoma cells during the glucosamine (Glc)-depleted recovery of melanization in Glc-treated B-16 amelanotic melanoma cells. To characterize the trafficking of other melanocyte-specific (melanosomal) proteins, such as TYR-related proteins (TRP) 1/2, Pmel17, MART-1 and Rabs27A/B in amelanotic GSH-induced B-16 (GSH-B-16) cells, we used subcellular fractionation, western blotting and confocal laser microscopy (CFLM) to examine differences in intracellular distribution of those melanosomal proteins compared with control melanotic non-GSH-treated B-16 (Non-B-16) cells. In the melanosome-rich large granule fraction, whereas the amelanotic GSH-B-16 cells had a significantly diminished protein/activity of TYR compared with the melanotic Non-B-16 cells, there was substantially no difference in the distribution of TRP-2, processed Pmel17 (HMB45) or Rab27A/B proteins, which suggested that melanosomes in the amelanotic GSH-B-16 cells contain those melanosomal proteins except for TYR. Analysis of merged images obtained by CFLM revealed that while all melanosomal proteins studied (TYR, TRP-1/2, processed processed Pmel17, MART-1 and Rab27A/B) co-localized with each other in the vicinity of the nuclei of the melanotic Non-B-16 cells, those proteins, except for TYR, also co-localized with each other in the amelanotic GSH-B-16 cells. These results suggest that the intracellular trafficking of TYR to melanosomes is selectively disrupted independent of the trafficking of TRPs-1/2, and Pmel17 in the amelanotic GSH-B-16 cells, providing a model similar to oculocutaneous albinism type 2.

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MicroRNA miR-125b expression in early progression of malignant melanoma.

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*M*icroRNAs (miRNAs) are are involved in the regulation of many cellular processes, including differentiation, proliferation, and apoptosis. Recent studies have suggested that deregulation of diverse miRNAs plays an important role in pathological processes such as cancer. The aim of this study was to identify potential miRNAs involved in progression of cutaneous malignant melanoma. Furthermore, we wanted to examine, the effect of transfection by a specific miRNA on melanoma cell lines. 28 archived melanoma FFPE samples (T2) were laser microdissected. MiRNA expression profiles established by use of microarrays and differentially expressed miRNAs were identified. Results were validated by qRT-PCR. Melanoma cell lines were transfected by miR-125b and the effect evaluated by use of proliferation and apoptosis assays. By using microdissection it was possible to obtain a pure sample of malignant melanoma cells. By looking at our microarray data, we recognized microRNA miR-125b to be downregulated in the sentinel-node positive malignant melanomas compared to sentinel-node negative. This was validated by qRT-PCR. In our functional studies, exogenenous expression of miR-125b resulted in lower levels of proliferation, whereas inhibition of miR-125b didn't had any effect. Regarding apoptosis, inhibition of miR-125b resulted in lower levels apoptosis, while mimicking of miR-125b had no effect. Laser microdissected cells of FFPE melanoma specimens seem suitable as a source for miRNA microarray profiling. MiR-125b may be involved in an early progression of cutaneous MM, with tumor suppressor potential.

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Tight Junction-specific proteins are absent from melanoma while ZO-1 is widely expressed

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Malignant Melanoma (MM) is the most aggressive form of skin cancer and is highly resistant to conventional chemotherapy, immunotherapy and targeted therapy. The presence of Tight Junctions (TJ) in these tumors might result in the encapsulation of specific areas which are no longer accessible for the immune system or for therapeutics, therefore contributing to this resistance. We investigated various TJ proteins, including members of the TJ specific claudin family and the multifunctional protein ZO-1 in the tumor and the tumor-microenvironment in MM and nevi, including borderline cases and MM cell lines. ZO-1 was clearly positive in MM and nevi, but its increased localization in invasive areas of MM was correlated with tumor progression (Breslow Index). Other TJ proteins were only expressed in a minority of cells or absent. Tracer assays in spheroids generated from cultured MM cells showed no stop of the tracer arguing for the absence of barrier forming TJs. But we found a correlation of ZO-1 protein levels and MM invasiveness. Investigating the tumor-microenvironment of nevi and melanoma including borderline cases we saw a striking difference in ZO-1 expression in the epidermis: While nevi exhibited only a slight upregulation compared to normal skin, in MM a broadened ZO-1 expression in all epidermal layers was found. We conclude that functional TJ reducing the accessibility for therapeutics are absent in MM but protein levels of ZO-1 might be involved in invasiveness of tumor cells. Expression of ZO-1 in the tumor-microenvironment might be used for diagnostic delineation between MM and nevi.

ABSTRACTS

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A New Reconstructed Human Epidermis to Address Challenges in Melanocyte Biology

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Studying human melanocyte biology either depends on in vitro cell culture models or on the availability of fresh skin biopsies, which harbour pathologically changed tissue in most cases. Most in vitro culture models do not allow physiological interactions between melanocytes and keratinocytes, since melanocytes need very special culture conditions for growth. Even in co-culture models there is no physiological spatial organisation of melanocytes together with keratinocytes. Here we present a new reconstructed epidermis with integrated melanocytes which is based on the already established technology of Epidermal Skin Test 1000 (EST1000, CellSystems, Germany). We tested different ratios of melanocytes and keratinocytes for the production of the skin models and used melanocytes from different ethnic groups. To demonstrate the physiological behaviour of this epidermis model we successfully stimulated the melanin synthesis with chemical inducers and also by UV-radiation. Blocking melanin synthesis by chemical inhibition of the tyrosinase activity demonstrated that tanning of the models is due to the specific activity of melanocytes. Finally the melanin content could be correlated with different tanning intensities. This epidermis model can be cultivated for at least 4 weeks and allows short term as well as long term studies. We conclude from these results that CellSystems´ newly reconstructed epidermis with integrated melanocytes is able to cover all necessary applications in studies where tanning and bleaching of the skin is of importance.

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A systems biology approach to tumor suppression in malignant melanoma

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The inactivation of tumor suppressor proteins like p53 and p16 is involved in the development of many malignant tumors including malignant melanoma. Recently, evidence has been provided that 14-3-3 sigma, a downstream target of p53, might play an important role in tumor suppression in malignant melanoma.To better understand the complex process of tumor suppression in malignant melanoma, we established a mathematical model integrating the effects of 14-3-3 sigma gene silencing, the dynamics of 14-3-3 sigma induction and intracellular compartmentalisation and the role of interacting proteins such as p53, MDM2, WEE1 and Cdc25. In vitro experiments with different melanoma cell lines were performed and a mathematical model was subjected to computer simulations to analyse different scenarios of protein activation and interaction. Our analyses showed that strong stimulations, exerted for example by genotoxic stress, are necessary to induce 14-3-3 sigma expression in malignant melanoma even in cases of intermediate levels of gene methylation. More importantly, our model suggests that downregulation of p53 expression via 14-3-3 sigma gene methylation and reduction of its nuclear localisation significantly affect p53 activity. Taken together, the complex regulation of tumor suppression in malignant melanoma is described by a mathematical model using a systems biology approach. The proposed model allowed us to predict tumor cell behavior under different conditions of stimulation and emphasized the important role of gene methylation of tumor suppressor molecules for tumor malignancy. These findings might open new perspectives for future treatment strategies of malignant melanoma.

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N-trans-Feruloyl-3-Hydroxytyramine: a substrate-mimicking inhibitor of human tyrosinase in vitro

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Melanin synthesis (melanogenesis) is under the control of complex regulatory pathways but tyrosinase is the rate limiting enzyme and thus remains the most efficient way to downregulate melanin production.The goal of our work was to develop a characterized molecule with efficacy comparable to that of existing products without long term toxicity. We focused our investigations on compounds with high structural similarities with tyrosine and L-DOPA - the natural substrates of tyrosinase - that would act as substrate-mimicking inhibitors. This way, we identified N-trans-Feruloyl-3-Hydroxytyramine, as the best candidate. The ability of N-trans-Feruloyl-3-Hydroxytyramine to inhibit tyrosinase activity *in vitro* was evaluated using the human and mushroom tyrosinase model systems as well as total melanin quantification in the murine B16 cell line. Moreover, using quantitative RT-PCR, we measured the mRNA expression of Pmel 17, a melanosome specific protein critical for melanin maturation. *In vitro*, this molecule inhibited human tyrosinase with higher efficacy than the reference inhibitors kojic acid and arbutin and without cell toxicity as measured in normal human melanocytes. Using this model, IC50 was estimated at about 38µM. Moreover the inhibition appeared to be specific to mammalian tyrosinases as shown by a very poor inhibition of mushroom tyrosinase but a significant decrease of total melanin in the murine B16 cell line. In this latter model, IC50 was evaluated at about 30µM. Finally, the derivative exhibited significant inhibition of Pmel-17 gene expression at 100µM. These *in vitro* data suggest that N-trans-Feruloyl-3-Hydroxytyramine could be an interesting ingredient for complexion lightening cosmetic formulas.

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Ionizing radiation induces serotonin release from mast cells: Implications for malignant melanoma cells

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Mast cells are key effector cells of the immune system and accumulate around the margin of cutaneous malignancies. Previous studies have demonstrated that exposure to ionizing radiation results in degranulation of dermal mast cells and release of mediators. The purpose of our study was to evaluate the specific effect of serotonin on malignant melanoma cells. Irradiation of the human mast cell line HMC-1 with 5 Gy and subsequent ELISA analysis revealed serotonin as an ionizing radiation-induced mast cell secretory product. The presence of serotonin receptors 5-HT2AR, 5-HT2BR and 5-HT2CR on the human melanoma cell line IPC-298 was verified using immunofluorescence. To investigate the effect of serotonin on cell proliferation of melanoma cells, IPC-298 cells were stimulated with serotonin and examined by proliferation assay. The results showed that serotonin dose-dependently inhibited melanoma cell proliferation. It is well established that melanoma metastasis is regulated by adhesion molecules. Therefore, we next aimed to determine the influence of serotonin on adhesion molecule expression by IPC-298 cells. As shown by gene array and flow cytometry, IPC-298 cells constitutively expressed a variety of adhesion molecules. Stimulation with serotonin caused a significant increase of the cell surface expression of intercellular adhesion molecule-1 (ICAM-1) and integrins beta1 (CD29), alpha2 (CD49b), alpha5 (CD49e) and alpha6 (CD49f) on melanoma cells.Taken together, our data imply ionizing radiation-induced serotonin release by mast cells to be a crucial factor in malignant melanoma development.

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The dual role of IFN-α in TRAIL-induced apoptosis of melanoma cells

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Human peripheral blood leukocytes acquire the cytotoxic molecule TNF-related apoptosis-inducing ligand (TRAIL) in response to IFN-α treatment. Since IFN-α is used adjuvantly in melanoma therapy, we asked the question whether the induction of TRAIL+ immune cells has a role in preventing disease progression. To this end we analyzed the susceptibility of established melanoma cell lines and such generated from metastases of stage IV melanoma patients to TRAIL. Overnight treatment of melanoma cells with soluble TRAIL induced apoptosis ranging from 6-54%, as determined by Annexin V/PI staining. Since TRAIL acts via TRAIL receptors (TRAIL-R) we analyzed the TRAIL-R expression pattern on melanoma cells by flow cytometry. We generally detected moderate to high levels of the pro-apoptotic TRAIL-R2, but little to no expression of TRAIL-R1, -R3 and -R4. This was reflected in the TRAIL-R2 mRNA levels, which were generally 2-log higher compared to the other receptors. Interestingly, the magnitude of TRAIL-induced apoptosis did not correlate with TRAIL-R mRNA levels or protein surface expression. Since the role of IFN-α in melanoma may not be confined to leukocyte effector cells, we also studied its effects on melanoma cells. Treatment of melanoma cells with IFN-α alone inhibited proliferation but did not induce apoptosis. IFN-α pre-treatment, however, enhanced subsequent TRAIL-induced apoptosis, suggesting effects on TRAIL-R expression or other molecules of the apoptotic cascade. In summary our findings suggest that by inducing cytotoxic TRAIL on effector leukocytes and enhancing melanoma cell susceptibility to TRAIL, the role of IFN-α in melanoma therapy may be a dual one.

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2,4,6-octatrienoic acid promotes melanogenesis in normal human melanocytes

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Skin pigmentation is the outcome of melanin synthesis, which acts as a filter against UV and possesses also scavenger properties capable to protect UV-related skin damage. Melanin synthesis is a complex process controlled by several regulators affecting intracellular signal transduction pathways. We have observed that parrodienes, a class of compounds sharing some structural features with carotenoids, natural precursors of retinoids, counteract senescence-like phenotype in fibroblasts and induce peroxisome-activated receptor gamma (PPARg), a receptor involved in an intracellular mechanism of retinoid-like molecules and exerting a crucial role in the control of cell proliferation and differentiation. Considering that PPARg agonists can stimulate melanogenesis, we selected 2,4,6-octatrienoic acid, a derivative of parrodienes, to study its ability to promote melanogenesis in normal human melanocytes (NHM). Exposure of NHM to Octa resulted in a significant induction of tyrosinase expression and activity, known to be a rate-limiting enzyme in melanin synthesis. The melanin content was also significantly increased by Octa treatment. Moreover we examined the expression level of PPARg and microphthalmia-associated transcription factor (MITF), the master transcription factor regulating melanocyte differentiation, proliferation and survival. The observed increase in pigmentation was associated with a stimulation of both PPARg and MITF expression. These results demonstrate that Octa is able to regulate melanogenesis in NHM, suggesting its promising use to enhance skin photoprotection and counteract pigmentary disorders.

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Protodynamic intracellular acidification by *cis*-urocanic acid promotes apoptosis of melanoma cells *in vitro* and *in vivo*

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The extracellular tumour microenvironment is acidified whereas the intracellular pH of tumour and stromal cells is neutral. *cis*-Urocanic acid (*cis*-UCA), an endogenous compound of the skin, can acidify the cytosol by transporting protons into the cells. This phenomenon termed the protodynamic concept was studied in human cancer cells. *cis*-UCA dose dependently reduced the number of viable human melanoma, cervical carcinoma, and fibrosarcoma cells at weakly acidic extracellular pH. The intracellular pH decreased by up to 0.5 pH units in a concentration-dependent manner with 0.3–30mM *cis*-UCA at extracellular pH 6.5 but not at pH 7.4. Under the same conditions, 30mM *cis*-UCA induced annexin-V binding and activation of caspase-3 in A2058 melanoma cells as signs of apoptotic cell death. Finally, growth of human melanoma xenografts in SCID mice was suppressed by 60% following intratumoural injection of *cis*-UCA. Accordingly, the percentage of tumour necrosis and active caspase-3-immunopositive cells increased whereas proliferation activity decreased. These results identify cis-UCA as an anticancer agent inhibiting melanoma growth by immediate intracellular acidification followed by apoptotic cell death *in vivo*.

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Melanoma cell-derived factors stimulate hyaluronan synthesis in dermal fibroblasts by upregulating HAS2 through AKT –signaling

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Hyaluronan is a large extracellular matrix molecule, which is produced by hyaluronan synthases (Has1, 2, 3) on the plasma membrane. Several cancers are associated with hyaluronan overproduction either by tumor cells or the surrounding stromal cells. This increased hyaluronan accumulation has been shown to enhance tumor cell invasion and metastasis leading to an unfavourable clinical prognosis. In the present work, we studied the effects of melanoma cell-derived factors on fibroblast hyaluronan synthesis. We found that melanoma cell-conditioned medium (CM) stimulated strongly fibroblast hyaluronan synthesis and changed the morphology of fibroblasts. The fibroblasts treated with melanoma cell CM were more elongated, contained numerous long protrusions and displayed a wide pericellular hyaluronan coat. The elevated hyaluronan production in CM-treated cultures was associated to upregulation of *HAS2* mRNA, approximately 7-fold, respectively as determined with qRT-PCR. The expression levels of *HAS1*, *CD44* and *Hyal2* did not change after treatment with melanoma CM and the expression of Has3 was slightly upregulated. To find out the signalling routes to HAS2 upregulation, we screened the phosphorylation profiles of 46 kinases with a phospho-kinase-array. Melanoma cell CM treatment strongly induced the phosphorylation of AKT, JNK, p38, CREB, HSP27 and cJUN in fibroblasts. An inhibitor of AKT suppressed the melanoma cell CM –induced hyaluronan production in fibroblasts.The data suggests that the induced hyaluronan synthesis by HAS2 upregulation in fibroblasts is, at least partly, due to activation of AKT signalling. In the future, prevention of hyaluronan overproduction in tumor stroma is a potential target for anticancer drugs.

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Risk for a second primary melanoma in patients with a history of melanoma and multiple melanocytic nevi: A 5 year follow up study

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To evaluate the risk for a second primary melanoma in patients with a past history of melanoma and multiple melanocytic nevi. Secondly, to compare the risk and risk factors with patients with multiple nevi but without a history of melanoma. Altogether 113 patients with a history of primary melanoma and 98 patients without a history of melanoma were followed for over 5 years. In both groups all patients had over 100 melanocytic nevi and on clinical grounds 5 or more atypical nevi. Additional risk factors, such as a family history of melanoma and former dysplastic nevi were equally present. Dermatologic examinations including dermatoscopy and whole body photographs were performed 1-2 times a year. 29 second primary melanomas were diagnosed in 20 (17,7%) patients of the melanoma group, while in the non melanoma group only 1 (1,0%) melanoma was diagnosed. The melanomas were superficial (average depth 0,39 mm) with a favourable prognosis. The melanoma group showed a 25 times higher risk for a second primary melanoma than the non melanoma group for a first primary melanoma. 86,2% of the melanomas were diagnosed in patients with no additional risk factors. In conclusion, patients with a past history of melanoma and multiple melanocytic nevi have a high risk for developing a second primary melanoma. A history of melanoma is the most important risk factor for a second primary melanoma and long-term follow-up of such patients is warranted. The follow up proved to be effective in diagnosing superficial melanomas with a good prognosis.

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Neuron navigator 3, a new cancer-associated gene, shows copy number changes in dysplastic nevi, primary melanomas and in metastasizing melanoma cells.

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Melanomas are heterogenic tumors and carry hundreds of mutations, the most common being those of the BRAF (50-60%), NRAS (15%) and c-kit (3%) genes. Melanocytic nevi frequently harbor these oncogenic mutations but only a minority progresses to melanoma. Neuron navigator 3 (NAV3) belongs to the group of Navigator proteins that bind to the + ends of microtubules and are involved in cell proliferation and motility. *NAV3* gene mutations or copy number changes have been reported in melanoma, glioblastomas and colorectal cancer, and downregulation of *NAV3* has been shown in some brain tumors and in adrenal carcinoma. We looked for *NAV3* aberrations, using FISH, in dysplastic nevi, primary melanomas, and corresponding sentinel lymph nodes or metastases. Also, two established melanoma cell lines were analyzed. Cut-off values for the FISH results were based on findings in benign nevi. We observed *NAV3* amplifications and/or deletions in 68% of the primary melanomas (17/25; range of deletion 6-98 % and amplification 10-79 % in the cell nuclei) and in 4/6 dysplastic nevi. *NAV3*-deleted cells were found in all involved sentinel lymph nodes examined. In the two metastases so far analyzed, one pair showed few *NAV3*-deleted cells in the primary tumor but up to 40 % in the metastases. The melanoma cell lines were heterogenic, WM-793 showing 20% of amplifications and 36% of deletions, whereas metastatic WM-239 showed 21% of deletions. We suggest that lack of NAV3 function could interrupt the normal behaviour of the cellular microtubule network and might induce a malignant phenotype.

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Identification of priming events in sentinel lymph nodes in a murine model of cutaneous melanoma

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Cutaneous melanoma first metastasizes into sentinel lymph nodes that control the lymphatic drain from the area of the primary tumor. Sentinel lymph node biopsy has therefore become an important diagnostic procedure in patients with primary melanomas at tumor stages ≥T2a. Successful colonization of tumor cells into sentinel lymph nodes is thought to require priming of this environment, but these priming factors are yet undefined. Using our previously described xenotransplantation model, in which human melanoma cells injected into the skin of SCID mice metastasize to sentinel lymph nodes, we aimed to identify and analyze a gene expression signature reflecting priming events. Tumor negative sentinel lymph node expression profiles have been compared to expression profiles i) of micro- and macrometastases and ii) of lymph nodes of control (= non tumor-bearing) animals. For analysis, Affymetrix® gene arrays have been used. Results obtained from these experiments have been validated by using RT-PCR based mRNA analysis and immunohistochemistry. With these techniques we were able to identify gene products which may serve as potential candidates responsible for initiating melanoma lymph node metastasis by altering the lymph node microenvironment.

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Characterization of *in vitro* cultured dedifferentiated melanocytes

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Previously we described that normal human adult epidermal melanocytes dedifferentiated when cultured *in vitro* in a chemical mitogen-free medium (Melmix®). These *in vitro* dedifferentiated cells are bipolar, proliferate rapidly, lose melanin content and TRP-1 and c-Kit expressions. To further characterize these cells we compared them to melanocytes grown in conventional chemical mitogen containing medium in which cells regain a differentiated phenotype: they are dendritic, contain pigment and express TRP-1 and c-Kit. The proliferation rate of the dedifferentiated cells was twice as fast as the differentiated ones based on CFSE analysis. Senescence-associated β-galactosidase assay showed similar rates of senescence in both cultures. Translocator protein (TSPO) and nestin mRNAs are expressed at similar levels in both cells, however nestin protein expression was higher in the dedifferentiated cells; both TSPO and nestin are characteristically expressed in neuronal precursor cells. Interestingly, p53 an important tumor suppressor in UV-induced cellular stress response showed a highly increased expression in the non-pigmented, dedifferentiated cells compared to pigmented, differentiated cells after UVB irradiation.

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Evaluation of the long-term efficacy of UVB-311nm-therapy in vitiligo patients

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Vitiligo is a common idiopathic acquired depigmentation disorder characterized by the loss of melanocytes from the epidermis. Narrow-band UVB therapy is a well-established treatment modality in this disease but there is only little data on long-term efficacy available. In this retrospective university-based hospital study we enrolled 73 patients (45 women and 28 men female) with vitiligo who had been treated with UVB-311nm-therapy twice a week during the 8-year period from 1999 to 2006. The median number of treatments was 34 and the median cumulative dose 33.9 J/cm2. A follow-up questionnaire was sent in 2008 to all patients in order to evaluate the efficacy of therapy and the course of the disease after treatment. Forty-seven (63%) patients returned the questionnaire. UVB-311nm treatment resulted in at least partial repigmentation of vitiligo lesions in 25 out of 47 patients (53%). Certain lesions showed complete repigmentation in 9 (19%) patients. Stable disease for at least 12 months was observed upon UVB-311nm treatment in 23 (49%) patients. At the present UVB-311nm-therapy remains the gold standard in the treatment of vitiligo. The therapy can induce repigmentation and stability of disease. It also may improve the quality of life of patients with vitiligo, as reported by many patients.

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Role and regulation of Raf kinases in melanoma

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Melanoma has become a major public health problem in many countries. In order to develop new therapies for this tumour, we are studying signaling pathways which play a role in proliferation, survival and differentiation of melanocytes and melanoma. The Mitogen Activated Protein Kinase (MAPK) pathway regulates melanocyte proliferation in culture and *in vivo*. Constitutive activation of the MAPK pathway in melanoma, either by mutations in the Ras gene or by mutations in B-Raf, has been reported in 15% and 50% of melanoma respectively. The presence of B-Raf mutations in approximately 50% of human melanoma highlights the oncogenic properties of this kinase in melanocytes and inhibitors of B-Raf are currently under clinical development. However, we have shown that B-Raf was inactivated in melanoma containing a Ras mutation. This inhibition was due to a down-regulation of B-Raf by its substrate Erk, which phosphorylates B-Raf on its amino-terminus to prevent its interaction with Ras. This mechanism of B-Raf negative regulation forces these melanoma cell lines to use the other Raf isoform, C-Raf. The inactivation of B-Raf in Ras mutated melanoma suggests that B-Raf inhibitors will be ineffective in these cancers. Moreover, we have demonstrated that an inhibitor inactivating both B-Raf and C-Raf, which is in clinical development (Sorafenib), paradoxically induced activation of these kinases by heterodimerization. Together, our results highlight novel Raf kinase regulation mechanisms, which will play an important role in the resistance of melanoma to the inhibitors currently in clinical development.

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The SCF/KIT signaling pathway is a key regulator of epidermal melanocytes migration and of the skin pigmentation homogenization.

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The stem cell factor (SCF) is the natural ligand of the KIT receptor which is a member of subfamily III of the Receptor Tyrosine Kinase family. In the skin, this pathway regulates melanocyte homeostasis and their epidermal distribution. In the present study, we analyzed the localization of SCF and KIT expression in the skin and in several skin-related cell lines, and evaluated their implication in the pigmentation process. To help us in our approach, we developed a specific compound (IV09.007) and studied its ability to modulate the SCF/KIT signaling pathway. IV09.007 treatment of *ex vivo* skin or cultured cells markedly increased the expression of both SCF and KIT (as revealed by immunohistological studies and western blotting). IV09.007 also increased melanin content in the basal layer of the epidermis (Fontana-Masson staining). These effects could contribute to a better protection of the epidermal basal layer. Interestingly, migration of cultured human melanocytes was enhanced in the presence of IV09.007 in the culture medium. The action of the compound on the global phosphorylation state of the skin and on players of the SCF/KIT signaling cascade (ERK1/2) was also investigated. Taken together, these studies confirm the importance of the SCF/KIT pathway in the skin and suggest that modulating this pathway could enhance the homogeneity of melanocyte repartition in the skin, and favor a higher production and content of protective me

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The Forkhead Box Transcription Factor FoxP3, a prognostic marker for progression-free survival in stage III melanoma

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Regulatory T cells have already been associated to poor prognosis in various types of cancer. It was previously reported, in ovarian carcinoma, that high expression of Foxp3 identified a subgroup of patients characterized by a significantly worse prognosis in terms of overall survival and progression-free survival. This result suggests that high expression levels of Foxp3 might represent a surrogate marker for an immunosuppressive microenvironment contributing to tumor immune escape. Regarding melanoma, it was previously shown that CD4+CD25^{high} T cells were overrepresented in metastatic lymph nodes. The aim of the present study was to evaluate the prognostic value of Foxp3 as a single marker regarding progression-free survival and overall survival in stage III (AJCC) melanoma patients. For this purpose, we analyzed Foxp3 expression in 102 tumor invaded lymph nodes, using quantitative real-time PCR, normalized on T lymphocytes. A Cox model was used to analyze patients' survival. High Foxp3 expression level was independently associated with poor prognosis in terms of progression-free survival (p=0.005) but not in terms of overall survival (p=0.87). In the group of patients receiving TIL, the expression level of Foxp3 was not a prognostic factor for progression-free survival or overall survival (p=0.36 and 0.8). No significant difference in Foxp3 expression was observed between patients with one invaded LN and patients with more than one invaded LN (p=0.3775). In conclusion, Foxp3 expression using qPCR might represent a prognostic factor in stage III melanoma for progression-free survival.

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Different patterns of micro RNA expression in cutaneous and uveal melanomas

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Uveal melanoma (UM) is the most common ocular primary tumour in adults. Metastasis, which develops in 50% of patients, has often a fatal outcome. In contrast to cutaneous melanoma, UM disseminates by hematogenous spread primarily to the liver. In this experiment we dissected 26 paraffin embedded UM samples for the microarray micro RNAs (miRNA) analysis. The samples originated from UM patients (mean age of 64,4 years) of whom 11 had metastatic disease. The control material was 28 microdissected samples of primary cytaneous melanomas (PCM) (stage T2). We found that miRNA expression profiles of UM differed radically from those of PCM. Although based on the hierarchical cluster analysis 4 different groups of UM was apparent, these groups did not correspond to the presence or absence of metastasis. In conclusion, the molecular miRNA signatures of UM and PCM are very different suggesting basic differences in the pathogenesis of these tumors. The metastatic behaviour of UM cannot be predicted from the miRNA expression pattern.

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Metastasizing preferences of melanoma subtypes

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Several cancer entities show distinct organ-preferences for their metastatic spread. In melanoma such affectation has not been systematically studied. In order to gain insights into correlations between tumor metastasis and genetic background of melanoma subtypes, a retrospective study was performed.137 patients of melanoma stage IV were included into the study. Two groups were formed according to their molecular background: Superficially Spreading (SSM) and Nodular (NMM) were pooled in group 1 and compared towards Acrolentiginous (ALM) and Mucosal (MM) melanoma (group 2). Group 1 shows a high frequency of BRAF mutations, while group 2 shows genetic aberrations in cKIT. Over-all survival was identical in both groups witsh mean survival of 10 months. LDH presented prognostic implications. The time interval until appearance of the first distant metastasis and the distribution pattern were analysed and correlated to the median survival time. Metastasis to distant organs was shown to be slightly faster in group 2 (p= 0.075). However, the preference to present bone metastases was significantly higher for group 2 in comparison to group 1 ($p = 0.002$), which revealed a preferential spread of metastases into the brain ($p < 0.0001$). This study has disclosed new insights into melanoma subtype distinction in the context of distant metastasis and prognosis. The differentiation of two melanoma groups confirmed the rearrangements of melanoma classification according to genetic similarities. Further knowledge about melanoma subtype specific characteristics might help to improve targeted therapeutic employment but and to understand and manage the disease in terms of prognosis.