The antimycotics suppress thymic stromal lymphopoietin production in human keratinocytes

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Thymic stromal lymphopoietin (TSLP) is produced by epidermal keratinocytes, and it induces Th2-mediated inflammation. TSLP expression is enhanced in lesions with atopic dermatitis, and is a therapeutic target. Antimycotic agents improve the symptoms of atopic dermatitis. We examined whether antimycotics suppress TSLP expression in human keratinocytes. Normal human keratinocytes were incubated with polyinosinic-polycytidylic acid (poly I:C) plus IL-4 in the presence of antimycotics. TSLP expression was analyzed by performing ELISA and real time PCR. Luciferase assays were performed to analyze NF-κB activity. IκBα degradation was analyzed by performing western blot analysis. Poly I:C plus IL-4 increased the secretion and mRNA levels of TSLP, which was suppressed by an NF-κB inhibitor, and also enhanced NF-κB transcriptional activities and induced the degradation of IκBα in keratinocytes. The antimycotics itraconazole, ketoconazole, luliconazole, terbinafine, butenafine, and amorolfine suppressed the secretion and mRNA expression of TSLP, NF-κB activity, and IκBα degradation induced by poly I:C plus IL-4. These suppressive effects were similarly manifested by 15-deoxy-A-12,14-PGJ2 (15d-PGJ2), a PGD2 metabolite. Antimycotics increased the release of 15d-PGJ2 from keratinocytes and decreased the release of thromboxane B2, a thromboxane A2 metabolite. Antimycotic-induced suppression of TSLP production and NF-κB activity was counteracted by an inhibitor of lipocalin type-PGD synthase. In conclusion, antimycotics itraconazole, ketoconazole, luliconazole, terbinafine, butenafine, and amorolfine may suppress poly I:C plus IL-4-induced production of TSLP by inhibiting NF-κB via increasing 15d-PGJ2 production in the keratinocytes. These antimycotics may block the overexpression of TSLP in lesions with atopic dermatitis.

The possibility of having the same pathogenesis of mycosis fungoides, large-and small plaque parapsoriasis

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Background: Large plaque parapsoriasis (LPP) and Small plaque parapsoriasis are general diseases, which are related to cutaneous T-cell lymphoproliferative disorders, whose biological distinction from early Mycosis Fungoides (MF) is still not clearly defined. Objectives: Some authors recently have found the preponderance of immature dendritic cells (CD209/DC-SIGN) in MF and syndrome Sezary lesions, while in the normal skin these cells were absent. The authors supposed that it is probably important for the mediation of immunological tolerance against malignant T-cells. Therefore, the purpose of our study is to show the expression of CD209/DC-SIGN markers in order to investigate the role of these markers in LPP and SPP. Methods: Three patients with LPP and seven patients with SPP were included in the study. Skin biopsy were obtained from lesions well-circumscribed round or oval pink patches. Patients with LPP lesions have patches with size more than 8-10 cm and patients with SPP lesions have patches with size less than 5-6 cm in diameter. Immunohistochemistry was performed on the paraffin tissue sections for CD209/DC-SIGN monoclonal antibodies. Results: The study showed a significant infiltration of CD209/DC-SIGN in dermal infiltrate in both conditions. Limitations: The number of patients was small. Conclusions: Our study possibility demonstrates that LPP and SPP have the same pathogenetic mechanisms like MF and syndrome Sezary. The presence of immature dentritic cells (CD209/DC-SIGN) in dermal infiltrate probably play important role in the mediation of immunological tolerance against malignant T –cells. For the representative results of these pathogenetic mechanisms it needs a control group of patients with non-chronic dermatitis.

003

Reduced CD18 levels drive Treg conversion into Th17 cells in the CD18hypo PL/J mouse model of psoriasis

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Defective development and function of CD4+CD25high+Foxp3+ regulatory T cells (Tregs) contribute to the pathogenesis of psoriasis and other autoimmune diseases. Little is known about the influence of adhesions molecules on the differentiation of Foxp3+ Tregs into pro-inflammatory Th17 cells, occurring in lesional skin and blood of psoriasis patients. In the CD18hypo PL/J mouse model of psoriasis reduced expression of CD18/β2 integrin to 2–16% of wildtype levels is associated with progressive loss of Tregs, impaired cell-cell contact between Tregs and dendritic cells (DCs) as well as Treg dysfunction as reported earlier. In the present investigation, Tregs derived from CD18hypo PL/J mice were analyzed for their propensity to differentiate into Roryt+ IL-17 producing Th17 cells *in vivo* and in *in vitro* Treg-DC co-cultures. Adoptively transferred CD18hypo PL/J Tregs were more inclined towards conversion into IL-17 producing Th17 cells *in vivo* in an inflammatory as well as non-inflammatory environment compared to CD18wt PL/J Tregs. Addition of neutralizing antibody against CD18 to Treg-DC co-cultures *in vitro* promoted conversion of CD18wt PL/J Tregs to Th17 cells in a dose-dependent manner similar to conversion rates of CD18hypo PL/J Tregs. Reduced thymic output of nTregs and peripheral conversion of Tregs into Th17 cells therefore both contribute to the loss of Tregs and the psoriasiform dermatitis observed in CD18hypo PL/J mice mice. Our data overall indicate that CD18 expression levels impact Treg development as well as Treg plasticity and that differentiation of Tregs into IL-17 producing Th17 cells is distinctly facilitated by a subtotal deficiency of CD18.

004

Programmed cell death1 (PD-1) regulates the effector function of autoimmune CD8 T cells and protects against immune-related disease

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PD-1 is an inhibitory molecule expressed by activated T cells. Its ligands (PD-L1 and -L2) are expressed by many leukocytes and non-leukocytes. Recent clinical trials have shown that anti-PD-1 or anti-PD-L1 blocking antibodies enhance cancer immunity. To assess the role of PD-1 in CD8 T cell-mediated diseases, we used PD-1 knockout (KO)-OVA-specific T cell-receptor transgenic (Tg) CD8 T cells (OT-I cells) in a murine model of graft-versus-host disease (GvHD). Mice expressing membrane-bound ovalbumin in skin and mucosae (mOVA mice) develop a GvHD-like disease, manifested by skin/mucosal lesions and weight loss, after transfer of $\geqq 5\times10^5$ OT-I cells. Recovery begins 14 days after adoptive cell transfer. We found that mOVA mice that received as few as 5×10^4 PD-1KO-OT-I cells developed GvHD with intense skin and mucosal lesions and with significant weight loss (-16.6%±2.7) by day 7, and mice required euthanasia by day 9. mOVA mice that received 5×10^4 wild-type OT-I cells did not develop lesions or weight loss (-1.1%±3.3, $p<0.05$; *U* test). FACS analysis showed that PD-1KO-OT-I cells accumulated in skin-draining lymph nodes and spleens (6.7% and 31.3%, respectively) of mOVA mice 7 days after cell transfer to a greater extent than wild-type OT-I cells (1.8% and 2.9%). Four days after cell transfer, >90% of OT-I cells, CD4 T cells and DCs expressed PD-L1, and 7 days after transfer 39.5% of CD4 T cells and 14.1% of DCs expressed PD-L2. Consistent with our prior studies, when mOVA×OT-I double Tg (dTg) mice received 1×10^6 OT-I cells they did not develop GvHD, whereas dTg mice that received 1×10^6 PD-1KO-OT-I cells developed disease with skin/mucosal lesions and weight loss (-15.3%±1.6, $p<0.01$; *U* test) at day 6. In aggregate, our data strongly suggest that the PD-1/PD-L1, -L2 system can influence the development and/or progression of CD8 T cell-mediated immune disease. Enhancement of PD-1 or PDL1/PDL2 expression by effector cells, accessory cells or target cells may attenuate CD8 T cell-mediated diseases, including GvHD.

005

Anti-inflammatory effect of a traditional Chinese medicine Qingpeng ointment on induced atopic dermatitis in mice

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Background. Atopic dermatitis (AD) is a recurrent pruritic and chronic inflammatory disease. Qingpeng ointment (QP) is a traditional Chinese medicine which has been used in the treatment of AD in China, but the mechanism is unknown. Aim. To analyze the anti-inflammatory effects of QP on induced AD in mice. Methods. AD lesions were induced in 108 eight-week-old BALB/c mice by repeated application of 2, 4-dinitrofluorobenzene on shaved backs and the mice were then equally divided into 6 groups to untreated group (model control), different concentration of QP (100%, 75% and 50%) treated group, vehicle of QP treated group and Mometasone Furoate cream (MF) (positive control) treated group respectively, and treated for two weeks. Eighteen mice receiving no sensitization or treatment served as blank normal control. Macroscopic and microscopic changes of the skin lesions were observed after the treatment. The levels of serum immunoglobulin (Ig) E, tissue interferon (IFN)-γ, interleukin (IL)-4 and IL-17A protein were measured with Enzyme-Linked Immunosorbnent Assay (ELISA) and tissue mRNA expression of IL-4, IFN-γ and IL-17A was measured with quantitative real-time polymerase chain reaction (PCR). Results. Similar to MF, QP could significantly inhibit the induced AD lesions in a dose-related pattern. Levels of serum IgE, tissue IL-4 and the mRNA expression of IL-17A was suppressed by QP treatment while the tissue IFN-γ was increased. Conclusions. QP could inhibit hapten induced AD lesions in mice by at least inhibition of IL-4, IgE and IL-17A production and by increasing the tissue level of IFN-γ.

006

Human Langerhans' cell migration can be inhibited by blocking β1 integrin function

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Langerhans' cells (LCs) are the outermost sentinels of the immune system, with dual immunostimulatory and immunoregulatory roles. Following stimulation with self or foreign antigen, LCs disengage from neighbouring keratinocytes and migrate to draining lymph nodes. Integrins are bidirectional signalling molecules associated with various aspects of cellular physiology, migration and survival. They exist as heterodimers, with α and β subunits. LC migration in mice is contingent upon functional α6 and α4 integrins with presumed β1 integrin partner subunits. We have investigated the role of β1 integrin function in human LC mobilisation using an epidermal explant assay. Four 6mm punch biopsies were taken from sun-protected skin of 6 healthy subjects (age 18-30y) and incubated in ethylenediaminetetraacetic acid (EDTA) for 2h before the epidermis was mechanically removed using forceps. For each subject, 1 epidermal sheet was fixed in acetone immediately (for baseline counts) and the remaining 3 sheets cultured at 37C for 24h in RPMI medium alone or in the presence of 10ug/ml functional or non-functional rat anti-β1 integrin IgG2a monoclonal antibody. The sheets were stained with CD1a-fluorescein isothiocyanate (FITC) antibody before LC counts were performed using immunofluorescent microscopy. Mean LC counts were 1131 +/- SD 253 per mm2 at baseline with approximately 18% LCs migrating at 24h in the presence of either culture medium alone (mean LC count 933 +/- SD 270) or added non-functional anti-β1 integrin antibody (mean LC count 922 +/- SD 223). In the presence of functional anti-β1 integrin antibody, LC migration was significantly reduced ($p<0.01$; mean LC count 1085 +/- SD 229; mean migration 3.5%). These findings implicate functional β1 integrin-containing heterodimers in the migration of LC from the epidermis. Future investigations will utilise the explant model to further explore integrin subunit involvement in LC migration.

007

Low numbers of memory T cells correlate with minor CCL27 expression in prenatal human skin

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CCL27, a chemokine constitutively expressed in the epidermis of adults, is a key chemo-attractant for CLA+ memory T cells. Recently, it has been reported that, in contrast to adult skin, prenatal skin harbors only few memory T cells. In this study we investigated whether this scarcity correlates with CCL27 levels during gestation in vivo and analyzed its expression in fetal and adult human primary keratinocyte (KC) as well as organotypic skin cultures in vitro. Immunofluorescence revealed no/low CCL27 expression in embryonic (9-14 weeks estimated gestational age (EGA)) and fetal (18-24 weeks EGA) human skin, respectively, as compared to the strong staining pattern observed in adult skin. In line with this in situ expression pattern, secreted CCL27 was present in supernatants of ex-vivo skin cultures derived from adult skin samples but was absent in supernatants of prenatal skin. Similarly, CCL27 was produced and secreted in vitro by adult primary human KC but not in fetal primary human KC. Stimulation with the TLR3 ligand poly (I:C) - a potent inducer of a variety of chemokines in KC - led to a strong induction of CCL27 secretion merely in adult but not in fetal KC. Given that a major difference between pre- and post-natal epidermis is the differentiation status of KC, we investigated the effect of KC-differentiation on CCL27 production and secretion in monolayer and organotypic skin cultures using adult KC. In both experimental settings, cell-differentiation strongly up-regulated CCL27 expression and secretion in KC. Together our findings suggest that CCL27 plays a major role for the influx of memory T cells during skin development. In addition, we demonstrated that epidermal CCL27 secretion is strongly dependent on KC differentiation.

008

Calcitonin gene-related peptide (CGRP) and norepinephrine (NE) each bias Langerhans cell (LC) antigen presentation towards generation of Th17 cells via actions on endothelial cells (ECs)

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We have recently found that the neuropeptide CGRP enhances production of IL-6, a cytokine important for Th17 cell differentiation, by stimulated ECs and others have shown that NE also has this effect. Thus, we asked whether CGRP alters the outcome of LC antigen presentation via effects on bystander ECs. We used the transformed BALB/c EC line, bEnd.3, to examine this question. bEnd.3 cells (10^4/well) were plated in round-bottom 96-well plates in medium containing 100 nM CGRP, 10^{-5} M NE or medium alone. After 3 h, plates were washed x 4. Then, 10^4 LCs (obtained from BALB/c epidermis by magnetic antibody separation) and $3x10^5$ T cells from DO11.10 mice (BALB/c background) were added per well followed by addition of a chicken ovalbumin fragment ($cOVA_{323-339}$) to 10 μM. DO11.10 mice express transgenes such that their T cells recognize $cOVA_{323-339}$. Other wells were set-up without bEnd.3 cells. After 48 h of culture, cells were harvested and $CD4^+$ T cells analyzed by FACS. In cultures containing CGRP- or NE-treated bEnd.3 cells, T cells expressing intracellular IL-17A were increased along with a decreased number of T cells expressing interferon gamma (IFNγ). In replicate cultures, T cells were isolated and RNA extracted for quantitative RT-PCR. Treatment of bEnd.3 cells with CGRP or NE resulted in enhanced mRNA expression for RORγt, IL-6 and IL-17A with decreased expression of IFNγ and T-bet. Supernatants from these cultures assayed for cytokine production by ELISA revealed that addition of CGRP- or NE-exposed bEnd.3 cells significantly enhanced IL-6 and IL-17A production while significantly inhibiting IFNγ production. Preliminary experiments examining IL-6 and IL-17A production with primary murine dermal microvascular ECs substituted for bEnd.3 cells yielded a similar result. As vessels within the dermis and secondary lymphoid organs are associated with sensory and sympathetic nerves, these results suggest a novel mechanism of regulation of cutaneous immunity by the nervous system.

009

IFN-α abrogates the regulatory function of human CD4+CD25+FOXP+ Treg via MAPK/PDE4 pathway-regulated cAMP repression

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IFN-α is well known for its anti-tumour activity and is therefore frequently used as a therapeutic agent in a multitude of malignancies, including malignant melanoma and cutaneous lymphomas. Previous reports in our group elucidated that IFN-α abolishes the suppressive function of human regulatory T cells (Tregs) in vitro and in a xenogenic GvHD model in vivo. In the present study, we found that IFN-α Tregs represses intracellular cAMP levels in Tregs. Blocking of cAMP degradation by administration of the non-selective phosphodiesterase inhibitor IBMX or the PDE4 (highly expressed in T Cells) specific inhibitor rolipram renewed cAMP levels accompanied by a restored suppressive activity of the Tregs, indicating IFN-α as inhibitor of Treg functions by cAMP repression. Activation of PDE4 is regulated by MAP kinases dependent pathways. Consequently, treatment with an Erk-specific inhibitor completely normalized cAMP accumulation in IFN-α-treated Treg, demonstrating that IFN-α activates an Erk-/PDE4 pathway resulting in cAMP reduction. In contrast, we did not observe an altered methylation state of the TSDR (Treg specific demethylated region) within the Foxp3-promoter, shown to be required for the stability of the FOXP3 expression, nor differences in the cytokine production and anergic state of Tregs in the presence of IFN-α, excluding an impact on the Treg differentiation program by the type I interferon. In conclusion, IFN-α acts as a potent regulator of Treg activity affecting the suppressive function but not differentiation and stability of human CD4+CD25high FOXP3+ Tregs.

010

Accumulation of varicella zoster specific CD4+Foxp3+Tregs after cutaneous Ag challenge in humans

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Regulatory T cells play an important role in control of immune responses and have been found in normal and inflamed human skin. The origin and antigen specificity of Foxp3+CD4+ regulatory T cells is a question of some debate. We induced a DTH response by intradermal injection of varicella zoster virus (VZV) skin test to investigate the relationship between VZV-specific CD4+ T cells and CD4+Foxp3+ regulatory T cells (Tregs) during a memory immune response in humans in vivo. CD4+Foxp3- and CD4+Foxp3+ T cells increase in parallel in the skin after VZV injection. The proportion of CD4+Foxp3+ T cell remained constant throughout the response at around 10% of CD4+ T cells. VZV-specific CD4+ T cells were identified with a MHC class II tetramer or by intracellular staining for either IFN-γ or IL-2 after antigen re-challenge in vitro. Using both methods, significant increase in VZV-specific T cells was found at the site of skin challenge compared to blood of the same individuals. Significant local proliferation was observed and peaked on day 7 after challenge when around 20% of total and >50% of tetramer defined VZV-specific CD4+ T cells in the skin expressed Ki67. CD4+Foxp3+ T cells that were found in the skin after VZV injection were CD25hiCD127loCD39hi and did not secrete IFN-γ or IL-2 after antigenic re-stimulation. In addition, increased numbers of Tregs in the skin were associated with a significantly decreased inflammatory reaction, suggesting that these cells had inhibitory activity in vivo. Foxp3+ T cells that were CD25hiCD127loCD39hi were also identified within the VZV tetramer population. In summary, our data indicate that Ag- specific regulatory cells accumulate at sites of immune response in parallel to the effector T cells of the same specificity. We hypothesise that CD4+Foxp3+ regulatory T cells specific for foreign antigens may be derived from an antigen-specific memory population at the site of an immune responses in vivo.

011

TNF inhibitors directly target Th17 cells in psoriasis

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TNF represents a key proinflammatory cytokine and plays a critical role in pathogenesis of psoriasis. Therefore, TNF inhibitors, such as infliximab, adalimumab and etenercept, show a great therapeutic efficacy on psoriatic lesions as well as patient's quality of life. It has been thought that TNF inhibitors attenuate psoriasis through inactivation of myeloid dendritic cells and subsequently the IL-23/Th17 pathway or direct inhibiting effect on keratinocyte activation. However, the detailed mechanism of TNF inhibitors still remains obscure. We experienced a patient with psoriatic erythroderma, who showed demarcated attenuation of lesions at the sites of adalimumab (ADA) injection. Immunohistochemical study using the lesional skin where ADA was subcutaneously administrated revealed that ADA predominantly bound to infiltrating T cells rather than epidermal keratinocytes, dendritic cells or macrophages. This finding prompted us to investigate any direct effect of TNF inhibitors on T cells. Peripheral blood T cells from normal healthy donor stimulated in vitro with immobilized anti-CD3/CD28 in the presence of IL-1beta and IL-23 resulted in production of IL-17A and TNF, indicating the Th17-skewing. In this experimental setting, inclusion of etanercept significantly inhibited gene expression of IL-17A and production of IL-17A. Inhibition of IL-17A production by etanercept was also observed in the peripheral blood T cells from psoriasis patients. Taken together, the present study suggests that TNF produced by Th17 cells is required, in an autocrine fashion, for full maturation of Th17 cells. Therefore, TNF inhibitors directly target Th17 cells, which play a crucial role in psoriasis pathophysiology.

012

Early defect in cutaneous immune responses to varicella zoster virus challenge in old individuals

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A marked increase in the susceptibility to skin infections and malignancies has been observed in older humans indicating that cutaneous immunity becomes defective with age. As Varicella Zoster Virus (VZV) often reactivates in the old individuals causing shingles we studied the kinetics of the cutaneous response in young and old subjects (<40 years old and >65 years old respectively) who were challenged with a VZV skin test antigen. We found that old individuals have reduced clinical responses to intradermal challenge VZV despite showing equivalent PBMC proliferative responses to VZV in vitro. In young individuals both CD4+ and CD8+ T cells accumulated at the site of VZV injection, peaking at day 7, whereas T cell accumulation was significantly reduced in the old, correlating with the reduced clinical score. There was also significant reduction in the accumulation of inflammatory CD11c+ DCs and CD163+ macrophages in the old individuals. The expression of E-selectin, VCAM and ICAM-1 was also significantly reduced in the old individuals which may contribute to the observed lack of cellular infiltrate. There was no difference between young and old individuals in the numbers of resident T cells, DCs and macrophages in normal skin or at 6hrs after VZV challenge. There was however an increase in proportion of Foxp3+CD4+ cells in the normal skin of old individuals and this inversely correlates with the magnitude of the clinical response. To understand the mechanism responsible for lack of responsiveness in the old skin we examined the transcriptome of young and old skin after VZV challenge. In agreement with the cellular data, reduced inflammatory signature was observed in the old skin suggesting inadequate early activation in response to VZV challenge.

013

Imiquimod with dacarbazine diferentiates B7H1-expressing MDSC into IFN-γ producing macrophages in B16F10 melanoma

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Myeloid-derived suppressor cells (MDSC) comprise a heterogeneous population of cells (CD11b+, Gr-1+) that can be found in tumor-bearing hosts. We previously reported that MDSCs suppress the activity of T cells together with Tregs through B7H1-mediated pathways. To further investigate the suppressive function of B7H1 in melanoma, first we subcutaneously injected mice with 2 x 105 B16F10, administered several doses of B7H1Ab and then assessed the suppressive capacities of B7H1Ab in vivo. The administration of B7H1 Ab significantly suppressed the B16F10 melanoma growth in a dose-dependent manner. FACS analysis revealed that the blocking of B7H1 in vivo induced CD69 expression on CD4 and CD8 cells in the tumor-bearing host. To analyze the suppressive function of MDSC, we isolated CD11b+ cells and co-cultivated them with syngeneic CD4+ T cells and allogeneic BMDCs. Actually, splenic, B7H1 highly expressing CD11b+ cells suppress the proliferation of CD4+ T cells. Next, to develop B7H1 targeting immunotherapy, we set up therapeutic models for B16F10 melanoma using imiquimod (IQM) and dacarbazine (Dac). The administration of IQM with Dac significantly suppressed the B16F10 melanoma growth. Moreover, we investigated the mechanisms of the suppressive function of this immuno/chemotherapy, especially focusing on B7H1-expressing MDSC. Interestingly, IQM/Dac therapy induced the differentiation of IL-10 producing, B7H1 expressing MDSC into IFN-γ producing macrophages, which might be related to the suppression of melanoma growth. Our results suggest that splenic CD11b+ cells highly express B7H regulatory molecules, and IQM/ Dac therapy differentiates these MDSC into immunogenic macrophages.

014

Induction of CD163+ M2 macrophages in the lesional skin of eosinophilic pustular folliculitis

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Eosinophilic pustular folliculitis (EPF) is a rare dermatitis of unknown etiology that is characterized by recurrent clusters of pruritic, follicular, sterile papules and pustules over the face, trunk and upper extremities. Recent reports suggested that EPF is often accompanied by peripheral blood eosinophilia, and a Th2 cytokine-dominant condition has been postulated as one of the underlying mechanisms. More recently, it was reported that Jumonji domain containing-3 (Jmjd3) is essential for M2 macrophage polarization and targeting transcription factor interferon-regulatory factor 4 (Irf4), which has been shown to be involved in Th2 polarization. Accordingly, the purpose of this study was to elucidate the involvement of M2 macrophages and Th2 related signals and cytokines in EPF. We employed immunohistochemical staining for CD163, pSTAT6, IL-10 and Foxp3 to 5 cases of classic, non-immunosuppression-associated EPF. Interestingly, immunohistochemical staining revealed dense infiltration of CD163+ M2 macrophages in the interstitial area of the dermis and perifollicular areas of the lesional skin. In addition, the pSTAT6-expressing infiltrating cells and IL-10 producing cells suggested the contribution of Th2 cells to the establishment of EPF. In parallel with M2 macrophages, Foxp3+ Tregs were also prominent in the interstitial area of the dermis and perifollicular areas of the lesional skin. Our case might suggest possible mechanisms in the development of EPF through CD163+ M2 macrophages and Th2 pathways.

015

Involvement of IL17 positive T cells in acne vulgaris

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To characterize the inflammatory infiltrates of acne vulgaris lesions, and to confirm previous transcriptomic results indicating the involvement of Th17 cells in the pathogenic process of acne, lesional and non-lesional human skin biopsies from twelve patients with inflammatory acne vulgaris were analyzed. qRT-PCR and Luminex assays were performed for mRNA and protein analysis, respectively. In parallel, histology and immunohistochemistry were performed to document the morphology of the lesions and to detect IL17-positive T cells in the inflammatory infiltrates. At the mRNA level, the most representative cytokines secreted by Th17 cells, including IL17A, IL17F and IL26, were found to be up-regulated in acne lesions. Furthermore, the protein levels of cytokines characterizing Th17 cells, such as IL6, IL17A, IL17F, IL22, IL23A, CCL20 and TNF, were up-regulated in lesional skin versus non-lesional skin ($p<0.05$). Histological examination was consistent with the diagnosis of acne vulgaris, most lesional biopsies showing perifolliculitis/folliculitis up to ruptured follicle walls. Infiltrates were more important in lesional biopsies when compared to non-lesional areas (10/12 and 0/12 patients with a score of slight to marked, respectively). IL17 positive cells were found to be correlated with the presence of infiltrates. IL17 positive cells were mainly CD3 positive cells in lesional as well as non-lesional samples (87+/- 14 % and 90% +/- 11 %, respectively). Our results identify acne vulgaris as an inflammatory disorder mediated among other factors by a Th17 response. These findings indicate new possibilities to target the inflammatory response in acne vulgaris.

016

A new model for evaluating topical treatments for Th17 mediated diseases using e*x vivo* human skin

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The activation of skin resident T cells and subsequent production of pro-inflammatory cytokines IL-17 and IL-22 are pivotal steps in the development and progression of autoimmune skin disease, such as psoriasis. Although several rodent models exist, none completely recapitulates human disease. Animal models also present a challenge for target validation when investigating effects of topical treatment because the properties of rodent and human skin differ. A novel tissue-based assay that focuses on T cell-mediated immune responses within the skin was developed. Resident T cells are activated in situ in freshly excised healthy human skin, resulting in Th17 differentiation, and significant cytokine production, including IL-17 and IL-22. Importantly, we show that T lymphocytes remain inside the dermal tissue throughout culture as stimulation-dependent cytokine expression is observed by PCR for up to 6 days post stimulation. After 4 days of stimulation under Th17-polarizing conditions, epidermal ballooning degeneration was observed. The data suggest that expression of IL-17 and IL-22 in the tissue caused the tissue damage. Indeed, similar degeneration of the epidermis was observed when recombinant IL-17A and IL-22 protein was added to the culture medium, in the absence of overt T cell stimulation. IL-17 production is highly dependent on the transcription factor RORgt. Therefore, the model was further validated using specific small molecule inhibitors of RORg. Pretreatment of human skin explant cultures with RORg inhibitors abrogated activation-induced il-17a and il-17f message expression, but not il-22. Our data show that T cells can be activated in situ in healthy skin explant cultures, which provide an ideal model system for assessing target engagement of immunomodulatory therapeutic compounds intended for topical use.

017

Expression of IL-23/Th17 pathway in early inflamed acne lesions

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Acne vulgaris is a common disease characterized by androgen dependence, follicular hyperkeratosis, increased sebum excretion, colonization with P. acnes and inflammatory events. The formation of microcomedone is preceded by mononuclear, mainly CD4 positive T-cell infiltrates and macrophages. We examined the biopsies obtained from early inflamed lesions and non-lesional skin of acne patients (n=20) and, as control, lesional and non-lesional skin of psoriasis patients (n=9) by RT-PCR and immunohistochemistry. RT- PCR showed significant similarities between cytokine profiles in lesions of acne and psoriasis. We found increased expression of Th17 cytokines: IL-17A increase was 22.7-fold ($p< 0.001$) in lesions of acne compared to the non-lesional skin and 20.1-fold ($p<0.001$) in psoriasis in lesional compared to non-lesional skin. IL-23p19 increased 3.1-fold in acne and 9.2-fold in psoriasis, $p<0.001$ in both diseases. Also Th1 markers CXCR3, T-bet and INF-gamma were significantly increased in acne and psoriasis compared to non-lesional skin. Foxp3 elevation was 2.5 fold in acne and 4.1 fold in psoriasis ($p<0.001$ in both) and also TGF-beta and IL-10 increased very significantly. IL-17 is known to be a potent inducer of antimicrobial peptides and chemotaxis of neutrophils. Pro-inflammatory cytokine IL-8, IL-1beta and TNF-alpha were significantly increased as well as the antimicrobial peptides S100A7, S100A9, LCN2, hBD2, hBD3 and hCAP18. Immunohistochemistry revealed IL-17A and Foxp3 positive cells. Naturally the localization of the positive cells was different in acne compared to psoriasis. This is the first study linking Th17 and Treg cells to early inflammatory events in acne lesions and shows similarities in cytokine profiles in acne and psoriasis.

018

Development of a novel immunotherapy for melanoma which inhibits interaction between CD155 on melanoma cells and TIGIT on activated CTL

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Durable complete responses observed in metastatic melanoma patients who received anti-CTLA-4 or anti-PD-1 antibody clearly show that blockade for T cell-suppressive molecules is a promising strategy to reject melanoma tumors. We have identified some novel, IFNγ inducible T cell-suppressive molecules expressed on melanoma cells like PD-L1, by gene expression profiling of melanoma cells. Also we have established the experimental system to test the impact of the candidate molecules on anti-tumor CTL response in vitro and in vivo, using highly antigen specific artificial CTLs and melanoma cells transfected with the candidate genes. CD155 is one of the candidate molecules, which is reported as a co-stimulatory molecule expressed by APCs and tumor cells. TIGIT is one of the receptor for CD155 expressed by activated T cells that suppresses activation of T cells. CD155 was expressed by human melanoma cell lines (6/6) and up-regulated by IFNγ. Also, it was expressed on melanoma tissues (5/5). Activation of the melanoma specific CTLs assessed by cytokine release was significantly suppressed by CD155 over-expressed on melanoma cells, and the suppressive effect was comparable to that of PD-L1 over expressed on melanoma cells. Also we could confirm that blockade for CD155-TIGIT interaction could enhance the activation of melanoma reactive CTLs on encountering CD155 expressing melanoma cells. Moreover, additive effect of CD155-TIGIT blockade and PD-1-PD-L1 blockade was observed in vitro. This is the first report showing the suppressive effect of CD155 over-expressed on melanoma cells in anti-melanoma CTL-response. In addition to blockade for CTLA-4-B7 and PD-1-PD-L1 engagement, blockade for CD155-TIGIT engagement is another immunotherapy to induce strong anti-melanoma immuno-response.

019

Interferon-γ and tumor necrosis factor control human cancer by induction of cellular senescence

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Recent studies from targeted cancer immunotherapies show that adaptive immunity can control human cancer. Cancer control eventually occurs in the absence of cytotoxic effects, e.g. induction of apoptosis or perforin/granzyme B-mediated cytotoxicity. Interestingly, we and others found that tumor-specific, interferon-γ (IFN-γ)- and tumor necrosis factor (TNF)-producing T helper 1 (Th1) cells arrest cancer growth, but the underlying mechanisms were unknown. As MHC class II-restricted Th1 cells can not directly interact with most cancer cells, we investigated the effect of soluble Th1 cytokines on human cancer cells. Proliferation was measured by BrdU incorporation, cell cycle regulation by Western blot analysis of the p16Ink4a/Rb signalling pathway, and senescence induction by an in vitro growth arrest assay. Treatment of A204 rhabdomyosarcoma cells with IFN-γ and TNF for 72 h strongly suppressed their proliferation. Growth inhibition was accompanied by severe hypophosphorylation of Rb at Ser795 indicating that the combined action of the two cytokines drives cancer cells into senescence. Indeed, the cell cycle arrest was permanent, as the cancer cells did not restart growing after IFN-γ/TNF removal. To study whether cytokine-induced senescence could be of broader relevance, we screened 11 human cancer cell lines for permanent growth arrest. All cancer cell types expressed the relevant IFN-γ and TNF receptors, and responded to challenge with the cytokines. Moreover, 55 % of the cell lines were permanently growth arrested. IFN-γ/TNF also induced senescence in primary human rhabdomyosarcoma and melanoma cells. Taken together, our data show that Th1 cytokines control human cancer by inducing irreversible growth arrest. Thus, cytokine-induced senescence can be considered as an intergral part of tumor surveillance by the immune system, and opens perspectives for innovative immunotherapeutic approaches.

020

Casein kinase II regulates the intracellular routing of the dendritic cell receptor DEC205 to MHC class II+ compartments and it's recycling to the cell surface

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The intracellular trafficking of the dendritic cell (DC) antigen receptor DEC205 is guided by an intracellular domain, that routes antigens into MHC-II+ compartments. This domain contains a putative casein kinase II (CKII) phosphorylation site and we asked whether this site is involved in intracellular targeting of DEC205 to late endosomes (LE). We generated fusionreceptors containing the HuIgG-binding, extracellular domain of human CD16 and the intracellular DEC205 domain (CD16:DEC) and established stably transfected the antigen presenting cell line DCEK. In pulse – chase experiments we incubated the celllines with HuIgG on ice for 1h, followed by a chase at 37°C for 20 to 60 min. We show, that CD16:DEC transfected cells bind and endocytose HuIgG efficiently, and after 30 min many vesicles start to fuse with Rab7+ LE. These vesicles were also positive for CKII indicating a role of CKII in DEC205 trafficking. When we applied the CKII inhibitor TBB, endocytosis of HuIgG by CD16:DEC was not affected, but instead the recycling of the DEC receptor from LE to the cell surface was blocked. Moreover, inhibition of CKII also lead to a prolonged half-life of endocytosed HuIgG within LE and in antigen presentation assays with HuIgG-specifc T-cells, TBB-treated DCEK cells induced reduced T cell proliferation as compared to controls. Finally we confirmed our results by deleting the CKII site in chimeric CD16:DEC receptors by site-directed mutagenesis yielding CD16:DECdelCKII receptors. These receptors accumulated in LAMP1/Rab7+ LE and showed severely reduced expression on the cell surface. Thus this data indicate that phosphorylation of the intracellular domain of the DEC205 antigenreceptor by the CKII is crucial for the transport of its ligands beyond the LE to MHC class II+ compartments and for the recycling of the DEC205 receptor back to the cell surface. Further investigations of this pathway may explain the efficiency of antigen targeting to DC in vivo.

021

The amino acid sequence EDExxL in the intracellular domain of the DEC205 receptor mediates antigen uptake in dendritic cells by interaction with adaptin-2

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The antigen receptor DEC205 mediates efficient endocytosis in dendritic cells (DC). Its intracellular domain harbours a putative targeting sequence (single letter code: EDExxL), which is highly homolog to a DExxxL sequence previously shown to route lipoprotein receptors to lysosomes. To analyze the function of this motive in DEC205, we generated fusion receptors containing the IgG-binding extracellular domain of human CD16 and the murine intracellular DEC205 domain (WT-DEC:CD16). By site directed mutagenesis we also mutated the EDExxL sequence to AAAxxL (AAA-DEC:CD16). Stably transfected cell lines of DCEK cells were established. When we analyzed the endocytosis of the ligand human IgG (hIgG) in the two different cell lines we found that WT-DEC:CD16 cells took up the ligand completely within 30 min, whereas AAA-DEC:CD16 endocytosed only 10% of surface bound hIgG. When analyzing the distribution of the DEC:CD16 receptors in steady state, i.e. without incubation with hIgG, we found that WT-DEC:CD16 receptors were present in vesicles within the cells, whereas AAA-DEC:CD16 receptors remained mostly close to the cell surface. Analysis of the intracellular transport of hIgG showed that in WT-DEC:CD16 hIgG moved through early endosomes (EE; Rab5+/EEA1+; 10 min after uptake) to late endosomes (LE; Rab7+; 30 min after uptake). In contrast, AAA-DEC:CD16 molecules remained mostly on the surface and only partly entered the EE. When analyzing the recruitment of adapter molecules to DEC:CD16 receptors we found colocalisation of adaptin-2 with WT-DEC:CD16 but not with AAA-DEC:CD16. These results could further be supported by co-immunoprecipitation of adaptin-2 with WT-DEC:CD16. Thus these data indicate that the intracellular targeting of the antigenreceptor DEC205 is initiated by the interaction of adaptin-2 with an EDExxL motive in it's intracellular domain. This interaction is crucial for the effective presentation of antigens by DC and may therefore influence the effective activation of T cells by MHC-peptide complexes.

022

GILT expression in medullary thymic epithelial cells is required for presentation of tissue-specific self antigens and deletion of autoreactive T cells

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Clonal deletion of thymocytes with a high avidity interaction for self-peptide:MHC complexes is critical for the maintenance of tolerance and prevention of autoimmunity. We have previously shown that gamma-interferon-inducible lysosomal thiol reductase (GILT) is required for thymic deletion of CD4+ T cells specific for the self and melanoma antigen, tyrosinase-related protein 1 (TRP1). To define GILT's role in the maintenance of central tolerance, we show that GILT expression is enriched in thymic antigen presenting cells (APCs) capable of mediating deletion, namely medullary thymic epithelial cells (TECs) and thymic dendritic cells. TRP1 expression is restricted to medullary TECs, consistent with Aire-mediated transcriptional regulation. GILT facilitates the MHC class II-restricted presentation of endogenous TRP1 by pooled thymic APCs (TECs and bone marrow-derived APCs). Using bone marrow chimeras, we demonstrate that TRP1-specific T cells are deleted in chimeras in which GILT is expressed in both TECs and bone marrow-derived APCs or in which GILT expression is restricted to TECs, demonstrating that GILT expression in TECs is sufficient for deletion of TRP1-specific thymocytes. In contrast, a large number of TRP1-specific T cells develop in chimeras which lack GILT expression in both TECs and bone marrow-derived APCs. Chimeras in which GILT expression is limited to the bone marrow-derived APCs have intermediate levels of TRP1-specific T cells, demonstrating that GILT expression in TECs is required for efficient deletion of TRP1-specific thymocytes. These findings suggest that GILT operates in mTECs to facilitate the presentation of tissue-specific self antigens and promote deletion of autoreactive T cells.

023

Pathodynamics of CD8 T-cell trafficking in a murine model of graft-versus-host disease (GvHD), implicate the CXCR3 axis as a crucial organizer of cutaneous inflammation and injury

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Though the skin is the most commonly affected organ in GvHD, the mechanisms underlying its development are incompletely characterized. To study early molecular processes, qRT-PCR was used to profile epidermal chemokine mRNA expression in a model of GVHD-like disease where K14-mOVA mice containing a self-antigen, ovalbumin (OVA), develop skin/mucosal inflammation and weight loss following adoptive transfer of OT-I (OVA-specific) CD8+ T cells. qRT-PCR of epidermal mRNA demonstrated marked upregulation of CXCL9 and CXCL10, IFN-γ inducible ligands sharing a common CXCR3 receptor, with 10 fold induction of CXCL10 mRNA detectable as early as 2 days post-OT-I transfer (dpt) and peak induction of CXCL9 and CXCL10 mRNA at 100 and 150-fold, respectively at 5 dpt. At 2 to 4 dpt, qRT-PCR of skin-draining lymph nodes (SDLN) revealed early upregulation of CXCL9 and CXCL10 mRNA from between 20-40 fold vs. untreated K14-mOVA SDLN mRNA. FACS analysis of the SDLN at 5 dpt demonstrated that greater than 95% of OT-I cells expressed CXCR3 while only 20% of recipient CD8 T-cells expressed CXCR3 suggesting upregulation of CXCR3 might facilitate trafficking to highly inflamed foci. Coincident with peak epidermal CXCL9/10 mRNA expression, OT-I cells were detected in the skin as early as 5 dpt via IF and progressive infiltration continued, persisting beyond 13 dpt. FACS of total skin suspensions showed that OT-I cells accounted for 31.8% of total skin lymphocytes. The kinetics of chemokine and receptor expression suggested that CXCL9/10 may accelerate migration of Ag-specific T-cells to antigen-replete tissues orchestrating target organ damage. Transfer of OT-I/CXCR3KO cells into K14-mOVA mice resulted in unaltered weight loss, but markedly attenuated skin disease vs. wild-type OT-I transferred K14-mOVA controls. These findings indicate that, in GvHD, epidermis expresses CXCL9 and 10 and attracts CXCR3+ T cells and suggest that targeting the CXCR3 axis may have clinical utility in GvHD and related skin diseases.

024

Characterization of house dust mite specific T cells in atopic dermatitis at the single cell level

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$CD4^+$ Tetramer-staining and T cell cloning of house dust mite (HDM) specific T cells have been reported recently and are described as central and effector memory T cells producing Th1, Th2 and Th17 cytokines in patients with allergic asthma. Here, we used the novel technique Chipcytometry to perform deep characterization of Der p 1 and Der p 2-tetramer-binding T cells at single cell level in blood of patients with atopic dermatitis (AD). By subsequent fluorescence staining/bleaching steps, immobilized cells are analyzed by a comprehensive marker set, providing information about the T cell subset. Furthermore, the staining is documented in a microscopy picture, allowing a qualitative analysis and discarding artefacts, which may influence experiments with low cell numbers significantly. In this approach, every single tetramer-positive T cell was stained with a deep-characterization marker panel including CD4, CD8, CD14, CD19, CRTh2, CCR-6, IL-18R, CD27, CD45R0, CD45RA and a vital stain. By this means, we describe house dust mite-specific memory T cells ($Tetramer^+/CD45R0^+$) in the peripheral blood of sensitized patients with AD. We confirm observations from asthma research and describe HDM-specific T cells of the Th2, Th17 and Th2/Th17 phenotype as detected via surface markers. Furthermore we can show that these cells are terminally differentiated ($CD27^-$) and therefore most likely contribute to the disease in AD-patients.

025

Anaphylaxis triggered by innate immune signals as co-factors is mediated by basophils independent of IgE

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Anaphylaxis is classically induced by cross linking of IgE bound to FcεRI on mast cell (MC) or basophil surfaces. Alternatively, IgG antibodies can also initiate anaphylaxis. Certain co- or augmentation factors, as best documented for wheat dependent exercise induced anaphylaxis, alcohol consumption or infections, can augment anaphylaxis. To understand how infections trigger anaphylaxis we sensitized mice with Ovalbumine (OVA) and mimicked infection by pretreatment with different pathogen associated molecular patterns (PAMPs) prior to challenge. Pretreatment triggers full-blown anaphylaxis as measured by significantly decreased core body temperature, significant reduction in systolic blood pressure and an increase in serum histamine levels. Experiments using basophil depleted or basophil deficient and mast-cell-deficient Kitw-sh/w-sh mice showed that PAMP triggered anaphylaxis is MC independent but basophil dependent. Most interestingly, IgE-knock-in mice, in which the exon for IgG1 is substituted by an exon coding for IgE leading to IgG1 deficiency and IgE overproduction, do not develop PAMP dependent anapyhlaxis. Finally, to understand how PAMPs act on basophils triggering IgG1 dependent activation, we generated knock-out mice deficient in the pathogen recognition receptors (PRR) TLR2 and NOD2, allowing the analysis of the PAMP PGN. In those mice PAMP dependent anaphylaxis was completely absent. In summary, we prove that PAMPs act as co-factors modulating the onset of IgG1 and basophil dependent anaphylaxis via binding to PRR. These data show for the first time a clinical relevance of IgE independent basophil-mediated anaphylaxis. This is of major clinical importance for the diagnosis and management of patients with anaphylaxis and may help to develop to new therapeutic strategies.

026

Nanpoarticle based intradermal formulation targeting DC skin subsets to increase vaccine potency against HIV-1

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Delivery of vaccine formulations into the dermis using antigen-coated micro needles is a promising approach due to the efficient antigen delivery and safety. There is evidence that intradermal delivery of vaccines is more effective than alternative routes, such as intramuscular. Vaccines preventing HIV infection are still lacking, effective prevention against this pathogen would represent a major advance in global health. The aim of this study is to evaluate the capacity of a new intradermal vaccine formulation using virus specific antigens conjugated to polypropylene sulfide nanoparticles (PPS-NP) to induce immunity against HIV infection. We tested the efficacy of PPS-NP formulations on primary Dendritic Cells (DC) including skin DC subsets. Firstly we used Monocyte-derived DC (MDDC) for preliminary developmental experiments. We further investigated these aspects on plasmacytoid (pDC) and myeloid (myDC) DC from the peripheral blood as well as on langerhans cells (LC) and dermal DC (dDC) from human skin samples. Primary DC subsets were incubated with the PPS-NP and PPS-NP-p24 formulations in the presence and absence of TLR agonists before analysis for cell viability, maturation and cytokine production. From these preliminary results we can conclude that the PPS-NP formulation is non-toxic to the DC and does not affect DC maturation or TNF-α induction by TLR agonists. Antigen presentation experiments indicate that PPS-NP-p24 is preferentially presented by DCs over unconjugated p24 protein. These results are in contrast to antigen presentation using B cells as antigen presenting cells (APC). We have determined that delivery of the HIV antigen p24 via PPS-NP is non-toxic to cells in vitro. The nanoparticle formulation does not affect the function of the antigen presenting cells with regards to response to stimulus. The formulation may however be more efficiently presented in DC subsets over p24 antigen alone. In conclusion nanoparticle based vaccines represent a potential avenue to increase delivery of antigens in the skin.

027

The specific IgE against sweat antigen in sera of patients with allergic diseases

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Background: We previously demonstrated that the semi-purified human sweat antigen (QRX) causes positive skin tests and histamine release from basophils via specific IgE in patients with atopic dermatitis (AD). In the last 37th JSID, we reported that the method of enzyme-linked immunosorbent assay (ELISA) to measure specific IgE against QRX by using sera of patients with AD. In this study, we examined the specific IgE against QRX in patients with other allergic diseases. Methods: QRX was prepared by anion-exchange and reverse-phase column chromatography from pooled sweat samples of healthy donors. Sera of patients with AD (n=63), cholinergic urticaria (ChU) (n=27), allergic rhinitis (AR) (n=11), asthma (n=15), and sera of normal controls (NC) (n=23) were used in this study. QRX was immobilized onto the wells of 96-well plates which were pre-coated with monoclonal IgG1-kappa against QRX. QRX-specific IgE in sera were bound to the immobilized QRX and detected by horseradish-conjugated anti-human IgE and substrates. To quantify the amount of IgE, the standard serum was created by pooling sera of 20 patients with AD whose basophils release histamine in response to QRX. Results: Positive rate of specific IgE against sweat antigen in sera of NC and patients with AD, ChU, AR, and Asthma are 26.1%, 71.4%, 55.6%, 63.6%, and 13.3%, respectively. The levels of the IgE against QRX in sera of patients with severe AD were significantly higher than those with mild AD. Conclusions: Quantification of specific IgE against QRX by ELISA is useful not only to diagnose sweat allergy but also to evaluate disease severity in patients with AD. Moreover, patients with ChU and AR have significantly higher positive rates of specific IgE against QRX than NC.

028

Identification of inducible lymph node-like structures in the skin: A key site to elicit contact hypersensitivity responses

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Epicutaneous sensitization is established in the draining lymph nodes where dendritic cells (DCs) and T cells colocalize, however it remains unclear how the elicitation of cutaneous immune responses are efficiently induced in the skin. To address this issue, we used murine contact hypersensitivity (CHS) as a delayed-type hypersensitivity model, which consists of the sensitization and elicitation phases. Initially, using CD11c-DTR (diphtheria toxin receptor) and Langerin-DTR knockin mice, we identified dermal DCs (dDCs), but not Langerhans cells, as a responsible DC subset for elicitation of CHS. This finding suggests that the dermis is the primary site for elicitation. Next, we visualized the dynamics of dDCs and memory T cells in the elicitation phase of CHS using two-photon microscopy, and found that dDCs accumulated in perivascular areas and interacted with skin-infiltrating memory T cells for several hours. This immunological synapse-like interaction was essential for memory T cells proliferation in situ in an antigen and integrin LFA-1 dependent manners. Intriguingly, dDC accumulation was abrogated by depletion of macrophages by means of LysM-DTR chimeric mice. Taken together, we have demonstrated for the first time that lymph node-like structures are induced at the post capillary venules in the elicitation phase of CHS, and that this complex consists of perivascular macrophages, dDCs and memory T cells which interact via immunological synapse formation to induce efficient cutaneous acquired immune responses.

029

Therapeutic potential of B and T lymphocyte attenuator expressed on CD8+ T cells for contact hypersensitivity

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Background: In the past decade, mechanisms underlying allergic contact dermatitis have been intensively investigated by using contact hypersensitivity (CHS) models in mice. However, the regulatory mechanisms, which could be applicable for the treatment of allergic contact dermatitis, are still largely unknown. Objective: To determine the roles of B and T lymphocyte attenuator (BTLA), a CD28 family co-inhibitory receptor, in hapten-induced CHS reaction and examine the therapeutic potential of an agonistic agent for BTLA on CHS reaction. Methods: BTLA-deficient (BTLA-/-) mice and littermate wild-type (WT) mice were subjected to dinitrofluorobenzene (DNFB)-induced CHS reaction. SCID mice injected with CD4+ T cells and CD8+ T cells from either WT mice or BTLA-/- mice were subjected to CHS reaction. In addition, the effects of an agonistic anti-BTLA antibody (6A6) on CHS reaction were evaluated. Results: BTLA-/- mice showed enhanced DNFB-induced CHS reaction and increased IFN-γ production of CD8+ T cells as compared to WT mice. SCID mice injected with WT CD4+ T cells and BTLA-/- CD8+ T cells showed enhanced CHS reaction as compared to those injected with WT CD4+ T cells and WT CD8+ T cells. On the other hand, SCID mice injected with BTLA-/- CD4+ T cells and WT CD8+ T cells exhibited similar CHS reaction to those injected with WT CD4+ T cells and WT CD8+ T cells. Moreover, in vivo injection of 6A6 suppressed DNFB-induced CHS reaction and IFN-γ production of CD8+ T cells. Conclusion: Stimulation of BTLA with the agonistic anti-BTLA antibody may have therapeutic potential for CHS reaction.

030

IL-6 and IL-10 secreted from B16 melanoma cells initiate the differentiation of CD4+/ CD8+ T cells in cancer microenvironment: Inhibition of STAT3-signaling cascades is prerequisite to cancer immunotherapies

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In established melanoma-bearing hosts, any acquired immune responses were severely restricted in part through phosphorylated-STAT3 (pSTAT3). We had generated recombinant R9-GRIM19 fusion protein (rR9-GRIM19) to inhibit the STAT3-mediated signaling cascades in cancer microenvironment, and investigated the mechanism of antitumor effects by intratumoral (i.t.) injections of rR9-GRIM19 in B16-bearing mice. In culture, we confirmed that IL-6 and IL-10 were the main soluble STAT3 activators secreted from B16 cells, and that IL-6 and IL-10 were significantly reduced by pretreatment with rR9-GRIM19. In vivo, pSTAT3 expression in all CD4+ and CD8+ T cells of tumor-draining LNs (DLNs) were elevated on day 5 after subcutaneous implantation of B16 cells, with differentiation into IL-6/ IL-21/ IL-10-producing CD4+/ CD8+ T cell phenotypes. I.t. injections of rR9-GRIM19 alone elicited marginal anti-B16 effects with partial pSTAT3 suppression in T cells of DLNs by promoting the T cell differentiation into IL-17-producing (but not IFN-gamma-producing) phenotypes. However, conventional active immunotherapies using with CpG-ODN and immunogenic antigens (e.g.; Trp2-peptide or rR9-OVA) did not have such inhibitory effects upon pSTAT3 in either T cells. Instead, complete B16 regressions with melanoma-specific CTL expansion and differentiation into IFN-gamma-producing phenotypes, as well as induction of memory effects, was elicited only when combined with i.t. injections of rR9-GRIM19 plus Th1/ Tc1-inducible adjuvants (CpG and rR9-OVA) (COG therapy) without melanoma-specific immunotherapies. We also confirmed that pSTAT3 expressions in all CD4+ and CD8+ T cells of DLNs from COG-treated B16-regression mice were completely reverted to the basal level. All these data indicated that rR9-GRIM19 is a novel and indispensable adjuvant for the strong antitumor effects in cancer-bearing hosts.

031

The expressions of T cell immunoglobulin and mucin domain molecules on peripheral blood mononuclear cells and skin lesions of sarcoidosis patients

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Sarcoidosis is a T helper (Th) 1-mediated inflammatory disease with unknown aetiology, characterized by the formation of noncaseating granulomas, and involving accumulations of monocyte/macrophage-lineage cells and T cells. T cell immunoglobulin and mucin domain (Tim) proteins regulate activation and function of $CD4^+$ T cells and are differentially expressed on Th1 and Th2 cells. In humans, Tim-1 protein is associated with Th2 cell responses and Tim-3 preferentially is expressed on fully differentiated Th1 cells but not on Th2 cells. Furthermore, Tim-3 is a regulator for key pro-and anti-inflammatory cytokine production in human monocytes. In this study, we investigated whether Tim molecules could play a role in the pathogenesis of sarcoidosis. $Tim\text{-}1^+$ T cells and $Tim\text{-}3^+$ T cells in peripheral blood were significantly increased in sarcoidosis patients compared with healthy controls. The relative frequency of both T cells was correlated with the serum ACE and soluble IL-2 receptor levels. On the other hands, the relative frequency of $Tim\text{-}3^+$ monocytes was decreased in the patients compared with controls, while $Tim3^-$ monocytes was significantly increased in the patients and expressed intracellular IL-12. $Tim\text{-}1^+$ cells and $Tim\text{-}3^+$ cells were present in and around the granulomas of sarcoidal skin lesions, respectively. These findings indicate the critical role of Tim molecules in the regulation of Th1/Th2 polarization in sarcoidosis.

032

CXCR3 deficiency prolongs Th1-type contact hypersensitivity

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Sensitization and challenge using dinitrofluorobenzene (DNFB) induce contact hypersensitivity (CHS) with Th1 cell infiltration, whereas those using FITC generate CHS with Th2 cell infiltration. In this study, we attempted to determine the role of CXCR3, a chemokine receptor, in Th1- and Th2-type CHS induced by DNFB or FITC using CXCR3-deficient (CXCR3-/-) mice. Ear swelling was prolonged after DNFB challenge in CXCR3-/- mice, which was accompanied by increased Th1 cytokines and decreased TGF-β and IL-10 expression at a late time point of CHS, while there was no significant difference between wild-type and CXCR3-/- mice in FITC-induced CHS. In Th1-type CHS, the number of regulatory T cells (Tregs) was decreased in the challenged ear of CXCR3-/- mice compared to that of wild-type mice, suggesting that CXCR3 would be important in migration of Tregs into the site of inflammation. Moreover, we examined the characteristics of CXCR3+ Tregs both in vitro and in vivo, revealing that CXCR3+ Tregs expressed high levels of TGF-β and IL-10 as well as IFN-γ compared to CXCR3- Tregs. DNFB-specific CD8+ T cells as well as Tregs expressed CCR4, which may mediate their migration to the skin in the absence of CXCR3. BrdU proliferation assay suggested that CXCR3+ Tregs function as potent immunoregulatory cells to suppress Th1-type immune responses. When CXCR3-/- mice were injected with CXCR3+ Tregs, the prolonged ear swelling induced by DNFB was normalized. Taken together, our results suggest that CXCR3+ Tregs play a key role for quenching Th1-type CHS.

033

Opposing immune reactions drive psoriasis and eczema

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Although innovative therapeutic approaches become available for both psoriasis and to a lesser extent for eczema, causative treatments do not exist. Here, we used our previously published model of patients with co-existing psoriasis and inducible eczema reactions towards house dust mite or nickel (n = 16) to investigate the pathogenesis of psoriasis and eczema simultaneously in the same patient. Biopsies of acute eczema reactions and active neighboring psoriasis plaques were clinically and histologically investigated. T cells isolated from lesional skin were phenotyped for cytokine secretion by ELISA and flow cytometry. To understand signaling networks underlying these two conditions, whole transcriptome analysis was performed. Clinically, all analyzed psoriasis patients developed typical eczema upon antigen challenge. Histological analysis revealed typical hallmarks for eczema and psoriasis, whereas the array analysis highlighted a clear cutting-edge picture of the pathogenesis of these two conditions. Bioinformatic analysis of the array results led to the identification of novel pathways, either unique or shared between psoriasis and contact eczema that might be translated into new therapeutics. Furthermore, a number of possible disease biomarkers were identified, thus paving the way to personalized medical treatment. In conclusion, we show that psoriasis and eczema are trigged by specific immune reactions rather than genetic factors.

034

Anti-inflammatory effect of cholecystokinin in skin disorders

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Cholecystokinin (CCK) is a major gastrointestinal peptide hormone, which induces the release of bile and other digestive enzymes to facilitate digestion of fat and protein. It is also known to possess immunomodulatory actions, and predominant anti-inflammatory effect has been suggested in pancreatitis and arthritis models. We have previously shown that topical application of CCK8S exerts an anti-pruritic effect in mice, demonstrating the potential for dermatological therapeutic usage. Nevertheless, studies relating to the interaction of this unique peptide with skin are still very limited. We sought to investigate the function of CCK on skin, by primarily focusing on inflammatory skin disorders. To evaluate the role of CCK in atopic dermatitis (AD), serum CCK8 level of AD patients was evaluated by ELISA. Various factors influence serum CCK concentration, and food intake is known to raise the value by approximately 10-folds. Therefore, we selected patients with serum CCK8 concentration below 100 pg/mL, and in this specific group of patients, a negative correlation was seen between serum CCK8 level and serum IgE concentration (R2=0.80). In contact hypersensitivity model using mice, topical application of CCK8S before sensitization and/or elicitation significantly attenuated the ear swelling in CCK8S applied groups compared to CCK untreated mice ($P<0.005$). Additionally, in the in vitro assay, cultured human vascular endothelial cells were prepared, and upon stimulation with TNF-α, marked expression of intercellular adhesion molecule-1 (ICAM-1) was observed using confocal microscopy. This augmented expression was profoundly depressed by the addition of CCK8S, particularly on the cell surface. Furthermore, RT-PCR analysis confirmed that the expression of mRNA for ICAM-1 was also decreased by the addition of CCK8S. The present study indicates that this novel peptide is capable of exerting strong anti-inflammatory function in the skin, and the association with inflammatory skin disorders such as AD is addressed.

035

TACE/ADAM17 deficiency in epidermis leads to IL-17A-associated inflammation with atopic dermatitis-like phenotype

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TACE (tumor necrosis factor-α converting enzyme), or ADAM17 (a disintegrin and metalloproteinase) is a membrane-bound proteolytic enzyme that regulates cell proliferation and differentiation. We recently developed *Tace*$^{flox/flox}$/*Sox9-Cre* mice, in which TACE/ADAM17 is absent in epidermis (TACE cKO), to study them in the context of hair follicle biology. Unexpectedly, TACE cKO manifested phenotypes that closely resembled atopic dermatitis (AD). They exhibited intense scratch behavior and chronic skin inflammation with mast cell and T cell infiltration. Skin barriers were disrupted as determined by Raman spectrometer, and serum IgE was increased. Major proteins in the epidermal differentiation complex including filaggrin were not remarkably changed, however. We identified via gene array that *Il-17a* expression was dramatically increased, and FACS analysis revealed prominent infiltration of Th17 cells as well as IL-17A producing-γδ T cells in epidermis, and increased numbers of Th1, Th2, and Th17 cells in skin-draining lymph nodes. IL-17A-producing cells in epidermis also expressed IL-22. Consistently, increased mRNA expression of the Th17-driven cytokines IL-1β and IL-23a were detected in TACE cKO epidermis. Crossing TACE cKO to Rag2 KO or c-Kit KO mice demonstrated that lymphocytes modestly, but significantly contributed to AD-like phenotype, whereas mast cells did not. Transplantation of WT bone marrow into TACE cKO mice failed to rescue phenotype, indicating that TACE-deficient epidermis, rather than hematopoietic cells, was responsible for the phenotype in TACE cKO mice. In conclusion, TACE-deficiency in epidermis led to AD-like chronic skin inflammation associated with IL-17A. TACE cKO mice provide a novel model to explore the mechanisms of aberrant inflammation accompanied by dysfunction of skin barrier, an aspect shared by human AD.

036

Inhibition of STAT6 signals exacerbates IgE-mediated, basophil-dependent prurigo-like reactions

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Prurigo is a common skin disease characterized by urticarial papules and/or nodules with severe itching. This condition occurs in association with various underlying diseases, such as chronic renal failure and internal malignancies. Atopic dermatitis is also occasionally complicated by prurigo lesions. The pathological mechanisms causing prurigo remain unclear, although Th2-type immunity has been implicated in its etiology and our recent study showed a number of basophils in prurigo lesions. The present study demonstrated that repeated intra-dermal injection of TNP-OVA to dorsal skin of IgE-transgenic mice resulted in the development of persistent, basophil-dependent, nodular skin lesions. Histopathologically, marked hyperkeratosis with irregular acanthosis was accompanied by dense cellular infiltrate comprising mononuclear cells, eosinophils, basophils, and increased numbers of mast cells. Marked sprouting of PGP9.5(+) nerve fibers in the epidermis was also observed. These lesions seemed to share morphological similarities with prurigo reactions in humans. Local cytokine profiles were characterized by elevated levels of interleukin (IL)-4, IL-13, IL-17, IL-22, IL-31, and nerve growth factor. In vivo depletion of basophils resulted in alleviation of skin reactions, indicating the basophil-dependent nature of this inflammation. Nuclear translocation of pSTAT6 and pSTAT3 was observed in epidermal keratinocytes. C57BL/6 mice also developed prurigo-like lesions when repeatedly challenged with TNP-OVA in conjunction with TNP-IgE. Unexpectedly, STAT6 (-/-) mice showed significant exacerbation of skin reactions compared with wild-type C57BL/6 mice. Consistent with this finding, local administration of STAT6 small interfering RNA resulted in exacerbated skin inflammation. Th2 immunity mediated by STAT6 thus appears to play protective roles in basophil-dependent prurigo-like reactions and therapies targeting Th2-type cytokines may risk aggravating inflammation from prurigo.

037

Regulation of Claudin-1 expression in Langerhans cells by EpCAM

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Langerhans cells (LC) express high levels of epithelial cell adhesion molecule (EpCAM; CD326), a cell-surface protein that has been implicated in homotypic cell–cell adhesion in epithelia and in metastasis in many carcinomas. LC also express claudin-1, a critical component of tight junctions (TJ). LCs take up antigen on skin surfaces by extending their dendrites between keratinocytes (KC) while forming claudin-containing TJ that maintain an effective barrier, and selectively promote IgG1–predominant (Th2) humoral responses. To determine if EpCAM might modulate TJ that form between LC and KC, conditional knockout mice with EpCAM-deficient LC (LC/EpCAM cKO mice) were examined. We found that claudin-1 protein was downregulated several fold ($p < 0.01$) in EpCAM-deficient LC in epidermis to almost background levels. EpCAM-deficient MHCIIhigh CD11c+ Langerin+ CD103- cells in skin-draining lymph nodes that represent epidermal LC that have migrated from epidermis also expressed reduced levels of claudin-1. We hypothesized that EpCAM-deficient LC would be unable to penetrate KC TJ and promote humoral responses to percutaneously applied protein antigen. Unexpectedly, activated EpCAM-deficient LC extended their dendrites through KC TJ, and TJ that formed between LC and KC contained ZO-1 and claudin-1 in amounts that were indistinguishable from those involving WT LC. Flow cytometry confirmed that claudin-1 signals in EpCAM-deficient LC under inflammatory conditions were higher than in steady state ($p < 0.05$), which is consistent with the accumulation of claudin-1 at TJ in docking dendrites. Numbers of TJ-docked dendrites that were formed by EpCAM-deficient LC after tape stripping did not differ from those formed by WT LC. OVA patch immunization also induced robust anti-OVA IgG1 responses in LC/EpCAM cKO mice that were comparable to those in control animals. These results indicate that activated EpCAM-deficient LC form functional TJ with surrounding KC and do not provide strong support for the concept that EpCAM plays a prominent role in functionally important acquisition of external antigen by LC.

038

Mechanistic correlations between two itch biomarkers, cytokine interleukin-31 and neuropeptide β-endorphin, via STAT3/calcium axis in atopic dermatitis

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Itch is the cardinal symptom of atopic dermatitis (AD). β-Endorphin, a neuropeptide, is increased in both AD skin and sera. Interleukin (IL)-31, an itch-relevant cytokine, activates IL-31 receptors in keratinocytes. However, how IL-31 and β-endorphin interact in AD skin remains elusive. This study aims to investigate the mechanistic interaction of IL-31 and β-endorphin in AD. This was a prospective cross-sectional study. We recruited adult patients with AD and controls according to Hanifin's AD criteria. Serum levels of IL-31 and β-endorphin were measured by enzyme-linked immunosorbent assay. Expressions of IL-31 receptor A (IL-31RA) and β-endorphin in the skin were assessed by immunohistochemistry. Their expression in the skin and blood was compared and correlated in patients with AD and in controls. We also treated primary keratinocytes with IL-31 and measured calcium influx, β-endorphin production and signalling pathways to define their mechanistic interactions. Our result showed β-Endorphin was increased in the supernatant from IL-31-treated keratinocytes. IL-31 receptor activation resulted in calcium influx and STAT3 activation; pretreatment with STAT3 inhibitor stopped the increase of β-endorphin. Notably, either replacement of extracellular calcium or treatment with 2-aminoethoxydiphenyl borate, an inhibitor for the store-operated channel, blocked STAT3 activation. We found higher levels of blood β-endorphin and IL-31, which were significantly correlated, in patients with AD. Moreover, IL-31RA and β-endorphin were increased and colocalized both in AD human skin and TPA-painted mouse skin. We concluded that IL-31 receptor activation in keratinocytes induces calcium influx and STAT3-dependent production of β-endorphin. These results might contribute to an understanding of the regulatory mechanisms underlying peripheral itch.

039

Epidermotropic resident memory T cells require hair follicle-derived cytokines to reside in epidermis

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Strong antigenic stimuli in skin leads to the generation of effector memory T cells (T_{EM}) that distribute not only to primary sites of inflammation, but also to the whole body surface as resident memory T cells (T_{RM}), and confer long term protection. Widespread distribution of T_{RM} represents steady-state T_{EM} trafficking, which mechanisms are yet to be clarified. Herein, we characterized T_{RM} in WT epidermis and evaluated cytokine requirement for their persistence in skin. Both $CD8^+$ and $CD4^+$ T cells of T_{RM} phenotype ($CD44^+$ $CD69^+$ $CD103^+$ $CD62L^-$ $CCR7^-$) were found in WT epidermis. Whereas $CD8^+$ T_{RM} distributed both in hair follicles (HF) and interfollicular epidermis, $CD4^+$ T_{RM} existed exclusively in HF. To simulate steady-state T_{RM} trafficking, we adoptively transferred T cells into Rag2-/- mice. T cells accumulated to HF from day 7, and distributed in epidermis or dermis as T_{RM} in the absence of any inflammation, around two weeks after transfer. Given the association of T_{RM} with HF, we analyzed via real-time PCR for factors that might affect T_{EM} generation. Langerhans cells (LC) expressed IL-15, and keratinocytes in the HF isthmus expressed both IL-7 and IL-15. Lack of LC did not affect T_{RM} distribution. To address the contribution of keratinocyte-derived cytokines, IL-7 KO and IL-15 KO mice were reconstituted with Rag2-/- bone marrow to obtain the setting where IL-7 and IL-15 deficiencies were limited to peripheral tissues. Splenic T cells were then adoptively transferred and skin was analyzed for T_{RM}. Numbers of epidermotropic $CD4^+$, but not $CD8^+$ T_{RM}, significantly decreased in IL-7-deficient epidermis. In contrast, epidermotropic $CD8^+$ T_{RM} were decreased in IL-15-deficient epidermis, whereas $CD4^+$ T_{RM} were increased. Collectively, epidermal distribution of $CD4^+$ and $CD8^+$ T_{RM} required HF-derived IL-7 and IL-15, respectively, demonstrating the importance of HF-T cell crosstalk in epidermal T_{RM} homeostasis.

040

Antigen-presenting dendritic cells maintain immunological tolerance in the oral cavity by inducing $Foxp3^+$ regulatory T cells

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Oral cavity is often affected by immunological skin disorders such as Stevens-Johnson syndrome and Behçet diseases. However, it is unclear how tolerance versus immunity is regulated in the oral cavity. We have previously shown that dendritic cells (DCs) excel in expanding and inducing antigen-specific $Foxp3^+$ $CD25^+$ $CD4^+$ regulatory T cells (T-regs). Here, we show that antigen-presenting DCs play a key role in inducing $Foxp3^+$ T-regs to maintain tolerance in the oral cavity. We found that $CD11c^+$ DCs from oral-cavity-draining cervical lymph nodes have the capacity to induce $Foxp3^+$ T-regs in the presence of antigen in vitro. Moreover, oral-cavity-draining cervical lymph nodes contained higher frequencies of $Foxp3^+$ T-regs and ROR-γt^+ $CD4^+$ T cells than other lymph nodes. The high frequency of $Foxp3^+$ T-regs in the oral-cavity-draining cervical lymph nodes was not dependent on the Toll like receptor (TLR) adaptor molecules, Myd88 and TICAM-1 (TRIF). In contrast, the high frequency of ROR-γt^+ $CD4^+$ T cells was dependent on Myd88 and TICAM-1. These data suggest that, antigen-presenting DCs may play a vital role in maintaining tolerance by inducing $Foxp3^+$ T-regs in the oral cavity where many commensals and food antigens exist.

041

Newly identification of a migratory subset of dermal γδ T cells that initiate contact hypersensitivity by producing TNF-alpha in the draining lymph nodes

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In the murine skin, there exist two subsets of γδ T cells; dendritic epidermal T cells (DETCs) and dermal γδ T cells. However, the role of skin γδ T cells in cutaneous acquired immune responses remains unclarified. In the present study, we generated mice lacking only dermal γδ T cells using bone marrow transplantation chimera and demonstrated that contact hypersensitivity (CHS) response to DNFB was significantly reduced by dermal γδ T cell-depletion. In vitro restimulation of sensitized lymphocytes revealed that dermal γδ T cells promote the sensitization phase of CHS. In addition, by means of photo-labeling system using photoconvertible protein-Kaede-transgenic mice, we demonstrated that a fraction of γδ T cells migrated from the skin to draining lymph nodes (DLNs) prominently during the sensitization phase. Majority of skin-derived γδ T cells in DLN expressed CCR6 and Vγ4 chain, suggesting that they were dermal origin. In contrast to other skin immune cells, the migration of γδ T cells to the DLNs was independent of Gαi-coupled receptors, since pertussis toxin treatment did not prevent their migration. Moreover, coculture of dendritic cells with Vγ4-positive γδ T cells led to a significant upmodulation of MHC class II molecules and IL-12 production in a TNF-α dependent manner, which suggests that migrating γδ T cells activate the dendritic cell functions in the DLNs. Taken together, we newly identified Vγ4-positive dermal γδ T cells, which migrate to the DLNs and establish the sensitization phase of CHS via enhancing dendritic cell functions.

042

Single cell tracking reveals the segregation of slow cycling memory CD8 T cells from the effector pool at the peak of the immune response

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Upon encounter of cognate antigen, naïve T cells undergo sequential multiple cell divisions associated with the acquisition of effector function. Following the eradication of antigen, the effector T cell population contracts and leaves behind memory T cell population. The kinetics and mechanisms that result in the generation of CD8 memory T cells have remained controversial. Here we made use of a novel approach to dissect the proliferative behavior of effector and memory T cells, that is single cell tracking of cell cycle progression of OVA-specific T cells transgenically expressing the fluorescent ubiquitination-based cell-cycle indicator (FUCCI). While G0/1 phase T cells are indicated by red fluorescence, early S and S/G2/M T cells turn to yellow and green. Following infection with OVA expressing influenza virus in vivo, FUCCI/OTI T cells proceeded divisions, with decreasing red+ cells from 99% to 5% and increasing green+ cells up to 44.6% during early infection. Surprisingly, red FUCCI/OTI cells with a memory phenotype reappeared at the peak of infection. These cells had a history of vigorous prior proliferation indicated by cell trace dye dilution, suggesting they developed from the fast dividing effector T cell pool. Next, we tracked divisions of single cells trapped in microwells. Mathematical modeling revealed inheritance of division times in progeny consistent with the development of distinct T cell lineages. Strikingly, in later divisions (generation ≥8), a slow cycling subpopulation appeared with smaller cell size and expression of memory markers. Collectively, we report a novel developmental process whereby at the height of the immune response T cells segregate into populations within distinct proliferative histories characteristic of terminal effector and memory T cells.

043

A system for differentiating human monocytes into antigen presenting cells

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In the context of physiological inflammation, human monocytes differentiate into a continuum of antigen presenting cells spanning from macrophages (MΦ) to dendritic cells (DC). However, in most experiments MΦ and DCs are produced and studied separately. This is despite multiple recent studies demonstrating that not only DCs but MΦ as well can migrate to lymph nodes and cross-present antigens to T cells. We therefore sought to develop a single method to differentiate monocytes into a range of antigen presenting cells (APCs). Experiments evaluated morphology using light microscopy, phenotype using ten parameter flow cytometry, and function using APCs co-cultured with autologous naïve CD8+ T cells (HLA-A201+ donors) ± a "long" MART-1 (16-43) melanoma peptide (MART-1 LP) requiring intracellular processing for effective cross-presentation of the HLA-A201/MART-1 (27-35) complex to CD8+ cells. After engaging activated platelets and serum proteins under flow followed by 18 hr culture in RPMI 1640/15% AB serum, we discovered that fresh human monocytes differentiated into mature DCs (3.96±0.61% of CD11c+ cells were CD14-HLADR++CD83+CD86++CD80+), immature DCs (30.27±4.41% of CD11c+ were CD14-HLADR+CD83-CD86+CD80-), and inflammatory MΦ (65.78±3.87% of CD11c+ cells were CD14+HLADR+CD86+CD83-CD80-). Morphologically, cells had properties of MΦ (large cells with pseudopodia extending to particles) and DCs (granular cells with limited branched projections). Functionally, these cells together in co-culture cross-presented the MART-1 LP to autologous naïve CD8+ T cells, as assessed by CD8+ cells co-stained with HLA-A2/MART-1 (27-35) *vs* control HLA-A2/gp100 dextramers following stimulation with MART-1 LP (*vs* no peptide). To the best of our knowledge, this is the first study to report a single method to concurrently produce cells across the inflammatory APC spectrum. Ultimately, since this system requires only a brief *ex vivo* intervention, cells can be returned to the patient quickly in possible future antigen presenting cell based immunotherapy.

044

New role of IL-17A as an inducer for Th2 in murine atopic dermatitis

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Atopic dermatitis (AD) is generally regarded as a T helper 2 (Th2)-mediated inflammatory skin disease. However, the number of IL-17 positive cells is increased in the peripheral blood and the acute skin lesion of AD patients. Therefore, it is important to evaluate the role of IL-17A in the development of AD. To this end, we used IL-17A deficient (IL-17-/-) mice in a hapten-induced AD-like mouse model. We found that IL-17A induced TSLP and TARC/CCL17 mRNA expressions in the lesional skin and promoted Th2 differentiation in the draining lymph nodes. Consistently, the serum IgE level in IL-17-/- mice was significantly lower than that in wild-type mice after repeated hapten application. We also found that the main producer of IL-17A in the lesional skin was dermal γ4+ CCR6+ γδT cells, which migrated to the epidermis. To further give in-depth consideration to the above findings, we used flaky tail (Flgft) mice that exhibit AD-like skin lesions with elevated IgE. We crossed Flgft mice with IL-17A-/- mice and generated Flgft mice deficient in IL-17A (IL-17A-/-Flgft mice). IL-17A-/- Flgft mice showed impaired serum IgE level compare to Flgft mice in the steady state. In addition, the number of IL-4 producing cells in the skin draining lymph nodes was significantly decreased in IL-17A-/- Flgft mice compare to that in Flgft mice. Moreover, epidermal thickness tended to be decreased in IL-17A-/- Flgft mice compared to Flgft mice. In line with the result of the hapten-induced AD model, the main producer of IL-17A in the skin of Flgft mice was γ4+ γδT cells. Taken together, our results suggest that dermal γ4+ γδ T cells migrating into the epidermis are the essential sources of IL-17A in the AD-like skin lesions, and that IL-17A promotes Th2 induction and antigen-specific IgE production in line with TSLP and CCL17 induction in keratinocytes. Therefore, IL-17A seems to play an important role in the development of AD.

045

Langerhans cell derived IL-6 is required for the differentiation of Th17 during skin infection with Candida albicans

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Langerhans cells (LC), CD11b+ dermal dendritic cells (dDCs) and CD103+ dDC acquire antigen in the skin and promote the initiation of adaptive immune responses in regional lymph nodes. We recently demonstrated using a Candida albicans skin infection model that these DC subsets all acquire antigen but LC are necessary and sufficient for the differentiation of naïve T cells into the Th17 phenotype while CD103+ dDC were required for the development of Th1. In other systems, Th17 differentiation requires the presence of TGFβ and either IL-6 or IL-1β depending on the tissue as well as IL-23 for the maintenance of the Th17 phenotype. Activated LC and dDC produce high levels of these cytokines. Langerin-Cre MyD88fl mice in which LC cannot response to most TLR agonists have reduced expression of many of these cytokines and fail to produce a robust Th17 response. To test the importance of individual LC-derived cytokines, we generated a series of mice in which LC were rendered unable to secrete a particular cytokine. WT→IL-1β-/- bone-marrow chimeras in which LC and non-hematopoetic cells cannot produce IL-1β, the development of Th17 in response to C. albicans skin infection was unaffected. Similarly, Th17 development was intact in WT→IL-12p40-/- chimeras indicating that LC-derived IL23 is not required. In contract, development of Th17 was significantly reduced in WT→IL-6-/- chimeras. Since TGFβ-/- and Langerin-Cre TGFβfl mice lack LC, we used Langerin-CreERT2 TGFβfl mice to test the requirement for LC-derived TGFβ. Tamoxifen treatment efficiently ablated TGFβ expression in LC but did not affect the development of Th17. Thus, although Th17-inducing cytokines are secreted by LC and dermal DC after infection with C. albicans, only LC-derived IL-6 appears to be non-redundantly required.

046

Langerhans cell and CD103+ dermal dendritic cell promote Tfh in the steady-state

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Directing antigen to dendritic cells via antibody/antigen conjugates that target antigen-uptake receptors is a highly efficient vaccination technique and also allows for in vivo examination of the effect of antigen presentation by particular DC subsets. Classic experiments determined that antigen presentation in the absence of DC activation led to deletional tolerance. Antibodies directed to the C-type lectin Langerin which is expressed by Langerhans cells (LC) and CD103+ dermal DC (dDC) have been shown in short-term studies to induce strong CD4 and CD8 T cell responses. To study the effects of antigen presentation isolated to LC or CD103+ dDC in the steady-state, we performed a series of experiments in which either 1) transgenic mice that express human Langerin only in LC were immunized with anti-human Langerin/antigen complexes or 2) mice lacking LC were immunized with anti-mouse Langerin/antigen complexes. In both cases the DC remained unactivated and antigen was exclusively found on either LC or CD103+ dDC, respectively. Both DC subsets, were able to promote proliferation of MHC-II tetramer binding endogenous CD4 T cells that peaked at day 7 and slowly declined over time without evidence of deletion. LC but not CD103+ dDC induced a small expansion of antigen-specific Foxp3+ Treg. In contrast, both subsets induced strong expansion of a population of antigen-specific CXCR5+, PD-1+ CD4 T cells that expressed IFNγ, IL-21 and IL-4 which is most consistent with follicular helper T cells (Tfh). In addition, immunization led to the formation of germinal centers in regional LN and was accompanied with a robust humoral response. Thus, presentation in the steady-state by LC and CD103+ dDC promotes Tfh and humoral responses but not deletional tolerance.

047

RNase7 regulates TH2 cytokine production by activated human T-cells

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One of the major antimicrobial peptides (AMPs) secreted by keratinocytes is RNase7, a member of the RNaseA family. RNase7 is constitutively expressed in the epidermis of healthy human skin and has been found to be upregulated in chronic inflammatory skin diseases such as atopic dermatitis and psoriasis. In addition to their antimicrobial activity immunregulatory functions have been published for several AMPs. In lesional skin of patients with atopic dermatitis and psoriasis activated T-cells might be directly exposed to RNAse7. Here we investigated the influence of RNase7 on activated T-cells. In the current study we demonstrate that treatment of activated human CD4+ T-cells and TH2 cells with RNase7 significantly reduced the production of the TH2 cytokines IL-4 and IL-13 by activated human T-cells while IFNγ and IL-2 were not regulated. This effect was was not due to enhanced apoptosis. To analyze if ribonuclease activity is required for the regulation of the TH2 cytokine release we repeated our experiments with a ribonuclease-inactive recombinant RNase7 mutant. Interestingly, our data show that RNase7 ribonuclease activity is dispensable for the observed regulatory effect. Furthermore, we were able to show that the inhibition of TH2 cytokine production by RNase7 is mediated by a down-regulation of GATA3 activity. Our data indicate that RNase7 has immunmodulatory functions on TH2-cells and might foster a TH1 cytokine environment in the skin.

048

A study on the heterogeneity of CD16+ myeloid cells in human blood that serve as precursors of inflammatory dermal dendritic cells

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Dermal dendritic cells (DCs) are important regulators of skin immune responses. Among these we previously identified slan (6-sulfo LacNAc) DCs as a population of TNF-alpha producing dermal inflammatory DCs (TIP-DCs) in psoriasis and lupus erythematosus. SlanDCs in blood share functional and phenotypic features with a larger cell population now categorized as non-classical monocytes (CD16high CD14low-negative). So far, there are no parameters or cell surface markers that allow studying the heterogeneity of these cells. To this end we started a program to generate monoclonal antibodies with specificity for CD16+ myeloid cells. We thereby obtained the monoclonal antibody DD3 that turned out to be highly specific for a not yet characterized subset of human CD16high CD14low-negative cells. Our phenotypic studies combining the anti-slan mAb (M-DC8) and the new mAb DD3 allowed us to distinguish for the first time between very distinct subsets of CD16high CD14low-negative cells. We next aimed for an extensive side by side study on the LPS-induced cytokine producing capacity of different DC and monocytes subsets. These studies clearly showed that in contrast to all other antigen presenting cells (APCs) investigated (CD1c+ DCs, CD141+ DCs, CD14 monocytes, CD14dim monocytes) slanDCs were the most prominent source of IL-12p40/p70, followed by the new mAb DD3-defined subset of non-classical monocytes. These studies underscore that the previously ill defined population of CD16+ myeloid cells in blood can be further dissected into functionally distinct subpopulations. Furthermore, we think that unraveling the heterogeneity of human inflammatory dermal DCs at the level of their precursor cells in blood will ultimately allow a better understanding of immune regulation in the skin.

049

Human Th9 cells: A novel skin homing T cell subset with potent autocrine and paracrine proinflammatory properties

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IL-9 producing Th9 cells have been proposed as a novel subset of helper T cells and animal studies suggest a role in tumor immunity and in allergic/autoimmune inflammation. Prior studies have been limited to genetically modified mice or to Th9 cells differentiated in vitro using TGF-β; studies of memory Th9 cells from human blood and tissues are conspicuously lacking. We isolated tissue resident T cells from healthy human skin, intestine and lung and found Th9 cells only in skin. IL-9 was also expressed in skin lesions of psoriasis and atopic dermatitis. We next sorted blood T cells into 3 populations (skin tropic (CLA+), gut tropic (α4β7+) or neither) and studied cytokine production and pathogen specificity. IL-9 was produced only by skin tropic T cells and many were specific for C. albicans. IL-9 production was transient after activation, was not dependent on TGF-β, and Th9 cells did not express other T lineage cytokines (IFN-γ, IL-17, IL-13) but did co-express TNFα/granzyme B, establishing their identity as a distinct T cell subset. IL-9 receptor was highly expressed by CLA+ T cells suggesting Th9 cells may be both a source and target of IL-9. Blocking IL-9 at the initiation of in vitro culture strongly inhibited up-regulation of IL-9, IFN-γ, IL-13, and IL-17 in CLA+ T cells, suggesting IL-9 production was needed for maximal inflammatory cytokine production of skin tropic Th1, Th2, Th9 and Th17 cells. In summary, human Th9 cells are preferentially skin-tropic or skin-resident. The C. albicans specificity of many Th9 cells suggests they may defend against extracellular pathogens. Given their ability to initiate and enhance production of other inflammatory cytokines, Th9 cells may also participate in initiating and maintaining cutaneous inflammation.

050

IRF8 and IRF4 may work cooperatively in CD8 T cell effector differentiation

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We previously reported that interferon regulatory factor 8 (IRF8), a transcription factor that belongs to the IRF family, integrates TCR/co-stimulation and γc-cytokine signaling pathways and drives effector differentiation of CD8 T cell. We demonstrated that these two signaling pathways synergistically promote the generation of cytotoxic T cells and IRF8 is persistently activated by these two signals. Blocking these signaling pathways by either ZAP70 or Jak3 inhibition in vitro abrogated IRF8 upregulation and inhibited effector differentiation of CD8 T cells. Using K14-mOVA Tg mice that develop graft-vs-host disease (GvHD) after transfer of syngeneic CD8/OT-I T cells we showed that OT-I/IRF8KO cells induced less severe GvHD and attenuated effector functions, including cytotoxicity and IFN-γ production. However, genetic depletion of IRF8 did not completely abrogate the GvHD response or effector functions in vivo, suggesting that other transcription factors may be involved. Since IRF8 exerts its function(s) by forming a dimer with other IRF family members, we assessed other IRF family members for pairing. Only IRF4 showed an expression pattern similar to IRF8. IRF4 was upregulated upon activation of CD8 T cells and was suppressed by blocking TCR/co-stimulation or γc-cytokine signaling pathways, suggesting that IRF4 may drive effector differentiation of CD8 T cells together with IRF8. To determine how IRF8 and IRF4 expression relates to that of T-bet and Eomes (two important transcription factors that also play a role in CD8 T cell effector function) expression, we performed real-time PCR using activated CD8 T cells cultured in the presence of a ZAP70 inhibitor or a Jak3 inhibitor. The results demonstrated that T-bet and Eomes did not show parallel expression patterns to IRF8 and IRF4 suggesting that IRF8 and IRF4 function independently of T-bet and Eomes and may be useful targets for modulating these types of immune responses.

051

HIV infection predisposes skin to toxic epidermal necrolysis via depletion of skin-directed CD4(+) T cells

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Infection with the human immunodeficiency virus (HIV) is a well-known risk factor for the development of adverse cutaneous drug eruptions (ACDEs); however little is known about the pathogenesis of ACDEs or the mechanism by which HIV infection predisposes individuals to a higher incidence of ACDEs. Additionally, the mortality of severe ACDEs remains high, and there is need for investigation into new treatment modalities and immunological mediators that may serve as potential targets for such new therapies. To assess whether an altered immunological state contributes to the development of ACDEs in HIV-infected individuals, skin biopsies of toxic epidermal necrolysis (TEN) lesions from HIV-positive and non-infected patients were examined via immunohistochemistry to characterize the associated inflammatory infiltrates. An 8-fold increase (p=0.006) in the ratio of CD8(+) to CD4(+) T cells was observed in the dermis of HIV-positive patients afflicted with TEN compared to HIV-negative controls. This increase was associated with a decrease in the absolute number of dermal CD4(+) T cells (p=0.044); however no significant difference was observed in the total number of CD8(+) T cells (p=0.351) between HIV infected individuals and controls. Furthermore, there was a trend towards a decreased number of CD25(+)T cells in the dermis of HIV infected skin (p=0.062). Our findings suggest that the increased risk of ACDEs in HIV-positive individuals may be attributed to a shift in the CD8(+) to CD4(+) ratio towards the effector CD8(+) T cell population. The tropism of the HIV virus for CD4(+) T cells may explain the substantial decrease in CD4(+) T cells but preservation of the CD8(+) T cell response among HIV patients with TEN. In addition, these results identify CD4(+)CD25(+) regulatory T cells as an important mediator in the pathogenesis of ACDEs and as a potential target for future cell-specific TEN therapy.

052

Characterisation of epidermal and dermal T cells in healthy human skin

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Healthy human skin contains a large number of T cells. Depending on the local microenvironment, skin derived T cells have the ability to act either as regulatory or effector T cells. Tissue resident memory T cells (Trms) are retained in murine skin after infection and are identified by CD103 and CD49a expression. Trms provide a first line of defence against secondary infection or viral reactivation. Similar T cells have been shown in human skin after HSV infection. In this study, we analysed the different subpopulations of CD4+ and CD8+ T cells present in healthy human skin. Cell suspensions prepared from epidermis, dermis and peripheral blood were analysed by flow cytometry or RNA expression within 24 hours of sample collection. Histology was performed on cryopreserved skin biopsies. Intracellular IFNγ and IL-17 production was analysed following PMA/Ionomycin stimulation by flow cytometry. We found a dominance of CD4+ over CD8+ T cells in healthy epidermis and dermis. A substantial number of epidermal CD4+ and CD8+ T cells expressed CD103+ whereas CD49a was only detected in a proportion of epidermal CD8+ cells. Dermal T cells contained a lower percentage of CD103 expressing T cells as compared to epidermis. The majority of epidermal and dermal CD4+ T cells were CD45RO+ CD62L-. However, a prominent population of CD45RO+CD62L+ CD4+ T cells was also present in dermis. Two distinct populations of CD45RA-RO+CD62L- or CD45RA+RO-CD62L- CD8+ T cells were present in epidermis and dermis. Skin T cells contained at least a ten-fold higher proportion of IL-17A expressing CD4+ and CD8+ T cells as compared to their circulating counterpart. CCR6, a chemokine receptor expressed on Th17 cells, was expressed on almost all CD103+ CD45RO+ CD4+ T cells and on a majority of CD103+ CD45RO+ CD8+ T cells in both epidermis and dermis. In conclusion, we showed a heterogeneous population of memory T cells present in healthy human epidermis and dermis. CD103+CCR6+ CD45RO+ expressing T cells may represent a subset of IL17A producing Trms residing in healthy human skin.

053

Beta arrestin-2 signalling attenuates contact allergic inflammation and inhibits proinflammatory chemokine production by keratinocytes

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Beta arrestins are ubiquitously expressed adapter proteins that participate in G-protein receptor signalling through receptor desensitization and internalization. They also act as multifunctional adaptor proteins that direct the recruitment, activation and scaffolding of various cytoplasmic signalling complexes including MAPK and non-receptor tyrosine kinases. In beta arrestin-2 deficient mice (Arrb2-/-) a decreased recruitment of T cells into allergic lung tissue and an increased infiltration of neutrophils into inflamed or wounded skin was described. Given these opposing effects in different immune cell subsets we investigated the role of beta arrestin-2 in the regulation of contact hypersensitivity (CHS) responses to the obligate contact sensitizer DNFB. We found significantly increased allergic ear swelling in beta arrestin-deficient mice compared to wildtype animals. Immunohistological analyses revealed a strikingly increased neutrophil infiltration with abundant subcorneal pustules in inflamed ear tissue of animals lacking beta arrestin-2. mRNA levels of neutrophil-attracting chemokines including CXCL1, CXCL-2 and CCL8 were elevated in inflamed ear tissue of Arrb2-/- mice. Experiments with adoptive transfer of sensitized lymphocytes and bone marrow chimeric mice demonstrated a role for beta arrestin-2 in radioresistant skin cells for the regulation of inflammatory responses during the challenge phase of CHS. We found that beta arrestin-2 deficient keratinocytes secreted higher levels of immune cell recruiting chemokines under basal and inflammatory conditions. Taken together, the experimental results suggest that beta arrestin-2 signalling attenuates allergic inflammation by inhibiting chemokine production in epidermal keratinocytes.

054

Time course effects of topical Dermatophagoides farinae applications on the progression of atopic dermatitis-like symptoms in NC/Nga mice

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Atopic dermatitis (AD) is not a simple inflammatory skin disease but is a quite complicated skin syndrome influenced by genetic background and different types of environmental factors such as allergens and microbes. Although various animal models have been suggested to analyze the pathogenesis of AD and the development of therapeutic drugs for the disease, there are few reports to evaluate each stage of the skin condition occurring in the course of AD. In this study, we identified the correlation between the expressions of immunologic factors and each stage of AD progression. To evaluate the time course effects of topical allergen stimulations on the onset and duration of AD-like symptoms, we applied Dermatophagoides farinae extract (DfE) together with skin barrier disruption on the upper dorsal skin of NC/Nga mice twice a week for 8 weeks. Repeated application of DfE rapidly elevated the dermatitis score followed by histologic changes of the skin at the each stage of AD progression in a time-dependent manner. From the 2nd week of the DfE application, the skin surface appeared the dryness and hemorrhage, and edema and excoriation occurred from the 3rd week. Interestingly, we found that skin barrier disruption with 4% Sodium dodecyl sulfate (SDS) twice a week failed to develop the AD-like skin lesions in NC/Nga mice. We also identified the correlation between the expressions of AD-related immune regulatory factors, such as immunoglobulin (Ig) subclasses and cytokines, and the each stage of AD progression. The changes in the expressions of total and D.farinae-specific Ig E, G1, G2a in plasma as well as splenic Th1, 2, 17 cytokines affected the change at the each stage of AD-like skin symptoms. In conclusion, our results suggest that we should pay attention to the characteristics of each stage of AD progression and choose the suitable stage of animal model not only to elucidate the pathogenesis of AD but also to develop and evaluate the therapeutic drugs for AD.

055

Human Langerhans cells potently induce Th1 cytokines in the presence of poly(I:C), but are less responsive than dendritic cells towards bacterial stimuli

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Dendritic cells (DC) are professional antigen presenting cells and provide a link between the innate and adaptive immune system. Langerhans cells (LC) represent a highly specialized subset of DC localized in the epidermis, an environment extensively exposed to pathogens. Due to their unique location it has been suggested, that resident LC are ideally positioned to capture invading viruses and possibly induce anti-viral immunity, but in contrast to dermal DC, contribute to tolerance towards bacterial commensals. Therefore, we analyzed the maturation and cytokine production of human immature monocyte-derived DC (MoDC) and LC-like cells (MoLC) upon stimulation with TLR ligands, pro-inflammatory cytokines and soluble CD40 ligand (CD40L). MoLC were distinguished from MoDC by the expression of E-Cadherin, Langerin and TROP-2. Pro-inflammatory cytokines as well as CD40L highly induced the expression of co-stimulatory molecule CD86 and activation marker CD83 after 24h of activation. MoDC, representing dermal DC subsets, were strongly activated by bacterial stimuli, leading to upregulation of CD83 and CD86, together with production of IL-6, IL-10, IL-12p70, IL-23 and IFN-γ. In contrast, MoLC showed impaired responsiveness to bacterial TLR ligands, but highly matured in the presence of poly(I:C), a molecular pattern associated with viral infection. The accompanied production of IFN-γ and IL-12p70 indicates the development of a Th1 response by activated MoLC. Additionally, we characterized the migratory capacity towards the CCR7 ligand, CCL21. Activation for 48h led to increased migration in both subsets according to their maturation status, but showed impaired migration of mature MoLC compared to MoDC, suggesting a crucial contribution of dermal DC in the initiation of immunity. Taken together, these data underline the pivotal role of the different DC subsets for the regulation of immune responses in human skin.

056

Effect of topical application of Quercetin on atopic dermatitis in NC/Nga mice

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Atopic Dermatitis (AD) is a chronic inflammatory skin disease that commonly begins in childhood. Quercetin-3-O-α-L-rhamnopyranosyl-2″-gallate (QRG), which was isolated from the bark of Acer ginnala Maxim grown natively in Korea, has been known to have several biological effects, including anti-oxidative, anti-inflammatory and anti-cancer activities. In this study, we examined the effect of topical application of QRG on skin inflammation and AD-like skin lesions in mouse models. Over a period of eight weeks, mice applied daily to QRG for 4 weeks after received twice a week application of 100 mg Dermatophagoides farinae (Df) ointment on back for 4 weeks. Topical application of QRG were down-regulated the development of AD-like skin lesions in NC/Nga mice. Interestingly, QRG markedly decreased inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 mRNA expression in skin. QRG also significantly suppressed the level of total plasma Immunoglobulin (Ig) E induced by topical Df-stimulation. We also showed that topical application down-regulated the expression of representative cytokines, Interleukin (IL)-4, -5 and -13, induced by Df stimulation. In the present study, we demonstrated that topical application of QRG was able to improve Df-induced AD-like inflammatory responses. These results demonstrate that QRG might be beneficial in the treatment of AD.

057

Induction of regulatory T cells by human antimicrobial peptides

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Antimicrobial peptides (AMP) are small molecules which are released by a variety of cells including keratinocytes. AMP were initially described according to their antimicrobial activity. They are essential components of the innate immune response and most responsible for antibacterial defense. Recently it was discovered that AMP exert additional activities beyond their antimicrobial capacities. They were demonstrated to influence the adaptive immune system by modulating antigen presenting cells. Recently we demonstrated that the murine beta defensin-14 (mBD-14) is able to induce regulatory T cells (Treg). mBD-14 appears to shift non-regulatory T cells into Treg by inducing the Treg-associated transcription factor Foxp3. Here, we studied whether human AMP exert similar immunosuppressive features. For this purpose human peripheral blood mononuclear cells obtained from buffy coats of healthy donors were separated into CD4+CD25+ and CD4+CD25- cells by magnetobead separation. The non-regulatory CD4+CD25- fraction was incubated with the human AMP hBD-2,-3, psoriasin and RNAse-7 respectively. Intracellular FACS analysis 24 hours later revealed upregulation of Foxp3 and neuropillin upon incubation with hBD-3. CTLA-4 was moderately increased as well. In addition, stimulation of CD4+CD25- cells with hBD-2, -3 and RNAse-7 resulted in the induction of glycoprotein A repetitions predominant (GARP), a transmembrane protein which is present on the surface of activated Treg. In contrast, psoriasin did not affect the expression of Treg-associated molecules. These data provide first evidence that similar to their murine analogues certain human AMP may exert similar immunosuppressive capacities. Through this ability, human AMP may protect the host from microbial attacks on the one hand, but tame T-cell-driven reactions on the other hand, thereby enabling an antimicrobial defense without collateral damage by the adaptive immune system.

058

Molecular characterization of ECP-induced monocyte-derived dendritic cells

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Extracorporeal photopheresis (ECP) in the treatment of cutaneous T-cell lymphoma, GVHD and autoimmune conditions continues to spur the question of how ECP is capable of inducing immunogenicity and tolerance. We previously demonstrated that ECP treatment leads to large-scale conversion of peripheral blood monocytes into functionally competent leukocytes with dendritic cell phenotypes, which may play key roles in the immunomodulatory capabilities of ECP. To characterize this population of cells on a molecular level, we assessed for differential surface expression of selected gene-products on monocytes after treatment with a model-ECP apparatus. Four gene-products (CXCL16, SIRPa, ICAM1, TNFR1) showed significant increases in surface expression after model-ECP treatment as compared to PBMC ($p < 0.01$ for all). To identify transcription factors (TFs) expressed by ECP-treated monocytes but not peripheral blood monocytes, rtPCR was performed. Interactions with platelets during ECP passage was also assessed by using model-ECP plates coated with low- and high-density platelets. Seven TFs demonstrated increases in mRNA after passage through the model-ECP plate (ΔRQ range 1.35- 6.78). Increased platelet density induced directional changes in expression ($RQ < 0.5$) for VDR, NFkB2, CDKN1A and BCL3. BTBD4, a transcription factor recently identified as being specifically expressed in classical DCs, demonstrated ~3-fold increase in expression after treatment. CREM, an important component of cAMP-mediated signal transduction and possible DC maturation marker, was significantly up-regulated with both platelet treatments (RQ = 6.78 and 5.9 for low- and high-density platelets, respectively). In summary, passage through the ECP plate apparatus caused activation of novel surface molecules and transcription factors that define and characterize a unique subset of "physiologically-induced" DCs. The effects of these cells on the immunobiology of ECP treatment will be further elucidated.

059

CD8 T cell epitopes in large and small T antigens of merkel cell polyoma virus exclusively elicit anti-viral T-cell responses in merkel cell carcinoma patients

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Merkel cell carcinoma (MCC) is a highly aggressive skin cancer with approximately 1600 new cases in the US each year and the incidence is increasing. Metastatic disease is insensitive to radio- or chemotherapy. The Merkel cell polyomavirus (MCV) is identified as the main causative agent for MCC, and together with several reports of spontaneous regression and prognostic value of CD8 T cell infiltration, is points to the use of immunotherapeutic treatment approaches. The viral oncoproteins are highly specific tumor targets and foreign antigens that represent the ideal targets for immunotherapy. However, today our knowledge of T cell recognition is restricted to one HLA-A24 restricted T cell epitope in the Large T antigen. We sat out to map T cell recognition of the polyoma virus by a broad range of HLA-restriction. We identified potential HLA-binding peptide-sequences in Large T, Small T and VP1 (capside protein) by in silico prediction, and verified the HLA binding experimentally among 398 candidate HLA-ligands. 236 verified HLA ligands were selected for T cell analyses. We analysed peripheral blood from both MCC patients and healthy donors for reactivity against this set of potential MCPyV derived T cell epitopes. We identified responses against 12 sequences from Large and Small T antigens and 23 sequences from VP1. Strikingly, T cell responses against Large and Small T antigens were found exclusively in MCC patients and not in healthy donors. We verified the antigen processing and functional recognition of epitopes restricted to HLA.A2, A11 and B7. The sequences that we have identified in this study are prime candidates for T cell based immunotherapy of Merkel Cell Carcinoma – and importantly this study shows that immune recognition of Large and small T antigens are restricted to Merkel cell carcinoma.

060

The PD-1 pathway is a potential drug target for squamous cell carcinoma in solid organ transplant recipients

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Immunotherapies with anti-PD-1 and anti-PD-L1 mAb have shown an impressive clinical response in patients with melanoma, and are currently being tested for other cancers. Squamous cell carcinoma (SCC) is a significant cause of morbidity and mortality in organ transplant recipients undergoing systemic cyclosporine A treatment, however, the involvement of the PD1/PD-L1 pathway in these patients is unknown. We therefore sought to determine the expression of the PD-1 pathway in cutaneous SCC and the potential effect of cyclosporine A on this pathway. Immunohistochemical staining of PD-1and its ligands (PD-L1& PD-L2) was observed in the stromal region of SCC in immune competent patients (n=6) as well as in immune-suppressed transplant recipients (n=4), but not in normal skin of healthy volunteers (n=3). Double immune-fluorescent staining on SCC biopsies (n=3) showed the positive expression of PD-L1 on CD11c+ dermal dendritic cells, forming cellular clumps in the SCC stroma. In contrast, no double positive cells were found in normal skin (n=2). To determine the impact of cyclosporine-A on PD-1 signaling, normal human keratinocytes (n=3) were treated with cyclosporine-A (100ng/mL) and induction of PD-L2 was observed at both 24h and 48h ($p<0.05$). In summary, we demonstrated the activation of the PD-1 axis in cutaneous SCC, and its induction by cyclosporine A. Peripheral tissue expression of PD-1 ligands is important for mediating immune tolerance and inhibiting the immune response. Thus, the impact of cyclosporine-A on PD-L2 expression in keratinocytes may underlie the successful immune evasion of SCC in transplant recipients. Although further functional analysis is required to delineate the role of PD-1 signaling associated with SCC progression, our data suggest that PD-1 pathway is a potential target for immunotherapy in transplant recipients with SCC.

061

Modulation of CD81 by hypersensitivity reaction inducer drugs

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CD81 is a widely expressed cell-surface protein involved in many biologic responses inducing the activation of the immune system and virus entry in the target cells. In B cells, CD81 is a component of the CD19/CD21 co-receptor complex that allows B cell activation and Epstein-Barr Virus (EBV) entry. EBV reactivation is frequently associated to drug reaction with eosinophilia and systemic symptoms (DRESS) which is a severe drug-induced hypersensitivity skin reaction. We previously show that only the culprit drugs trigger the production of EBV in DRESS patients. Interestingly, recent studies show that CD81 overexpression could be modulated by drugs such as phenobarbital. Thus, we focused on the drug-induced modulation of CD81 protein expression. B lymphoblastoid cell lines from 5 DRESS patients and 6 healthy controls were incubated or not with sulfamethoxazole, valproic acid, carbamazepine, allopurinol, amoxicillin or gentamicin, the latter being an irrelevant drug which does not induce hypersensitivity reaction. CD81 and CD19 cell surface expression were evaluated with fluorescence activated cell sorter (FACS) analysis. Our results show that there is no drugs' effect on the Mean Fluorescence Intensity (MFI) of CD19, which decreases over time during the culture. On the contrary, the MFI of CD81 is increased by sulfamethoxazole and valproic acid. However, there is no significant difference between DRESS patients and healthy controls. In conclusion, these findings suggest that CD81 is a target of certain hypersensitivity reaction inducer drugs, such as sulfamethoxazole and valproic acid, and may facilitate viral entry in B cells.

062

Genetic variations in IL6 and IL12B as protective markers for psoriasis

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Recent studies associate a number of genetic polymorphisms to psoriasis. In this direction, we aimed to assess the protective potential of three genetic variants of interleukin genes. In the present case-control study we genotyped a group of 67 psoriasis patients and 69 healthy subjects for IL6 rs1800795, IL12B rs3212227 and IL12B rs6887695. All data was than statistically analyzed using SPSS. Statistical significance was present for IL6 rs1800795, IL12B rs6887695 (p=0,001, p=0,028, respectively). In the case-control analysis, IL6 rs1800795 CC vs. GG carriers showed a strong protection against the disease (OR=0,072, p=0,018), so did IL12B rs6887695 CC vs. GG carriers (OR=0,198, p=0,025). Combining these markers in a multivariate analysis increased the protective value to 95,5% for CC vs. GG in both IL6 rs1800795 and IL12B rs3212227 (p=0,006). Concluding, we report of the minor alleles of IL6 rs1800795 and IL12B rs6887695 as potentially protective markers for psoriasis. In daily clinical practice, such markers could be integrated in the management of psoriasis, increasing the accuracy of diagnosis, or offering a more appropriate counseling to patients with familiarity for the disease.

063

Human CD141+ dermal dendritic cells integrate danger signals to modulate their function

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As an organ the skin is subject to colonization by a wide range of microorganisms each of which contain myriad molecules, which may be detected by innate receptors such as Toll-like receptors (TLRs). Dendritic cells (DCs) are the key sensors of danger signals in the environment, and integrate these signals to regulate adaptive immune responses. We recently characterised a tissue resident population of dermal DCs in human skin identified by the expression of CD141 (Chu, Ali et al. J Exp Med 2012). In the steady state these DCs are capable of cross presenting antigens and directing immunoregulatory responses via production of IL-10 and induction of regulatory T cells (Treg). However, the relative contribution of distinct tissue resident DCs to immunity and tolerance remains poorly understood. Here we isolate the DCs from human skin samples and sort them into three major populations of cells (CD141+ dermal DCs, CD1c+ dermal DCs and epidermal Langerhans cells), and analyse their response to TLR ligands and microbial products. In the steady state CD141+ dermal DCs have a unique cytokine response including production of IL-6, IL-23 and IL-10, they stimulate weak proliferation of alloreactive CD4 T cells and induce potent Treg. However, following TLR ligation proliferation of alloreactive CD4 T cells is restored and there is a marked increase in production of IL-6 and IL-23. In parallel we have been studying monocyte derived vitamin D induced $CD141^{hi}$ immunoregulatory DCs. Like their skin resident counterparts these DCs upregulate IL-6 and IL-23 production in response to LPS and imiquimod and also increase expression of molecules known to be important in the induction of productive T cell responses including CD80, CD86 and CLEC9A. These results suggest that under pro-inflammatory conditions CD141+ tissue resident DCs may be key in switching the response from immunoregulation to immunity, with potential relevance to protective immune responses of the skin and inflammatory disorders such as psoriasis.

064

***In vivo* induction of human cutaneous inflammation results in the accumulation of neutrophils expressing RORγ and IL-17, while IL-17+RORγ+ T cells are absent**

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Recent, successful clinical trials using antibodies targeting IL-17 in psoriasis support the importance of IL-17 in the pathophysiology of psoriasis. However, there is some debate concerning the cell source of IL-17 in the inflamed skin. The objectives of this study were to characterize the IL-17-producing innate and adaptive cell populations in time, before and during early clinical manifestation of skin inflammation. Therefore, we utilized two established models for the induction of human skin inflammation *in vivo* that share many histological features with psoriasis: application of leukotriene B4 (LTB4) (n=10) and tape stripping (n=10). Biopsies were taken in healthy volunteers at 5-6 different time-points between 0 and 72 hours and were processed for immunohistochemistry. Application of LTB4 first led to an influx of neutrophils, with a peak incidence at t=24-32 hours, followed by an influx of T cells at t=48 hours. Tape stripping caused an early influx of neutrophils and T cells at t=16 hours. Presence of mast cells remained steady during the skin inflammatory process. Characterization of IL-17 associated cells, led to the finding that IL-17 was produced by neutrophils and mast cells in both models. Notably, only the neutrophils co-expressed the IL-17 associated transcription factor RORγ. Surprisingly, none of the T cell subsets expressed RORγ or IL-17. These observations challenge the classical opinion that IL-17 is predominantly associated with T cells in psoriasis. It is attractive to hypothesize that IL-17+/RORγt+ neutrophils might contribute to the development of psoriasis.

065

Differential migration of Langerhans' cells from patients with early- and late-onset psoriasis

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Psoriasis is an immune-mediated inflammatory disease with two distinct ages of onset, defined as early-onset psoriasis (EOP) and late-onset psoriasis (LOP), presenting, respectively, before and after 40 years (y) of age. Langerhans' cells (LC) are epidermal dendritic cells that, after activation, migrate from skin to draining lymph nodes and play roles in initiating and regulating immune responses. We have shown previously that, in EOP, LC mobilisation is impaired in response to tumour necrosis factor (TNF)-α and interleukin (IL)-1β; cytokines that induce 20-30% LC migration in healthy individuals. In LOP patients, however, intradermal injection of IL-1β induces LC migration, whereas TNF-α does not. In an *ex vivo* explant model, unlike healthy individuals, epidermal sheets from EOP patients did not display spontaneous LC migration. This study sought to characterise spontaneous LC migration in LOP individuals. In LOP patients (1 male, 3 female; mean age 59.5y; mean age of onset 48.5y), two 6 mm punch biopsies were taken under local anaesthetic from uninvolved skin on the buttocks. The epidermal sheet was removed from one biopsy and fixed in acetone immediately (T0). The other was floated on 500 µl RPMI medium containing 10% foetal calf serum for 24 h before being fixed (T24). The frequency of LC in epidermal sheets was assessed in blinded fashion using fluorescence microscopy. The mean density of LC in the T0 of LOP patients was 592±83 LC/mm^2. T24 was significantly lower; 476±57 LC/mm^2 ($p<0.05$). The mean decrease in LC frequency in T24 compared with T0 was 18.7%, a level comparable with that seen in healthy individuals (19.6%). This is in contrast to EOP, in which we have previously shown that spontaneous migration of LC from explanted uninvolved skin is absent or minimal. These data confirm that the explant model is a useful system to investigate LC migration *ex vivo* and further highlights the differential behaviour of LC in EOP and LOP.

066

Tubacin, a histone deacetylase 6 inhibitor, suppresses contact sensitivity via impairment of the immune synapse

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Histone deacetylase (HDAC) enzymes affect the acetylation of histones and other cellular proteins that are therapeutic targets for many diseases. HDAC inhibitors (FDA-approved) have been reported to have immune modulatory properties as well as anti-cancer effects; however, the mechanism(s) are largely unknown. HDAC6 deacetylates a unique substrate, α-tubulin, a major cytoskeleton protein. Since acetylated α-tubulin is involved in cell-cell adhesion and HDAC6 is concentrated at the immune synapse (IS), the contact site between T cells and antigen-presenting cells, we hypothesized that HDAC6 may be essential for IS function leading to T cell activation. To test this hypothesis, we utilized a murine model of 2,4,6-trinitrochlorobenzene (TNCB)-induced contact sensitivity and in vitro assays using tubacin, a specific HDAC6 inhibitor. Balb/c mice were given tubacin IP before sensitization and/or challenge with TNCB. Tubacin (2.0mg/kg) given before challenge, but not before sensitization, significantly suppressed the ear swelling response (-71.8±10.1%, $p<0.05$; U test). In vitro, proliferation of hapten specific T cells obtained from TNCB-sensitized mice and stimulated with mitomycin-C-treated spleen cells conjugated with 2,4,6-TNBS, a water-soluble analog of TNCB, was inhibited by 5 µM of tubacin (-52.7±3.81%, $p<0.05$; t-test). To analyze mechanism(s) involved in this suppression, naive T cells were stimulated with anti-CD3/CD28 Ab. Tubacin (at 4 or 5 µM) inhibited cell proliferation (-47.3±1.06% and -76.1±0.64%, respectively, $p<0.05$; t-test). In contrast, tubacin at the same concentrations did not inhibit ionomycin and PMA-induced cell proliferation. Finally, 5 µM of tubacin inhibited ZAP-70 phosphorylation, an activation marker of CD3 signaling, in the anti-CD3/CD28 Ab-stimulated T cell proliferation assay (-40.2±5.73%, $p<0.05$; t-test), suggesting that inhibition of HDAC6 may impair IS function leading to disruption of CD3 signaling. These findings suggest that HDAC6 may be a novel target for the treatment of contact sensitivity and other immune-mediated diseases.

067

The regulatory T cell population is altered in atopic dermatitis

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Atopic dermatitis (AD) is considered as a dermatologic disease resulting from skin barrier disorders and overreacting skin immune response. However the role of immunosuppression and especially of regulatory T cells (Tregs) remains unclear. In this work, we aimed to characterize Tregs in AD patients and in two mouse models of AD. Adult patients with AD lesions show elevated demethylation of CpG dinucleotides in a conserved region of FoxP3 intron 1 in peripheral blood, which demonstrates more Tregs exhibiting suppressive function as compared to healthy controls and non-lesional patients. Similarly, both mouse models i.e. topically treated with vitamin D3 (VitD3) or overexpressing TSLP under a K14 promotor, exhibit increased ICOS-expressing CD4+ CD25+ FoxP3+ Tregs in skin draining lymph nodes (sdLNs). Moreover, proportions of natural Tregs are increased compared to induced Tregs, suggesting that AD favors expansion of natural Tregs. Direct upregulation of Tregs by VitD3 was excluded by removal of the application site and instead, skin-derived dendritic cells (DCs) are required for Treg induction. Indeed, Langerhans cells (LCs) are the first skin-derived DC subset to reach the sdLN and to induce expansion of Tregs on day 3 of VitD3 treatment, while numbers of Langerin+ and Langerin- dermal DCs and other DCs were increased only on day 5. However, depletion of Langerin-expressing DCs indicates that other DCs can step in for the induction of cytokine-producing, largely natural, Tregs in AD. In conclusion, although skin-derived DCs expand activated Tregs in AD, resulting immunosuppression is unable to counteract the ongoing skin inflammation.

068

The role of reactive oxygen species produced in XS-106 dendritic cells by the irritant, benzalkonium chloride

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Contact dermatitis is an inflammatory skin condition resulting from cutaneous contact with materials. Dendritic cells (DCs)-derived reactive oxygen species (ROS) during antigen presentation were reported to play important roles in the pathogenesis of contact dermatitis. Previously, we have shown that allergen-induced ROS increase immune responses in XS106 DCs and human monocyte-derived DCs (MoDCs). However the roles of irritant-induced ROS in XS106 DCs are not fully characterized. Therefore, herein, we have evaluated the roles of ROS produced in XS106 DCs by irritant, benzalkonium chloride (BKC). ROS were produced by BKC in XS106 DCs and ROS production was increased in correlation to the incubation time. When pretreated with GSH, catalase and vitamin E, ROS productions were blocked. Apoptosis was increased in BKC-treated XS106 DCs in concentration dependent manner. However, antioxidants did not prevent apoptosis which implies that apoptosis by BKC is not related to ROS produced by BKC stimulation. Cell surface molecules, such as CD80, CD86 and MHC II, were expressed on XS106 DCs both before and after BKC treatment without significant differences. Cytokine production from BKC-treated XS106 DCs was checked by ELISA. IL-12 and IL-4 secretions were not increased in XS106 DCs by BKC.TNF-α was produced in XS106 DCs in correlation to the concentration of BKC. TNF-α secretion was blocked by GSH and vitamin E, but not by catalase. In summary, the role of BKC-induced ROS in XS106 DCs is different from TNBS-induced ROS. BKC-induced ROS in XS106 DCs do not have a role related to the immune response but probably only related to inflammation.

069

Human intraepidermal T cells: A phenotypically and functionally distinct population of skin resident T cells

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In mice, epidermis is colonized by invariant γδ dendritic epithelial T cells and CD8 αβ T cells but human intraepidermal T cells remain largely uncharacterized. We find that T cells are as numerous as Langerhans cells in the epidermis of healthy human skin. In contrast to mice, all intraepidermal T cells (IET) were αβ, γδ T cells were not evident, and both CD4 and CD8 T cells were present, in a ratio of 3:1. 44% of IET CD4 cells expressed CD103, as compared to 13% in the dermis and central memory T cells were rare. IET contained both FOXP3$^+$ Treg and effector T cells. IET had distinct cytokine profiles with significantly increased TNFα and IL-22 and decreased IL-4 production as compared to human CD4 dermal T cells (DT). IL-17 and IFNγ were elevated but not significantly higher in IET. In contrast to DT, IET responded poorly to mitogens, showed limited proliferative capacity and underwent apoptotic cell death, prevented by the caspase inhibitor Z-VAD-FMK, after removal from the epidermis. We infused allogeneic human CD103$^-$ blood T cells into NSG mice grafted with human foreskin, which lacks resident T cells. Human T cells migrated specifically into the grafted human skin and T cells within the epidermis upregulated CD103 expression but those in the dermis did not (17% IET CD103$^+$ vs. 1% DT). Stimulation in direct contact with keratinocytes, but not in transwells and not with fibroblasts, significantly upregulated CD103 expression on human blood T cells in vitro. CD103$^+$ IET persisted in CTCL patients treated with alemtuzumab, which depletes recirculating but not sessile skin T cells, suggesting CD103$^+$ IET do not recirculate. In summary, we find human epidermis contains a distinct population of nonrecirculating CD103$^+$ intraepithelial lymphocytes that are functionally and phenotypically distinct from other skin T cells and may play a critical role in protecting the skin from infection.

070

Deciphering the role of Langerin+ Dermal DC in the immune response inducing by skin scarification (S.S.) with vaccinia virus (VACV)

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The murine Langerin Diphteria Toxin Receptor (Lang-DTR transgenic mice) allows us to dissect the relative roles of Langerhans cells (LC, late repopulators) and Langerin+ dermal DC (Ln+dDC, early repopulations) after administration of diphtheria toxin. Mice skin scarified (s.s.) with VACVova showed rapid proliferation of OT-1 cells in skin draining nodes, rapid distribution of OT-1 cells to skin by day 3, peaking at day 7, strong cytotoxicity to ova-expressing tumors (EG7) in vitro, and protection against tumor growth in vivo. Mice depleted only of LC were indistinguishable from normal mice in all these parameters. However, mice depleted of both LC and Ln+dDC showed delayed >4 day proliferation in draining nodes, only 20% of control numbers of skin OT-1 cells at day 7, and poor in vitro cytotoxicity. However, their ability to induce E and P selectin ligands on OT-1 cells was unimpaired. A third dermal population of DC exists, that are CD11c+ and Ln-; these were unaffected by our treatments. In tumor growth experiments, mice depleted of LC and Ln+dDC still showed significant delay in tumor growth (300mm3 at day 3 in normal mice, vs day 21 in LC and Ln+dDC deficient mice). We therefore conclude that Ln+dDC's, and LC play a non-dominant role in tumor immunity in this model, and that Ln-DC and lymph node resident DC play a pivotal role. The major role of Ln+dDC may be in protection against acute viral infection, while other Ln-DC's may play a more important role in tumor rejection.

071

Merkel cell carcinoma-specific T cells fluctuate with tumor burden and express therapeutically targetable PD-1 and Tim-3 exhaustion markers

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Purpose: The persistent expression of Merkel cell polyomavirus (MCPyV) oncoproteins in Merkel cell carcinoma (MCC) provides a unique opportunity to characterize immune evasion mechanisms in human cancer. We isolated MCC-specific T cells and determined their frequency, functional status and response to inhibitory receptor blockade. Experimental Design: Multi-parameter flow cytometry panels and HLA/peptide tetramers were used to identify and characterize T cells from tumors (n=7) and blood (n=18) of MCC patients and control subjects (n=10). PD-1 ligand (PD-L1) and CD8 expression within tumors were determined using mRNA profiling (n=35) and immunohistochemistry (n=13). Results: MCPyV-specific CD8 T cells were detected directly ex vivo from the blood of 7 of 11 (64%) patients with MCPyV-positive tumors. In contrast, 0 of 10 control subjects had detectable levels of these cells in their blood ($p<0.01$). MCPyV-specific T cells in serial blood specimens increased with MCC disease progression and decreased with effective therapy. MCC-specific CD8 T cells expressed higher levels of both PD-1 and Tim-3 inhibitory receptors compared to T cells specific to other human viruses ($p<0.01$). PD-L1 was present in 9 of 13 (69%) MCCs and its expression was correlated with CD8 lymphocyte infiltration. Antibodies targeting these inhibitory receptors augmented MCPyV-specific T cell cytokine production in response to the cognate antigen. Conclusion: MCC-specific T cells expand with tumor burden and show evidence of inhibition by PD-1 and Tim-3 exhaustion mechanisms. Reversal of these inhibitory pathways is therefore a promising therapeutic approach for this cancer.

072

Propionibacterium acnes induced IL-17 response in acne vulgaris: A potential inflammatory response targeted by all trans retinoic acid and vitamin D3

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Acne vulgaris is the most common skin disorder affecting millions of people worldwide and inflammation resulting from immune responses targeting P. acnes plays a significant role in its pathogenesis. Our recent findings reveals that, P. acnes is a potent inducer of Th17 and Th1, but not Th2 responses in human PBMCs. P. acnes stimulated expression of genes known to directly signal Th17 responses including IL-17A, RORα, RORc, IL-17RA and IL-17RC. The subset of T cells identified to secrete IL-17 was primarily CD4+ and not CD8+ T cells. Supernatants from P. acnes-stimulated PBMCs were sufficient to promote the differentiation of naïve CD4+CD45RA T cells into Th17 cells. Furthermore, we found that the combination of IL-1β, IL-6 and TGF-β neutralizing antibodies completely inhibited IL-17 production. Importantly, we demonstrate that IL-17-expressing cells were present in skin biopsies from acne patients but not from normal donors suggesting that IL-17-induced inflammation may have clinical relevance. Finally, we found that both all-trans retinoic acid (ATRA) and the biologically active form of vitamin D3 (1,25D3) inhibited P. acnes-induced Th17 differentiation. Together, our data suggest that IL-17 may play a role in acne pathogenesis and that both ATRA and 1,25D3 could be effective tools to modulate Th17-mediated diseases such as acne.

073

Efficacy of RG1-VLP vaccination against genital and cutaneous human papillomaviruses *in vitro* and *in vivo*

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Licensed human papillomavirus (HPV) vaccines, based on virus-like particles (VLP) self-assembled from major capsid protein L1, prevent infection with HPV16 and 18, which cause 70% of cervical carcinomas and a subset of other genital and oro-pharyngeal cancers. However, they may not protect against infections with less prevalent mucosal or cutaneous HPV. In contrast immunizations with minor capsid protein L2 induce low-titer but potentially cross-neutralizing antibodies. We have previously generated chimeric RG1-VLP by genetic insertion of HPV16L2 amino acids 17-36 (RG1) within the DE-surface loop of HPV16 L1. Vaccinations induced cross-neutralizing antibodies against HPV16/18/31/45/52/58/6/11/5. Immunization of 10 rabbits with RG1-VLP adjuvanted with human-applicable alum-MPL induced robust L2 antibodies (ELISA titers 2,500-12,500). Antisera cross-neutralized mucosal high-risk HPV26/33/35/39/68/59/68/73/69/53/34, low-risk HPV6/11/32/40/44/70, and cutaneous HPV2/27/3/76, by pseudovirion- or native virion-based neutralization assays (titers 25-1,000). Boostable antibody titers over 1 year and ELISPOT assays demonstrated the induction of B-cell-memory and CTL responses. Using a mouse genital challenge model, passive transfer with 20µl RG1-VLP immune serum efficiently protected mice against PsV infection with hr HPV16/18/45/31/33/52/58/35/39/51/59/68/56/73/26/53/66/34 and lr HPV6/43/44. RG1-VLP is a promising new generation HPV vaccine with broad-spectrum efficacy against mucosal and cutaneous types.

074

Endothelial activation in localized oedema with sclerodermatous evolution in skin chronic graft versus host disease

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Clinical forms of skin chronic Graft Versus Host Disease (cGVHD), an allo-immune reaction after hematopoietic stem cell transplantation, are mainly lichenoid or sclerodermatous. Up to date, oedematous forms of skin cGVHD have never been described. We retrospectively describe localized skin oedema with rapid sclerodermatous evolution in 5 patients in the setting of cGVHD. Mean Soluble Vascular Adhesion Molecule-1 (sVCAM-1) concentration, as measured by ELISA, in oedematous cGVHD was 2045 ng/ml, versus 282 ng/ml in sclerodermatous cGVHD, 526 ng/ml in allograft patients without cGVHD and 360 ng/ml in control healthy donor group ($p<0,05$ for all three measures). In lichenoid cGVHD, mean sVCAM level was 1612ng/ml. These results suggest an endothelial activation in oedematous and lichenoid skin cGVHD which may be the first phase of a more complex process sometimes leading to skin fibrosis. Though rare, this reversible clinical form of oedematous skin cGVHD must be recognized and treated before sclerosis is formed.

075

Development and functional competence of dermal dendritic cells in the human fetus

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Dendritic cells (DCs) are critical regulators of immune responses to pathogens, tumours and vaccines, and tolerance to self. Immunological competence in the human fetus is poorly understood with scant attention paid to the contribution of fetal DC network to tolerance and immunity. Our study aims to map the development and functional maturity of DCs in the human fetal dermis and how this impacts immune competence. Flow cytometry analysis of fetal skin revealed the presence of three myeloid DC subsets characterised by the expression of CD141, CD1c and CD14 from 12 weeks of gestation. These subsets correspond phenotypically to the adult cutaneous DC complement, although the frequency of CD141+ DCs was higher in fetal skin. Gene expression profile of FACS-purified DC subsets from 18-22 weeks fetal skin and spleen were obtained by microarray. Comparative transcriptomics analysis showed temporally conserved alignment of fetal CD141+, CD1c+ and CD14+ DCs with their respective adult counterparts from skin and blood. These findings highlight the early establishment of a comprehensive cutaneous DC network in the human fetus. Ongoing studies are focused on dissecting their specific immunological functions and competence.

076

Microanatomical dissection of the human dermis: The relationship between antigen presenting cells, lymphatic and blood vessels

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Dendritic cells (DCs) and macrophages (Macs), collectively defined as antigen presenting cells (APC), are abundant in the human dermis. DCs and Macs maintain immune homeostasis and generate rapid responses to inflammatory challenges. The dermis has a rich supply of blood and lymphatic vessels that enable leukocytes to enter and migrate from the skin during steady state and inflammation. Hitherto, there is limited data on the spatial relationship of APCs, lymphatic and blood vessels in situ and the temporal demonstration of DC migration via skin lymphatics. We performed immunofluorescent microscopy analysis of serial skin sections cut with a dermatome and demonstrate the presence of small LYVE1+ initial lymphatic vessels and CD31+ capillaries 100-150 µm beneath the skin surface. Larger well-connected initial lymphatic and blood vessels were visible at a depth of 250 µm followed by LYVE1lo Podoplanin+ collecting vessels with valves in the luminal surface at >300 µm. DCs and Macs were peri and intervascularly distributed in the papillary dermis. However, DCs were more abundant apically while Macs dominated the lower section of the papillary dermis. In contrast, CD3+ T cells were primarily localised in the lower papillary dermis and entirely perivascular in distribution. We assessed DC lymphatic migration by serial time point analysis and live microscopy of skin cultured ex vivo. Very few HLA-DR+ DCs were detected within lymphatic channels at t=0 but their frequency increased after 24 and 32 hours of culture. FXIIIa+ macrophages were never observed within the lymphatic vessels. Our findings on the distinct microanatomical network of APCs, lymphatic and blood vessels speculate the existence of functional immune niches within the dermis. The dynamic studies of DC migration via lymphatics will contribute towards the knowledge base required to facilitate future DC-based immunotherapy strategies.

077

The protective capacity of CD8+ T_{RM} cells in distant skin after cutaneous virus infection

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We have previously shown that repetitive infections of skin at different sites leads to an accumulation of protective T cells that encompass all sites of skin, including those distant from the infected sites. Additional central memory cells also accumulate in lymph nodes. These protective skin homing T cells presumably move out of lymph nodes draining the infected skin, enter the blood stream, and are distributed throughout the body, where they preferentially home to sites that express low constitutive levels of their ligands—E selectin, CCL19, and ICAM-1, all of which are expressed on normal skin. In order to test this theory, we repetitively infected a mouse, and thirty days later we parabiotically joined it to a normal mouse. After four weeks, parabionts were separated, rested for two weeks, and challenged with vaccinia virus (VACV) on distant skin. The previously infected parabiont rapidly cleared VACV infection from distant skin, while the uninfected parabiont, although it contained abundant central memory T cells and antibody, could not efficiently clear infection. This indicates that prior skin infection at different sites in the same organ by the same pathogen multiple times through a subject life leads to subsequent resistance to infection throughout that organ, in a process mediated by skin-resident memory T (T_{RM}) cells generated by multiple infections.

078

Dermal γδ T cells and their role in contact hypersensitivity

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Recent studies have shown that γδ T cells not only exist in murine epidermis (Dendritic Epidermal T cells, DETC) but also populate in the dermis. DETC have been well-studied for decades. However, little is known about the homeostasis of dermal γδ T cells and their roles in skin immune responses such as in contact hypersensitivity (CHS). In this study, we first analyzed the phenotype of dermal γδ T cells and found they have a memory phenotype (CD62L-CD44hi), express high levels of CD69, CD103, and E- and P-selectin ligands, but have heterogeneous γδ TCR repertoires (30% TCR Vγ2+). Upon activation, these dermal γδ T cells produce large amounts of IL-17 and IL-22. GFP+:GFP- parabiosis experiments further showed that dermal γδ T cells are constantly circulating, whereas DETC are non-recirculating. In order to explore their roles in CHS, we first sensitized and challenged TCR δ-/- mice with 2, 4-dinitrofluorobenzene (DNFB). A remarkable reduction of CHS responses in these pan-γδ T cell-deficient mice was observed, suggesting γδ T cells play a pivotal role in CHS responses. We next generated dermal γδ T cell-deficient chimeric mice by bone marrow transfer with injection of neonatal thymocytes to irradiated mice. These mice were then sensitized and challenged with DNFB and CHS responses were measured. Our results showed that CHS responses in dermal γδ T cell-deficient mice was significantly lower than that of wild type mice, demonstrating dermal γδ T cells do participate in CHS responses. Furthermore, we depleted IL-17-producing Vγ2+ dermal γδ T cells by using neutralizing antibodies and found that CHS responses were also significantly reduced in these mice. Therefore, our data suggest that circulating IL-17-producing γδ T cells in dermis play an important role in establishing CHS response.

079

IL-22 single-producing CD4+ T lymphocytes: Candidate effector cells in acute cutaneous graft-versus-host disease

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Graft-versus-host disease (GVHD) is the major clinical complication of allogeneic hematopoietic stem cell transplantation (HCT) and can present in an acute and chronic form. The question whether similar or different pathomechanisms are operative in acute (aGVHD) and chronic GVHD (cGVHD), has not yet been resolved. To address this issue, lesional skin biopsies were obtained from aGVHD (n=25) and cGVHD (n=16) patients. The cellular infiltrate was assessed by immunofluorescence stainings; interleukins and chemokines were measured by real-time RT-PCR. Cytokine secretion profiles of stimulated T cells from collagenase-digested lesional skin biopsies were analyzed by intracellular flow cytometry. While CD4+ and CD8+ T cells dominated the inflammatory infiltrate in both acute and cGVHD, the analysis of the quality of the T cell-mediated immune response revealed striking differences. In aGVHD lesions, there was a predominance of Th2 cytokines (IL-4, IL-13) and chemokines (CCL17, CCL22). In accordance with these findings, we observed an increase of IL-4-producing T cells in aGVHD lesions. Remarkably, levels of IL-22 mRNA were highly increased in aGVHD but not in cGVHD, while IL-17 was up-regulated in both forms of the disease. Cytokine-producing cutaneous T cells in aGVHD lesions showed an increased percentage of IL-22 and IL-17 single-producing CD4+ T cells. The immune response occurring in cGVHD skin lesions was characterized by a Th1/Th17 pattern, as evidenced by a relative increase of Th1 chemokines (CCL5, CCR5, CXCL9, CXCL10) as well as Th1/Th17 cytokines (IFN-γ, IL-12/IL-23p40, IL23p19). Our findings shed new light on the pathomechanisms operative in the different manifestations of cutaneous GVHD and, furthermore, identify molecular signatures to more accurately predict and verify the occurrence of this disease.

080

Global circulating CD4+ and CD8+ T cells are more differentiated in old melanoma patients compared to healthy controls

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Melanoma is highly immunogenic but disease progression occurs despite the presence of tumour specific T cells. Constant antigen exposure may drive the melanoma specific T cells to an end-stage, as is the case in some chronic virus infections such as CMV. This is particularly pertinent amongst the elderly where melanoma incidence and mortality are highest. This study sought to assess T cell differentiation in melanoma patients aged 65 years and above. Flow cytometry was used to analyse T cell differentiation (CD27 and CD45RA expression) and CMV T cell responses in PBMCs of stage I – III melanoma patients and age matched healthy controls. Compared to the controls, melanoma patients had an inverted CD4:CD8 ratio. In the CD4+ T cell compartment there was a significant decrease in naïve (CD27+CD45RA+; $p<0.005$) cells that was accompanied by an increase in the central memory (CD27+CD45RA-; $p<0.005$) and effector memory (CD27-CD45RA-; $p<0.05$) fractions. Among the CD8+ T cells a reduction in central memory cells ($p<0.05$) and an increase in effector memory cells re-expressing CD45RA (CD27-CD45RA+; $p<0.05$) was observed. These trends were CMV independent and likely to be a true attribute of melanoma burden. However, no significant increase in frequency and differentiation of specific CD8+ T cells against Melan-A and NY-ESO-1 could be measured by MHC dextramers among the HLA-A2+ participants. In summary, global circulating CD8+ T cells are driven towards an end-stage in old melanoma patients. This is a process which is partially reversible and could be exploited as a therapeutic target.

081

TIM-3 does not act as a receptor for galectin-9

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T cell immunoglobulin and mucin protein 3 (TIM-3) is a type I cell surface protein that was originally identified as a marker for murine T helper type 1 cells. TIM-3 was found to negatively regulate murine T cells responses and galectin-9 was described as a binding partner that mediates T cell inhibitory effects of TIM-3. Moreover, it was reported that like PD-1 the classical exhaustion marker, TIM-3 is up-regulated in exhausted murine and human T cells and TIM-3 blockade was described to restore the function of these T cells. Here we show that the activation of human T cells is not affected by the presence of galectin-9 or antibodies to TIM-3. Furthermore, extensive studies on the interaction of galectin-9 with human and murine TIM-3 did not yield evidence for specific binding between these molecules. Moreover, profound differences were observed when analysing the expression of TIM-3 and PD-1 on T cells of HIV-1 infected individuals: TIM-3 was expressed on fewer cells and also at much lower levels. Furthermore, whereas PD-1 was preferentially expressed on CD45RA-CD8 T cells, the majority of TIM-3-expressing CD8 T cells were CD45RA+. Importantly, we found that TIM-3 antibodies were ineffective in increasing anti-HIV-1 T cell responses in vitro, whereas PD-L antibodies potently reverted the dysfunctional state of exhausted CD8 T cells. Taken together our results are not in support of an interaction between TIM-3 and galectin-9 and yield no evidence for a functional role of TIM-3 in human T cell activation. Moreover, our data indicate that PD-1 but not TIM-3 are promising targets to ameliorate T cell exhaustion.

082

DEC-205 targeted nanoparticles for improved DC-based antigen delivery and cross-presentation

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Nanoparticles (NP) prepared from the biodegradable polymer poly(D, L-lactide-co-glycolide) (PLGA) have been extensively used in clinical settings for drug delivery and are currently the subject of intense investigation as antigen (Ag) delivery vehicles for vaccine applications. The goal of our present work is to increase the potency of dendritic cell (DC)-based anti-tumor vaccines to overcome inherent limitations in Ag stability and cross-presentation. Here we describe a nanoparticulate delivery system with the ability to simultaneously carry a high density of protein-based Ag while displaying DC targeting ligands on its surface. Utilizing a targeting motif specific for the DC-associated surface ligand DEC-205, we show that targeted NP encapsulating a MART-1 peptide epitope are both internalized and cross-presented with significantly higher efficiency than unlabelled or isotype control NP. In addition, utilizing a combination of confocal, electron microscopy and Imagestream analysis to determine intracellular localization of incorporated NP, we report that DEC-205 labeled NP rapidly escape from the DC endosomal compartment and do not co-localize with markers of early (EEA-1) or late endosome/lysosome (LAMP-1). This indicates that encapsulated Ags delivered by NP may have direct access to the cytoplasmic MHC Class I loading machinery, overcoming the need for "classical" cross-presentation and facilitating heightened DC stimulation of anti-tumor CD8+ T cells. Finally, preliminary data utilizing CpG oligonucleotide-labeled NP also indicated that these reagents can act as "multifunctional" Ag carriers, delivering both Ag and adjuvant in the same delivery vehicle to maximize DC immunostimulatory capacity. These results indicate that this delivery system provides a flexible and versatile methodology to deliver tumor-associated Ag to DC with high efficiency and with the possibility of simultaneously targeting virtually any DC stimulatory motif.

083

Subcutaneous immunization to HIV gag is potentiated by Flt3L and develops through classical rather than migratory dendritic cells

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Immune modulatory agents are needed to potentiate subcutaneous (s.c.) vaccination and improve T cell immunity. In this study, we evaluated the dendritic cell (DC) hematopoietin, Flt3L and HIV gag protein immunization. Flt3L was detected in serum early after protein vaccine immunization to HIV gag with adjuvant. Flt3L treatment improved the T cell response to s.c. protein vaccination by 4.5 fold, and deletion of Flt3 reduced this by 50%. Flt3L enhanced CD4+ and CD8+ IFNγ effector responses, proliferation, and humoral immunity to gag, with markedly improved mucosal immunity in the lungs and lamina propria. Administration of Flt3L expanded several tissue migratory DC (migDC) populations that capture and traffic antigens to the skin-draining lymph node (LN), in addition to LN-resident classical (cDC) and plasmacytoid (PDC) DC. However, in contrast to prior data supporting a role for both migDC and LN-resident subsets in s.c. vaccination, surprisingly we observed DC migrating from the skin were not required for immunity, and could temper the immune response. These results indicate that with a protein vaccine, the immune response to protein vaccine is controlled by the resident or cDC in the lymph node.

084

The dual RAR and RXR agonist alitretinoin modulates leukocyte recruitment pathways and suppresses dendritic cell functions *in vitro* and *in vivo*

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Retinoids have shown beneficial effects in treatment in a variety of diseases. The effects of retinoid acids are mediated by binding to retinoic acid receptors (RAR) and the retinoic X receptors (RXR). Alitretinoin, a retinoid binding to both RAR and RXR, demonstrated significant efficacy in the treatment of chronic hand eczema. To investigate the mode of action of alitretinoin in vitro and in vivo we analyzed structural cells as well as leukocyte subsets and performed a clinical study determining the skin-homing phenotype and activation status of leukocytes before and under treatment with alitretinoin. In direct comparison with the RAR agonist acitretin, alitretinoin showed markedly enhanced suppression of chemokine production in keratinocytes as well as inhibition of dendritic cell maturation and activation. Moreover, the T cell-activating capacity of alitretinoin-treated dendritic cells was significantly lower than those treated with acitretin. In vivo, patients undergoing altitretinoin treatment showed a marked downregulation of the "skin-homing" abilities of effector T cells. Furthermore, mixed leukocyte reactions in patients were significantly reduced during alitretinoin therapy. Taken together, these results suggest that the dual RAR and RXR agonist alitretinoin is superior to the RAR agonist acitretin in modulating skin inflammation.

085

Granzyme B plays a central role in delineating subsets and functional differences in human Tregs in blood and skin

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In the study of human Tregs, the cells are most commonly isolated from the blood. These Tregs have been shown to exhibit defective suppression when isolated from patients with a large number of different autoimmune diseases. When isolating Tregs from the blood, we demonstrate that they basically belong to three functionally distinct Treg populations. The most striking feature that distinguishes these Treg populations is how the cells respond to, or utilize Granzyme B (GzmB). To clarify the role that GzmB plays in Treg biology, we examined its role in suppression ex vivo by three populations of human CD25hiFoxP3+ Tregs from the circulation. We show that one subset of Tregs expresses and utilizes GzmB to suppress by killing, while the two other Treg populations typically lack GzmB and are inhibited by its activity. Thus, although GzmB has been described to represent a component of the Treg suppressive repertoire, we show that it actually inhibits suppression by the majority of circulating FoxP3+Tregs. Whether GzmB would negatively impact Treg suppression, depends on the type of Treg that is involved in the specific interaction at the site of immune-regulation. As we show that IL-6 induces human CD4 T cells to express GzmB, and that GzmB contributes to the Treg resistance exhibited by responder T cells derived from autoimmune patients, we propose that GzmB may play a novel role in regulating the activity of specific subsets of human regulatory T cells. Furthermore, as many of the cells in skin can express GzmB and IL-6, it is crucial to understand how the Tregs of the skin differ from those in the circulation and the role they may play in skin autoimmunity and cancer.

086

Impaired CCR7-mediated migration of MUTZ-3-derived Langerhans-like cells in response to pro-inflammatory stimuli

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Epidermal dendritic cells (DC), Langerhans cells (LC), are recognized to play an essential role as first-line defence against the invasion of pathogens. Here, we investigated LC obtained from the human myeloid leukaemia cell line MUTZ-3 and human peripheral blood-derived monocytes, respectively, as in vitro model systems to study LC differentiation and activation. After cytokine-dependent differentiation Langerhans-like cells generated from MUTZ-3 progenitor cells (MUTZ-LC) as well as monocyte-derived LC (MoLC) displayed an immature phenotype and showed comparable expression levels of the DC-specific markers CD1a and Langerin. However, flow cytometry analysis revealed higher expression of maturation markers (CD83, CD86) on MUTZ-LC compared to MoLC. Furthermore, a similar but distinct Toll-like receptor (TLR) mRNA expression pattern was observed by quantitative RT-PCR. Immature MUTZ-LC expressed lower levels of TLR transcripts involved in viral sensing (TLR3, 7, 8), but showed higher TLR1 and 2 expression when compared to MoLC. Upon stimulation with standard maturation cocktails (TNF, IL-1β together with IL-6, PGE2 or LPS, respectively) and soluble CD40 ligand, a significantly lower CCR7-mediated migration towards CCL21 was found for MUTZ-LC in transmigration chemotactic assays. Similar results were obtained after stimulation with different TLR ligands. Our results demonstrate that MUTZ-LC and MoLC share many phenotypic and functional properties although MUTZ-LC show lower migration rates which may be explained by a limited maturation phenotype. Further investigations will allow deciphering the mechanisms involved in LC differentiation and activation to gain a more detailed insight into LC function in vivo by using human in vitro model systems.

087

Assessment of nickel sensitivity using a T-cell immune function assay

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We conducted a pilot investigation of an adenosine triphosphate (ATP) based in-vitro assay for diagnosing nickel sensitivity, one of the most common causes of contact allergy, using a T-cell mediated Immune response assay (Cylex Inc., Columbia, Maryland). While patch testing is the gold standard to diagnose nickel sensitivity, cytokine and lymphocyte proliferation assays have been studied to monitor T-cell immune status. We utilized this ATP based assay to measure T-cell activation in the presence of nickel in CD3 lymphocytes. A total of 8 individuals were enrolled following patch testing, 4 patients with positive reactions to nickel, and 4 controls with negative reactions. Whole blood samples from each person were collected into sodium heparin vacutainer tubes and tested within 10 hours. The whole blood was added to a microtiter well and incubated with Con A and tetanus antigens as positive controls, blood alone as baseline, and 100, 75, 50, 25 and 12.5 microM solutions of nickel sulfate hexahydrate (NiSO4.6H2O) to determine a dose response curve. Following incubation for 15 to 18 hours in a 37°C, 5% CO2 incubator, CD3 cells were selected using magnetic particles coated with anti-human CD3 monoclonal antibody, washed to remove residual cells, and lysed to release intracellular ATP. ATP was measured with a luciferin/luciferase system and a luminometer (Berthold, Knoxville, TN). An upward trend of ATP values from baseline was observed in all 4 patients with nickel sensitivity. Increase in ATP values varied widely from 6.72 fold to 1.04. One patient with the strongest patch test reaction had the highest increase in ATP values (from 85 to 571 ng/ml). Optimum responses were seen with 25 and 50 microM solutions. ATP values did not follow specific trends in controls. Limiting factors of this study are the small cohorts, limited number of lymphocytes per microwells and using a non-specific marker (CD3) for memory T cells. To our knowledge this is the first attempt to introduce ATP-release assays for in-vitro diagnosis of metal sensitivity.

088

Genetic or chemical RORγ blockade profoundly suppresses hapten mediated contact hypersensitivity responses

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Retinoid related orphan receptor-gamma (RORγ) is a lineage specific transcription factor for the Th17 cell generation. Pathogenic Th17 cells that produce IL-17, IL-22 and express IL-23R are localized in the inflamed skin of psoriasis and allergic contact dermatitis patients. While critical roles of IL-17 are reported in hapten induced contact hypersensitivity (CHS), roles of RORγ as well as Th17 cells in the development of CHS responses have never been directly assessed. In addition, selective blockade of IL-17 alone moderately suppresses CHS immune reactions. Here we demonstrate that deficiency of RORγ inhibits the expression of IL-17 together with other Th17 related genes (IL-22 and IL-23R), and therefore profoundly suppresses the development of CHS immune reactions in a murine model of hapten induced CHS. The decrease in inflammation correlated with fewer total lymphocytes, T cell, and neutrophil infiltration to the site of skin inflammation. Interestingly, there was comparable CHS induced ear inflammation in Tbet-/-bone marrow chimeric mice (Tbet-/-ch) and TNFα-/- ch mice, which were deficient in Th1 cells and TNFα+T cells respectively. However, there was moderate suppression of CHS immune reactions in IFNγ-/- and TNFα-/- mice as well as in mice which were systemically treated with neutralizing anti-TNFα antibody. Finally, therapeutic blockade of RORγ using a specific RORγ inverse agonist (SR1001) also suppressed the DNFB induced CHS reactions very significantly. Our data suggest that RORγ blockade will be a more effective in suppression of CHS compared to the selective blockade of IFNγ, IL-17 or TNFα individually.

089

Melanoma-silencing through CD4$^+$ T helper (Th1) cell cytokine-induced senescence

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Adoptive T-cell transfer is a promising therapeutic option for patients with metastatic malignant melanoma. Recent evidence suggests that CD4$^+$ T helper (Th1) lymphocytes are of great importance for those therapeutic strategies. Besides melanoma killing, the secretion of IFN-γ and TNF-α by Th1 lymphocytes seems to be crucial for efficient tumor control. Yet the mechanisms underlying T cell-mediated control of melanoma growth remain enigmatic. To analyse the effects of the combined application of IFN-γ and TNF-α, we treated a panel of melanoma cell lines with these two cytokines. By FACS-analysis we found that IFN-γ and TNF-α can cause apoptosis and various types of cell cycle arrest, including a predominant G0/G1 arrest. In some melanomas the cell cycle arrest remained stable over >5 passages after withdrawal of IFN-γ and TNF-α, what defines senescence. Moreover, the cells had a senescence-associated secretory phenotype with the production of IL-6, IL-8, IP-10 and CCL-2. Thus, the two Th1-cytokines induced senescence in various melanomas, as defined by stable growth arrest and functional phenotype. To investigate whether it is possible to generate melanoma-specific Th1 cells, that are capable to drive melanomas into senescence under therapeutic conditions, we developed a protocol to induce melanoma-antigen specific Th1 lymphocytes. We primed peripheral blood mononuclear cells with a NY-ESO-1 peptide mix and developed stable Th1 cell lines. We restimulated the Th1 cells with NY-ESO-1 and harvested the supernatant. We incubated various melanomas with the T cell supernatant and analyzed the effect of this supernatant on the melanoma cell cycle. The supernatant induced both apoptosis and cell cycle arrest, including a G0/G1 arrest as seen in senescent cells. Thus, melanoma-specific Th1 cells can be generated that produce cytokines driving human melanomas in a senescence–defining stable growth arrest. This seems to be of great relevance, as modern immunotherapies rather induce stable growth arrest in vivo than complete cancer eradication.

090

Tissue resident and central memory T cells share a single clonal origin but play distinct and non-overlapping roles in long-term memory responses in skin

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Tissue resident memory T cells (T_{RM}) provide rapid and effective anti-viral protection, far superior to that of circulating central memory T cells (T_{CM}). In our latest work, this emerging paradigm shift in understanding immune memory can now be extended to non-viral antigens. We used functional in vivo and in vitro assays, parabiotic mice, and high throughput TCR sequencing, to show that similar populations of long lived T_{RM} cells mediate a rapid response to contact sensitizers (e.g., DNFB). In normal mice, the local skin response of sensitized mice to DNCB is ultra-fast, site specific and migration-independent. The antigen specific T_{RM} cells implicated express the same surface markers as virally-induced T_{RM}. In contrast, mice in which development this T_{RM} cell compartment is blocked develop a slower, diffuse and migration-dependent response, which can be blocked by FTY720. Importantly, this T_{CM} response gave rise to a new population of T_{RM} that subsequently provided a site-specific ultra-fast and migration independent response upon rechallenge. Finally, using high throughput TCRβ sequencing to delineate the clonal response to three distinct antigenic challenges to skin: DNFB, an ovalbumin peptide (OVA) and Modified Vaccinia Ankara virus (MVA, a non replicating poxvirus), in both normal and parabiotic mouse tissues, we find that expanded clones of T cells carrying the identical antigen specific TRCβ are found in high quantities in local and distant skin, as well as the draining and distant lymph nodes. Taken together, our results indicate, for the first time that we are aware, that a single primed naïve T cell gives rise simultaneously to T_{RM} and T_{CM} that have complementary and non-overlapping roles in immune memory, and fundamentally redefine our understanding of not only delayed type hypersensitivity and skin immune responses in general.

091

Systems analysis of pneumococcal vaccine response in psoriasis patients

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"Exercising" the immune system by vaccination allows to dynamically investigate the human immune system in an ethically acceptable manner and make immune alterations, not visible at steady state, measurable. We here investigated patients with chronic plaque type psoriasis receiving either ustekinumab (n=15), which simultaneously blocks the IL-12 and IL-23 axis, or who were untreated with systemic immunosuppressants or biologicals for a minimum of 8 weeks (n=18); untreated patients were selected to match demographics and PASI scores of treated patients (median age 45 years, median PASI 3.4). Patients received the 23-valent pneumococcal polysaccharide vaccine. Using systems approaches, we collected in addition to detailed clinical data and complete blood counts also samples for whole blood microarray, fresh whole blood 10-color polychromatic flow cytometry and serology. Measuring binding antibodies to 14 polysaccharides, no significant difference in serological response between psoriasis patients without systemic treatment or receiving ustekinumab, or a healthy adult control group (n=12) was noted. The quality of the antibody response (opsonizing antibodies titers) will be further investigated. While a spike in circulating CD19+CD20lo+CD27+CD38hi plasmablasts was observed on day 7 following vaccine administration in all groups, this immune response was significantly smaller in patients receiving ustekinumab compared to healthy adults ($p<0.05$). Likewise a day 7 increase in circulating ICOS+ CD4+T lymphocytes was diminished both in ustekinumab treated and untreated patients compared to healthy controls ($p<0.05$) while no such difference was apparent on day 0 before vaccination. This affected both CXCR5+ and CXCR5- CD45RA- memory CD4+T cells. In summary, we here show that although changes in immune cell composition in the blood may not be visible in psoriasis patients with mild disease activity in the steady state, such alterations can become apparent when "exercising" the immune system by vaccination.

092

Induction of a long-lived protective mucosal T cell response by inactivated Chlamydia trachomatis coupled to a TLR7 agonist by nanoparticles

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The obligate intracellular pathogen *Chlamydia trachomatis (Ct)* is the most commonly reported sexually transmitted bacterial infection and the world's leading cause of infectious blindness and female infertility. We designed a vaccine consisting of inactivated *Ct* and a TLR7 agonist covalently linked to nanoparticles that were attached to the microbial surface and, using a mouse model of *Chlamydia* genital tract infection, tested its ability to induce protective immunity. *Chlamydia* loads of the uterus were determined by qPCR and the *Ct*-specific $CD4^+$ T cell response was analyzed by flow cytometry. Genital and intranasal, but not subcutaneous, immunization with the construct resulted in protection when mice were genitally challenged with infectious *Ct* up to six months after immunization. Protection after immunization with our vaccine construct was observed in antibody-, B cell-, and CD8-deficient mice, while RAG-$2^{-/-}$ mice were not protected against *Chlamydia* challenge. Furthermore, we found a robust *Chlamydia*-specific $CD4^+$ T cell response as determined by rate of T cell proliferation and secretion of IFN-gamma and were able to transfer to naïve mice protection against genital *Chlamydia* challenge by injecting purified $CD4^+$ T cells from immunized mice. With the new technologies described here, we were able to modify inactivated *Chlamydia* with nanoparticles to induce a protective T cell response. We observed transmucosal protection as we could specifically induce $CD4^+$ memory cells within the genital mucosa by intranasal immunization. This is essential for translating this vaccine against *Chlamydia* and possibly other sexually transmitted infections to humans.

093

Diverse cutaneous dendritic cell subsets play distinct roles in the transport of soluble proteins to draining nodes and subsequent cross-presentation to naïve CD8 T cells

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We have developed a system in which dendritic cells (DC) isolated from the skin-draining lymph nodes of cutaneously immunized mice present their in vivo-acquired antigen to naïve CD8 T cells ex vivo. Whole ovalbumin protein was used as the topical immunogen, as it requires processing within DC to cross-prime naïve ovalbumin-specific OT-I CD8 T cells. DC populations from immunized wild-type (WT) mice stimulated robust proliferation of OT-I cells. This system has allowed us to explore the relative contribution of various DC subsets and DC-expressed molecules to the process by which cutaneous antigen is delivered to antigen-specific naïve CD8 T cells. We used various mouse breeding and cell sorting techniques to acquire greatly enriched populations of several distinct DC subsets. DC populations from immunized CCR7-deficient mice were unable to stimulate OT-I proliferation, suggesting that migratory DC are required for cross-priming CD8 T cells, and implying that lymph node resident DC by themselves are inadequate to perform this function. This notion was supported by our finding that magnetic-bead-purified CD8α+ DC (a LN-resident subset) from immunized WT mice were also incapable of cross-priming CD8 T cells by themselves. DC from CCR7-deficient hosts and purified CD8α+ DC from WT hosts were efficient at cross-presenting soluble ovalbumin added to the culture, implying that an element of Ag transport is required in vivo that is bypassed ex vivo. Electronic sorting of migratory DC from LN-resident DC demonstrated that migratory DC from immunized hosts were by themselves capable of cross-priming CD8 T cells. Further sorting of the migratory DC into CD11b+ versus CD103+ subsets demonstrated that the vast majority of cross-priming function lies within the CD11b+ population of cutaneously immunized mice. Thus, our in vivo/ex vivo system has allowed us to identify the specific DC subset most likely responsible for presenting soluble skin-derived Ag to naïve CD8 T cells.

094

Transcriptional and functional conservation between human Langerhans cells and mouse thymic DCs

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The characterization of human DC subsets is essential for the design of new vaccines and for better translation of mouse immunological data to the clinic. Previously, we showed that human epidermal Langerhans cells (LCs) were highly efficient at priming effector CD8+ T cell responses. However, the ability of mouse LCs to cross-present antigens and drive potent CTL responses is controversial. We used expression profiling to find the human LC equivalent in the mouse. Our studies suggest that the mouse equivalent to the human LC are the two mouse thymic DC subsets. Our studies also reveal a distinct genetic signature of over 25 genes, that could identify and predict which DC subsets can cross-present. The cross-presentation signature was present in the human blood CD141+ DCs and correctly identified the ability of other distinct human and mouse DC subsets to cross present. We confirmed these predictions with the functional ability of DCs to efficiently cross-prime effector CD8+ T cells. Our studies may help bridge differences between mouse and human DCs and facilitate the application of these data to the clinic.

095

Longitudinal characterization of an antigen-specific T cell response

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During the primary immune response antigen-specific T cells are activated and T cell proliferation and differentiation occurs. Competition among different T cells specific for the same antigen helps shape the T cell response. Following antigen clearance the immune response waynes and many T cells under apoptosis. However, a memory response remains. These memory T cells are poised to respond quickly and robustly to antigen rechallenge. In general, it is believed that the most high affinity cells are selected to become components of the memory pool. By using T cell repertoire analysis to follow the endogenous immune response to model antigens (e.g. hen eggwhite lysozyme and myelin basic protein) in two different mouse strains (BALB/c and C57BL/6) we demonstrate that the same T cells are generated repeatedly throughout the life of an animal and that a strikingly similar repertoire is regenerated following myeloablation and syngeneic bone marrow transplantation. Finally, we demonstrate that, in healthy animals, a profound repertoire shift can be observed in the secondary immune response following antigen rechallenge. This repertoire shift depends on the conditions used to prime the animals. In contrast to current dogma, we observed that high affinity antigen-specific CD4+ T cells are often entirely lost during the secondary immune response, even though they are well-represented in the primary immune response.

096

Next generation T cell receptor sequencing for the identification and monitoring of pathogenic T cells in alopecia areata

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Alopecia Areata (AA) is one of the most common autoimmune diseases, characterized by non-scarring hair loss. T cells are considered the critical pathogenic cells, since they abundantly infiltrate the hair follicles in AA, and are both necessary and sufficient to transfer disease in the C3H/HeJ mouse model of AA. Next generation T cell receptor (TCR) sequencing provides a platform to identify and track the frequency of pathogenic TCR clonotypes, representing a functional biomarker of disease activity. Using this technique for TCR β chain sequencing, we have identified strikingly expanded T cell clones in lesional skin from five AA patients, which represented up to 9.5% of the total TCR sequences, supporting an antigen-driven process in AA, and providing evidence of dominant pathogenic T cell clones. Comparative data from the C3H/HeJ mouse model of AA also strongly supports this notion, as identical TCR sequences were dramatically expanded in new AA lesions of recipient mice grafted with lesional skin from the same donor. In human AA patients, we found that some T cell clones that were expanded in affected skin, also circulate at significant frequencies (>0.1% of total blood sequences) in the peripheral blood of the patient. By correlating the disease severity with the frequencies of circulating pathogenic clones, we are now testing the hypothesis that circulating pathogenic TCR frequency distinguishes patchy Alopecia (AAP) and generalized Alopecia (AU), and/or correlates with baseline disease severity or disease trajectory. In a longitudinal study, we will determine if increased circulating pathogenic TCR frequency precedes or occur concomitantly with disease progression. The unique accessibility of clonally expanded pathogenic T cells within the hair follicle end organ represents an ideal clinical setting to examine the broad applicability of next generation sequencing to identify and track pathogenic TCR clonotypes in human autoimmunity.