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BIDIRECTIONAL INTERACTIONS BETWEEN MAST CELLS (MCs) AND FIBROBLASTS. Stephen J. Galli, M.D.
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Several lines of evidence indicate that MC products can regulate fibroblast function and that fibroblasts, in turn, can influence MC survival, maturation, and phenotype.

Interactions between a receptor expressed by MCs (c-kit) and a fibroblast product, the c-kit ligand, stem cell factor (SCF), can account, at least in part, for many of the effects of fibroblasts on MC survival, development, and phenotype, and can also significantly regulate MC function. Moreover, studies in genetically MC-deficient W/W^v (or Kit^W/Kit^{W-v}) mice, the congenic normal (+/+) mice, and "mast cell knock-in Kit^W/Kit^{W-v} mice", show that MCs are essential for virtually all of the increased expression of Type I collagen mRNA by dermal fibroblasts at sites of IgE-dependent cutaneous responses in mice. This work defines model systems that can be used to investigate many potential interactions between mast cells and fibroblasts.

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STEEL FACTOR AND ITS RECEPTOR C-KIT MEDIATE CELL-CELL INTERACTIONS. Leonie K. Ashman, Gabriella W. Aylett, Antony C. Cambareri, Jean-Pierre Levesque and Paul J. Simmons.
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Steel Factor (SLF) is an essential growth factor for mast cell proliferation and differentiation. In addition, SLF is chemotactic for mature mast cells, promotes their survival, and primes them for mediator release in response to other stimuli. SLF exists in membrane-bound and soluble forms which deliver subtly different signals to target cells. The balance of these forms, which are generated by alternate mRNA splicing and proteolytic cleavage, is regulated in a tissue-specific fashion as well as by external stimuli. In addition to acting as a classical cytokine-receptor system, SLF-c-Kit mediates cell-cell adhesion directly and by influencing other adhesion pathways. Modulation of the level of c-Kit expression may be an important regulator of adhesive interactions and cell signalling.

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MAST CELLS AND FIBROBLASTS: DIFFERENT FORMS OF HUMAN TRYPTASE. Lawrence B. Schwartz.
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Mast cell precursors emerge from the bone marrow, and home to tissues where they complete their differentiation and maturation, largely under the influence of Stem Cell Factor, typically in close association with fibroblasts and connective tissue components. Mast cells also influence the micro environment by release of preformed, lipid and cytokine mediators. The major preformed protein component of mast cell secretory granules is tryptase, a neutral protease. Although tryptase may influence the turnover of connective tissue by activating prostromelysin, and directly stimulate the proliferation of fibroblasts, its capacity to perform these tasks *in vivo* is uncertain. Two gene products, α-tryptase and β-tryptase appear to be expressed by all human mast cells. However, while β-tryptase is stored in secretory granules and released during degranulation, α-tryptase appears to be secreted constitutively, and is the predominant form present in blood at baseline. Distinct processing mechanisms for α- and β-tryptase may be responsible for their different cytoplasmic trafficking pathways. Two immunoassays, one recognizing primarily β-tryptase, and the other β- and α-tryptase, suggest high levels of β-tryptase in systemic anaphylaxis and of α-tryptase in systemic mastocytosis. Thus, different forms of tryptase are useful indicators of mast cell involvement in various human conditions.

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MAST CELLS AND ANGIOGENESIS. Klas Norrby.
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Activation of connective tissue mast cells (MCs) *in situ* causes *de novo* neovascularisation. In fact, MC-mediated angiogenesis (MCMA) is more vigorous and of longer duration than angiogenesis induced by heparin-binding growth factors such as bFGF and $VEGF_{165}$. As secreting MCs (a) release and generate potent mediators, cytokines, arachidonic acid metabolites, enzymes and chemotactic factors and (b) stimulate cells metabolically and mitogenically paracrinically, MCMA may in part be mediated by factors produced by activated cells other than MCs. However, MC-histamine is angiogenic via occupancy of membrane receptors of the H_1 and H_2 type and angiostatic through occupancy of intracellular receptors (H_{ic}). TNF-alpha, produced and secreted by MCs, is also a potent angiogen at low concentrations. Moreover, heparin, a specific MC product, can systemically modify angiogenesis, probably primarily by affecting heparin-binding growth factors: high-molecular-weight fractions can stimulate some angiogenesis reactions whereas low-molecular-weight fractions suppress angiogenesis. MCMA may well play a role in inflammation, angiogenesis diseases and in the growth of cancer tumours which are angiogenesis dependent.

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Immunologic and Biochemical Characterization of Human Heart Mast Cells.

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Cardiac mast cells have been implicated in the pathogenesis of coronary spasm, atherosclerosis, and cardiomyopathy. Despite this, nothing is known about the immunological and biochemical characteristics of the human heart mast cell (HHMC). We have isolated and partially purified HHMC and compared them with mast cells isolated from lung (HLMC) and skin (HSMC) tissues. Cross-linking of the high-affinity receptor for IgE ($Fc_\epsilon RI$) caused the release of preformed (histamine and tryptase) and *de novo* synthesized mediators (peptide leukotriene C_4 and prostaglandin D_2). The tryptase content of HHMC (19.4 ± 1.5 $\mu g/10^6$ cells) was lower than that of HSMC (33.4 ± 2.5 $\mu g/10^6$ cells) and higher than HLMC (10.6 ± 1.9 $\mu g/10^6$ cells). Maximal stimulation of HHMC with anti-IgE led to the release of LTC_4 (17.5 ± 5.1 ng/10^6 mast cells) and PGD_2 (17.8 ± 5.0 ng/10^6 mast cells), whereas HSMC synthesized more PGD_2 (65.0 ± 6.8 ng/10^6 mast cells) and much less LTC_4 (< 5 ng/10^6 cells). Recombinant human C5a anaphylatoxin- and protamine-induced histamine release from HHMC and HSMC, but not from HLMC. Substance P and morphine selectively induced the release of histamine from HSMC, but not from HHMC and HLMC. Compound 48/80 caused histamine release from HSMC and HHMC, but not from HLMC. The pattern of mediators synthesized and the responsiveness of HHMC to different secretagogues appear unique.

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LYMPHOCYTE INTERACTIONS WITH MAST CELLS AND PLATELETS IN DTH. **P.W. Askenase, MD, Yale University School of Medicine, New Haven, CT,**

Murine DTH is dependent on release of serotonin (5-HT), a vasoactive amine stored in mast cells, and in platelets. Morphological and 5-HT antagonist experiments confirmed that T cells activate mast cells to release 5-HT to activate the vasculature, allowing recruitment of T cells and then leukocytes. Factors responsible for activation of mast cells in DTH are IgE antibodies and antigen-specific factors, analogous to IgE. The role of mast cells versus platelets has been clarified recently. Experiments demonstrated that antibody *depletion of platelets* in mast cell deficient mice, inhibited DTH, suggesting that both mast cells and platelets are important. Studies of platelet *repletion* with immune T cells transferred with platelets passively sensitized with IgE, reconstituted 5-HT release for recruitment of T cells, and inflammatory cells. Recent studies suggest that receptors on platelets are both $Fc\epsilon R1\alpha$ and $Fc\epsilon R2$. **In summary,** mast cells and platelets are central to DTH in mice, where they release 5-HT to act on endothelial cells; aiding the recruitment of DTH-effector T cells.

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Mast cell involvement in murine hair growth as a model for studying the role of mast cells in physiological tissue remodelling Ralf Paus,
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Circumstantial evidence suggests that skin mast cells (MC) may be involved in the tissue remodelling events that characterize the cyclic growth (anagen) and regression (catagen, telogen) activity of the hair follicle. MC-hair follicle interactions might be exploited therapeutically, and point to as yet unclear physiological functions of MC in general. Using the murine hair cycle (C57BL/6) as an attractive model for studying MC-hair follicle interactions, we have shown that the number and preferential cutaneous localization of histochemically and immunohistologically detectable skin MC fluctuate significantly during the murine hair cycle: a minimum of MC is detectable during early anagen and during the anagen VI-catagen transformation, and a maximum during anagen IV. Anagen induction and the anagen VI-catagen transformation were associated with a significant increase in the percentage of degranulated MC. The inhibition of MC degranulation (cromoglycate i.c.) retarded both anagen and catagen development, and antagonists of MC products (clemastin, ranitidin, ketanserin i.p.) inhibited induced anagen development. Vice versa, the i.c. application of compound 48/80 or capsaicin, or of endogenous, IgE-independent mast cell secretagogues (ACTH , substance P), induced hair growth (anagen). Histomorphometry revealed that both anagen and catagen development in MC-deficient mice (W/W^v -/-) were significantly retarded compared to +/+ control littermates. Given the battery of potent growth-modulatory agents that strategically located skin MC are equipped with or can generate, we speculate that MC serve as "switchboards of tissue remodelling", which contribute to hair growth regulation mainly by modulating hair cycle-associated angiogenesis, perifollicular cytokine milieu and proteolysis as well as matrix remodelling.

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Mast Cells and Fibrosis. Henry N. Claman, M.D.
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Increased mast cell numbers are seen in a variety of fibrotic situations, including early would healing, keloid activity and neurofibromas. We have been interested in scleroderma. In a mouse graft-versus-host disease (GVHD) system, used as a model for scleroderma, we noted extreme mast cell activation and degranulation. In human scleroderma, we found increased numbers of mast cells and of activated "phantom mast cells" (i.e. mast cells without granules) in involved and not-yet involved skin. We postulated a link between mast cell activation and excess collagen production in GHVD and scleroderma. The putative pathway involves chronic secretion of heparin from mast cells leading to increased biological activity of heparin-binding growth factors, including tryptase and firboblast growth factors.

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MAST CELLS AND SENSORY NERVES IN AXON REFLEX VASODILATATION IN HUMAN SKIN. John C. Foreman
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Endothelin-1 is a potent vasoconstrictor in human skin but the vasoconstriction it induces is surrounded by an area of vasodilatation. Using capsaicin to render primary afferent neurones non-functional and by studying the effects of endothelin-1 in patients with diseases characterized by primary afferent neuronal damage (Raynaud's disease and vibration white finger) we have demonstrated that the vasodilator response to endothelin-1 in human skin in mediated by an axon reflex in neuropeptide-containing, primary afferent neurones.

Endothelin-1 does not release histamine from human cutaneous mast cells in vitro but the vasodilator response that it produces in human skin is partly blocked by selective H_1 histamine receptor antagonists. Furthermore, activation of the axon reflex in human skin by capsaicin, which itself does not release histamine, causes mast cell depletion in human skin.

These observations which support a role for mast cells at the effector end of the axon reflex vasodilatation will be discussed together with evidence which does not support this hypothesis.

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SKIN MAST CELL ACTIVATION BY STRESS-INDUCED CORTICOTROPIN RELEASING HORMONE (CRH). T.C. Theoharides, Dept. of Pharmacology Tufts University School of Medicne, Boston, MA USA

Mast cells are ubiquitous in the body, mostly perivascularly and often in close apposition to neurons. They secrete many vasoactive, nociceptive and proinflammatory molecules in response to immunoglobulin E and specific antigen, as well as to anaphylatoxins, leukocyte-derived factors, cytokines and neuron-derived molecules. Mast cell mediators, in turn, can sensitize sensory neurons which further activate mast cells by releasing neurotransmitters or neuropeptides. In addition to allergic and anaphylactic reactions, therefore, mast cells can participate in neurogenic inflammation indirectly and through tissue damage by releasing proteases and attracting inflammatory cells. Stress induces CRH release which has pro-inflammatory actions outside the brain. CRH induced secretion from rat skin mast cells over a concentration range from 10^{-4} to 10^{-10} M. This effect was documented both by extravasation of intravenously injected Evans blue, as well as morphological evidence of mast cell degranulation from the site where CRH was administered subcutaneously. The free acid analog of CRH which has no biological activity had no effect on mast cells. Treatment of rats neonatally with capsaicin did not reduce mast cell secretion to CRH indicating it was not due to CRH-induced release of neuropeptides from sensory nerve endings. These findings may have implications for the pathophysiology and possible therapy of neuroinflammatory skin disorders such as neurogenic pruritus, eczema and psoriasis which are exacerbated by anxiety and stress.

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CONCORDANT ABSENCE OF SKIN MAST CELL MARKERS AND SKIN INFILTRATES IN MALIGNANT MASTOCYTOSIS.

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Although mast cells (MC) in various organs are of hemopoietic origin, organ-specific diversity of MC has been described. We analyzed the phenotype of MC in 4 pts with malignant mastocytosis, selected on the basis of bone marrow (bm) origin of the pathologic clone, kit-ligand independent MC growth, multiorgan-involvement and absence of skin infiltrates. Malignant MC in all 4 pts expressed tryptase but not chymase (MC_T). They expressed c-kit (CD117), CD9, CD43 and CD44, but not IL-3R, CD11b, CDw17 (basophil Ag), or myelomonocytic Ag (CD14, CD15). Malignant MC also lacked the skin MC marker C5aR/CD88. Electron microscopy confirmed the presence of MC-precursors with granules containing scrolls, but not crystals (hallmark of skin MC). Together, in 4 pts with malignant mastocytosis the absence of skin infiltrates was associated with absence of skin MC markers.

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The mast cell proteases tryptase and chymase inhibit the mitogenic effect of thrombin on keratinocytes by cleavage of the 'tethered ligand' domain of the thrombin receptor.

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Tryptase and chymase are the main serine proteases in skin mast cells. Recently, we demonstrated that both proteases inhibit the mitogenic potential of thrombin on human HaCaT keratinocytes. In the present study, we investigated the mechanism which could be responsible for this inhibition of proliferation.

The 'tethered ligand' binding domain of the thrombin receptor $TRAP^{42-55}$ was incubated with tryptase (human, lung) and chymase (mouse, skin) under standard conditions. Reactions were stopped by ultrafiltration, the filtrates were tested for biological activity, and the molecular weight of the fragments was determined after separation by reversed phase HPLC. Digestion of $TRAP^{42-55}$ by tryptase resulted in two (FT^{42-45}, FT^{46-52}) and by chymase in three fragments (FC^{42-45}, FC^{46-52}, FC^{53-55}). In contrast to the other generated fragments, only FT^{42-45} induced mitogenic effects similiar to $TRAP^{42-55}$ on human HaCaT-keratinocytes.

Our data suggest that tryptase and chymase are able to cleave the extracellular domain of the thrombin receptor at a most sensitive part. This domain represents the 'tethered ligand' domain which is responsible for the activation of the thrombin receptor and for the mitogenic effects of thrombin.

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MULTIFUNCTIONAL CYTOKINES DO NOT EFFECT SKIN MAST CELL HISTAMINE SECRETION.

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The influence of various cell types, through the release of soluble cytokines, on mast cell activation in chronic allergic inflammation or late phase reactions in the skin still remains unclear. The aim of our study was therefore to investigate the capacity of a diverse range of cytokines to prime or directly stimulate enzymatically isolated human skin mast cells (HSMC) to release histamine. Being aware of mast cell-nerve/fibroblast interactions, we were especially interested in examining the effects of NGF-ß and SCF on HSMC histamine releasability. Furthermore, a number of cytokines that have recently reported to be produced from T_H2-lymphocytes as well as mast cells themselves, which are known to be crucially associated with the augmentation of allergic processes, were also examined. HSMC were preincubated with the cytokines for 10 min and subsequently activated with suboptimal concentrations of anti-IgE, A23187 or substance P. Surprisingly, other than SCF, neither IL-3, IL-4, IL-5, IL-6, IL-8, PF-4, MCP-1, RANTES, GM-CSF, G-CSF nor NGF-ß had significant influence on the histamine release from HSMC. With respect to the present data, HSMC do not appear to be targets of these cytokines.

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MAST CELLS IMMUNOPOSITIVE TO TYROSINE HYDROXYLASE (TH) AND DOPAMINE (DA) CONTACT SYMPATHETIC NERVE FIBERS IN THE RAT DURA MATER.

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Rat dura mater contains numerous mast cells of various phenotypes. We have previously shown that most of these cells contain histamine and serotonin (5-HT) and that some of them make close contacts with sensory and autonomic nerve terminals. Some dura mast cells also contain tryptophan hydroxylase (TPH, the rate-limiting enzyme of 5-HT synthesis). TPH can be localized only in a juxta-nuclear organite ressembling the Golgi apparatus. These cells could contact TPH-I nerve fibers.

During studies on the sympathetic innervation, we have noticed that a large number of mast cells could be immunopositive to TH (TH-I) and that these cells were frequently contacting TH-I (sympathetic) nerve fibers. These cells were also immunopositive to DA but not to noradrenaline. They could be differentiated morphologically (larger size, shape of granules) from TPH-I and 5-HT-I mast cells. As for TPH, TH was sometimes localized only in a juxta-nuclear organite.

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SCF-MEDIATED DOWNREGULATION OF c-kit IN HUMAN MAST CELLS.

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Recent data suggest that local expression of SCF is associated with accumulation of mast cells (MC) and decreased expression of c-kit. This study was performed to demonstrate the effect of rhSCF on expression of c-kit mRNA and surface c-kit protein in isolated MC and a human MC-line, HMC-1. Incubation of lung MC (purity: 90%) with rhSCF, 100 ng/ml for 120 min resulted in decreased expression of c-kit mRNA (OD, control: 8.4 *vs* SCF: 3.1). The decrease of c-kit mRNA was associated with decreased expression of cytoplasmic c-kit and surface c-kit on MC (HMC-1: MFI[control]: 83.1±6.9 versus MFI[SCF: 100 ng/ml for 12 hrs]: 22.0±2.9). The effect of rhSCF on c-kit expression was dose- and time- dependent with maximum effects observed with 10-100 ng/ml of rhSCF after 12 hrs. Together SCF induces downregulation of its receptor in human lung mast cells and HMC-1 cells.

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NUMBER, PHENOTYPE AND FIXATION-SENSITIVITY OF DUODENAL MAST CELLS IN NORMAL PERSONS, GASTRITIS AND CROHN`S DISEASE.

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The number and phenotype of MC in normal (n=9) and inflamed (n=13) human duodenum was studied. In normal duodenum, the staining and fixation properties of mucosal MC correspond to the rat mucosal MC (MMC) type (Safranin O^-, Berberine sulfate$^-$, Alcian Blue$^+$, formalin fixation-sensitive). Using Irani`s protocol, these MC mostly are MC_T (tryptase+/chymase-). Submucosal MC show the rat connective tissue (CTMC) type (Safranin$^+$, Berberine sulfate$^+$) and were MC_{TC}. In gastritis pts, elevated numbers of mucosal MC were found (controls: 187±23/mm^2; gastritis: 413±139/mm^2). A subset of mucosal MC was fixation insensitive, but still Safranin$^-$ and Berberine sulfate$^-$. In Crohn's disease, mucosal MC decreased (34±30/mm^2) and most were fixation insensitive. Together, normal human duodenal mucosa contains MC with staining and fixation properties of rat MMC. The number and fixation properties of mucosal MC may change under pathologic conditions.

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PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF THE HUMAN RENAL MAST CELL. Waltraud J. Beil, Wolfgang Füreder, Helene Wiener, Ulrich Maier, Klaus Lechner, Peter Valent.
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We have characterized the phenotype and functional properties of human renal MC (RMC) and compared RMC with other MC types. RMC were isolated from 17 pts suffering from renal tumors by enzyme-digestion. As assessed by combined toluidine blue/immunofluorescence staining with mAbs, RMC expressed c-kit/CD117, CD9, CD43, CD44, CD54 and CD63. RMC did not express CD2,3,4,8,19,23,11b,14,15, 16,w17 or CD35. RMC also lacked IL-3Rα/CD123, GM-CSFRα /CDw116, IL-1RII/CD121b, IL-6R/CD126, IL-7R/CD127, IL-8R/ CD128 and IL-10R as well as the skin MC marker C5aR/CD88. Activation of RMC through IgER or SCFR resulted in histamine secretion, whereas rhC5a, IL-1 through -10 or EPO showed no effects ($p>0.05$). By in-situ stains, most RMC were tryptase+/chymase- (MC_T) and Berberine sulfate-. Electron microscopy confirmed the presence of MC in renal tissue and revealed a scroll+/cristal- granule- type. Together, RMC are tryptase$^+$, C5aR$^-$ MC with phenotypic and functional properties similar to human lung MC.

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A simple immunofluorescence-technique for co-visualizing mast cells and peptidergic nerve fibers in skin *V. A. Botchkarev, S. Eichmüller, M. Maurer, O. Johansson*, B. M. Czarnetzki, and R. Paus.*
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Although mast cells (MC) and peptidergic nerve fibers can be co-visualized by double-labeling and histochemical methods, this is notoriously difficult in practice, e.g due to background problems, distinct fixation requirements, and availability of suitable antibodies. Here, we describe a simple immunofluoresence-technique for the simultaneous demonstration of nerve fibers and skin MC, based on the immunohistological detection of neuropeptides or neuronal markers combined with non-specific staining of glycosaminoglycans in connective tissue-type MC by avidin-FITC. Cryostat sections of perfusion-fixed C57BL/6 mouse back skin were incubated with antibodies to a neuronal marker (PGP 9.5, neuron-specific enolase, tyrosine hydroxylase, neurofilament 150, or S-100), or a neuropeptide (CGRP or substance P). The sections were then incubated with secondary antibodies, labeled with rhodamine, followed by FITC-avidin. This demarcated all glycosaminoglycan+ skin MC by green, and nerve fibers by red fluorescence. MC identity was verified by double-staining with anti-histamine antibody, showing 100% co-localization. Perfusion fixation was needed when neuropeptides were co-stained; for NF 150 and PGP 9.5, acetone fixation sufficed. This technique, which can be extended to co-visualizing MC with multiple other antigens and which worked in murine, rat and human skin, allows to estimate e.g. the percentage and granulation status of MC located next to specific peptidergic nerve fibers, and to dissect differences in the association of certain nerve fiber-subtypes with avidine+ MC under physiological, pathological and experimental conditions.

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MAST CELLS IN LICHEN PLANUS; MORPHOLOGICAL AND FUNCTIONAL CHARACTERIZATION. Ewa Brzezińska-Błaszczyk, Anna Zalewska, Anastazy Omulecki.
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Lichen planus is a skin disease of unexplained etiology. It is assumed that mast cells might play an active role in pathomechanism of this condition. The purpose of our study was to describe some morphological and functional properties of lesional mast cells in comparison with cutaneous mast cells from healthy subjects. Our work was performed on skin speciments from 11 patients with lichen planus and from 11 healthy subjects. Morphological analysis has showed some increase in mast cell number, confined to the inflammatory infiltrate, in lesional skin. Functional studies, which were performed *in vitro* on mast cells obtained by enzymatic dispersion technique, have pointed out that mast cells from lichen planus skin: (1) had the same "releasability" as mast cells from healthy skin; (2) showed a similar reactivity to anti-IgE as those of control group; (3) released histamine in response to TNF-α-activation at the same level as control cells; (4) had slightly high substance P-induced histamine release than mast cells of healthy subjects.

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MAST CELLS IN MALIGNANT SCHWANNOMA. Toshio Demitsu, Satoru Murata, Tomoharu Kiyosawa, Hideo Yaoita
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We found mast cell accumulation and the activation in malignant schwannoma. To determine the cell to cell interactions between human skin mast cell (HSMC) and malignant schwannoma (MS) cell, we investigated the HSMC survival and the morphological alterations when cultured with MS-derived cells from the patient. HSMCs at a cell purity of 50-70% obtained from normal human skin were cocultured with MS-derived cell feeder layer grown on the coverslips. At 1 wk highly granulated HSMCs were observed and they demonstrated various shapes such as round, spindle and dendritic form in close contact with the adjacent MS-derived cells. Coculture with MS-derived cell feeder layer revealed significantly increased number of HSMC compared to the culture with normal human skin fibroblast layer at 1 wk and 2 wk. We failed to detect SCFmRNA on cultured MS-derived cell by RT-PCR. These results suggest that MS-derived cell was helpful for HSMC culture in vitro and some factor(s) other than SCF might be implicated in this effect.

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THE ENDOGENOUS A_3 ADENOSINE RECEPTOR AND THE TRANSFECTED M_1 MUSCARINIC RECEPTOR USE SPECIFIC G PROTEINS TO INDUCE INCREASE IN INTRACELLULAR CALCIUM IN THE RAT BASOPHILIC LEUKEMIA CELL LINE RBL-2H3. Edgar Dippel, Frank Kalkbrenner, Rita Haubold and Günter Schultz.
Institut für Pharmakologie, FU Berlin, Berlin, F.R.G.

Adenosine is known to modulate through A_3 adenosine receptors (A_3R) the immediate hypersensitivity reaction induced by mast cells. We studied the question which specific G protein is used by the A_3R in the rat mast cell model RBL-2H3 to activate phospholipase C (PLC). The A_3R-agonist NECA(N-ethylcarboxamidoadenosine) induced in RBL-2H3 cells pertussis toxin (PTX)-sensitive increase in inositolphosphates and release of calcium from intracellular stores. We injected antisense oligonucleotides directed against the α-, β-, and γ-subunits of G proteins into the nuclei of RBL cells and,48 hours later, measured the increase in $[Ca^{2+}]_i$ by single cell imaging in the injected cells loaded with FURA-2. In addition to the endogenous A_3R we investigated the stably transfected M_1 muscarinic receptor (M_1R) coupling via a PTX-insensitive G protein to PLC by using the subclone RBL-hm1. The results indicate that the M_1 receptor uses heterotrimeric G proteins composed of $G\alpha_q/\alpha_{11} \cdot \beta_1/\beta_4 \cdot \gamma_4$ and the A_3R uses Gi_3 to stimulate PLC. Thus our results indicate that Gi_3 is important for the adenosine-mediated potentiation of serotonin release induced by antigens in mast cells.

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THE NEUROPEPTIDE GALANIN STIMULATES PHOSPHOLIPASE C VIA PERTUSSIS TOXIN-INSENSITIVE G PROTEINS IN THE RAT BASOPHILIC LEUKEMIA CELLS RBL-2H3. Edgar Dippel, Rita Haubold, Günter Schultz and Frank Kalkbrenner.
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The neuropeptide galanin regulates or modulates different biological systems by inhibition of adenylyl cyclase, stimulation of ATP-sensitive K^+ channels and inhibition of voltage-dependent Ca^{2+} channels. All these effects are mediated by pertussis toxin (PTX)-sensitive G proteins of the G_i/G_o family. In RINm5F and GH_3 cells and in two lung cancer cell lines, a galanin-induced release of calcium was shown. Our results demonstrate that in the rat mast cell model RBL-2H3 galanin also induces a rapid, transient and concentration-dependent increase in the intracellular calcium concentration which was inhibited by the specific receptor-antagonist galantide. Chelation of extracellular calcium by EGTA did not abolish this effect, indicating that galanin induces release of calcium from intracellular stores. In addition, the effect was PTX-insensitive, indicating that G proteins of the Gq family are involved. Galanin also induced an increase in inositolphosphates in these cells, but neither galanin nor the more potent analogue galanin (1-16) induced secretion of serotonin from RBL-2H3 cells. The results suggest a role of the neuropeptide galanin as a signal molecule between nerve endings and mast cells.

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CYCLOSPORIN A AND FK506, BUT NOT RAPAMYCIN, INCREASE DNFB INDUCED TNF-ALPHA EXPRESSION IN HUMAN EPIDERMAL SHEETS.

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The potential use of immunosuppressive compounds like FK506 and rapamycin or SDZ 241-180 in the topical use of dermatologic diseases (e.g. eczema, psoriasis) is currently investigated in clinical trials. In attempt to further characterize their mechanism(s) of action, we studied the effect of FK506, rapamycin and cyclosporin A (CsA) in a human ex-vivo model and a murine in-vivo model for contact hypersensitivity. METHODS: I. Human epidermal sheets were generated by dispase treatment from freshly obtained skin. These samples were exposed to either DNFB or DNFB plus 1. FK506, 2. rapamycin or 3. CsA for 1 hr. RNA was isolated and RT-PCR was performed. TNF-α expression was assessed by liquid hybridization. II. Earswelling was assessed in mice treated with DNFB, or DNFB plus FK506, rapamycin or CsA. RESULTS: FK506 and CsA increased the DNFB induced TNF-α expression in human epidermal sheets markedly, whereas rapamycin, a structural analogue of FK506 did not. FK506 and CsA could prevent contact hypersensitivity in the murine in-vivo model, but rapamycin could not. CONCLUSION: These data suggest: I. FK506 and CsA differentially regulate TNF-α expression in the skin (upregulation of TNF-α in DNFB treated human epidermal sheets versus TNF-α inhibition in lymphocytic lines or mast cell lines). II. Increased expression of TNF-alpha in human epidermis by FK506 and CsA correlates with the inhibition of DTH in mouse skin.

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MAST CELL RECONSTITUTION OF W/W^v MICE IMPROVES TNF DEPENDENT SURVIVAL AFTER EXPERIMENTAL PERITONITIS

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TNF depletion of infected animals has been shown to lead to aggravation in many experimental infections. Accordingly, survival of mice after sublethal CLP (cecal ligation and puncture) leading to peritonitis and sepsis depends critically on TNF as demonstrated earlier. Neutralization of endogenous TNF with anti-TNF antibodies was lethal in this infection model. Mast cells seem to be the only resident cells able to store preformed TNF in their granules. W/W^v mice showed decreased serum TNF levels 2 h after LPS injection. The mast cell-deficient W/W^v mice that underwent CLP were more sensitive to CLP than normal mice resulting in 92 % mortality in W/W^v vs. 8 % mortality in normal littermates. 60 % W/W^v mice could be protected from lethal CLP by i.p. injection with recombinant mouse TNF after CLP. Reconstitution with cultured mast cells 2 weeks before CLP protected 70 % of the reconstituted W/W^v mice. Survival of the reconstituted W/W^v mice also depends on TNF because none of these mice survived CLP after injection of anti-TNF antibodies. Currently, we are investigating the influence of mast cell activators and mast cell products on survival after CLP.

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TRYPTASE, A POTENT MITOGEN FOR KERATINOCYTES.

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Psoriasis is a inflammatory skin condition characterized by a marked hyperproliferation of keratinocytes and by increased numbers of mast cells and their degranulation. To investigate whether tryptase, a proteinase released by mast cell degranulation, is a mitogen for keratinocytes we studied the effect of isolated human tryptase. Tryptase markedly stimulates the proliferation of both keratinocytes in primary culture and the HaCaT cell line in a concentration-dependent fashion with a threshold of ~1 pM. At a concentration of 1 nM, tryptase increases ^{3}H-thymidine incorporation into HaCaT cells by 84 ± 3% (mean ± SEM, n = 6). Related proteinases (e.g. thrombin and trypsin) have no effect on keratinocytes in primary culture although they are mitogens for transformed HaCaT cells. The tryptase-induced proliferation is only observed in the presence of heparin and blocked by active site directed inhibitors, indicating that the response depends on the catalytically active tryptase-tetramer.

Our results suggest that tryptase which is present near the dermo-epidermal junction in lesional skin may play an important role in the pathogenesis of psoriasis.

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EXTRACTS FROM *ASCARIS SUUM* LARVAE INDUCE IL-4 RELEASE FROM HUMAN BASOPHILS.

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Parasitic worms are potent inducers of the synthesis of IL-4 and of IgE. Since human basophils have been shown to produce IL-4 after stimulation with certain agents, we asked whether basophils might release IL-4 in response to stimulation with extracts from larvae of the intestinal nematode *Ascaris (A.) suum*. Basophils were purified from freshly obtained peripheral blood or from buffy coats from healthy human donors by a ficoll density gradient, countercurrent elutriation and a percoll density gradient (mean purity 70%). L2 larvae of *A. suum* were grown from embryonated eggs by *in vitro* culture. Aqueous larval extract was incubated with basophils in the presence of IL-3. The extract triggered IL-4 release (median 0.5 ng/10^6 basophils, range 0.06-1.5 ng/ml) in 9 of 9 donors. As assessed by ultrafiltration, the molecular weight of the IL-4 inducing parasite material was above 30 kD. These data show that *A. suum* can induce IL-4 release from basophils which may contribute to the elevated IgE found in *Ascaris* infection. Whether this effect is IgE-mediated is currently under investigation.

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INTERACTION OF TRYPTASE AND BPTI IN BOVINE MAST CELLS.

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We performed recently a biochemical and ultrastructural analysis of the content and subcellular localization of BPTI and tryptase in bovine liver capsule mast cells (L. Fiorucci et al., Bioch. Biophys. Acta, in press). The results indicate the colocalization of the enzyme and the inhibitor within the mast cell granules. In order to have more information on the interaction of tryptase and BPTI in the secretory granules, activation of mast cells and analysis of the exocytosed products were performed.

Mast cells suspensions were obtained from bovine liver capsule with a combination of mechanical fragmentation and collagenase digestion. Further purification of mast cells was obtained using density gradients of Percoll. The supernatants of calcium ionophore-activated mast cells were electrophoresed and blotted. Immunoblot analysis was performed using antibodies directed against tryptase and BPTI, in the attempt to reveal the presence of the inhibitor-enzyme complex.

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HUMAN LUNG MAST CELLS EXPRESS AND RELEASE VASOACTIVE INTESTINAL PEPTIDE, VIP.

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Vasoactive intestinal peptide (VIP) is a multipotent mediator regulating functional properties of various cells. VIP is well known as a vasoactive substance, but the physiologic source of VIP has not been fully clarified. We report that human mast cells (MC, lung, n=4) and HMC-1 cells (mast cell line) are a rich source of VIP. These cells express higher levels of VIP (lung MC: 15.8-54.0 ng/10^7, HMC-1: 1.3-4.0 ng/10^7), compared to PMN (0.2-0.3 ng/10^7), or other leukocytes (<0.2 ng/10^7). Release of VIP from MC could be induced by IgE-dependent stimuli (control <5%; αIgE, 1 μg/ml: 39.4% VIP release) as well as by the ligand of c-kit, rhSCF (100 ng/ml: 25.4%). VIP and histamine secretion showed almost identical dose- and time- response curves suggesting a granular location of VIP molecule. MC-derived VIP may contribute to allergic and other MC-dependent reactions.

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INTERACTIONS OF HUMAN LUNG MAST CELLS WITH TISSUE FIBRONECTIN. Kirstin Goldring and Jane Warner,
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Within the tissues human lung mast cells (HLMC) are in contact with a range of neighbouring cells and matrix proteins which may influence their subsequent responses. It is known that HLMC and peripheral blood basophils express fibronectin receptors and so we have examined the effects of tissue fibronectin on both cell types. Fibronectin (1-100µg/ml) failed to initiate the release of histamine from HLMC. Release was 1±1% in 5 preparations of HLMC. In contrast, 100µg/ml tissue fibronectin triggered the release of 13±5% of the total cellular histamine from the basophils of 8 non-asthmatic subjects. Both HLMC and human basophils released histamine following crosslinking of the cell surface IgE by anti-IgE. Histamine release was 17±3% in the HLMC and 30±8% in the basophils. Isolation of the HLMC involves proteolytic digestion and so we compared HLMC obtained via mechanical dispersion with cells from collagenase treatment. We found that they too failed to respond to tissue fibronectin and their anti-IgE response was unchanged. In summary, tissue fibronectin initiates histamine release in human basophils but not HLMC.

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ALTERATIONS IN GANGLIOSIDE EXPRESSION ARE ASSOCIATED WITH MAST CELL DIFFERENTIATION

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Gangliosides are physiological cell membrane components. One known function is their role as mainly unspecific receptors for toxins and hormones or in cell adhesion. Furthermore, the ganglioside membrane composition is changed during physiological and neoplastic growth, and enhanced expression of the ganglioside GM3 has been described to be associated with the monocytic differentiation of HL 60 cells. In this study we have investigated changes in the ganglioside expression during the differentiation of peripheral blood cells into mast cells. After Ficoll centrifugation, the monocytic fraction from buffy coats was incubated in medium containing horse serum and murine fibroblast supernatant for 15 days. Ganglioside expression was compared before and after this incubation period. Gangliosides were extracted from the total lipid fraction in multiple steps using organic and inorganic solvents and were finally evaluated by quantitative TLC.
After 15 days of incubation, the cells showed the morphologic appearance of mast cells with a positive immunohistological staining for the high affinity IgE-receptor and tryptase. Comparison with undifferentiated cells revealed a highly enhanced expression of GM3 in differentiated cells (1.2 µg GM3/10^7 cells vs. 0.08 µg GM3/10^7 cells before differentiation, means of 4 experiments). From these preliminary results, it is concluded that ganglioside GM3 expression may be involved in mast cell differentiation. GM3 might act as a receptor molecule, but its specific action needs to be further investigated.

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DIFFERENTIAL ACTION OF CC CHEMOKINES ON MEDIATOR RELEASE FROM HUMAN SKIN MAST CELLS AND BASOPHILS. Karin Hartmann, Florian Beiglböck, Beate M.Czarnetzki, Torsten Zuberbier
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Chemokines are involved in the activation of various inflammatory processes. In human basophils, different CC chemokines are known to cause histamine and LTC4-release. In the present study, we have evaluated the effect of RANTES, monocyte chemotactic protein (MCP)-1, MCP-2, MCP-3, macrophage inflammatory protein (MIP)-1α and MIP-1β on human skin mast cells in comparison to basophils. All investigated CC chemokines caused dose-dependent basophil histamine release in mixed human leukocyte suspensions. In contrast, none of them was able to induce direct release of histamine, tryptase or prostaglandin D_2 from human skin mast cells, nor did priming with these substances enhance IgE-mediated mast cell mediator release. In addition, all chemokines failed to promote changes in the cytosolic free calcium level in the human mast cell line HMC-1. These results add further evidence for the differences between human mast cells and basophils regarding cytokine-dependent activation.

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MAST CELLS MODULATE THE BALANCE OF SERINE PROTEINASES (SERPS) AND SERINE PROTEINASE INHIBITORS (SERPINS). Hartmut Heine
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Mast cells (MCs) control extracellular matrix (ECM) functions. Main constituents of ECM are proteoglycans (PGs) and glycosaminoglycans (GAGs). Structure and function of these macromolecules are strongly influenced by SERPS, their activators and inhibitors. We have examined the influence of MCs on this regulatory network.
Cryostat sections (5µm) of normal human connective tissue were incubated with the SERPIN aprotinin (10.000 KIE/kg) coupled to FITC. Sections were counterstained with toluidine blue and viewed with a Leitz Orthoplan Microscope (Filtercombination BG12 (517)). Control sections were incubated with uncoupled aprotinin.
MCs showed a specific fluorescence of their granules. This suggests a receptor mediated endocytotic uptake of aprotinin into MCs. Flourescence of ECM was faint but compared to controls distinct. Aprotinin as an ECM bound natural SERPIN forms stoicchiometric complexes with SERPS. In the presence of heparin, a GAG released by MCs, plasminogen is very rapidly converted by plasminogen activators to plasmin. Uptake or release of aprotinin from ECM by MCs therefore seems to balance the conversion of plasminogen at a physiological level.

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MAST CELLS VERSUS ACTIVATED T-LYMPHOCYTES AS INDUCERS OF CD40-LIGAND ASSOCIATED IGE-SYNTHESIS. Hermes B, Kroczeck RA[#], Kolde G, Nowack F, Dallenbach F[±], Stein H[±], Haas N, Czarnetzki BM, Dept. Dermatol, Virchow Klinikum, Humboldt Universität; [#]Mol.Immunol., Robert Koch Institute; [±]Dept. Pathol, UKBF, Freie Universität, Berlin, Germany. Since IgE synthesis has been shown to require CD40/CD40L interactions, and since CD40L has been described also on mast cells, we have studied CD40L expression in various IgE- and mast cell- associated skin diseases (atopic dermatitis (N=12), scabies (N=11), chronic urticarid (N=12), normal skin (N=7), normal and dermopathic lymphnodes and tonsils (N=10) by immunohistochemistry, some by immunoelectronmicroscopy and HMC-1 mast cell and KU812 cell lines by FACS analysis. CD40, CD23, CD30, L26, FcεRI and mast cell tryptase expression were studied as well. CD40L was markedly expressed on lymphocytes in atopic dermatitis, scabies, and particularly (>10x) in lymphnodes and tonsils, but not in chronic urticaria or normal skin. No CD40L expression was noted on tissue mast cells or in cultured mast and basophilic cells. These data suggest that in the conditions studied, CD40L-associated IgE synthesis occurs most likely in lymphnodes and not in skin and that lymphocytes rather than mast cells are primarily involved in this process.

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GLUCOCORTICOIDS AS WELL AS ALL-TRANS-RETINOIC ACID SUPPRESS THE PRODUCTION OF MULTIPLE CYTOKINES IN ACTIVATED MOUSE BONE MARROW-DERIVED MAST CELLS. Lothar Hültner, Christoph Hüls°, Stephan Kölsch°, Hannelore Broszeit, and Edgar Schmitt°. GSF-Institut für Experimentelle Hämatologie, Munich, and °Institut für Immunologie, Johannes Gutenberg Universität, Mainz, Germany.
It has been reported that IgE receptor-mediated or Ca-ionophore-induced activation of mouse bone marrow-derived mast cells (BMMC) can result in the expression of a Th2-like cytokine pattern including the interleukins (IL) 3, 4, 5, 6, and 13 as well as GM-CSF and TNF-α. However, as demonstrated by Kölsch et al. and Kaspers et al. at this meeting, BMMC cultured in IL-3 plus IL-4 or IL-3 plus kit ligand are clearly able to produce also Th1-type cytokines (IFN-γ and IL-2) following activation. Glucocorticoids have been reported to suppress the production of Th1-type cytokines in T cells as well as TNF-α release and synthesis in activated BMMC. On the other hand, the expression of Th2-type cytokines by T lymphocytes was shown to be upregulated by glucocorticoids. For all-trans-retinoic acid similar effects have been demonstrated in activated CD4+ T lymphocytes and consequently a shift from a Th0/Th1- to a Th2-like pattern of cytokine expression has been observed in T cells treated with retinoic acid. In the present study we analyzed the effects of several glucocorticoids (dexamethasone, corticosterone, hydrocortisone) as well as the action of all-trans-retinoic acid on the cytokine secretion pattern of primary mouse BMMC activated by ionomycin or ionomycin plus IL-1. Here we show that glucocorticoids can suppress both the production of Th2-type cytokines (IL-4, IL-6, IL-9) and Th1-type cytokines (IFN-γ, IL-2) as well as IL-3 in activated BMMC. In contrast, all-trans-retinoic acid did not significantly modulate IL-4 production but also suppressed the generation of IL-3, IL-6 and IL-9 as well as the production of IL-2 and IFN-γ by BMMC activated by ionomycin or ionomycin plus IL-1. Under the conditions tested neither glucocorticoids nor retinoic acid did exert toxic effects on activated BMMC. In conclusion our results suggest that glucocorticoids and retinoic acid may be of potential therapeutic value in pathological conditions where overwhelming cytokine production by mast cells is an important pathogenetic feature, e.g. in inflammatory reactions such as atopic allergy.

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MAST CELLS INHIBIT KERATINOCYTE GROWTH. Maria Huttunen, Gunnar Nilsson*, Maija Horsmanheimo, Ilkka Harvima. Dept. of Dermatology, Univ. of Kuopio, Finland, Dept. of Pathology*, Univ. of Uppsala, Sweden.

We used HMC-1 leukemic mast cells, histamine, heparin and purified skin tryptase to investigate the effect of mast cells on human keratinocyte (KC) growth. HMC-1 cells were stimulated with 0.1 μM TPA and lysed by sonication to prepare HMC-1 extract. KCs were cultured in serum free medium using 24-well plates, and their growth response was measured with ^{3}H-thymidine incorporation.

Histamine inhibited thymidine incorporation dose-dependently reaching 29% and 89% inhibition at the concentration of 1 mM and 5 mM, respectively. Heparin showed maximal inhibition (26-32%) at the concentration of 200 ng/ml. Tryptase (0.0285 to 2.85 μg/ml) together with heparin (0.5 to 20 μg/ml) did not affect KC growth. Lysate from non-stimulated HMC-1 mast cells (1:1 corresponding to 50 000 cells/well, 1:3, 1:10, 1:30 and 1:100) inhibited thymidine incorporation in a dose-dependent fashion, and maximal inhibition (47%) was reached at 1:3 dilution. Furthermore, addition of lysate from TPA-stimulated HMC-1 cells to KC culture resulted in even higher inhibition (68%) at 1:1 dilution. These results suggest a controlling role for mast cells in keratinocyte proliferation.

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MURINE MAST CELL CHEMOATTRACTANT PRODUCED BY A KERATINOCYTE-DERIVED SQUAMOUS CELL CARCINOMA CELL LINE Yoshikazu Kameyoshi, Susumu Shinoda, Toshihiko Tanaka, Eishin Morita, Shoso Yamamoto.
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Accumulation of mast cells (MC) is often observed at the site of skin tumors. This suggests local production of factors which 1)attract MC from surrounding tissue, and/or 2)enhance growth of MC at the site. As to the latter, we have recently reported that a murine keratinocyte-derived squamous cell carcinoma cell line (KCMH-1) cells release a fibroblast-dependent mast cell growth enhancing factor. In this study, we examined whether KCMH-1 cells produce factors which attract MC. Migration of MC was evaluated by modified Boyden chamber method using a 48-well chemotaxis chamber. Conditioned medium of KCMH-1 induced migration of murine bone marrow-derived cultured MC in a dose dependent fashion. Migrated cell number at optimal concentration was similar to that induced by recombinant murine IL-3. It is consequently speculated that chemoattraction of MC by tumor-derived local factor is also an important mechanism for the accumulation of MC at the site of skin tumors, besides enhancement of growth of MC.

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IL-2 PRODUCTION IN ACTIVATED MOUSE BONE MARROW-DERIVED MAST CELLS IS UPREGULATED BY IL-1 AND SUPPRESSED BY IL-10. Uwe Kaspers, Reinhard Mailhammer, Cathy Kim°, Jesus Bujia°, Jean-Pierre Kremer, Edgar Schmitt", Monika Welle* and Lothar Hültner. GSF-Institut für Experimentelle Hämatologie, °HNO-Klinik, Universitätsklinikum Großhadern, Munich, "Institut für Immunologie, Johannes Gutenberg Universität, Mainz, and *Institut für Veterinärpathologie, Freie Universität Berlin, Germany.

It has been reported that IgE receptor-mediated or Ca-ionophore-induced activation of mouse bone marrow-derived mast cells (BMMC) can result in the production of different cytokines including the interleukins (IL) 3, 4, 5, 6, and 13 as well as GM-CSF and TNF-α. As yet, however, production of the Th1-type cytokine IL-2 by BMMC has not been documented at the protein level. Here we show that homogeneous populations of primary BMMC (*in vitro* age: 4 to 5 weeks; > 97% Alcian blue$^+$) were able to secrete low amounts of IL-2 upon activation with ionomycin. When in addition to the Ca-ionophore also human or murine recombinant IL-1 (α or ß) had been applied during the induction period, significantly higher amounts of IL-2 were detected in 24 or 48h supernatants. As compared to BMMC cultured in the presence of IL-3 alone, BMMC grown in IL-3 plus kit ligand (KL) displayed a morphologically more mature, connective tissue mast cell-like phenotype with a considerable number of safranin$^+$ and tryptase$^+$ mast cells and higher amounts of IL-2 secreted upon activation. IL-2 activities have been measured in a bio-assay with IL-2-dependent CTLL-2 cells and specified with a neutralizing dose of anti-mouse IL-2 mAb. In addition we have confirmed IL-2 production by activated mast cells at the protein level with a mouse IL-2-specific ELISA and at the mRNA level by RT-PCR as well as Northern blot analysis. When murine or human recombinant IL-10, cytokines known to indirectly inhibit the development and functional activity of Th1 cells, were added to ionomycin/IL-1-activated BMMC, IL-2 production was almost completely suppressed. Mast cells may provide a local source for IL-2 in the microenvironment of inflamed tissues and/or the draining lymph nodes, thereby stimulating the survival, proliferation and functional activity of T lymphocytes and NK cells.

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Cultivation of bone marrow-derived mast cells (BMMC) in the presence of IL-4 leads to the development of IFN-γ producing BMMC. S. Kölsch*, U. Kaspers#, S. Jin*, R. Mailhammer#, L. Hültner# and E. Schmitt*.
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The secretion of the Th1 cytokine IFN-γ by mast cells has not been documented at the protein level to date. Here we report that bone marrow-derived mast cells (BMMC) grown for four weeks in IL-3 (10 U/ml) and IL-4 (8 U/ml) secreted significant amounts of IFN-γ and IL-4 upon stimulation with ionomycin or FcεRI-crosslinking. In contrast, BMMC cultured in IL-3 alone failed to release IFN-γ, but produced IL-4. Because IL-4 and IFN-γ are antagonists concerning many immunologic functions, our data suggest that the IFN-γ production of mast cells generated in the presence of IL-4 may represent a negative feedback mechanism to neutralize the effects of IL-4.

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MUCOSAL MAST CELL ACTIVATION AND EARLY VASCULAR PERMEABILITY CHANGES IN A DTH REACTION IN THE RAT SMALL INTESTINE. Aletta D. Kraneveld, Dicky van Huven-Nolsen, Thea Muis, Andries Sj, Koster* and Frans P. Nijkamp. Department of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, P.O. Box 80082, 3508 TB Utrecht, The Netherlands

In this study, the role of mural mast cells (MMC) in the small intestinal DTH (delayed type of hypersensitivity) reaction was investigated. Male rats were skin sensitized with dinitrofluorobenzen (DNFB) and challenged intragastically with dinitrobenzene sulfonic acid (DNBS). Depletion (short- term dexamethasone treatment) and stabilization (doxantrazole treatment) of MMC before and at time of challenge were very effective in reducing edema formation, 48 hours after the challenge. This suggested that the MMC plays a role in initiating the DTH reaction. MMC activation and vascular leakage during the initiating phase of the DTH reaction (0-60 min after challenge) was investigated by measuring release of rat mast cell protease II (RMCP and Evans Blue tissue accumulation, respectively. MMC stabilization was effected by doxantrazole treatment. In addition, the influence of sensory nerves was studied by means of neonatal capsaicin-induced depletion of sensory neuropeptides. In the initiating phase, a significant increase in vascular permeability was found in DNFB-sensitized rats, associated with RMCP-II release. Doxantrazole treatment resulted in a significant reduction of vascular leakage and RMCP-II release. Neonatal capsaicin pretreatment abolished the DTH-induced early vascular response as well as MMC activation. The findings of this study are consistent with an important role of the MMC. In the initiating phase of the DNFB-induced DTH reaction in the small intestine of the rat. The results with neuropeptide depletion indicate that MMC activation early after the challenge is under sensory nervous control.

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ADHESION OF HUMAN MAST CELLS TO EXTRACELLULAR MATRIX PROTEINS. Sabine Krüger-Krasagakes, Andreas Grützkau, Razmik Baghramian, Beate M. Czarnetzki. Depatment of Dermatology, Virchow Klinikum, Humboldt Universität zu Berlin, Berlin, Germany.

Since mature mast cells reside within the connective tissue, interactions with extracellular matrix (ECM) proteins is of central importance for the migration, differentiation and localization of mast cells in tissues. We were thus interested in analyzing the expression of adhesion receptors on the human mast cell line HMC-1 and to study the ability of the cells to attach to ECM proteins. HMC-1 cells expressed various adhesion receptors for matrix proteins on their surface including β_1, α_2, α_3, α_4, α_5, α_6, $\alpha_v\beta_5$ integrins and CD44, but not $\alpha_v\beta_3$ and CD26 as could be shown by FACS analysis. In adhesion assays, we observed significant spontaneous binding of HMC-1 cells to fibronectin (FN), laminin (L), vitronectin (VN) and collagen I (Col), minimal binding to hyaluronic acid, but no binding to Col III and IV. Binding to FN and VN was dose-dependent and could be inhibited by anti-β_1 or anti-$\alpha_v\beta_5$ antibodies, RGD-containing peptides and divalent cation chelation. Adhesion to the other ECM proteins could not be inhibited by blocking antibodies directed to integrin chains. These findings indicate that the expression of adhesion receptors on human mast cells may be of general importance for the tissue localization of mast cells.

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EXPRESSION AND FUNCTIONAL ACTIVITY OF THE IL-8 RECEPTOR IN THE HUMAN MAST CELL LINE HMC-1. Undine Lippert, Metin Artuc, Andreas Grützkau, Dirk Schadendorf, Annelie Möller, Beate M. Czarnetzki, Sabine Krüger-Krasagakes.
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Mast cells have been shown to play a central role in diverse acute and chronic inflammatory diseases. In order to investigate mechanisms involved in mast cell accumulation at tissue sites, we have examined the potent neutrophil chemotactic factor IL-8 and its homologues MGSA and NAP-2 for their effects on mast cell migration, induction of calcium-flux and actin polymerization using the human mast cell line HMC-1. During chemotaxis assays, HMC-1 cells showed a significant chemotactic response towards all three chemokines, with maximal effects between 10^{-8} M and 10^{-9} M. Furthermore, we found an actin polymerization which peaked within 20 sec as measured by FACS, and a rapid increase of intracellular calcium flux up to 70 nM measured with FURA-2AM. In addition, HMC- I cells expressed mRNA for both types of the IL-8 receptor, as evidenced by PCR, and bound all three ligands, as shown by Scatchard-plot and competition experiments. By the use of specific monoclonal antibodies, we could show that HMC-1 cells express both types of the IL-8 receptor on their cell surface. These findings suggest that IL-8 might be an important factor for mast cell activation and migration of mast cells into inflamed tissues via specific IL-8 receptors on their cell surface.

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HISTAMINE RECEPTOR EXPRESSION BY THE HUMAN MAST CELL LINE HMC-1 AND THE HUMAN BASOPHILIC CELL LINE KU812. Undine Lippert, Sabine Krüger-Krasagakes, Reinhard Wanner, Andreas Grützkau, Beate M. Czarnetzki.
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In the past, basophils and possibly also mast cells have been shown to be influenced pharmacologically by histamine. H2 receptors and most recently also H3 receptors have been postulated to be involved. Furthermore, we have recently shown that an H1 receptor antagonist, loratadine, can inhibit cytokine release from human leukemic mast cells (HMC-1) and KU812 basophilic leukemic cells. In order to examine whether histamine receptors might be present on these cells and whether they might mediate these effects, we have investigated the expression of histamine H1 and H2 receptors on both cell lines by polymerase chain reaction. We found the transcripts of both receptors using specific H1 and H2 receptor primers. The specifity of the amplification products was verified by sequencing. These findings demonstrate for the first time the expression of both types of histamine receptors on the surface of these cell lines. The functional significance of these findings will have to be investigated in further studies.

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PEPTIDE TRANSLOCATION - AN INITIAL EVENT IN PEPTIDE-INDUCED MAST CELL ACTIVATION?
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If peptide-induced mast cell (MC) activation requires direct contact between peptide and G-protein, as proposed by Landry (1992), the peptides' ability to be translocated across the membrane should contribute to their histamine releasing activity. Therefore, we investigated the translocation of substance P(SP)-peptides across the membrane of MC using CLSM. Moreover, using RT-PCR we searched for tachikinin receptor mRNA. Our results show that (i)-SP (NK1) receptors are not expressed in rat peritoneal MC, supporting the view of a receptor-independent activation mechanism; (ii)-fluorescence labelled SP is imported into pertussis toxin treated MC and (iii)-intracellularly applied SP analogues induce degranulation of patch-clamped MC. Whether the translocation of peptide represents a prerequisit for peptide-induced MC activation remains to be clarified, because the ability of several peptides to perturb lipid membranes does not correlate with their MC activating potency.

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MAST CELLS LOCATED IN THREE COMPARTMENTS OF HUMAN PLACENTA. Danuta Maslinska, Maria Chciuk-Gornicka, Dariusz Szukiewicz.
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In the human term placenta following degranulation some of mast cells /MCs/ may be poorly stained with the routine histochemical methods. Therefore, in the present study MCs were detected by means of the specific biological markers / anti-tryptase, anti-chymase mAbs /. MCs in three different compartments of the placenta were found : 1/ tryptase-positive MCs /progenitors/ were found in the lumen of the placental blood vessels, 2/ in the tissue MCs were located in the vicinity of capillaries and blood vessels. These cells were Alcian blue stained and immunopositive with tryptase and chymase monoclonal antibodies. 3/ the same types of MCs were located within intravillous space.

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CAPSAICIN-INDUCED CUTANEOUS INFLAMMATION IN MAST CELL-DEFICIENT MICE: Marcus Maurer[1], Ralf Paus[1] and Stephen J. Galli[2]. [1]Department of Dermatology, Virchow-Klinikum, HU Berlin, 13344 Berlin, Germany, [2]Departments of Pathology, Beth Israel Hospital and Harvard Medical School, Boston, USA.

Activation of cutaneous sensory nerves by capsaicin (CPS) causes release of neuropeptides such as substance P and CGRP, as well as vasodilatation and increased vascular permeability. Since these neuropeptides are potent mast cell (MC)-secretagogues, we examined the possible involvement of MC in CPS-induced inflammation by applying CPS to the skin of genetically MC-deficient $WBB6F_1$-W/W^v or $WCB6F_1$-$S1/S1^d$ mice and the congenic normal +/+ mice. Topical treatment of +/+- mice ears with 0.1% CPS caused some degranulation of cutaneous MC 6h after treatment, as assessed by light microscopy. However, significant CPS-induced swelling occurred in the ears of both +/+ and congenic MC-deficient mice. While the magnitude of the swelling response was somewhat greater at early intervals after CPS in +/+ vs MC-deficient mice, our findings indicate that MCs are not required for the full expression of CPS-induced cutaneous inflammation.

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RECEPTOR TYROSINE KINASES EXPRESSED ON MOUSE BONE MARROW-DERIVED MAST CELLS. Eishin Morita, Susumu Shinoda, Toshihiko Tanaka, Shoso Yamamoto
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C-*kit* receptor is expressed on mast cells, and the activation of the c-*kit* signal transduction pathways play a key role in the development of mast cells. However, we have observed that activated NIH/3T3 fibroblasts support the survival of *W/W*^{v} mast cells, which lack the expression of functional c-*kit* receptor. On the other hand, receptor tyrosine kinases (RTKs) are a large family of proteins that play an important role in regulating the proliferation and differentiation of a variety of cells. In order to know the mechanism of fibroblast-dependent mast cell growth, we analyzed the RTKs expressed on mouse bone marrow-derived mast cells (BMMC) by using RT-PCR technique. We found that several RTKs, including c-*kit*, $PDGFR_\beta$, *MRK*, *TEK* and *RSE* are expressed on BMMC. This suggests that some growth factors apart from c-*kit* ligand regulate the growth of mast cells.

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SYNTHESIS OF SEROTONIN IN CULTURED NEONATAL RAT HEART CELLS: EVIDENCE FOR THE PRESENCE OF A HIGH NUMBER OF MAST CELLS. Rosemarie Morwinski, Gerd Wallukat, Uwe Karsten
Max Delbrück Centre, Berlin, Germany

Cell cultures prepared from neonatal rat heart ventricles include muscle and nonmuscle cells (the latter are usually specified as fibroblasts and endothelial cells).
Using a monoclonal antibody (F2) directed to surface determinants of rat connective tissue mast cells we found binding to about 30% of freshly isolated cells from neonatal heart ventricles. During cultivation these $F2^+$ cells appeared either large and flat with partly long processes or round to spindle-shaped, and they developed vesicles and granules of different size and number. They were often found in intimate contact with myocytes. We examined the presence of serotonin as a marker of mast cells in rodents by immunocytochemistry. In cultures with well developed muscle cells, serotonin was detected in rather small quantities in single greater granules of both flat and spindle-shaped assumed mast cells and their processes. However, in some cultures a great number of mast cells packed with small granules were stained with the antiserotonin antibody. Interestingly, in these cultures destruction of muscle cells was often observed. Our results unequivocally demonstrate the presence of a high number of mast cells in rat heart cell cultures, and we believe that similar processes could occur in vivo and participate in some heart diseases.

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ANALYSIS OF HMC-1 MASTCELL-LINE DERIVED EOSINOPHIL ATTRACTANTS. Norio Noso, Sabine Krüger-Krasagakes, Beate-M. Czarnetzki, Jens-M. Schröder.
Dpt. of Dermatology, University of Kiel and UKR Berlin, Germany

Skin mastcells are believed to actively participate in dermal eosinophil (Eo) accumulation often seen in allergic inflammation. In order to investigate which Eo-attractants are released by human mastcells we cultured the human mastcell-line HMC-1 and investigated Eo-chemotactic activity in supernatants of these cells after stimulation with Ca-ionophore A 23187 together with phorbol-myristate-acetate. As a result we detected Eo-chemotactic activity. For further characterization supernatants were separated by heparin-sepharose HPLC, followed by reversed phase HPLC of heparin-binding and non-binding proteins. Interestingly we did not find any heparin-binding Eo-chemotactic activity such as RANTES, MCP-3 or MIP-1α, which indicates that HMC-1 cells do not release Eo-chemotactic chemokines under these conditions although masses of neutrophil-chemotactic IL-8 could be detected. Instead strong Eo-chemotactic activity was seen in the non-heparin binding fractions. Purification of this activity to apparent homogeneity lead to a single 11 kD protein, which however rather may represent a co-purifying contaminant. Further experiments will show, whether this activity is identical with GMCSF. In conclusion HMC-1 cells produce upon stimulation a single Eo-chemotaxin, which needs to be further characterized and most likely may represent GMCSF.

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HUMAN MAST CELLS MODULATE PROLIFERATION AND CYTOKINE PRODUCTION BY $CD8^+$ T CELLS. F.L. de Pater-Huijsen, M.J. de Riemer, R.M.R. Reijneke, M. Pompen, R. Lutter, H.M. Jansen and T.A. Out. Dpt. of Pulmonology and Clinical Immunology Laboratory, Academic Medical Center, Amsterdam, and Laboratory for Exp. and Clin. Immunol., University of Amsterdam, Amsterdam, the Netherlands.

In patients with allergic asthma, both mast cells and T cells are believed to be involved in the immunological processes in the lungs. To obtain information on the interactions between mast cells and $CD8^+$ T lymphocytes we investigated the modulatory capacity of irradiated mast cells (human mast cell line HMC-1; Dr. J.H. Butterfield) on $CD8^+$ T cell proliferation and cytokine production *in vitro*. We performed our study using a highly purified polyclonal $CD8^+$ T cell population (over 99% $CD8^+$ T cells) and $CD8^+$ T cell clones (from peripheral blood from healthy subjects). The proliferation of a freshly established polyclonal $CD8^+$ T cell line was specifically decreased by mast cells (44%). IFN-γ production, however, was specifically increased (10-fold at 1 day), whereas no IL-4 and IL-5 could be detected. One $CD8^+$ T-cell clone, typical of the Th0 phenotype, responded to stimulated HMC-1 by a decreased proliferation and an increase in the ratio IFN-γ : IL-4 production. IL-5 production was markedly (43%) decreased. These results show that mast cells are able to modulate $CD8^+$ T cell responses *in vitro*.

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c-kit expression during postnatal hair follicle development and cycling. R. Paus, B. Handjiski, S. Eichmüller, B. M. Czarnetzki, M. Maurer.
Dept. of Dermatology, Virchow Hosp., Humboldt-Universität, D-13353 Berlin

It is widely held that the skin expression of c-kit (=SCF receptor) is restricted to melanocytes (MelC) and mast cells. However, several non-lymphoid, human normal and neo-plastic tissues also show c-kit expression, including breast epithelium and not clearly identified basal layer epidermal cells (*Cancer Res* 52:6139 + 54:3049, *Arch Dermatol Res* 424:135). Therefore, cutaneous c-kit expression may be less restricted than thought. Here, we report the distribution of c-kit immunoreactivity (ir) during the morphogenesis of Y-population hair follicles in neonatal mice, and during the adolescent murine hair cycle (C57BL/6). Already during the earliest stage of hair germ formation, strong c-kit ir was seen in the epithelium. During outer root sheath (ORS) and hair bulb morphogenesis, individually located, apparently epithelial c-kit+ cells appeared here and in the epidermal basal layer (in mature truncal mouse skin, all MelC are confined to the hair follicle). During the adolescent hair cycle, c-kit ir changed strikingly: in resting (telogen) follicles with absence of histochemically visible MelC, clusters of 1-5 c-kit+ cells were seen in the most distal telogen hair germ, just above the dermal papilla. During the construction of a new, melanogenically active hair bulb (anagen), individual c-kit+ cells were noted in ORS and hair matrix. By anagen VI, several dozen c-kit+ cells were labeled in the most distal hair matrix, sharply demarcated from the more proximal, accepted location of follicle MelC. We are currently studying whether these c-kit+ cells are truly epithelial in nature, as their phenotyp and location suggests, or whether they are non-melanogenically active MelC-related cells. Since we never saw an intraepithelial mast cell by histochemistry, the c-kit ir cells described above may represent epithelial stem cells (or their direct progeny), or the long elusive MelC precursors from which mature MelC are regenerated during each new hair cycle.

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HISTAMINE IS RELEASED IN THE WHEAL BUT NOT THE FLARE FOLLOWING CHALLENGE OF HUMAN SKIN IN VIVO: A MICRODIALYSIS STUDY. **Lars J. Petersen, Martin K. Church, and Per S. Skov. Department of Dermatology, Bispebjerg University Hospital, the Reference Laboratory, Copenhagen, Denmark, and Immunopharmacology Group, Southampton General Hospital, Southampton, U.K.**

The mediator mechanisms of the cutaneous flare response are controversial. The flare is neurogenic in origin, but do the neuropeptides released from the nerve endings cause the vasodilatation directly or do they induce the further release of mast cell histamine? We have addressed this question by inserting thin microdialysis fibers into the dermis within the areas of the wheal and flare to monitor changes in histamine levels provoked by intradermal injections of histamine, allergen, codeine or substance P. The histamine concentration in unprovoked skin was 10-11 nM. All stimuli released large amounts of histamine (15.8 to 38.3 pmol/20 min) at the injection site. Diffusion of histamine within the wheal was poor, levels at 2.3 mm and 3.7 mm being 6-29% and 0-6% respectively of those 1 mm from the injection site. No elevations of histamine levels were detected in the flare with any stimuli. These findings support the theory that the flare reaction does not involve histamine release from skin mast cells.

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SUBSTANCE P AND CAPSAICIN CAUSE HISTAMINE RELEASE FROM MURINE SKIN MAST CELLS *IN VITRO*. C. Pfrommer, M. Maurer, T. Zuberbier, T. Heinzelmann, S. Eichmüller, B. M. Czarnetzki, and R. Paus. Department of Dermatology, Virchow-Klinikum, HU Berlin, D-13344 Germany.

Increasing evidence suggests the regulation of skin-mast cell (MC) activation by substance P (SP), a neuropeptide released from sensory nerve endings. Pharmacological induction of SP-secretion from nerves by capsaicin (CPS) is a widely used model in the characterization of neurogenic inflammation. Here we examined the potential histamine-releasing effect of SP or CPS on enzymatically dispersed MC derived from the telogen back skin of 6-8 weeks old, female C57BL/6 mice. Supernatant and pellet histamine content was measured by EIA, and histamine release, following stimulation with anti IgE (2000 I.U./ml), SP (10^{-5}M-10^{-13}M), CPS (10^{-5}M-10^{-13}M), or vehicle as control (phosphate-buffer), was calculated as percentage of total histamine after correction for spontaneous release. Most notably, stimulation with SP in micro- to subnanomolar concentrations resulted in a highly significant release of histamine ranging from 40-60% of total histamine as compared to controls (20%), and was comparable to values observed with anti-IgE. These data support the concept of neuropeptide-mediated activation of murine skin MC by products released from sensory nerves, and that SP-induced histamine release is receptor-mediated rather than due to non-specific membrane effects. Surprisingly, a significant release of histamine in a dose-dependent manner was also observed after stimulation with CPS. Maximal stimulation occurred with CPS-concentrations of 10^{-9}M to 10^{-11}M, pointing towards the intriguing possibility of vanilloid receptor-mediated mechanisms in the stimulation of histamine release from murine skin MC.

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AIRWAY EPITHELIAL CELL PRODUCTS INFLUENCE PROLIFERATION AND DIFFERENTIATION OF A HUMAN MAST CELL LINE.

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Introduction: In the airways of asthmatic patients high numbers of activated mast cells are present nearby the epithelium. Epithelial cells are able to secrete mediators like cytokines. The effects these mediators may have on mast cells and their functions are unknown. We studied the effects of epithelial products on proliferation and differentiation of a human immature mast cell line, HMC-1 (Dr. J.H. Butterfield, Mayo Clinic, Rochester, USA).

Results: Medium conditioned for 48 hours by a human airway epithelial cell line (H292) in the absence of fetal calf serum (FCS) increased the proliferation of HMC-1 with a 130% after five days of exposure to conditioned medium. In contrast, conditioned medium obtained in the presence of 10% FCS resulted in almost no increase or even a slight decrease, whereas the tryptase content, as a measure of differentiation, increased 5 to 7-fold after 15 days of exposure. The effects excerted by conditioned epithelial medium could be due to kit ligand, which is produced by H292 cells at conditions studied here (shown by ELISA). However, addition of rh-kit ligand to HMC-1 can not explain the observed effects on proliferation, so other mediators may be involved too.

Conclusion: Conditioned medium derived from airway epithelial cells alters proliferation and differentiation of the human immature mast cell line, HMC-1.

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MAST CELLS AND EXPERIMENTAL AMYLOIDOSIS. Pawel Poppe Jaroslaw Kalbarczyk, Elzbieta Wojtecka-Lukasik, Danuta Maslinska.
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We have previously observed , that distribution of the mast cells in organs of hamsters is altered in the course of experimental amyloidosis .
In our recent research the histamine H1 and H2 receptor blockers were used and the plasma histamine levels as well as distribution of the mast cells were examined under the same experimental conditions.
Amyloidosis in hamsters was induced by casein.In liver , spleen , kidneys and pancreas amyloid deposits were detected with Congo red staining. Histamine serum blood level measured spectrofluorometrically was decreased to about 5 % of the control level in the group of animals treated with with H1 receptor blocking agent-cetirizine Only in animals receiving H1 and H2 receptor blockers but not in the intact or control animals / with amyloidosis / the penetration of the mast cells to the examined organs was observed . The relationship between mast cells , connective tissue cells and histamine is discussed .

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ACTIN-DEPOLYMERIZING TOXINS AFFECT PROTEIN TYROSINE PHOSPHORYLATION AND SECRETION IN RBL CELLS

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We studied the influence of the actin-depolymerizing toxins *C. botulinum* C2 and *C.difficile* ToxB on protein tyrosine phosphorylation (Tyr-P) and subsequent serotonin release induced by DNP-BSA and calcium ionophore A23187.
C2 toxin, which ADP-ribosylates actin (Aktories, Nature 322: 390, 1986), reduced DNP-BSA-stimulated Tyr-P of several proteins in a concentration- and time-dependent manner. ToxB, which glucosylates small GTP-binding proteins of the Rho family (Just, Nature, 1995, in press), inhibited A23187-induced Tyr-P of 70-75 and 110-130 kDa proteins. After antigen-stimulation, Tyr-P of an 110 kDa protein was strongly decreased by ToxB.
DNP-BSA- and A23187-mediated [^{3}H]serotonin release increased about 4-fold by C2 toxin. ToxB treatment inhibited DNP-BSA- and A23187-induced secretion by about 90% and 50%, respectively.
The data indicate different functions of the actin cytoskeleton in parallel signal-secretion-coupling pathways of RBL cells and suggest a crucial role of small GTP-binding proteins in these events.

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MAST CELLS AND NEUROTROPHIC FACTORS: Wendy Purcell, Claire Westgate, Chris Atterwill
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Rat brain (thalamic) mast cells (RBMC), isolated using collagenase digestion, and rat peritoneal mast cells (RPMC) were exposed to neurotrophic factors (NTFs). NGF induced histamine release (HR) from RBMC and RPMC in a concentration dependent manner (15 and 70% at EC50s of 0.01 and 0.5 µg/ml respectively). However, while RPMC were refractory to the effects of BDNF and CNTF, both these NTFs induced HR from RBMC: BDNF and CNTF induced 10% HR from RBMC with EC50s of 2.2 and 10 ng/ml, respectively. RPMC possess high affinity receptors (TrkA) for NGF. Our results indicate additional functional receptors on RBMC for BDNF (TrkB) and CNTF (CNTF receptor complex). This heterogeneity likely reflects functional differences in the mast cell subsets. NTFs as therapeutics in both peripheral neuropathies and neurodegenerative diseases induce inflammation and hyperalgesia, apparent upon dosing with NTFs in animal and clinical studies. This may reflect the ability of NTFs to activate mast cells to secrete vasoactive, chemotactic and hyperalgesic mediators. Since levels of NTFs increase in various autoimmune and neuroinflammatory diseases; mast cell interaction with NTFs may orchestrate active pathological episodes.

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Influence of autonomic and sensory neuromediators on cerebrovascular mast cells. Reynier-Rebuffel A-M (1), Dimitriadou V (1)Seylaz J (1) and Aubineau P(2).
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We have shown that autonomic and sensory nerves make close contacts with adventitial mast cells (MC) of meningeal arteries. In vitro assays of agonists and antagonists of these nerves gave the following results: MC exocytosis and 5-HT release was induced by -(1) acetylcholine (blocked by atropine),-(2) SP and CGRP (SP+CGRP can act synergistically) -(3) capsaicine-released endogenous SP and CGRP.

On the contrary, noradrenaline (NA) that had by itself no effect on MC exocytosis and 5-HT release, inhibited the exocytosis induced by acetylcholine or SP+CGRP through beta-1 adrenergic receptors.

Unbalance between sympathetic and parasympathetic or sensory nerves could lead to mast cell degranulation and inflammatory events such as vascular headache, in which autonomic dysfunction is likely to be involved.

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MAST CELLS AND THE ANGIOGENIC RESPONSE INDUCED BY PYOGENIC GRANULOMA

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The association of mast cells (MC) with neovascularization was found in both chronic inflammatory diseases and neoplasms. In this study ten samples of pyogenic granuloma (PG), a neoplastic disorder in pediatric dermatology, characterized by a tumultuous angiogenesis, and ten samples of normal skin were grafted onto the chick embryo chorioallantoic membrane (CAM), a useful in vivo model to study angiogenesis, with the aim to investigate their possible vasoproliferative activity. The angiogenic response was assessed on histologic sections by a morphometric method, four days after grafting. The vascular counts in the area underlying the PG was four times higher than those of normal skin and higher number of mucosal MC was detected in the CAM's intermediate mesenchyme of the pathological samples in comparison to controls. The role played in angiogenic response by the inflammatory cells, mainly MC, forming the perilesional infiltrate was supported by this study.

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MOUSE MAST CELLS GROWN IN STEM CELL FACTOR (SCF) EXPRESS TYPE II PITUITARY ADENYLATE CYCLASE POLYPEPTIDE (PACAP) RECEPTORS Anjona Schmidt-Choudhury, E.J. Goetzl, M.Xia, S.P.Sreedharan, G.T.Furuta, S.J. Galli, W.E. Schmidt, B.K. Wershil.

Depts. of Pediatrics and Medicine, Univ.of Kiel, Pediatric GI & Nutrition and Dept. of Pathology, Harvard Medical School, Boston, MA, Dept.of Medicine, USCF, CA

We have shown that the neuropeptides PACAP or vasoactive intestinal polypeptide (VIP) induce mediator release from mouse mast cells (MCs) grown in SCF (Gastroenterol.106: A664,1994).Two types of high affinity receptors for PACAP have been characterized:Type I receptors interacting with PACAP only and type II receptors interacting with either VIP or PACAP. In this study, we determined wether the reactivity of MCs grown in SCF to PACAP or VIP might reflect the expression of PACAP receptors. MCs grown in SCF for 6-8 weeks expressed an increased amount of the VIP receptor (Type II PACAP receptor) as determined by Western Blot. Using RT-PCR with oligonucleotides specific for mouse type I PACAP receptors, mouse brain, but not MCs grown in SCF, was found to express mRNA for the Type I receptor. These data demonstrate that SCF enhances the expression of the Type II PACAP/VIP receptors on mouse MCs in vitro and suggest a mechanism whereby endogenous SCF may regulate MC function *in vivo*.

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METHACHOLINE INDUCES WHEAL AND FLARE REACTIONS IN HUMAN SKIN BUT DOES NOT RELEASE HISTAMINE AS ASSESSED BY SKIN MICRODIALYSIS TECHNIQUE. Lars J. Petersen and Per S. Skov. Department of Dermatology, Bispebjerg University Hospital, and the Reference Laboratory, Copenhagen, Denmark.

Investigations have indicated that cholinergic agonists release histamine from isolated mast cells. The purpose of this study was to investigate if the cutaneous wheal and flare reaction induced by methacholine challenge in human skin involves histamine release as measured by skin microdialysis technique. Five hollow dialysis fibres were inserted intradermally in forearm skin in eight subjects. Each fibre was perfused with Krebs' Ringer (3.0 µl/min). Samples were collected in 2-min fractions before skin challenge and for 20 min after intradermal injection of methacholine 10^{-3} to 10^{-1} M, the vehicle, and a positive control, codeine 0.3 mg/ml. Histamine was assayed spectrofluorometrically. Methacholine caused a significant dose-related wheal and flare reaction, the flare to methacholine 10^{-1} M being comparable with that seen with codeine 0.3 mg/ml. Methacholine did not cause any significant histamine release, the release induced by methacholine 10^{-1} M being 0.1±0.3 pmol/20 min. Histamine release by codeine was 26.3±4.1 pmol/20 min. Thus, methacholine-induced wheal and flare reactions in human skin appeared not to involve histamine release.

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LDTI, A TIGHT-BINDING INHIBITOR OF HUMAN MAST CELL TRYPTASE. C.P. Sommerhoff, C. Söllner, G.P.Piechottka, G. Matschiner, and H. Fritz.

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In contrast to most other serine proteinases human mast cell tryptase (EC 3.4.21.59) is not inhibited by any proteinaceous inhibitor known so far. We have now isolated and characterized an inhibitor of human tryptase from the medical leech *Hirudo medicinalis*. Amino acid sequencing of LDTI (Leech-derived Tryptase Inhibitor; 46 amino acids, M_r 4738) revealed a high degree of similarity to the non-classical Kazal-type inhibitors bdellin B-3 and rhodniin. LDTI is a tight-binding (K_i ~1.4 nM) and specific inhibitor of human tryptase; it inhibits only pancreatic trypsin and chymotrypsin with similar affinities. LDTI effectively inhibits the tryptase-induced cleavage of biologically relevant substrates (e.g. vasoactive intestinal peptide, peptide histidine-methionine, and kininogen). Similarly, LDTI blocks direct cellular effects of tryptase, e.g. the mitogenic activity (IC_{50} ~1 nM). LDTI appears useful as a prototype of human tryptase-inhibitors, and as a pharmacological tool for the investigation of the (patho)physiologic role of tryptase in health and disease.

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OVINE CUTANEOUS RESPONSES TO INTRADERMALLY INJECTED SUBSTANCE P (SP), COMPOUND 48/80 (48/80) AND CALCIUM IONOPHORE (A23187). Gordon Sture*, John Huntley#, Anne MacKellar#, Hugh Miller*.

* Royal (Dick)School of Veterinary Studies, Edinburgh, UK. # Moredun Research Institute, Edinburgh, UK.

Dose response and time course studies were performed with SP, 48/80 and A23187 on the flank skin of 12 Blackface ewes. SP and A23187, but not 48/80, evoked a statistically significant weal response in ovine skin ($P<0.05$; Mann-Whitney U test). However, all three agents evoked statistically significant extensive mast cell degranulation and dermal neutrophil influx by six hours post-injection ($P<0.05$). In time course studies, mast cell degranulation was first demonstrated at the time of the maximal weal response (SP 15 min, A23187 60 min; $P<0.05$). Given the limitations of assessing direct secretagogue activity histologically, this preliminary study has identified SP, 48/80 and A23187 as putative ovine cutaneous mast cell secretagogues. Further studies using these agents and lymphatic cannulation techniques will thus allow the direct effect of mast cell activation on dermal cell trafficking to be investigated.

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SHEEP MAST CELL PROTEINASE (SMCP), A CHYMASE PRESENT IN OVINE DERMAL MAST CELLS, EVOKES DERMAL NEUTROPHIL INFLUX IN THE HOMOLOGOUS HOST. Gordon Sture*, John Huntley#, Alan Pemberton*, Anne MacKellar#, Hugh Miller*.

* Royal (Dick)School of Veterinary Studies, Edinburgh, UK. # Moredun Research Institute, Edinburgh, UK.

Dose response and time course studies were performed using SMCP on the flank skin of four Blackface ewes. In comparison to diluent controls, the intradermal injection of 36µg, 3.6µg, 360ng, and 36ng SMCP evoked a dose-dependant effect on both weal volume and on dermal neutrophil influx. In time course studies using 3.6µg SMCP the peak weal volume (median 68.9mm^3, range 20.2-95.1mm^3) occurred at 180 minutes post-injection ($P<0.03$; Mann-Whitney U test). Statistically significant dermal neutrophil influx was demonstrated histologically at 360 minutes post-injection ([$P<0.05$]; 3.6µg SMCP:median 816.9 neutrophils/mm^2 skin, range 461.5-1014.2 neuts./mm^2 skin; diluent control:median 100.8 neuts./mm^2 skin, range 50.2-186.4 neuts./mm^2 skin). This suggests that SMCP, released following dermal mast cell activation, may directly affect ovine dermal cell trafficking *in vivo*.

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INHIBITION OF TRYPTASE BY BENZAMIDINES. Jörg Stürzebecher Peter Wikström*, Helmut Vieweg*, Christian P. Sommerhoff+. Zentrum f. Vask. Biol. & Med., Universität Jena, Erfurt, Germany; *Pentapharm AG, Basel, Switzerland; +Abtg. Klin. Chem. & Klin. Biochem., Universität München, Germany.

Besides guanidino compounds and amines structurally related to Arg and Lys, compounds with other cationic groups are synthetic inhibitors of trypsin-like serine proteinases. We studied systematically the structure-activity relationships for inhibition of trypsin-like enzymes by benzamidine derivatives. These studies were extended to tryptase. For inhibition studies we used tryptase isolated from human lung tissue and the chromogenic substrate Tos-Gly-Pro-Arg-pNA.

In our initial screening we found only medium inhibitors of tryptase among simple benzamidines and bis-benzamidines exerting K_i-values in the micromolar range. Now, novel inhibitors containing 3-amidinophenylalanine [(3-Am)Phe] as key building block are presented. Two types of inhibitors were synthesized: Nα-arylsulfonylated piperazides of (3-Am)Phe and bis-benzamidines containing two (3-Am)Phe moieties. Some of the newly designed compounds are potent inhibitors of tryptase with K_i-values up to the 10^{-8} mol/l range. Therefore, (3-Am)Phe and other benzamidine-containing amino acids appeared to be promising lead structures for new tryptase inhibitors.

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HISTAMINE FROM PLACENTAL MAST CELLS AND THE RELEASE OF LABOUR. Dariusz Szukiewicz, Danuta Maśliński, Elżbieta Wojtecka-Łukasik, Jerzy Stelmachów.
Dept. of Pathophysiology, Medical Academy, Warsaw, Poland

Placentas obtained from 22 women whose full-term pregnancies differed in the method of delivery (spontaneous labour, elective cesarean section) were divided into two equal groups. All placentas were perfused with modified Krebs-Ringer-phosphate solution buffered to pH 7,4. The immuno-cytochemical method revealed mast cells in all placentas. Five specimens from each of the placentas were excised: 2 from the region contiguous to fetal surface of the placenta and 3 from the region contiguous to maternal surface. After homogenization histamine and 5-HT concentration were determined fluorometrically. Histamine levels in placental cuts obtained from maternal surface of the placenta were significantly higher in the case of delivery without contractile activity of the uterus (elective cesarean section). It is postulated that the simultaneous release of mast cell mediators (especially histamine) in placental tissue could be an important factor for evoking contractile activity of the human uterus, initiating labour.

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ENHANCEMENT OF FIBROBLAST-DEPENDENT MAST CELL GROWTH BY A FACTOR RELEASED FROM SQUAMOUS CELL CARCINOMA CELL LINE CELLS. Toshihiko Tanaka, Eishin Morita, Yoshikazu Kameyoshi, Shoso Yamamoto
Department of Dermatology, Hiroshima University School of Medicine, Hiroshima, Japan

Increased number of mast cells are often observed at the site of skin tumors. To investigate tumor-associated mast cell growth, we established a murine keratinocyte-derived squamous cell carcinoma cell line (KCMH-1), and found that the presence of KCMH-1 conditioned medium in the culture of bone marrow-derived mast cells (BMMC) enhanced fibroblast-dependent mast cell growth in mice. The factor inducing this enhancement was different from already known mast cell growth factors in mice. In addition, BMMC obtained from W/W^v mice could survive on NIH/3T3 fibroblast monolayer in the presence of the KCMH-1 conditioned medium. Although SCF is thought to play a key role in fibroblast-dependent mast cell growth, the results suggest the existence of novel pathway different from c-*kit*-SCF interaction in mast cell growth.

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HUMAN LEUKEMIC (HMC-1) AND NORMAL SKIN MAST CELLS EXPRESS β_2-INTEGRINS. Sylvia Weber, Magda Babina, Gabriele Feller, Beate M. Czarnetzki.
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Mast cells are resident cells of diverse tissues and are usually not found in the peripheral blood, although they derive from circulating progenitor cells. The mechanisms of migration and tissue distribution as well as the mechanism of mast cell interaction with other inflammatory cells are unclear so far. Possibly, β_2-integrins play an important role in these processes. In the present investigation, we have therefore studied the expression of the β_2-integrins CD11a (LFA-1), CD11b (complement receptor 3), CD11c (p150,95), CD18 (β_2-chain) and their counterreceptor CD54 (ICAM-1) on HMC-1 cells (subclone 5C6) by flow cytometry, and on human mast cells of normal skin (tissue sections) by an APAAP/toluidin-blue double staining technique. HMC-1 cells clearly expressed CD11a, CD18 and CD54, whereas the expression of CD11b and CD11c was close to the negative control. PMA induced an up-regulation of CD11a, CD11b, CD11c, CD18 and CD54. The effect of PMA showed a strong dose-response dependency. When investigating the time course of PMA-mediated up-regulation, a shedding of CD11a, CD18 and CD54 was observed. The presence of CD11a, CD11b, CD11c, CD18 and CD54 on individual skin mast cells could be confirmed by immunohistochemistry. In conclusion, mast cell β_2-integrins play possibly a more important role during interaction with other inflammatory cells than has hitherto been suspected.

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HUMAN HMC-1 MAST CELLS EXCLUSIVELY EXPRESS THE FcγRII SUBTYPE OF IgG RECEPTOR. Bettina Wedi, Alexander Kapp.
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Recent experiments in rodent mast cells indicated an important role of the Fcγ receptor-family for signal transduction through tyrosine phosphorylation that may result in cell activation via release of mediator(s) or cytokine(s). Thus we performed a flow cytometric analysis to study the profile of Fc receptors for IgG on the human mast cell line HMC-1. It was shown that HMC-1 mast cells did not express FcγRI (CD64) nor FcγRIII (CD16) on their surface. However, HMC-1 reacted with a mAb specific for the low-affinity FcγRII (CD32). This exclusive expression of the FcγRII subtype of IgG receptor is similar to basophils, although concerning cell surface molecules HMC-1 rather seem to resemble monocytes. Since in monocytes IL-4 was shown to downregulate all FcγR subtypes while PMA upregulated FcγRII-mRNA in further experiments the modulation of FcγR was studied. However, in this study 24 hour incubation of HMC-1 with IL-4 (500 U/ml), PMA (50 ng/ml) or A23187 (20 µM) did not change the profile or level of FcγR expression. In vivo, human uterine mast cells have been described to express FcγRII. Thus it remains to be clarified whether this low affinity receptor subtype for IgG is involved in antigen-dependent sensitization of mast cells.

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REGULATION OF MAST CELL DIFFERENTIATION BY FIBROBLAST AND KERATINOCYTE DERIVED GROWTH FACTORS. Pia Welker, Jürgen Grabbe, Beate M. Czarnetzki.
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In previous studies, we have shown that supernatants from the murine fibroblast L-cell-line (LCS) and the human keratinocyte cell line HaCaT (HKS) can upregulate mast cell characteristics. In order to identify specific mast cell growth, we analysed LCS and HKS for such factors by ELISA and/or Western-Blotting. Furthermore, the SCF-unresponsive human mast cell line HMC-1 was cultured for 10 days with horse serum (HS) alone or supplemented with LCS, NGF, TGF-β, GM-CSF, bFGF or PDGF. Tryptase activity was measured spectrophotometrically, histamine content by fluorescence-spectrophotometry and expression of FcεRIα by immunohistochemistry. HKS was found to contain NGF, TGF-β and GM-CSF and LCS NGF and TGF-β, but no GM-CSF. In HMC-1 cultures, no significant effects were observed with bFGF and PDGF, and TGF-β caused only a minor increase of FcεRIα expression. Cultures with GM-CSF resulted in downregulation of all mast cell markers studied. In contrast, NGF markedly increased tryptase activity, expression of FcεRIα and histamine content. These effects were paralleled by changes in the expression of FcεRIα and mast cell tryptase specific mRNA, as detected by semiquantitative PCR. Therefore, NGF may partially be responsible for the mast cell differentiating activity released both by fibroblasts and keratinocytes, whereas GM-CSF might play a role as suppressive regulator of mast cell maturation.

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T1, A NOVEL MAST CELL RECEPTOR, IS A POSSIBLE MODULATOR IN METASTATIC TUMOR GROWTH. Elisabeth Thomassen, Lothar Hültner and Anne Katrin Werenskiold
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An involvement of mast cells in tumorigenesis has been suggested both from the frequent observation of mast cells in tumor tissue and the observation of specific cellular interaction of mast cells with tumor cells *in vitro*. We have identified a novel mast cell-specific receptor, T1, which is structurally related to type I IL-1 receptor. Expression of the T1 receptor is restricted to primary and factor dependent continously growing bone marrow mast cells whereas it is absent from autonomous malignant lines. Invasively growing mammary and bone tumors secrete an extracellular soluble variant of the T1 receptor representing its isolated ligand-binding domain. This protein is deposited in the tumor extracellular matrix. These findings suggest, that soluble T1 expression by invasively growing tumors may represent a signal for accumulation of mast cells in the tumor microenvironment. The suspected T1/T1-ligand dependent interaction of mast cells and tumor cells is currently analysed. Metastatic and non-metastatic tumor cellines have been genetically engineered to express either increased or lower amounts of the T1 protein. Interaction of these cellines with mast cells is investigated both *in vitro* and *in vivo*.

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Investigation of the C5a receptor (C5aR) on the human mast cell line HMC-1 and on human skin mast cells (MC).

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The expression of the C5aR (CD88) on MC was studied with four novel anti-C5aR monoclonal antibodies (mab) directed to the N-terminal domain of the receptor. All mab bound to HMC-1 cells. The binding could be blocked by rC5a and by peptide EX-1 representing amino residues 1-31 on the N-terminal domain of the C5aR. C5aR-specific mRNA was detected in HMC-1 cells by RT-PCR. C5a but not C5a desArg led to a transient mobilization of intracellular calcium in HMC-1 which could be inhibited by preincubation of C5a with a neoepitope-specific anti-C5a mab. Anti-C5aR mab selectively stained c-kitR+ cells on normal human skin on sequential 2 μm sections which were metachromic after toluidine blue staining. The binding was inhibitable by peptide EX-1. A similar expression of C5aR epitopes was found on MC in psoriatic plaques while C5aR were not detectable in wheal and flare reactions.

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ROLE OF THE ACTIN CYTOSKELETON IN HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS.

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In suspended mast cells, histamine release induced by antigen, PMA and compound 48/80 was inhibited by *C.botulinum* C2 toxin, which depolymerizes actin by ADP-ribosylation. In contrast, A23187-induced secretion was not affected by toxin treatment. C2-induced inhibition was paralleled by depolymerization of F-actin. Attachment of mast cells to plastic or glass reduced secretion and increased submembranous F-actin. Under these conditions, C2 toxin increased mediator-induced secretion. *C.difficile* toxin B, which inactivates F-actin-regulating small GTP-binding proteins of the Rho family, inhibited antigen-compound 48/80-, PMA- and A23187-induced histamine release in suspended and adherent mast cells. The data indicate diverse functions of the actin cytoskeleton and a crucial role of Rho protein in stimulus-secretion-coupling in mast cells.

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CORRELATION OF MAST AND INFLAMMATORY CELLS IN SUBCUTANEOUS WORM NODULES OF ONCHOCERCIASIS PATIENTS.

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Nodules from patients with onchocerciasis were stained immunohistologically using antibodies against human mast cell (mc) tryptase and markers for eosinophils, neutrophils and macrophages to elucidate the localization and frequency of mc. They appeared predominantly singly or in accumulations perivascularly and in mixed inflammatory infiltrates between the adult worms and in the capsular area. Scattered mc were found in fibrous zones. They were increased with stronger infiltration and related to the inflammatory cells. No mc were detected in the pulpy cystic parts, dominated by neutrophils and macrophages. Mc were never localized directly at the adult worms or at microfilariae. A correlation of mc distribution to the occurrence of eosinophils was observed regarding higher numbers of mc and eosinophils in the capsular area of nodules with microfilariae producing females. Nodules with male worms only revealed more mc than those with non-producing females, although both contained very few eosinophils. Around dead worms numerous giant cells and few eosinophils but remarkably more mc were observed than around live filariae. In conclusion, the frequency of mc depends on the distribution of effector cells as well as the productivity and vitality of the worms.

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EFFECTS OF T HELPER 2-TYPE CYTOKINES, IL-3, IL-4, IL-5, AND IL-6 ON THE SURVIVAL OF CULTURED HUMAN MAST CELLS.

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We examined the effects of several cytokines on the survival of human mast cells of almost 100% purity, generated from umbilical cord blood cells in the presence of SCF and IL-6. Mast cells died after the withdrawal of SCF and IL-6 showing apoptotic changes. Treatment of mast cells with each of SCF, IL-3, IL-4, IL-5, and IL-6 prolonged their survival dose-dependently. On the other hand, IL-2, IL-9, IL-10, IL-11, TNF-α, TGF-β1, and NGF, showed no effect. PCR amplification of IL-3R, IL-4R, IL-5R and IL-6R yielded products of the correct size predicted from the sequence of each normal receptor. These findings suggest that IL-3, IL-4, IL-5, and IL-6, which are mainly produced by Th2 lymphocytes, might activate human mast cells *in vivo* via specific receptors in allergic reactions.

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ULTRASTRUCTURE OF SKIN MAST CELLS IN MASTOCYTOSIS.

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The aim of our study was to describe the ultrastructural features of skin mast cells in cutaneous and systemic mastocytosis and compare them with skin mast cells from healthy subjects. Biopsies from patients with mastocytosis were taken from lesional skin. The mast cells from healthy skin contained predominantly scroll-poor secretory granules, which were rich in amorphous material and "cores" of considerable electron density, gratings and/or lattice-like structures. We observed single mitochondria, poorly developed Golgi apparatus and tubules of endoplasmic reticulum. These mast cells did not show evidence of degranulation. Mastocytosis mast cells presented a vast diversity of secretory granules morphology. We observed numerous mitochondria and well developed tubules of endoplasmic reticulum as well as long, frequently interdigitated cytoplasmic projections. Secretory granules were very often encountered in the extracellular space. Ultrastructural analysis of skin mast cells in mastocytosis, especially in the systemic type, indicated that these cells were in a state of activation.

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ALTERATIONS IN ADHESION MOLECULE EXPRESSION IN CHRONIC AND DERMOGRAPHIC URTICARIA.

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In both chronic and dermographic urticaria, superficial perivascular leukocytic infiltrations are seen histologically in lesional skin. We have therefore investigated the role of endothelial adhesion molecule expression in the disease process. E-selectin, P-selectin, ICAM-1 and VCAM-1 expression was examined immunohistologically, and the serum levels of the soluble forms of these adhesion molecules were determined by EIA in patients with chronic urticaria (n=8), dermographic urticaria (n=5) as well as in healthy controls (n=8) and subjects with symptomatic allergic rhinitis (n=7) for comparison. Compared to controls and rhinitis subjects, a marked increase was observed for soluble P-selectin in dermographic urticaria (median 380 ng/ml) and chronic urticaria (median 497 ng/ml) compared to healthy controls (median 130 ng/ml) and rhinitis subjects (median 139 ng/ml). In contrast, the other adhesion molecules were not significantly elevated in both urticaria groups (median serum levels in dermographic urticaria vs healthy controls: sE-selectin 30 vs. 25 ng/ml, sICAM-1 359 vs. 299 ng/ml, sVCAM-1 780 vs. 663 ng/ml). Immunohistologically, a strong expression of P-selectin was found in superficial vessels of lesional and nonlesional skin in dermographic urticaria in contrast to the other adhesion molecules studied, supporting the findings for the soluble forms. As an alteration of sP-selectin was not seen in symptomatic allergic rhinitis, an unspecific effect due to inflammation appears to be unlikely. These results may therefore point to a pathologically relevant role of endothelial P-selectin expression in the disease.