

# International Mast Cell & Basophil Meeting 2010

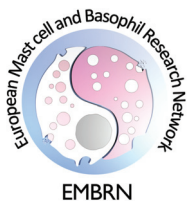
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*Scientific Programme*

*General Information*

*Book of Abstracts*



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## **WELCOME**

Dear friends and colleagues,

On behalf of the local organizing committee, the scientific committee and the program committee I welcome you to MCBM 2010, the International Mast Cell and Basophil Meeting. This Meeting is co-hosted and coorganized by the European Mast Cell and Basophil Research Network (EMBRN), by the Arbeitsgemeinschaft Mastzellen und Basophile (AGMZB), which is the Mast Cell and Basophil Section of the Society of Experimental Dermatology in German speaking countries (ADF, Arbeitsgemeinschaft Dermatologische Forschung) and of the German Society of Allergy and Clinical Immunology (DGAKI, Deutsche Gesellschaft für Allergologie und Klinische Immunologie), and the DFG-funded Priority Program on Mast Cell Research, the SPP 1394 "Mast cells – Promoters of health and modulators of disease").

We are very happy that so many of you came to join us to discuss the biology and the role of mast cells and basophils in health and disease. We are looking forward to 46 exciting lectures including four keynote and workshop lectures as well as posterwalks and roundtable discussions. Please contribute to making this meeting successful and fun with your questions, your contributions to the discussion and with sharing your knowledge and expertise and interest in mast cells and basophils.

Welcome again and may your time here include many learning, networking and enjoyable moments!

For the organizing committee, the scientific committee and the programme committee

Marcus Maurer

## Local Organizing Committee

**Prof. Dr. med. Marcus Maurer**

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## Scientific Committee

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Allergie-Centrum-Charité  
Charité - Universitätsmedizin Berlin

**Prof. Dr. med. Tilo Biedermann**

Universitätshautklinik Tübingen  
Eberhard-Karls-Universität Tübingen

**Prof. Dr. med. Stephan Bischoff**

Institut für Ernährungsmedizin  
Universität Hohenheim

**PD Dr. med. Knut Brockow**

Klinik und Poliklinik für Dermatologie und Allergologie am Biederstein  
Technische Universität München

## **Programme Committee**

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Allergie-Centrum-Charité  
Charité - Universitätsmedizin Berlin

**Dr. med. Frank Siebenhaar**

Allergie-Centrum-Charité  
Charité - Universitätsmedizin Berlin

## **Conference venue**

**Lecture Hall of the Department of Dermatology and Allergy**

(Hoffmann-Hörsaal)  
Charité - Universitätsmedizin Berlin  
Luisenstraße 2  
10117 Berlin

## **Evening Programme**

**Clärchens Ballhaus**

Auguststrasse 24  
10117 Berlin  
Tel: 030-30642268/69  
[www.ballhaus.de](http://www.ballhaus.de)

# Agenda

## Thursday, 9 December 2010

8.00 Registration

8.30 – 9.00 Welcome

### **Session I – Mast cell and basophil interaction with other cells**

Chairs: Ulrich Blank (France), Martin K. Church (United Kingdom / Germany)

9.00 – 9.07 Mast cell derived APRIL supports survival B lymphocytes  
*Ulrich Beuscher (Germany)*

9.10 – 9.17 Turnover of peripheral mast cells is extremely slow in the steady state but new precursors can be recruited from the bone marrow under inflammatory conditions  
*Anke Petzold (Germany)*

9.20 - 9.27 Ultrastructural evidence for human mast cell-eosinophil interactions *in vitro*  
*Yael Minai-Fleminger (Israel)*

9.30 – 9.37 Mast cell-derived Amphiregulin enables regulatory T-cells to suppress local inflammation  
*Dietmar Zaiss (Netherlands)*

9.40 – 9.47 Conditional knock-out of SH2 domain containing Phosphatase 2 (SHP2) in mast cells reveals a key role for SHP2 in mast cell development  
*Namit Sharma\* (Canada)*

9.50 – 9.57 Mast cells boost neutrophil effector functions  
*Anastasija Michel (Germany)*

### **Session II – Mast cell and basophil activation**

Chairs: Knut Brockow (Germany), Francesca Levi-Schaffer (Israel)

10.00 – 10.07 IgE immune complexes stimulate mast cell progenitor recruitment in a mouse model of allergic airway inflammation  
*Joakim Dahlin (Sweden)*

10.10 – 10.17 CD84 negatively regulates IgE high affinity receptor signalling in human mast cells  
*Damiana Alvarez-Errico (Spain)*

\* Recipient of the 2010 EMBRN Travel Award

- 10.20 – 10.27                      The dominant inhibitory effects of low-affinity IgG receptors in human and murine basophils  
*Friederike Jönsson (France)*
- 10.30 – 10.37                      Immunoglobulin free light chains are potential therapeutic targets in tumor-associated inflammation in mice and humans  
*Tom Groot-Kormelink (Netherlands)*
- 10.40 – 10.47                      Novel  $\alpha,\beta$ -unsaturated lactones inhibit mast cell activation Induced by intracellular calcium increase  
*Alicia Penissi\* (Argentina)*
- 10.50 – 10.57                      Lactobacillus induced attenuation of mast cell degranulation is associated with inhibition of K<sup>+</sup> channel (KCa3.1) current  
*Paul Forsythe (Canada)*
- 11.00                                      *Coffee break*

### **Keynote lecture I**

Chairs: Introduction by Gianni Marone (Italy), Gunnar Pejler (Sweden)

- 11.30 – 12.30                      Linking signaling and cell behavior to the clinical allergic response: a numbers game  
*Donald W. MacGlashan (USA)*

### **Session III – Mast cell and basophil mediators**

Chairs: Tilo Biedermann (Germany), Gunnar Nilsson (Sweden)

- 12.30 – 12.37                      Mast cell activation causes protein kinase C-dependent upregulation of the nuclear receptor 4a family of transcription factors  
*Anders Lundequist (Sweden)*
- 12.40 – 12.47                      Degradation of interleukin-13 by mast cell serine proteases  
*Iulia Karlsson (Sweden)*
- 12.50 – 12.57                      Human intestinal mast cells are a potent source of multiple Chemokines  
*Katrin Feuser (Germany)*

\* Recipient of the 2010 EMBRN Travel Award

- 13.00 – 13.07            Activation of basophils by anti-immunoglobulin E or auto-antibodies in serum from chronic spontaneous urticaria patients induces tumor necrosis factor alpha release  
*Sidsel Falkencrone (Denmark)*
- 13.10 – 13.17            The histamine H4 receptor as a novel drug target in inflammatory conditions  
*Ekaterini Tiligada (Greece)*
- 13.20 – 13.27            Basophils as source of hepatocyte growth factor (HGF) in chronic myeloid leukemia: possible implications for disease acceleration  
*Sabine Cerny-Reiterer (Austria)*
- 13.30                      *Lunch*  
*Press Conference*

### **Keynote lecture II**

Chairs: Introduction by Marcus Maurer (Germany), Bernd Echtenacher (Germany)

- 14.30                      Uncovering new roles for mast cells and basophils in allergy and autoimmunity  
*Juan Rivera (USA)*

### **Workshop - Methods in mast cell and basophil biology**

Chairs: Stephan Bischoff (Germany), Clemens Dahinden (Switzerland)

- 15.00                      How to study mast cell receptor signal transduction  
*Petr Draber (Czech Republic)*
- 15.20                      How to coculture and investigate the interaction of mast cells and other cells  
*Carlo Pucillo (Italy)*
- 15.40                      How to make use of mouse models for elucidating mast cell functions  
*Martin Metz (Germany)*
- 16.00                      How to obtain and work with human mast cells  
*Peter Bradding (United Kingdom)*

16.20 Questions and answers

16.30 *Coffee break*

### ***Postersession***

17.00 - 19.00 Posterpresentations and Posterwalks

### ***General Assemblies***

18.00 – 18.15 AGMZB

18.30 – 19.30 Kompetenznetzwerk Mastozytose

20.00 *Dinner & Dance*

### **Friday, 10 December 2010**

7.00 – 8.00 SPP breakfast

8.15 Welcome

### ***Session IV – Mast cell and basophil biology***

Chairs: Karin Hartmann (Germany), Petri Kovanen (Finland)

8.20 - 8.27 PEST Domain-Enriched Tyrosine Phosphatase (PEP) positively regulates mast cell antigen-mediated signalling and anaphylaxis  
*David Obiri (Germany)*

8.30 – 8.37 Evidence for a hyper-responsive mast cell phenotype  
*Madeleine Radinger (Sweden)*

8.40 – 8.47 Mast cells are critical for the limitation of thrombin-induced inflammation  
*Cathleen Sünder (Germany)*

8.50 – 8.57 Rab GTPases and mast cell exocytosis  
*Ronit Sagi-Eisenberg (Israel)*

9.00 – 9.07 IL-33 is a product rather than an initiator of acute IgE-mediated responses in humans  
*Michaela Fux (Switzerland)*

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- 9.10 – 9.17 Mast cell functions revisited in Cre recombinase-mediated mast cell eradication (Cre-Master) mice  
*Thorsten Feyerabend (Germany)*
- 9.20 – 9.27 Munc18-2 controls granule translocation in mast cells through dynamic interactions with the fusion machinery and the microtubule cytoskeleton  
*Ulrich Blank (France)*
- 9.30 9.37 Mast cells in human thyroid cancer  
*Maria Rosaria Galdiero (Italy)*
- 9.40 – 9.47 Importin beta plays an essential role in the regulation of the LysRS-Ap4A pathway: Ag-dependent nuclear translocation of Ap4A hydrolase  
*Irit Carmi-Levy (France)*
- 9.50 – 9.57 Insulin like growth factor-1 and insulin increase Toll like receptor-induced Tumor necrosis factor- $\alpha$  and Interleukin-6 but reduce Interleukin-1 $\beta$  production in mast cells by activating the Phosphatidylinositol 3-Kinase pathway  
*Thomas Hochdörfer (Germany)*
- 10.00 – 10.30 Histamine and its Receptors: Celebrating a 100 Year Journey  
*Martin K. Church (United Kingdom)*
- 10.30 Coffee break

### **Session V – Mast cell and basophil driven diseases**

Chairs: Axel Lorentz (Germany), Peter Valent (Austria)

- 11.00 – 11.07 Co-factor dependent anaphylaxis driven by innate immune signals is mediated by basophils  
*Florian Wölbing (Germany)*
- 11.10 – 11.17 Mast cells foster adenocarcinoma development while contrasting anaplastic variants in prostate carcinogenesis  
*Paola Pittoni (Italy)*
- 11.20 – 11.27 BAT-on-a-chip: Optimisation of conditions for the use of a cell line in a live basophil allergen array  
*Franco Falcone (United Kingdom)*
- 11.30 – 11.37 Chronic Idiopathic Urticaria – clinical experience in a research clinic setting at Royal Adelaide Hospital  
*Robert Heddle (Australia)*
- 11.40 – 11.47 Clinical presentation of clonal and non-clonal Systemic Mast Cell Activation Disorders  
*Luis Escribano (Spain)*

11.50 - 11.57                      Pediatric mastocytosis is a clonal disease associated with c-kit extracellular domain mutations that have different functional and signalling properties compared with kit-phosphotransferase domain mutations  
*Patrice Dubreuil (France)*

12.00                                      Lunch  
EMBRN Council Assembly

### **Keynote lecture III**

Chairs: Introduction by Franco Falcone (United Kingdom), Frank Siebenhaar (Germany)

13.00                                      Mechanistic insights gained from the analysis of FcERI signaling modulation of basophils during clinical studies  
*Wayne Shreffler (USA)*

### **Session VI – Mast cell and basophils: clinical implications**

Chairs: Ilkka Harvima (Finland), Julia Trosien (Germany)

13.30 – 13.37                      Novel molecular and therapeutic concepts in mast cell disorders  
*Peter Valent (Austria)*

13.40 – 13.47                      Phosphoregulation in Janus kinase/STAT-pathway of human Thelper2-lymphocytes and HumanMastCell-1-line – application in the histamine liberation test  
*Friedhelm Diel (Germany)*

13.50 – 13.57                      The antimicrobial activity of mast cells against staphylococcus aureus and pathogen countermeasures  
*Eva Medina (Germany)*

14.00 – 14.07                      Infections affect mast cell reactivity – possible implications for asthma exacerbations  
*Rohit Saluja (Sweden)*

**Round table      *Academia-Industry interaction:  
What we need and what we get***

Moderator: Marcus Maurer (Germany)

14.15 – 15.15

Academia  
Gianni Marone (Italy)  
Stephan Bischoff (Germany)

Industry  
Paola Lovato, Leo Pharma (Denmark)  
Robert Maykut, Novartis (Switzerland)  
Gary Jennings, Jado Technologies (Germany)  
Ursula Sonnenschein, Bühlmann Laboratories AG  
(Switzerland)

15.15

*Closing remarks*

15.30 - 16.30

EMBRN General Assembly

## Order of the abstracts according to the poster list

### Activated Human Lung Mast Cells Modulate Airway Smooth Muscle Cell Proliferation in Asthma

Hatem Alkhouri<sup>a</sup>, Fay Hollins<sup>b</sup>, Lyn M Moir<sup>c</sup>, Christopher E Brightling<sup>b</sup>, Carol L Armour<sup>a</sup>, J Margaret Hughes<sup>a</sup>

Respiratory Research Group, <sup>a</sup> Faculty of Pharmacy and <sup>c</sup> Woolcock Institute of Medical Research, The University of Sydney, NSW, Australia. <sup>b</sup> Department of Respiratory Medicine, University Hospitals of Leicester, Leicester, UK.  
Hatem.Alkhouri@Sydney.edu.au

Background: Activated mast cell (MC) densities are increased on the airway smooth muscle (ASM) in asthma. There they may modulate ASM functions leading to airway remodelling. MC mediators released simultaneously often have opposing effects on ASM function. However, their combined effects on ASM function are unknown.

Aim: To determine the combined effects of human lung MC products released at different times after activation on the proliferation of ASM cells from donors with and without asthma.

Methods: MC freshly isolated from human lung were stimulated with IgE/anti-IgE. The culture supernatants (SN) were collected after 2 and 24h and then the MC lysed. The SN/lysates were added at different concentrations to serum deprived, sub-confluent human ASM cells treated with IL-4R alpha antibody or prostanooids and thromboxanes receptor antagonists or left unstimulated. DNA synthesis was then quantified by a 5h [<sup>3</sup>H]-thymidine pulse and the effects on proliferation confirmed by cell counting after 3 and 5 days. Intracellular signalling was investigated 15 and 120 minutes after the MC 24h SN were added using phospho-protein arrays. Results: MC 2h SN and lysates had no significant effects on asthmatic and non-asthmatic ASM cell DNA synthesis. Only the MC 24h SN reduced DNA synthesis in asthmatic, but not non-asthmatic, ASM cells. As well, the 24h SN significantly reduced asthmatic ASM cell numbers over 5 days. The 24h SN also inhibited the sustained activation of ERK1 and 2 and Akt1, 2 and 3 at 120 minutes. However, prostaglandins, thromboxanes, IL-4 and IL-13 were not involved in the inhibitory effects of the MC 24h SN.

Conclusions: Surprisingly, MC newly synthesized products released by 24h differentially modulated the proliferation asthmatic and non-asthmatic ASM cells. They prevented the sustained activation of pro-proliferative signalling pathways in the asthmatic ASM cells. Thus MC not directly in contact with ASM cells may counteract ASM cell proliferative responses to mitogens in their immediate vicinity in asthma.

Funded by: NHMRC Australia, H Alkhouri is supported by an NHMRC Biomedical PhD Scholarship, and CE Brightling by a UK DoH Clinician Scientist Award and Wellcome Senior Fellowship.

### Mast cells contribute to the immune response against murine cytomegalovirus

Marc Becker<sup>\*</sup>, Stefan Ebert<sup>†</sup>, Pamela Friedrich<sup>\*</sup>, Rafaela Holtappels<sup>‡</sup>, Matthias Reddehase<sup>‡</sup>, Christian Taube<sup>‡</sup>, and Michael Stassen<sup>\*</sup>

<sup>\*</sup> Institute for Immunology

<sup>‡</sup> Dept. of Pulmonary Medicine, III. Medical Clinic

<sup>†</sup> Institute for Virology

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Mast cells are important effector cells in bacterial or parasitic infections, but information about the involvement of mast cells in antiviral immunity is sparse.

In this project we investigate the role of mast cells during acute infection with murine cytomegalovirus (mCMV) using mast cell-deficient Kit<sup>W-sh/W-sh</sup> mice and their wildtype littermates.

After systemic infection with mCMV, viral load in different organs was determined. Especially in the lungs, mast cell-deficient Kit<sup>W-sh/W-sh</sup> mice developed higher virus titres compared to wildtype mice. This implies impaired viral clearance in the absence of mast cells, which can be restored after reconstitution of mast cell-deficient mice with BMDC. Most likely, this phenomenon is due to lower numbers of virus-specific CD8<sup>+</sup> T cells found in the lungs of Kit<sup>W-sh/W-sh</sup> mice.

These findings suggest an important function for mast cells in antiviral immunity against mCMV, although the precise role of mast cells in the immune response is currently unknown.

### Probiotics (Ecologic 825) modulate mast cell degranulation and reduce stress-induced barrier dysfunction *in vitro*

Anders H. Carlsson<sup>1</sup>, Femke Lutgendorff<sup>1,2</sup>, Louis M.A. Akkermans<sup>2</sup>, Derek M McKay<sup>3</sup>, Johan D Söderholm<sup>1</sup>

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<sup>2</sup>Gastrointestinal Research Unit, Department of Surgery, University Medical Center, Utrecht, The Netherlands.

<sup>3</sup>Gastrointestinal Research Group, Department of Physiology & Pharmacology, The Calvin, Phoebe and Joan Snyder Institute of Infection, Inflammation and Immunology, University of Calgary, Calgary, Canada

Mail to presenting author: anders.h.carlsson@liu.se

Background: Stress has deleterious effects on intestinal barrier function, and stressful life events may contribute to the course of inflammatory bowel disease. Mast cells play a pivotal role in pathogenesis of stress-induced barrier dysfunction due to the release of barrier disruptive content. Data from recent animal studies in our laboratory suggested that mast cells may also contribute to the barrier protective properties of probiotics (Ecologic 825; mixture of 10 strains, lactobacilli and bifidobacteria), possibly via the release of 15d-PG<sub>2</sub> and enhanced PPAR-γ activity.

Aim: Here, by a reductionist *in vitro* approach, we further elucidated whether probiotics can modulate mast cell mediator release, and by which mechanisms this may result in amelioration of stress-induced barrier dysfunction.

Methods: Confluent monolayers of the human colon-derived T84 epithelial cell line were co-cultured with rat basophilic leukemia (RBL)-2H3 mast cells and pretreated with probiotics (125x10<sup>4</sup> CFU/ml), one hour before addition of 100nM CRH to activate mast cells. Mast cell release of β-hexosaminidase, TNF-α and 15d-PG<sub>2</sub> was determined. Transepithelial resistance (TER), and permeability to microspheres were measured. To determine the dependence of PPAR-γ, the specific PPAR-γ antagonist T0070907 was used.

Results: CRH-induced activation of mast cells resulted in decreased TERs and increased permeability to microspheres in the T84 monolayers. Both pretreatment with probiotics and filter-sterilized probiotic supernatant resulted in lower levels of mast cell-released β-hexosaminidase and TNF-α, but increased release of 15d-PG<sub>2</sub>. Probiotic pretreatment of mast cells ameliorated CRH-induced epithelial barrier dysfunction in monolayers, but this effect was limited when mast cells were incubated with T0070907. However, when the T84 monolayers were pretreated with probiotics before being exposed to conditioned medium of CRH-activated mast cells no protective effect was seen.

Conclusion: Probiotics modulate mast cell mediator release to a more barrier protective profile, resulting in amelioration of stress-induced epithelial barrier dysfunction via PPAR-γ dependent pathways.

### Mast cell derived APRIL supports survival B lymphocytes

Jasmin Baumgartl, Nicole Hannemann, Christian Bogdan, H. Ulrich Beuscher

Institut for Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen, Wasserturmstrasse 3, 91054 Erlangen, Germany.

Mast cells (MCs) have been implicated in regulating B-cell growth through the release of cytokines including interleukin (IL)-4 or CD40 ligand. This study investigated the ability of MCs to produce the B-cell survival factors, B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL). The results showed that murine bone marrow derived MCs (BMMCs) stimulated with phorbol myristic acid (PMA) in combination with ionomycin released a B cell survival activity, which, however, was not detectable in supernatants (SN) of BMMCs prepared from APRIL deficient mice. In addition, the B cell survival activity in SN was dose dependently blocked in the presence of soluble neutralizing receptors for APRIL (TACI-Ig) but not for BAFF (BAFF-R-Ig), indicating that BMMCs are a cellular source for APRIL. Moreover, APRIL activity was not detectable after stimulating BMMCs with IgE/DNP or after stimulation with either PMA or ionomycin alone, indicating that APRIL is not released upon degranulation, but requires an additional stimulus for synthesis and /or processing. Finally, because no APRIL activity was found in SN of PMA/ionomycin-stimulated bone marrow derived dendritic cells, it is proposed that BMMCs not only release APRIL, but also provide the appropriate milieu to form bioactive APRIL, i.e. ligand oligomerization via BMMC-derived proteoglycans. The results offer the intriguing possibility that mast cell activation feeds

back to sustained antibody production in infection or even in autoimmune diseases such as rheumatoid arthritis.

### **Mast cells promote the lymph node hypertrophy and joint inflammation in type II collagen induced arthritis**

Anne Dudeck<sup>1</sup>, Anke Petzold<sup>1</sup>, Julia Scholten<sup>2</sup>, Katrin Peschke<sup>1</sup>, Jan Dudeck<sup>1</sup>, Ari Waisman<sup>2</sup> and Axel Roers<sup>3</sup>

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<sup>3</sup> Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany

Mast cells (MCs) play a critical role in innate immunity and in the induction of adaptive immune responses. Increasing evidence suggests that MCs also have an important impact on the pathogenesis of autoimmune diseases including rheumatoid arthritis (RA). Several studies using the K/BxN serum transfer model of antibody-induced arthritis have shown that mast cells are critically involved in the effector phase of arthritis characterized by a severe joint inflammation. However, the role of mast cells in the initiation phase of arthritis remains to be evaluated. Here, we used a new Cre/loxP-based model of inducible mast cell deficiency to study the impact of mast cells on the initiation of type II collagen induced arthritis (CIA). Immunization of mice with type II collagen/CFA resulted in a lymph node hypertrophy reflecting the massive expansion of B lymphocytes. Interestingly, this B cell expansion and lymph node hypertrophy was markedly diminished in the absence of mast cells. Moreover, the antigen-specific release of IFN- $\gamma$  and IL-17 by lymph node cells of immunized mice was significantly reduced in mast cell-depleted animals compared to mast cell-proficient littermates suggesting an impact of mast cells on the polarization of T cells towards Th1 and TH17 subtype. Finally, the reduced immune response was reflected by a significant alleviation of the type II collagen-induced joint inflammation in mast cell depleted mice in comparison to mast cell proficient littermate controls.

### **Mast cell dependent priming of Th2 responses during intestinal helminth infection**

Matthew Hepworth<sup>1</sup>, Emilia Danilowicz<sup>1</sup>, Martin Metz<sup>2</sup>, Richard Lucius<sup>1</sup>, Marcus Maurer<sup>2</sup> and Susanne Hartmann<sup>1</sup>

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<sup>2</sup> Allergie Centrum Charite, Charite-Universitätsmedizin Berlin, Germany.

Signals derived from infected tissues are vital in determining optimal expansion and programming of the adaptive immune response and expansion of innate effector cell types. In the mucosal tissues several key cytokines have been identified (IL-25, IL-33 and TSLP) which modulate tissue residing antigen presenting cells in order to stimulate a Th2 response, however relatively little is known about which other cell types may contribute essential signals in the initial stages of the response. Mast cells are potentially important in the early stages of infection as they are distributed throughout mucosal tissues and can respond quickly via the release of preformed granules containing inflammatory mediators. Using mast cell deficient mouse models we observed a profound deficiency in the priming of Th2 immune responses following infection with the intestinal helminths *Heligmosomoides polygyrus* and *Trichuris muris*. Production of IL-25, IL-33 and TSLP in the gut tissues was also dramatically reduced in the first week of infection in mice lacking mast cells suggesting an important upstream role for these cells in early tissue derived events. Subsequently this resulted in a reduction in IL-25 dependent Nuocyte expansion in the draining lymph node. Both Th2 and Nuocyte responses could be restored in mast cell deficient mice if recombinant IL-25 was applied during the first week of infection or by replenishment with WT bone marrow. Thus, we identify an essential role for mucosal tissue mast cells in determining the onset and priming of Th2 immunity.

### **Fibroblast-induced mast cell differentiation promoted by Kit-dependent and Kit-independent pathways requires direct adhesion via VCAM-1 and $\alpha_4\beta_7$ integrin**

Mandy Leist<sup>1</sup>, Cathleen Sünder<sup>1</sup>, Martin Metz<sup>1</sup>, Anne Dudeck<sup>2\*</sup> and Marcus Maurer<sup>1</sup>

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The mechanisms of mast cell (MC) homeostasis in peripheral tissues are largely unknown and may involve proliferation, apoptosis, migration, and differentiation of MC precursors. Bone marrow derived cultured MCs (BMCMCs) exhibit increased proliferation and a phenotypical change towards the connective tissue type MCs (CTMCs) when cocultured with fibroblasts (Fbs). Aim of our study is to evaluate the influence of Fbs on MC differentiation. Since BMCMCs exhibited adhesion to *Swiss albino 3T3* Fbs, we analysed the regulation of proliferation and differentiation of BMCMCs with focus on the impact of this directed adhesion. Surprisingly, the proliferation of BMCMCs was markedly increased only if MCs underwent direct cell-to-cell contact to Fbs. Furthermore, the increase in histamine content and mast cell protease 4 (MCPT4) mRNA expression, i.e. indicators of differentiation towards CTMCs, in BMCMCs was dependent on direct adhesion as well. Most notably, MCs deficient for the SCF-receptor Kit, also showed a marked, albeit lesser increase in proliferation, histamine content and MCPT4 expression when cocultured with Fbs. These findings suggest an SCF/Kit-independent pathway for the modulation of MC biology by Fbs. However, Kit-deficient BMCMCs showed no differences concerning their adhesion to Fbs as compared to wildtype BMCMCs. Furthermore, we found that an interaction of Vascular Cell Adhesion Molecule 1 (VCAM-1) expressed by Fbs and its ligand  $\alpha_4\beta_7$  integrin on BMCMCs is largely responsible for the adhesion. Moreover, we could show that the VCAM-1/  $\alpha_4\beta_7$  itself does not induce BMCMC differentiation. Thus, our data show that BMCMC proliferation and differentiation towards CTMCs induced by Fbs is dependent on cell adhesion, in part induced by VCAM-1 /  $\alpha_4\beta_7$  interaction, but mediated by at least two separate pathways, one Kit-dependent and the other Kit-independent. The identification of membrane bound receptors other than Kit that induce MC proliferation and differentiation may provide interesting therapeutic targets for MC-driven diseases.

### **Mast cells boost neutrophil effector functions**

Anastasija Michel<sup>1\*</sup>, Fatma Doener<sup>2\*</sup>, Sebastian Reuter<sup>3</sup>, Marc Becker<sup>4</sup>, Pamela Friedrich<sup>5</sup>, Stefan Tenzer<sup>6</sup>, Matthias Klein<sup>7</sup>, Tobias Bopp<sup>8</sup>, Edgar Schmitt<sup>9</sup>, Hansjörg Schild<sup>10</sup>, Markus P. Radsak<sup>11</sup>, Christian Taube<sup>12</sup>, and Michael Stassen<sup>13</sup>

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Mast cells are able to trigger life-saving immune responses in murine models for acute inflammation. In such settings, several lines of evidence indicate that the rapid recruitment of neutrophils initiated by the release of mast cell-derived pro-inflammatory mediators is a key element of innate immunity. Herein, we investigate the impact of mast cells on critical parameters of neutrophil effector function. In the presence of activated murine bone marrow-derived mast cells, neutrophils freshly isolated from bone marrow rapidly lose expression of CD62L and upregulate CD11b, the latter being partly driven by mast cell-derived TNF and GM-CSF. Mast cells also strongly enhance neutrophil phagocytosis and generation of reactive oxygen species. All these phenomena partly depend on mast cell-derived TNF and, to a greater extent, on GM-CSF. Furthermore, spontaneous apoptosis of neutrophils is greatly diminished due to the ability of mast cells to deliver antiapoptotic GM-CSF. Finally, we show in a murine model for acute lung inflammation that neutrophil phagocytosis is impaired in mast cell-deficient *Kit<sup>W-sh</sup> / Kit<sup>W-sh</sup>* mice but can be restored upon mast cell engraftment. Thus, a previously unrecognized feature of mast cells is their ability to boost neutrophil effector functions in immune responses.

### **Ultrastructural Evidence For Human Mast Cell-Eosinophil Interactions *In Vitro*.**

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Background: The two main effector cells of allergic inflammation are mast cells and eosinophils, which co-exist in the inflamed tissue during the late and chronic reactions, forming an "Allergic Effector Unit". Eosinophil peroxidase (EPO) is an abundant leukocyte basic protein secreted into the extracellular space after degranulation by activated eosinophils. This mediator contributes to the cytotoxic potential of the eosinophil and causes tissue injury during allergy. Previous work has found that EPO has the potential to influence mast cell function.

In this study, we have investigated the existence of short-term (60 min) *in vitro* interactions between human peripheral blood eosinophils and cord-blood-derived mast cells by transmission electron microscopy (TEM), and examined the influence of these interactions on the levels of EPO released by eosinophils.

#### Methods:

Cell-cell interactions, as well as EPO translocation, were detected by TEM following 60 minute incubation of peripheral blood eosinophils and mature cord blood mast cells in co-culture and mono-culture at 2:1 ratio.

EPO release was determined by a colorimetric assay following 45 minute incubation of peripheral blood eosinophils and mature cord blood mast cells in co-culture and mono-culture at either 10:1, 5:1, 2:1, or 1:1 ratios.

Results: We have found that mast cells and eosinophils adhere to each other; the lipid body content and the granule morphology of co-cultured mast cells and eosinophils, respectively, are altered, and the level of EPO released by co-cultured eosinophils is elevated. Moreover, we found that EPO was transferred from eosinophils to mast cells in co-culture.

Conclusions: Our results thus indicate that, when co-cultured, mast cells and eosinophils show signs of physical contact and activation. This is the first *in vitro* evidence of functional physical interactions between human mast cells and eosinophils, interactions that might also occur *in vivo* during allergic diseases.

### Turnover of peripheral mast cells is extremely slow in the steady state but new precursors can be recruited from the bone marrow under inflammatory conditions.

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Mast cells are tissue resident hematopoietic cells that are particularly frequent in tissues lining inner and outer body surfaces. The dynamics of peripheral mast cell populations are incompletely understood. We developed a novel genetic model that allows induced depletion of mature connective tissue mast cells in adult mice resulting in a reduction of skin mast cells to few percent of normal numbers. Unexpectedly, the numbers of cutaneous mast cells were still low 12 months after induced depletion. Moreover, in congenic bone marrow chimeras, donor derived mast cells were hardly detectable in the skin 14 weeks months after bone marrow transplantation. These findings indicate that release of mast cell precursors from the bone marrow occurs at a very low rate or not at all in the adult animal in the absence of inflammatory stimuli. Mast cell numbers rapidly recovered in mast cell-depleted mice upon induction of irritant dermatitis. Skin inflammation induced a rapid accumulation of mast cells that were primarily derived from non-bone marrow sources in mast cell-competent mice. In contrast, irritant-induced mast cell hyperplasia was primarily due to recruitment of new mast cell precursors from the bone marrow in mast cell-depleted animals. Collectively, our findings show that only under conditions of inflammation, skin mast cell numbers are regulated by a feedback-loop between skin and bone marrow.

### Human basophils and detection of major histocompatibility complex class II expression

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#### Background:

To initiate an allergic response, the allergen needs to be presented to a T cell, by an antigen-presenting cell (APC). The APC internalizes, processes, and subsequently presents the allergen on the surface by the major histocompatibility complex II (MHC II) molecule. Until recently, the dendritic cell (DC) has been considered the cell responsible for allergen presentation in connection to induction of an allergic response. However, recently it has been published that mouse basophils

can express MHC II, internalize, process, and present soluble antigen,

These findings need to be confirmed in human basophils, thus the aim of this study was to investigate MHC class II expression in the human basophil population.

#### Methods:

Peripheral blood mononuclear cells were isolated from fresh buffy coat blood (< 5 h old) from 15 donors. Following, the cells were analysed by flow cytometry and gating on basophils using FcεRIα vs. an exclusion panel (CD3, CD14, CD19, CD56). Further, basophils expressing MHC class II were identified using the isotype for the specific antibody. The ability of three different antibodies to detect MHC class II expression on human basophils was tested.

#### Results:

Using an antibody specific for all three MHC class II subtypes (HLA-DR, -DP, and -DQ), significant higher amount of MHC class II<sup>+</sup> basophils were detected compared to antibodies specific for the subtype HLA-DR only. However, a significant difference was also observed between the HLA-DR specific antibodies.

The detected amount of basophils expressing MHC class II varied between donors from 0% to >7%.

#### Conclusion:

The experiment shows that human basophils can express MHC class II, however the amount of basophils detected as MHC class II<sup>+</sup> depends on the antibody used.

### Conditional knock-out of SH2 domain-containing phosphatase 2 (SHP2) in mast cells reveals a key role for SHP2 in mast cell development

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SHP2 (PTPN11) is a positive effector of growth signaling pathways downstream of Kit receptor tyrosine kinase, that functions in mast cell development and survival. Defining the roles of SHP2 in adults has been aided by the development of Cre recombinase/LoxP conditional knock-out (KO) mouse models that trigger SHP2 KO in specific tissues and cell types. In this study, we describe the phenotype of mast cell-specific SHP2 KO mice (MC-SHP2 KO), which were created using transgenic mice driving Cre expression under the control of the Mast cell protease-5 gene (Mcp5-Cre). We detect *shp2* null alleles in connective tissue mast cells (CTMCs) from peritoneum and skin, and their progenitors in spleen, but not in bone marrow. Interestingly, MC-SHP2 KO mice lack detectable peritoneal mast cells, and display a significant reduction in within the skin compared to control mice. These results are consistent with SHP2 being a key player in the Kit Y567/Src family kinase/Gab2/phosphatidylinositol 3' kinase axis of CTMC development in mice. *In vitro* CTMC differentiation assays were performed to identify the step at which SHP2 functions in mast cell development. Surprisingly, CTMCs lacking SHP2 showed no difference in CD81 upregulation, proliferation, or degranulation in response to basic compounds. Thus, the *in vivo* role of SHP2 may involve mast cell recruitment or survival within connective tissues.

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### Human mast cell tryptase stimulates neutrophil migration through bronchial epithelium.

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Infiltration of neutrophils into the lumen of the airways is a common feature of the respiratory inflammatory process. Mast cells may play a certain role in the regulation of this neutrophil migration by release of specific products as histamine, serine proteases, and multiple cytokines. A bilayer of cultured endothelial and bronchial epithelial cells was used as a model for the blood-air barrier, in which the migration of neutrophils was studied in the presence of different stimuli. Neutrophils were obtained from normal, non-smoking volunteers and labeled with a fluorescent marker. Confluent bilayers of cultured cells, pre-treated with 10 ng/ml TNF-α and IL-1β, showed a low spontaneous migration of neutrophils (1.9 ± 0.4%, n=4). Pre-incubation followed by fMLP (10<sup>-6</sup> M) stimulation resulted in 45.6 ± 5.8% migration (positive control). TNF-α (10 ng/ml) and histamine (10<sup>-5</sup> and 10<sup>-6</sup> M) were able to induce neutrophil migration through the cellular layers (13.3 ± 2.5% and 17.4 ± 0.6%, resp.). This induction was independent of pre-incubation of the bilayer. Tryptase (10 μM/ml) induced neutrophil migration (8.6 ± 1.2%, n=4),

which was significantly increased to  $21.8 \pm 0.9\%$  after pre-treatment of the bilayer with TNF- $\alpha$  and IL-1 $\beta$ . A number of mast cell products were able to stimulate migration of neutrophils through a bilayer of endothelial and bronchial epithelial cells.

### Mast cells in the proximal and distal airways

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**Introduction:** Mast cells may be involved in inflammation in both proximal and distal airways. We have examined mast cell numbers in the two compartments and assessed the effects of airways obstruction or being a current smoker.

**Methods:** Matched tissue from 14 patients (6F/8M, average age  $66.6 \pm 2.4$ ) was processed into GMA and mast cells enumerated by immunostaining with AA1. Sections were also stained for macrophages, neutrophils, B and T lymphocytes. None of the patients were on inhaled or systemic steroids.

**Results:** Mast cells were the most numerous inflammatory cell type we identified in the distal and proximal airways though some individual patients had high levels of macrophages and neutrophils. The number of lymphocytes was low. There were significantly more mast cells in the distal airways with a median (IQR) of  $60.5(37.4-70.7)$  mast cells/mm<sup>2</sup> compared with  $14.4(6.7-16.5)$  mast cells/mm<sup>2</sup> in the upper airways ( $P < 0.05$ ). There was no correlation between mast cell numbers in the two compartments suggesting that local rather than systemic factors are regulating mast cell density. We divided the patients into those with no evidence of airways obstruction (4F/4M, average age  $65.1 \pm 3.8$ , FEV<sub>1</sub>/FVC =  $0.76 \pm 0.02$ ) and those with mild/moderate COPD (2F/4M, average age  $68.7 \pm 2.4$ , FEV<sub>1</sub>/FVC =  $0.57 \pm 0.02$ ) we found no evidence that the presence of disease altered mast cell numbers in either the upper or lower airways. Six of our patients were current smokers and again we found no evidence that this modified mast cell numbers in either the upper or lower airways.

**Summary:** Mast cells were the most numerous inflammatory cell identified in the upper and lower airways with a significantly higher mast cell density in the lower airways. Mast cell numbers were not altered in patients with mild/moderate airways disease or in current smokers.

### Mast cell-derived Amphiregulin enables regulatory T cells to suppress local inflammation:

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Immune regulatory functions of mast cells have become increasingly appreciated in recent years. So have they for example been shown to be "essential intermediaries in regulatory T cell tolerance". During inflammation mast cell precursors infiltrate the site of inflammation, and proliferate and differentiate there. One of the most up-regulated genes upon activation of mast cells is the EGF-like growth factor, Amphiregulin (AREG). Here we show that AREG gene-deficient mice have a dysfunctional local immune regulation, which correlates with a missing expansion of a population of FoxP3<sup>pos</sup> CD4 T-cells that expresses the EGF-R under inflammatory conditions. We find this novel, AREG-mediated immunoregulatory pathway for regulatory T-cell function to be important in standard in vitro T-cell proliferation assays and, in vivo, in models of chronic inflammatory diseases, such as dermatitis and colitis, and in a tumor vaccination model. We further identified mast cells as the indispensable source of AREG within inflamed tissues. So could inflammation be prevented by reconstitution of c-Kit<sup>w-sh</sup> mice with wt - but not AREG-deficient bone marrow-derived mast cells. Moreover, disease outcome directly correlated with EGF-R expression on the FoxP3<sup>pos</sup> CD4 T-cell population in the draining lymph nodes. Taken together, we show that in inflamed tissues, mast cell-derived AREG is of critical importance to enable regulatory T-cells to suppress local immune responses.

### CD84 negatively regulates IgE high affinity receptor signaling in human mast cells

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CD84 is a self-binding receptor from the CD150 family that is broadly expressed in hematopoietic cells. High levels of CD84 are present in human mast cells and we analyzed its role in the

regulation of the IgE high affinity receptor (Fc $\epsilon$ RI). It has been described that in the absence of the adaptor SAP, CD150 family members exert inhibitory rather than activating signals. We observed that human mast cells express CD84 but lack SAP, so we explored a potential inhibitory role for CD84 in this context. We found that CD84 gets tyrosine phosphorylated upon Fc $\epsilon$ RI engagement in LAD2 and CD34+ derived mast cells (huMCs), and that release of granule contents is reduced when Fc $\epsilon$ RI is co-engaged with CD84. Supporting CD84 mediated weaker degranulation, we found that actin cytoskeleton depolymerization is also negatively regulated by co-engagement of Fc $\epsilon$ RI and CD84 as compared to Fc $\epsilon$ RI alone. In addition, we observed that CD84 also dampens Fc $\epsilon$ RI-mediated calcium mobilization after its co-crosslinking with the receptor. In order to understand CD84 inhibitory mechanism, we analyzed signalling pathways affected by CD84 engagement that could explain this receptor inhibitory effect in human mast cells. Consistent with the observed calcium mobilization reduction, our results show that the Syk-LAT-PLC- $\gamma$ -1 axis activity is downregulated after CD84 stimulation, since we observed lower tyrosine phosphorylation of the three mediators, compared to Fc $\epsilon$ RI or Fc $\epsilon$ RI plus Ig control. We found that inhibitory kinase Fes more gets phosphorylated after CD84 and Fc $\epsilon$ RI co-crosslinking than with Fc $\epsilon$ RI and Ig control con-engagement, which suggests that this kinase can be involved in CD84 inhibitory mechanism. Moreover, CD84 gets strongly tyrosine phosphorylated by Fes and Fes which gets activated after substrate binding is also hyperphosphorylated when co-expressed with CD84 in COS cells. Taken together, our results show a negative regulatory role for CD84 in human mast cells.

### Investigating the cytokinergic activity of monoclonal human IgE antibodies by human mast cell degranulation assay.

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#### Abstract:

Allergy is mediated by activation of mast cells via the high-affinity IgE receptor (Fc $\epsilon$ RI). Classically, IgE-bound Fc $\epsilon$ RI must be cross-linked by multivalent antigen to initiate signal transduction. However, it has been reported that some murine monoclonal IgE antibodies (IgE mAbs) can induce this signal transduction in mast cells in the absence of allergen. The majority of these IgE mAbs increase cell survival, whilst others stimulate cytokine release, and some initiate mast cell degranulation and the release of pro-inflammatory mediators. IgEs have therefore been described in a hierarchy of "poorly cytokinergic" to "highly cytokinergic".

A human mast cell degranulation assay has been used to successfully demonstrate the varying activities of a number of murine cytokinergic IgE mAbs. This assay will now be used to evaluate the cytokinergic activity of recombinant human IgE mAbs produced by transient transfection of HEK293E cells, which will enable further investigation of the structural elements or properties that may be responsible for the activity of a highly cytokinergic IgE.

Furthermore, this research will consider whether there is pathological relevance to the differences between highly cytokinergic and poorly cytokinergic IgEs. It may be that allergic patients exhibit increased levels of highly cytokinergic IgEs rather than poorly cytokinergic IgEs, compared to non-allergic individuals, and that mast cell activation by highly cytokinergic IgEs results in increased class-switching to IgE in local B cells and perhaps accounts for "intrinsic" allergic disease where no specific allergen can be identified.

### Mast cells as a new target of asbestos fibres.

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Asbestos is a strong pro-inflammatory and mutagenic compound, however its mechanism of action is still unclear. The interaction between asbestos fibres and professional phagocytes has been widely studied. Among cells with properties shared with professional phagocytes, mast cells have been recently enrolled. These cells are present in both human and rat lung (as much as 2% of the human alveolar wall is occupied by mast cells). Despite of the fact that in the entry site of the asbestos fibres together with professional phagocyte mast cells are also present, nothing is known about the mast cell-asbestos fibres interaction. Many years ago it has been reported that following asbestos fibre injection, pleural mast cells decrease in number, possibly due to their complete degranulation, suggesting that asbestos may be a strong stimulus for mast cell granule secretion. We report here that the interaction between asbestos fibres (crocidolite) and rat peritoneal mast cells differs from that of professional phagocytes. Asbestos fibres strongly triggered the

degranulation reaction, while failed to induce fibre engulfment and superoxide production. The morphological appearance of mast cells stimulated by asbestos suggested that fibres induce the expulsion of mast cell granules/granule remnants. We found that crocidolite fibres appear completely covered by these granules. While the soluble granule components such as histamine and  $\beta$ -hexosaminidase increase in supernatant of the asbestos-stimulated cells, chymase did not, remaining tightly bound to the extracellular granule which adhere to asbestos fibres. Accordingly we investigated if after mast cell interaction the fibres acquire new properties capable of accumulating other pro-inflammatory mediators giving them a strong pro-fibrotic power. We put forward the hypothesis that mast cells through release of soluble mediators and by arming the asbestos fibres with granule remnants, should be considered as a key factor in the pathogenesis of lung induced asbestos pathology.

#### **The dominant inhibitory effects of low-affinity IgG receptors in human and murine basophils**

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Basophils are increasingly understood as major players in immunity. Like mast cells, they express not only high-affinity IgE receptors (Fc $\epsilon$ RI) whose engagement by IgE and antigen triggers the release and/or secretion of pro-inflammatory mediators and cytokines, but also low-affinity IgG receptors (Fc $\gamma$ RII/III) whose function is poorly known. We investigated the expression and biological properties of IgG receptors in human and murine basophils. Basophils express both activating (Fc $\gamma$ RIIA in humans; Fc $\gamma$ RIIA in mice) and inhibitory (Fc $\gamma$ RIIB in both species) IgG receptors. Except in B cells, Fc $\gamma$ RIIB expression is higher in human basophils than in other leukocytes, including human mast cells which express no or little Fc $\gamma$ RIIB. Fc $\gamma$ RIIA could hardly activate human basophils, whereas Fc $\gamma$ RIIA activated mouse basophils as efficiently as Fc $\epsilon$ RI. Upon activation, human basophils released large amounts of histamine but secreted small amounts of IL-4, whereas murine basophils released small amounts of histamine but secreted large amounts of IL-4. Co-engaging Fc $\gamma$ RIIB with Fc $\gamma$ RIIA or Fc $\gamma$ RIIA abrogated IgG-induced human and murine basophil activation, respectively. Fc $\gamma$ RIIB also inhibited Fc $\gamma$ RIIA-dependent IgG-induced basophil-mediated passive systemic anaphylaxis. Co-engaging Fc $\gamma$ RIIB+Fc $\gamma$ RIIA or Fc $\gamma$ RIIB+Fc $\gamma$ RIIA with Fc $\epsilon$ RI markedly decreased IgE-induced human and murine basophil activation. Inhibition of IgE-induced activation was observed in basophils from all (n=35) normal donors tested, and it was reduced by anti-Fc $\gamma$ RIIA+B antibodies. Inhibition of Fc $\gamma$ RIIA-dependent and Fc $\epsilon$ RI-dependent murine basophil activation was reduced by anti-Fc $\gamma$ RIIB antibodies in basophils from wild-type mice and abrogated in basophils from Fc $\gamma$ RIIB-deficient mice. Fc $\gamma$ RIIB-dependent negative regulation is therefore dominant over Fc $\gamma$ RIIA- or Fc $\gamma$ RIIA-dependent basophil activation, in normal donors and wt mice, and inhibitory signals generated by Fc $\gamma$ RIIA+IIB or by Fc $\gamma$ RIIA+IIB affected activation signals generated by Fc $\epsilon$ RI in human and murine basophils. Low-affinity IgG receptors thus appear as a regulatory module which, when engaged by IgG immune complexes, generates dominant inhibitory signals that can control IgE-induced basophil activation in mice and humans.

#### **IgE immune complexes stimulate mast cell progenitor recruitment in a mouse model of allergic airway inflammation**

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Allergic asthma is associated with increased levels of antigen specific IgE and higher numbers of mature mast cells in the lung that can be mimicked using mouse models of allergic airway inflammation. Other studies have shown that the increment in mature mast cells is preceded by antigen-driven recruitment of mast cell progenitors to the lung. Moreover, antigen specific IgE forms immune complexes locally in the lung that is known to regulate the immune response, but the possible role of IgE immune complex formation on mast cell progenitor recruitment to lung during allergic airway inflammation is largely unknown. To study this, BALB/c mice were sensitized to ovalbumin (OVA) and challenged intranasally with a low dose of OVA-trinitrophenyl (TNP) alone or together with IgE-anti-TNP. We show that IgE immune complexes stimulate a specific 3-fold increase in recruitment of mast cell progenitors to the lung, compared to OVA-TNP alone. To elucidate what receptor was responsible for the recruitment of mast cell progenitors, we studied the effect of IgE immune

complexes in mice lacking either the CD23 receptor, or the common Fc $\gamma$  chain. The recruitment of mast cell progenitors to the lung in CD23<sup>-/-</sup> mice challenged with IgE immune complexes was similar to wild type mice treated in parallel, while Fc $\gamma$ RI<sup>-/-</sup> mice had an impaired IgE immune complex driven recruitment. These findings suggest that IgE immune complexes stimulate recruitment of mast cell progenitors through either Fc $\epsilon$ RI or Fc $\gamma$ RIV or both.

#### **Calcium influx pathways in human mast cells**

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Mast cells play an acute role in the pathophysiology of type 1 hypersensitivity reactions and allergies. Activation occurs via cross-linking of the Fc $\epsilon$ RI receptor by antigen-IgE complexes that, via a signalling cascade, elevate the intracellular calcium concentration. This calcium signal is necessary for the release and production of pro-inflammatory mediators that contribute to inflammation. The best characterised calcium channel in immune system cells is the store-operated calcium release activated calcium channel (CRAC), activated by Ca<sup>2+</sup> release from ER stores via the STIM1 protein. The expression of this channel in human mast cells has not yet been fully investigated and could be an important therapeutic target.

The aim of this study was to identify whether the LAD 2 human mast cells functionally express CRAC channels. RT-PCR showed LAD 2 cells express mRNA for Orai1, Orai2, STIM1 and STIM2, but not Orai3. Under whole cell patch clamp recording conditions, using intracellular dialysis of IP<sub>3</sub> and BAPTA to store deplete, these cells displayed an I<sub>CRAC</sub>-like current. Current-voltage relationships were inwardly rectifying with positive reversal potentials (+49.6mV n=10). The current at -80mV was reduced by Ba<sup>2+</sup> substitution (18% n=3) and potentiated in divalent free solution (301% n=7); this sodium current was partially inhibited by 10 $\mu$ M Gd<sup>3+</sup> (47.2% n=3). In summary, these data support a role for CRAC channels in calcium influx in human mast cells.

#### **Lactobacillus induced attenuation of mast cell degranulation is associated with inhibition of K<sup>+</sup> channel (KCa3.1) current.**

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Background: There is increasing evidence that ingestion of certain lactic acid bacteria (LAB) can modulate systemic immune responses and provide potentially therapeutic benefits at tissues sites beyond the gastrointestinal tract. In particular, it is suggested that LAB may aid in the prevention and/or treatment of allergic disorders. Despite the central role of mast cells in allergic disease little is known about the effect of LAB on the function of these cells. To address this we assessed changes in rat mast cell activation following oral treatment with a strain of Lactobacillus known to attenuate allergic responses in animal models.

Methods: Sprague Dawley rats were fed with L.reuteri (1x10<sup>9</sup>) or vehicle control for 9 days.  $\beta$ -Hexosaminidase and TNF release from peritoneal mast cells (RPMC) was determined in response to a range of stimuli. Passive cutaneous anaphylaxis (PCA) was used to assess mast cell responses in vivo. The Ca<sup>2+</sup> activated K<sup>+</sup> channel (K<sub>Ca</sub>3.1) current, identified as critical to mast cell degranulation, was monitored by whole cell patch-clamp.

Results: L.reuteri treatment lead to significant inhibition of mast cell degranulation in response to calcium ionophore, substance P, 48/80 and IgE mediated activation. Furthermore, the PCA response was significantly reduced in L.reuteri treated rats. The attenuation of mast cell response to stimuli was not mimicked by in vitro co-culture of mast cells with bacteria. Patch-clamp studies revealed that the RPMC from treated animals were much less responsive to the K<sub>Ca</sub>3.1 opener, 1-EBIO.

Conclusion: These studies identify mast cells and K<sub>Ca</sub>3.1 as immunomodulatory targets for LAB and suggest such actions may contribute to the beneficial effect of these organisms in allergic disease. Studies are ongoing to identify the mechanisms underlying these effects and to further characterize LAB induced changes in mast cells function.

#### **Human Basophil Activation is Dependent on the Nature of High-Affinity IgE Receptor Engagement.**

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Human basophils rapidly generate important pro-inflammatory and immunomodulatory mediators upon high-affinity IgE-receptor crosslinking with allergens. Here, we show that the ability of basophils to respond to IgE-mediated stimuli is negatively associated with the level of constitutive SHIP-1 phosphorylation as well as the type of IgE-dependent stimulus employed. Basophils were obtained from buffy coats and purified by negative selection and magnetic cell sorting. Cells were incubated with a variety of IgE-dependent triggers together with unstimulated controls for varying periods in order to determine rate and magnitude of histamine/IL-4 release and intracellular signalling (by Western blotting). We observed that constitutive SHIP-1 phosphorylation (but not total SHIP-1 or Syk expressions) in unstimulated basophil preparations correlated with maximum basophil histamine release to IgE-dependent activation. SHIP-1 was also associated with limiting basophil responses following supraoptimal concentrations of IgG-anti-IgE, a crosslinking agent that produced striking bell-shaped dose response curves. In contrast, there was no marked reduction in histamine release or increased SHIP-1 phosphorylation following supraoptimal stimulation of basophils with either concanavalin A or IgM-anti-IgE antibodies. Differential bell-shaped responses were also seen using basophils sensitised with various Der p 2-specific recombinant IgE antibodies and subsequent allergen-dependent triggering. Here, histamine and IL-4 releases were reduced more markedly with cells that had been sensitized with allergen-specific IgE of high affinity and with increasing clonality. Thus, the overall basophil sensitivity to IgE-dependent activation depends on constitutive SHIP-1 control but the pattern of mediators generated by various IgE-mediated triggers differentially involves SHIP-1 input, depending on the properties of the crosslinking agent. These properties may determine the severity of allergic reactions and serve as useful tools in understanding mechanisms controlling the activation and desensitization of allergic effector cells.

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#### Mast cells as target in cancer therapy

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**Purpose:** Cancer growth and inflammation are closely related processes. At present it is believed that infiltration of immune cells strongly contributes to tumor cell proliferation and metastasis. Recently mast cells have been identified as an important cell type in growth of pancreatic carcinoma in a murine model. We have investigated human cancer biopsy specimens for the presence of mast cells as a target for therapeutic intervention.

**Methods:** On tissue microarrays of cancer biopsies, also known as tissue chips, the clinical relevance of potential biological targets for diagnosis and therapy can be evaluated. The tissue chips (Biomax, USA) we have used are made from cores (range 12-210 cores/slide), of 5µm thickness and diameters of 1.5mm or 1mm. Each core represents one specimen providing an overview of influence of different ages (range 20-90), comparison of normal, malignant, metastasized, benign, adjacent, and inflamed tissues. Tissue arrays were immunostained for mast cells. Positive cells were quantified under a microscope.

**Results:** Mast cells were especially prominent in breast cancer, lung cancer, skin cancer and pancreatic cancer.

**Conclusion:** Breasts, lung, skin and pancreatic cancer types seem especially promising for mast-cell directed anticancer therapy.

**Keywords:** Mast cells, Tissue microarrays, cancer

**Key References:**

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#### Serglycin proteoglycans contribute to inflammatory process in experimental arthritis

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Rheumatoid arthritis is an autoimmune disease leading to swelling of synovial joints and with time causing diffused inflammation and nodular lesions in the body. Role of the acquired immune system in chronic inflammation has usually been the focus in RA research. However, recent studies link the innate immunity (mast cells, neutrophils etc) with the disease progression. MC derived mediators have now been shown to induce edema, connective tissue destruction, and contribute to fibrosis in arthritic joints. In a previous study we showed the beneficial effect of mast cell chymase deficiency in RA (Magnusson et al. *FASEB J* 2009). Serglycin (SG) is expressed by many different cell types and plays important roles in the intracellular storage of different mediators as well as a possible chaperon/transport vehicle of mediators in the extracellular environment. In this study, we used the SG knockout (KO) mouse strain as a model for studying the activity of SG-proteoglycan dependent mediators when challenged with bovine collagen to induce experimental arthritis, i.e. chronic inflammatory disease.

SG<sup>+/+</sup> and SG<sup>-/-</sup> mice on the DBA/1 background, 6-8 weeks old, were challenged by two different protocols. 1) Collagen induced arthritis (CIA) is an active immunization model where both the innate and acquired immune system is activated. The protocol induces arthritis after 3 weeks and a full-blown disease occur after 5 to 10 weeks. 2) The use of an antibody cocktail against Collagen II (anti CII) gives a passive immunization model which induces arthritis in 22-25 days.

We observe milder histopathology and lower cytokine and antibody production in the SG KO arthritic animals. Among differences in the joint morphology, inflammation, pannus formation and bone destruction were significantly lower in arthritic SG. The results indicate late arthritic onset accompanied with less severe and milder disease in SG KO compared to WT.

**Keywords:** mast cell • arthritis • serglycin • inflammation • synovial joints

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#### OxLDL-IgG immune complexes induce expression and secretion of proatherogenic cytokines by cultured human mast cells

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**Objective:** Human atherosclerotic lesions contain mast cells and IgG immune complexes containing oxidized LDL lipoprotein particles. Here we studied whether such oxidized LDL IgG immune complexes can activate human mast cells and induce them to express and secrete proinflammatory cytokines that are potentially capable of inducing and amplifying atherogenic processes.

**Methods and results:** Incubation of cultured human mast cells in the presence of oxidized LDL IgG immune complexes led to a significant dose-dependent upregulation of the expression and secretion of tumor necrosis factor-α and interleukin-8, and the chemotactic cytokine monocyte chemoattractant protein-1. The secretory responses were dose-dependent and associated with moderate release of histamine and tryptase, which are preformed mast cell mediators contained in the cytoplasmic secretory granules of the cells. Also native LDL IgG immune complexes induced similar proinflammatory cytokine response, suggesting that Fc gamma receptors, rather than LDL receptors or scavenger receptors, were involved in the IgG immune complex-induced mast cell activation.

**Conclusion:** Mast cells in atherosclerotic lesions which also contain oxidized low-density lipoprotein immune complexes may become activated by the immune complexes and secrete many proinflammatory cytokines. Our results suggest that intimal mast cells act as a cellular link between oxidized LDL

immune complexes and the inflammatory response in atherosclerosis

### **IgE dependent cytokine expression in human intestinal mast cells is decreased by arginine and glutamine**

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**Introduction:** Arginine and glutamine are essential amino acids with immunomodulatory functions, e.g. pharmacological doses of arginine and glutamine caused a reduced cytokine release of colonic biopsies from patients with active Crohn's disease. Mast cells are not only main effector cells of allergic reactions, but also well known for their capacity to participate in the regulation of inflammatory and immune responses, e.g. in the course of intestinal inflammation. The aim of our study was to examine effects of arginine and glutamine on mediator release and cytokine expression of human intestinal mast cells. **Methods:** Mucosal mast cells were isolated from surgery intestinal tissue specimen by mechanic and enzymatic digestion and purified by positive selection of c-Kit expressing cells. Isolated mast cells were cultured in the presence of stem cell factor and IL-4. Pre-stored  $\beta$ -hexosaminidase and de novo synthesized leukotriene C<sub>4</sub> (LTC<sub>4</sub>) were measured by enzymatic assay and ELISA. Cytokine mRNA expression was assessed by real-time RT-PCR. Signaling molecule activation was determined with Proteome Profiler™ antibody array. **Results:** Following over night incubation with combined pharmacological doses of arginine (2 mmol/l) and glutamine (10 mmol/l), IgE dependent mRNA expression for IL-8, MIP-1 $\beta$ , MCP-1 and TNF $\alpha$  was decreased compared to incubation with low doses of arginine (0.1 mmol/l) and glutamine (0.6 mmol/l). However, the release of pre-stored  $\beta$ -hexosaminidase was not affected, whereas the release of de novo synthesized LTC<sub>4</sub> was reduced in response to pharmacological doses of arginine and glutamine. Moreover, we found reduced phosphorylation levels of the transcription factors CREB, STAT1 and STAT4 in response to high doses of combined arginine and glutamine compared to low doses. **Conclusion:** Immunomodulatory nutrients such as the amino acids arginine and glutamine are capable of modifying IgE dependent cytokine expression of human mucosal mast cells.

### **Characterization of syk expression in human lung mast cells: relationship to function**

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#### **Abstract**

Previous studies indicate that the protein tyrosine kinase, syk, is critical in transducing Fc $\epsilon$ R1-mediated signals. In human basophils, 'releasability' has been linked to the extent of syk expression. Human lung mast cells, like basophils, are also found to be variably responsive to IgE-dependent activation. The aim of the present study was to determine whether the wide variability in human lung mast cell responses, following IgE-dependent activation, has a relationship to syk expression. Mast cells were isolated from human lung tissue and 'releasability' was determined by activating the cells with a maximal releasing concentration of anti-IgE. Syk levels in mast cells were determined by immunoblotting and flow cytometry. Histamine release from mast cells, challenged with a maximal releasing concentration of anti-IgE, ranged from 0 to 69% (mean $\pm$ SEM, 24 $\pm$ 2%, n=53). A proportion of these preparations (9 out of 53) released very low levels of histamine ( $\leq$  5%) in response to anti-IgE. Preparations that failed to respond to anti-IgE, ably responded to the calcium ionophore, ionomycin. Flow cytometry of a subset of preparations indicated that a weak response to anti-IgE was not related to a lack of surface IgE (n=13). Immunoblotting (n=24) and flow cytometry (n=9) studies demonstrated that, compared to mononuclear cells, human lung mast cells express low and variable levels of syk. However, there was no correlation between syk expression and mast cell releasability. Nonetheless, a number of putative inhibitors of syk including NVP-QAB205 (EC<sub>50</sub>, 0.2  $\mu$ M) effectively attenuated the IgE-dependent release of histamine from mast cells. These studies indicate that although syk may play an important role in mediating degranulation, syk expression does not govern mast cell releasability.

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### **NOVEL $\alpha,\beta$ -UNSATURATED LACTONES INHIBIT MAST CELL ACTIVATION INDUCED BY INTRACELLULAR CALCIUM INCREASE**

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**Background:** In previous work we have demonstrated that a sesquiterpene lactone isolated from *Artemisia douglasiana* Besser (dehydroleucodine, DhL), a xanthanolate isolated from *Xanthium cavanillesii* Schouw (Xt) and a synthetic butenolide (3-benzyloxymethyl-5H-furan-2-one, But), exhibit a strong antiinflammatory activity and prevent gastrointestinal damage elicited by necrosis-inducing agents. The mechanism of action of these drugs remains unclear. The present work was designed to examine the effect of DhL, Xt and But on calcium ionophore A23187-induced mast cell degranulation, with the goal of testing the hypothesis that such molecules act as mast cell stabilizers. **Methods:** LAD2 and rat peritoneal mast cells were incubated with: 1) Buffer (basal group) or 2) A23187 (mast cell secretagogue) or 3) DhL+A23187 or 4) Xt+A23187 or 5) But+A23187. Mast cell serotonin and  $\beta$ -hexosaminidase release studies, evaluation of mast cell morphology by light microscopy (toluidine blue stain and differential interference contrast), study of mast cell ultrastructure by transmission and scanning electron microscopy, dose-response (10  $\mu$ M to 1600  $\mu$ M) and time-response (5 min to 60 min) curves, cell viability evaluation (tripan blue stain), and comparative studies with the reference compounds ketotifen and sodium chromoglycate, were carried out. **Results:** A23187 increased serotonin and  $\beta$ -hexosaminidase release from LAD2 and rat peritoneal mast cells, and elicited evident granule ultrastructural changes. These effects were inhibited by DhL, Xt and But in a dose- and time- dependent manner. The inhibitory effects exhibited by DhL and Xt were stronger than those of ketotifen and sodium chromoglycate. **Conclusions:** DhL, Xt and But inhibit calcium ionophore A23187-induced mast cell activation, acting thus as mast cell stabilizers. These lactones seem to block signaling pathways downstream of cytosolic calcium increase. Our findings may provide an insight into the design of novel pharmacological agents which may be used to regulate the mast cell response.

### **Functional Genomics High-throughput Analyses of Mast Cell Activation**

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Mast cells are specialized secretory cells of the immune system that are implicated in allergic and inflammatory responses. These cells are packed with secretory granules (SGs), which contain allergic and inflammatory mediators. When triggered, multiple signaling events are activated resulting eventually in fusion of the SGs with the plasma membrane (degranulation). Efforts are being undertaken to develop novel therapies that will specifically target mast cell activation. Adopting screening approaches aiming to unveil stimulus-secretion coupling networks in mast cells has been limited due to their low transfection efficiency. Hence, genetic manipulations are unlikely to leave an impact on the actual readouts of average secretion measured by conventional methodologies. We have established a technology that overcomes this obstacle and allows functional genomics high-throughput analyses of mast cell degranulation. Specifically, we are using a fluorescence-based nanosystem, based on live cell array chips, that allows us to monitor and quantify mast cell exocytosis by imaging simultaneously hundreds of individual cells that were genetically manipulated. Using this technology, we have screened the family of RABs, small GTPases that localize to distinct membrane-bound compartments, where they regulate transport and fusion events. We have identified 22 RAB GTPases as significant modulators of mast cell degranulation. The involvement of 18 RABs was heretofore not recognized. Currently, we are combining multidisciplinary approaches including cell and molecular biology, genetics, and computational techniques to elucidate the mechanisms by which these proteins affect mast cell degranulation and decipher the multi signaling network leading to degranulation. Relevant Rabs, which serve as positive regulators, may serve as cellular targets for future developments of new therapeutic means to treat allergic and inflammatory diseases.

## Immunoglobulin free light chains synergistically enhance IgE-mediated mast cell activation

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Mast cells are key effector cells in allergic diseases. The most prominent pathway to activate mast cells is via crosslinking of IgE bound to the FcεRI resulting in degranulation and mediator release. In previous work, we have shown that activation via immunoglobulin free light chains (IgLC) may be an alternative pathway of antigen-specific mast cell activation (1). In recent studies we found that local or circulating IgLCs are increased and may be of importance in triggering mast cell activation in (non-IgE mediated) inflammatory diseases such as non-atopic asthma, inflammatory bowel disease, food allergy, rhinitis and rheumatoid arthritis (2,3,4,5). Since we found that IgLC are also often increased in allergic (IgE-driven) diseases, we investigated if IgLC could also influence IgE-mediated mast cell activation.

Primary cultured bone marrow-derived mast cells and the mast cell lines MC/9, CFTL15 and RBL-2H3 were stimulated by IgE in presence of various concentrations of IgLCs. Mast cell activation was monitored by the release of beta-hexosaminidase and IL-6 production.

Mast cell activation by IgE/FcεRI was greatly enhanced when simultaneously IgLC were crosslinked on the mast cell surface. When IgE and IgLC was titrated to concentrations which induced only limited to no mast cell activation (<5% beta-hexosaminidase release) combined crosslinking of IgE plus IgLC resulted in a synergistic activation of all tested mast cells. Both degranulation and IL-6 production were greatly increased in such combinations of IgE and IgLC. Mast cell activation was completely inhibited by syk kinase inhibitors.

This study indicates that combined crosslinking of IgE and IgLC results in synergistic activation of mast cells. Our results suggest that production of IgLC in IgE-mediated diseases may be of clinical relevance for the induction of allergic symptoms.

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## Basophil Sensitivity Decreases During the Updosing Phase of Subcutaneous Immunotherapy (SCIT) in Subjects Allergic to Grass Pollen

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### Background:

SCIT reduces the specific type-1 allergic response and is associated with significant relief of symptoms. Basophil sensitivity, defined as half maximum activation (LC50) after crosslinking of specific IgE by relevant allergen, is thought to reflect clinical intensity of allergic disease. We hypothesized that changes in basophil sensitivity can be used to evaluate the magnitude of the humoral component of these specific changes.

### Methods:

We measured changes in basophil activation in 24 subjects (18 patients on standard SCIT, 6 patients in a control group) with rhinoconjunctivitis due to grass pollen allergy. Basophil activation was measured by flow cytometry as the percentage of CD63 expression on the surface of CD193+ blood basophils activated by 8 log<sub>10</sub> dilutions of grass pollen extract (0,00001-100 SQU7ml). This was done on washed cells and cells reconstituted with plasma from the baseline and present visit, respectively. LC50 was the primary outcome measure.

### Results:

The LC50 in the samples reconstituted with present plasma changed from a median LC50 of -2.34 at baseline to a median LC50 of -1,10 after reaching maintenance dose (n=24, p<0,001). No significant changes were observed in the control group or in the samples reconstituted with baseline plasma or the washed cells samples.

### Conclusion:

We found the LC50 a useful and accurate tool to follow the development of tolerance during SCIT. As sensitivity decreased 14-fold in samples reconstituted with present plasma but not in the other groups, the main mechanism leading to allergen tolerance involves humoral factors.

## Phospholipid Micropatterns as a Tool for the Study of Rapid Non-Genomic Actions of Glucocorticoids in Mast Cells

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**Background:** Steroid hormones function by binding to intracellular receptors and subsequently modulating the transcription of target genes. These processes typically take 30-60 min and are termed genomic effects. In addition, very rapid actions of steroids have been described that are clearly incompatible with the genomic effects and are therefore classified as rapid nongenomic effects. These nongenomic actions do not depend on gene transcription and most likely involve modulation of signal transduction pathways that emanate from the plasma membrane. We have observed that glucocorticoids rapidly (~ 5 min) repress increased intracellular Ca<sup>2+</sup> levels triggered by cross-linking the high affinity IgE receptor in mast cells. Work presented here is aimed at investigating whether this rapid action of glucocorticoids is triggered by the glucocorticoid receptor (GR) or involves alteration of membrane properties of mast cells by the hormone. These studies are carried out using Dip-Pen Nanolithography technique where allergens are immobilized on surfaces and cross-linking of the IgE receptor and recruitment of a GR fused to a Green Fluorescent Protein (GR-GFP) is visualized at the single cell level.

**Methods:** We have exposed RBL-2H3 mast cells that express a GFP-GR fused protein to planar surfaces decorated with the activating ligand (2, 4-dinitrophenyl (DNP)-capped phospholipid). Deposition of the DNP was carried out by Dip-Pen Nanolithography (DPN). DPN employs an Atomic Force Microscope (AFM) tip to deliver molecular inks onto planar surfaces. Using phospholipids as ink allowed subcellular scale definition of the ligand templating and its mobility in the fluid lipid bilayers. Using glass as a substrate for DPN patterning enabled observation of the fluorescent ligand and dynamic reorganization of the fluorescent cell membrane components upon cell activation and hormone treatment.

**Results:** Spatial distribution of the lipid patterns containing antigenic phospholipids enabled evaluation of the cellular responses in RBL-2H3 mast cells to steroid hormones and visualization of the GR-GFP mobility at sites of cell- allergen contact.

**Conclusion:** These experimental conditions provide a unique opportunity of determining whether the rapid nongenomic action of glucocorticoids requires the recruitment of the GR to lipid rafts, where the IgE receptor clustering and early mast cell signaling event take place or through changes in membrane properties.

## The role of actin dynamics in mast cell degranulation

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Over the past years actin has emerged as a key player in calcium-regulated exocytosis, i.e. secretion. Although actin reorganization is a prerequisite for secretion, its precise role has not been elucidated yet. We aim to unravel the function of the actin machinery in mast cell degranulation using the rat basophilic leukemia RBL-2H3 cell line as a model. We have stimulated this cell line to degranulate either by cross-linking the IgE receptor with IgE and anti-IgE DNP or by treating the cells with A23187 and PMA. FRAP experiments demonstrated that actin is highly mobile in resting RBL-2H3 cells. Upon stimulation, however, actin polymerization is induced, resulting in a less dynamic actin population. In addition, podosome-like actin structures appeared within 3-20 min post-stimulation. Live cell imaging of degranulation using the v-SNARE VAMP8-pHluorin construct and LifeAct-RFP show that the fusion event itself is independent of actin. Combined TIRF and epifluorescence microscopy with VAMP8-pHluorin revealed that the majority of fusion events take place basally. The fusion events were significantly increased after treatment with latrunculin B (20 μM) as measured by a beta-hexosaminidase release assay. Interestingly, live cell imaging proposed that secretion, after disassembly of actin filaments, occurred both basally and apically. Even though nocodazole (3 μM) treatment impaired secretion efficiently, the data suggests that secretion of VAMP8 positive vesicles primarily occurs from pre-docked vesicle pools. Collectively, the results suggest that although actin is not involved in the vesicle fusion per se, it may fine-tune the potency of secretion and direct the releasable pool of vesicles to the correct surface of the cell.

## The soluble form of OX40 molecule mimics regulatory T cell inhibition on mast cells

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We have previously demonstrated that regulatory T cells (Tregs) affect mast cell (MCs) degranulation but not cytokine production through an OX40:OX40 ligand axis. Little is known about OX40 ligand transduction pathway in MCs, so we developed the soluble OX40 molecule (sOX40) to trigger MCs without the physical presence of Tregs to study the largely unknown signals following to these interaction sOX40 was shown to resemble the physiological effect of Treg on IgE-dependent-MC degranulation, calcium influx and cytokine production. Then we investigated whether modification in phosphorylation of proteins of the degranulation pathway were affected in the presence of sOX40.

Interestingly, we found that Fyn pathway activation but not LAT and Syk pathway was impaired following to the interaction through OX40:OX40L, confirming the idea that this axis is crucial in influencing mast cell activation. In conclusion sOX40 could be considered a useful tool to study OX40 ligand-dependent signal on MCs as well as an interesting molecule to influence the MC behavior in allergic or autoimmune diseases.

## Osmotic Mast Cell Activation and Prostaglandin E<sub>2</sub> role

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Mast cells are critical mediators of allergic disease, hypersensitivity reactions and asthma. In some asthma patients exercise produces airway hyperreactivity. The mechanism for exercise-induced bronchoconstriction has been suggested to be related to an increased airway fluid osmolarity. It has been reported that exposure of mast cells to hyperosmolar stimuli triggers degranulation and release of mediators. Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) has been shown to have a protective effect in patients with exercise-induced bronchoconstriction. Our main goal was to establish how PGE<sub>2</sub> and their antagonists alter the events occurring during osmotic stimulation in mast cells. Cells from human mast cells line LAD2, lung human mast cells, and peripheral blood CD34+ derived human mast cells were osmotically stimulated with mannitol (0.5M). The percentage of beta-hexosaminidase-release induced by mannitol for each type of mast cells was as follows: LAD2 mast cells (25%), Human lung mast cells (22%) and 33% for peripheral blood CD34+ derived human mast cells. PGE<sub>2</sub> acts through EP receptors (EP1-EP4), all except EP1 are expressed in mast cells and its effects were evaluated with the use of specific pharmacological antagonists. PGE<sub>2</sub> modulated mannitol-induced mast cell activation through interaction with EP receptors, especially EP3. We observed that mannitol treatment activates MAP kinase pathways, particularly pJNK and p38. MAP kinase activation as well as calcium mobilization was reduced after pretreatment with an EP3 receptor antagonist. These results are consistent with clinical observations and would help to interpret the mechanisms involved in the protective role of PGE<sub>2</sub> in osmotic mast cell activation.

## TRPC channels in human mast cells

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Inappropriate activation of mast cells is associated with a wide range of chronic allergic diseases including asthma, which affects 5.4 million people in the UK<sup>1</sup>. As the secretion and synthesis of mast cell mediators is controlled by Ca<sup>2+</sup> signalling, identifying the ion channels responsible for Ca<sup>2+</sup> influx is important for human health and will provide insight for development of new mast cell stabilising drugs. Whilst it is known that the store-operated Orai1 channel mediates the essential Ca<sup>2+</sup> influx for mast cell activation<sup>1</sup>, the identity and function of other Ca<sup>2+</sup> influx channels in mast cells is still unclear. We are investigating the role of TRPC channels in human mast cells. TRPC channels are less Ca<sup>2+</sup> selective than Orai channels, allowing passage of Ba<sup>2+</sup> and Sr<sup>2+</sup> ions. TRPC3, 6 and 7 channels are activated by diacylglycerol; TRPC1, 4 and 5 channels are also activated downstream of phospholipase C, possibly by Ca<sup>2+</sup> store depletion<sup>2</sup>.

Calcium imaging in human mast cells loaded with Fura2-AM showed that both Ca<sup>2+</sup> and Ba<sup>2+</sup> ions enter the cells after passive store depletion with thapsigargin, or following activation of FcεRI and P2Y purinoceptors (n>100 cells, N=3 independent experiments). β-hexosaminidase release assays showed that Ba<sup>2+</sup>

and Sr<sup>2+</sup> can support mast cell degranulation in the absence of extracellular calcium (n=3), suggesting that TRPC channels may contribute to mast cell function. Stimulation of cells with the diacylglycerol analogue, OAG, produced Ca<sup>2+</sup> influx in 35% of LAD 2 cells (n=120 cells, representative of 3 separate experiments), suggesting the involvement of the TRPC3/6/7 subgroup. Whole-cell patch-clamp recording techniques showed that OAG activated an outwardly-rectifying current in LAD 2 cells. RT-PCR confirmed the presence of mRNA for TRPC1, 3, 5 and 6 channels (n=3). Taken together, this data suggests that TRPC channels may be involved in calcium influx and mast cell function.

1. www.asthmauk.org

## Expression of IL-17 in mast cells of synovium of anti-citrullinated protein antibody-positive and -negative rheumatoid arthritis patients

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In mouse models as well as human rheumatoid arthritis (RA), mast cells have been implicated to play a functional role, especially in anti-citrullinated protein antibody-positive (ACPA+) disease. Recently, increased levels of histamine within the synovial fluid of ACPA+ RA-patients as well as increased numbers of mast cells with a degranulated phenotype in the synovia of the ACPA+ patients were observed compared to ACPA negative (ACPA-) patients. IL-17A has also been shown to play a functional role in RA. Recent data shows that - in synovial tissue - the mast cell is the major producer of IL-17A.

The objective of this study was to identify whether IL-17A expression by mast cells in synovium is different in ACPA+ and ACPA- RA patients.

Using histological and immunohistochemical staining with Chloroacetate Esterase (CAE) and IL-17A, there is no difference in total number of IL-17A+ cells, as well as in percentage of IL-17A+ mast cells between ACPA+ (n=23) and ACPA- (n=20) RA patients. These data are verified by double staining of IL-17A with ckit, CD3 and CD68. We also confirm that the mast cell is the major producer of IL-17A in synovium. The presence of IL-17 producing mast cells in synovium of ACPA+ and ACPA- RA patients implies that blocking IL-17 as treatment is justified for both patient groups.

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## The histamine H<sub>4</sub> receptor as a novel drug target in inflammatory conditions

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Histamine, one of the most important inflammatory mediators in mammals, is primarily derived from mast cells and basophils in peripheral tissues and elicits pleiotropic actions through binding to four G-protein-coupled receptor subtypes, designated H<sub>1</sub> to H<sub>4</sub> (HxR). Several preclinical proof-of-principle studies characterise the recently identified H<sub>4</sub>R as a major immunomodulatory histamine receptor playing a key role in the development and perpetuation of atopic and inflammatory pathologies. H<sub>4</sub>R is largely expressed on immune cells, including mast cells, basophils, monocytes, eosinophils, dendritic and T cells. H<sub>4</sub>R involvement in T<sub>H</sub>1/T<sub>H</sub>2 balance, eosinophil chemotaxis and mast cell trafficking presents exciting possibilities in potentially modulating effector cell pro-allergic function and histamine actions on various tissues commonly affected by allergic inflammation. Additionally, H<sub>4</sub>R functional expression on keratinocytes, chondrocytes, endocrine cells and neurons indicates an extensive biological role and provides insights into its implication in autoimmune diseases, pain and itching. Even though the interpretation of preclinical testing is limited by the species heterogeneity of H<sub>4</sub>R properties and the complex pharmacological profile of its ligands, a growing interest is directed towards the H<sub>4</sub>R exploitation in novel therapeutic strategies. The high patent filing activity in this field resulted in the advancement of the

first H<sub>4</sub>R antagonist to Phase I clinical trials for the control of asthma and rhinitis, while numerous H<sub>4</sub> receptor-targeting agents show promise for potential entry into clinical studies for the control of pain and inflammation. Taken together, the data strongly point to the H<sub>4</sub>R as a novel target for the pharmacological modulation of histamine-transferred signals and offer an optimistic perspective for the therapeutic exploitation of H<sub>4</sub>R ligands in inflammatory disorders such as allergy, asthma, chronic pruritus, arthritis, autoimmune diseases and possibly cancer. This work is part of the EU-FP7 COST Action BM0806: Recent advances in histamine receptor H4R research.

### **N-3 polyunsaturated fatty acids reduce allergic type mediator release by human mast cells in vitro via inhibition of reactive oxygen species**

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The increased ratio of n-6/n-3 polyunsaturated fatty acids (PUFA) in Western diets may contribute to the rapid increase in prevalence of allergic diseases. Mast cells (MC) are the key effector cells in allergy. The effect of different PUFA on MC activation and mediator release was investigated and the contribution of reactive oxygen species (ROS) and mitogen-activated protein kinase (MAPK) signaling routes was studied. Human MC lines (LAD2, HMC-1) were incubated for 24 hours with long chain n-6 (arachidonic acid, AA) or n-3 (eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA) PUFA. Effects of PUFA on degranulation, mediator secretion (PGD<sub>2</sub>, TNF- $\alpha$ , IL-4 and IL-13), and generation of ROS was measured. Incubation with PUFA did not reduce IgE-mediated degranulation by LAD2 cells. However, mediator release of ionomycin/PMA stimulated HMC-1 cells was differentially regulated. AA incubation increased PGD<sub>2</sub> and TNF- $\alpha$  ( $p < 0.05$ ) secretion whereas IL-13 ( $p < 0.01$  for all PUFA) and IL-4 ( $p < 0.05$  for EPA and DHA) secretion were inhibited. The reduction in IL-13 and IL-4 release by PUFA was associated with a reduction in ROS generation ( $p < 0.01$ ). Use of specific ROS inhibitor superoxide dismutase showed that IL-4 secretion is under regulation of superoxide, while IL-13 release was strongly decreased by general ROS inhibitor 1,3-dimethyl-2-thiourea (DMTU). MAPK signaling was found to contribute to IL-13 secretion by activated HMC-1 cells. MC incubation with MAPK inhibitors and PUFA showed differential effects for n-3 vs n-6 PUFA. MAPK inhibitors reduced IL-13 secretion by HMC-1 and only combined incubation of n-3 PUFA with p38 or JNK MAPK inhibitors further suppressed IL-13 production. In conclusion, n-6 PUFA enhanced production of proinflammatory mediators from mast cells, while n-3 PUFA most effectively suppressed ROS generation and IL-13 and IL-4 release. This suggests that an increase in dietary n-6/n-3 PUFA ratio may increase the susceptibility to develop allergic disease and enhance allergic inflammation.

### **Mast cell chymase modulates the response to house dust mite exposure**

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Chymase is stored as mature enzyme in mast cell granules and is released in large amounts upon cell activation and degranulation. A chymase polymorphism has been associated with allergic asthma but the role of chymase in the pathogenesis is not fully understood. In previous studies we have demonstrated that mouse mast cell protease-4 (mMCP-4) is the main chymotryptic enzyme in murine airways and that mMCP-4 can prevent the development of airway hyperreactivity in a model of ovalbumin-induced inflammation (Waern et al. J Immunol 2009, 183:6369). We here assessed the immune response and airway inflammation in mMCP-4<sup>-/-</sup> and wild type mice given repeated intranasal instillations with house dust mite (HDM) extract. We found significantly higher serum levels of IgE and higher numbers of airway eosinophils in mMCP-4<sup>-/-</sup> mice than in WT mice. Moreover, there was an increased production of IL-17A, and a tendency of increased IL-13, in cultures of HDM-restimulated splenocytes from mMCP-4<sup>-/-</sup> mice. These results suggest a regulatory role for mMCP-4 in the early sensitization process when release of mast cell proteases in response to IgE cross-linking would be

minimal. However, we found that HDM extract *per se* induced a low but significant degranulation in cultured mast cells derived from both mMCP-4<sup>-/-</sup> and WT mice. Together these results suggest that mast cell chymase is released upon HDM exposure and that chymase has a protective role in HDM-induced airway inflammation by acting as a negative regulator of allergic sensitization.

### **Airway Smooth Muscle CXCL1 Inhibits Mast Cell Migration**

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Background: Activated mast cell (MC) numbers on the airway smooth muscle (ASM) are increased in asthma. *In vitro*, more MC migrate towards the supernatants (SN) of cytokine stimulated ASM cells from asthmatics than non-asthmatics [1, 2]. In addition, non-asthmatic cell SN inhibit MC migration towards the asthmatic cell SN [2]. Thus, MC migration towards the ASM maybe under the control of inhibitory factors that are reduced or inactivated in asthma.

Aim: To identify the factor(s) released by non-asthmatic ASM cells that inhibit MC migration. Methods: Confluent, serum-starved ASM from donors with and without asthma were stimulated with Th1 (IL-1 $\beta$ , TNF $\alpha$  and IFN $\gamma$ ) or Th2 (IL-4, IL-13 and IL-1 $\beta$ ) cytokines (10ng/ml each) or left unstimulated. SN were collected after 24h and cells were counted. To identify potential inhibitory factors, cytokine profiling was conducted using cytokine protein arrays and confirmed by ELISA. HMC-1 chemotaxis towards ASM cell SN was then re-investigated after adding an identified chemokine of interest, neutralising it or blocking its receptor on MC.

Results: Only the chemokine CXCL1 was produced in greater amounts by non-asthmatic than asthmatic ASM cells. Non-asthmatic Th1 and Th2 stimulated ASM cells released 22088 $\pm$ 5975 and 21260 $\pm$ 3564 pg/10<sup>5</sup> cells whereas asthmatic cells released 4953 $\pm$ 1939 and 6943 $\pm$ 2763 pg/10<sup>5</sup> cells respectively. Adding rh-CXCL1 (50 and 100pg/ml) to either Th1 or Th2 stimulated asthmatic ASM SN inhibited MC migration significantly. Neutralising CXCL1 in asthmatic SN or blocking its receptor CXCR2 on MC did not affect MC migration. However, neutralising CXCL1 in non-asthmatic SN or blocking its receptor significantly promoted MC migration.

Conclusions: CXCL1 is a possible factor regulating MC migration to ASM. Although, further studies are needed to investigate the differential expression of CXCL1 between asthmatic and non-asthmatic cells, its reduced production by asthmatic ASM cells may directly contribute to the increased localization of activated MC on the ASM in asthma.

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### **Munc18-2 controls granule translocation in mast cells through dynamic interactions with the fusion machinery and the microtubule cytoskeleton**

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Stimulation of mast cells through high affinity IgE receptor (Fc $\epsilon$ RI) triggers the secretion of pre- and neformed inflammatory mediators through vesicular carriers. Recent studies have uncovered a variety of new effectors that link Fc $\epsilon$ RI signaling to the sophisticated machinery of vesicular trafficking and soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE)-dependent membrane fusion. This includes SNARE-interacting Sec1/Munc18 (SM) proteins shown to represent essential effectors in this process. However, it is still unknown how their interaction with SNAREs supports fusion. We show here in mast cells that specific silencing of the expression of the isoform Munc18-2 inhibits degranulation but not cytokine/chemokine release by a step that was partly independent of its SNARE partner Syntaxin3. In the absence of Munc18-2 activation-dependent secretory granule (SG) translocation to the plasma membrane was impaired, while Syntaxin3 downregulation directly inhibited fusion. Ultrastructural analysis indicated that Munc18-2 is

located on SGs and in the cytoplasm in small clusters that are part of the cytoskeletal meshwork adjoining SGs. During stimulation Munc18-2 and Syntaxin3 increasingly colocalize with clusters appearing predominantly at fusion sites. In addition, Munc18-2 interacted with the microtubule cytoskeleton in a stimulation-dependent manner, a process that was uncoupled by the microtubule-depolymerizing drug Nocodazole. Our data support a role of Munc18-2 in the organization of the topological arrangements of the membrane fusion apparatus and coupling to the cytoskeleton thereby facilitating granule translocation and SNARE-mediated membrane fusion.

### **Importin beta plays an essential role in the regulation of the LysRS-Ap<sub>4</sub>A pathway: Ag-dependent nuclear translocation of Ap<sub>4</sub>A hydrolase.**

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### **Mast cell functions revisited in Cre recombinase-mediated mast cell eradication (Cre-Master) mice**

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Keywords: mast cells, mast cell-deficient mice, gene targeting, arthritis, sepsis

Mast cell-deficient mice represent a major tool to elucidate the *in vivo* roles of mast cells. Beyond their crucial roles in anaphylaxis, mast cells are thought to be important immunological players under many physiological and pathological conditions including innate defense, antigen presentation, T-helper cell polarization, autoimmunity, or tolerance. Up to now, *in vivo* experiments were adversely affected by the fact that currently available mast cell-deficient mice are based on mutations in the pleiotropic growth factor receptor Kit. However, *Kit<sup>W/W<sup>v</sup></sup>* or *Kit<sup>Wsh/Wsh</sup>* mice suffer from multiple hematopoietic and non-hematopoietic defects, and are hence not selectively mast cell-deficient. Here, we present a novel mast cell-deficient, Kit-proficient mouse strain, generated by targeted knock-in of Cre-recombinase into the mast cell carboxypeptidase A (*Cpa3*) locus. In these Cre-mediated mast cell eradication mice (termed Cre-Master) mast cells are abrogated by Cre genotoxicity involving a p53-dependent mechanism. Connective tissue and mucosal mast cells are completely absent while other immune cells remain unaffected. Contrasting previous studies on enterobacterial peritonitis in Kit-mutant mice, Cre-Master mice respond very similar to wildtype mice with respect to inflammatory cytokine production, neutrophil recruitment, and survival. Hence, the major factor governing survival in this sepsis model is Kit rather than the presence of mast cells. Moreover, in a serum-transfer autoimmune arthritis model, Cre-Master mice are equally susceptible as wildtype littermates, while *Kit<sup>W/W<sup>v</sup></sup>* mice are protected. These major differences between Kit-mutant and Cre-Master mice call for a careful reassessment of mast cell functions *in vivo*.

### **IL-33 is a product rather than an initiator of acute IgE-mediated responses in humans**

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**Background:** Our previous studies showed that IL-33 is a potent basophil activator, enhancing IgE-induced mediator release and inducing cytokine expression in synergy with IL-3 without ever promoting degranulation by itself. (BLOOD: 113, p1526-34). A study in mice and with cultured human immature mast cells (MCs) however indicated that in the presence of high IgE IL-33 induces anaphylaxis *in vivo* and exocytosis of MCs/basophils *in vitro* (PNAS: 106, p9733-78; 2009). **Results:** To better understand the role of IL-33 in IgE-mediated immediate or late responses, we measured IL-33 in bronchoalveolar lavage (BAL) after allergen challenge of asthmatic patients. Furthermore we analyzed the potential of IL-33 to induce anaphylactic degranulation of basophils and MCs of allergic donors with high IgE titers (523-1394 ± 418 kU/l) using whole blood basophil degranulation and skin prick tests. Interestingly, after allergen provocation, IL-33 was detected early (after 10 minutes) together with tryptase but not in the late phase (after 18 hours) when IL-13 and granzyme B is elevated. This data show that IL-33 can be released rapidly and argue against the current view of IL-33 being an alarmin that requires cell necrosis for extracellular release. They are also consistent with involvement of IL-33 in regulating exocytosis. However, we did not observe basophil degranulation in whole blood with high IgE levels, nor any wheal and flare reactions in skin upon exposure to IL-33, demonstrating that basophils and skin MCs do not degranulate in response to IL-33. In conclusion our study demonstrates that IL-33 is released in early allergic reactions. However in humans, IL-33 might promote late allergic responses by acting on basophils and eosinophils rather than by inducing an acute anaphylactic degranulation of IgE-expressing cells, although we cannot exclude that MCs at other sites may behave differently.

### **Mast cells in human thyroid cancer**

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There is increasing evidence that mast cells infiltrate different human tumors. To assess the role of mast cells in human thyroid cancer, we compared the density of tryptase-positive mast cells in 96 papillary thyroid carcinomas (PTCs) versus normal thyroid tissue from 14 healthy individuals. Mast cell density was higher in 95% of PTCs (n=91) than in control tissue. Mast cell infiltrate correlated with extrathyroidal extension (P<0.001) of PTCs. Conditioned media (CM) from the thyroid carcinoma cell lines TPC1, NIM and 8505-C induced *in vitro* chemotaxis of human lung mast cells (HLMC) and both LAD-2 and HMC-1 cell lines. CM from normal thyroid primary cultures did not induce mast cell migration. CM from mast cell lines (HMC-1 and LAD2) and primary HLMC increased thyroid cancer cell invasive ability, survival and DNA synthesis *in vitro*. The latter effect was mainly mediated by two mast-cell-derived mediators: chemokines (CXCL1/GRO $\alpha$  and CXCL10/IP10) and histamine. Xenografts of thyroid carcinoma cells (8505-C) recruited mast cells LAD-2 and HMC-1 injected into the tail vein of BALB/c nu/nu mice. Co-injection of human mast cells and thyroid carcinoma cells (8505-C) accelerated the growth of thyroid cancer cell xenografts in athymic mice. This effect was mediated by increased tumor vascularization and cell proliferation and was inhibited by treating mice with sodium cromoglycate. In conclusion, our study data suggest that mast cells are recruited into thyroid carcinomas and promote proliferation, survival and invasive ability of cancer cells, possibly contributing to thyroid carcinoma growth and invasiveness.

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Selected presenting form: poster presentation.

### **Insulin like growth factor-1 and insulin increase Toll like receptor-induced Tumor necrosis factor- $\alpha$ and Interleukin-6 but reduce Interleukin-1 $\beta$ production in mast cells by activating the Phosphatidylinositol 3-Kinase pathway**

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Recognition of bacterial constituents by mast cells (MCs) is dependent, in part, on the presence of pattern recognition receptors, e.g. Toll-like receptors (TLRs). The final cellular response, however, depends on the influence of environmental factors. In the current study we tested the hypothesis that insulin-like growth factor-1 (IGF-1) and insulin modulate the TLR4-mediated production of proinflammatory cytokines, IL-6, TNF- $\alpha$ , and IL-1 $\beta$ , in murine MCs. IGF-1/insulin costimulation caused increased LPS-triggered secretion of IL-6 and TNF- $\alpha$ , but attenuated production of IL-1 $\beta$ , though all three cytokines were produced in an NF $\kappa$ B-dependent manner. The PI3K-specific inhibitor Wortmannin reverted the altered production of these cytokines in response to LPS + IGF-1 to levels obtained after LPS stimulation alone. In agreement, MCs deficient for SHIP1, a dominant negative regulator of the PI3K pathway, showed augmented secretion of IL-6/TNF- $\alpha$  and reduced production of IL-1 $\beta$  in response to LPS alone. The differential effects of IGF-1 on TLR4-mediated cytokine production were also observed in the context of TLR2 and IL-33 receptor-mediated MC activation. Importantly, these effects were observed with both bone marrow-derived MCs and peritoneal MCs, suggesting general relevance for MCs. Finally, costimulation of MCs via TLR4 and other PI3K-activating receptors, like Fc $\epsilon$ R1 and c-kit, resulted in PI3K-dependent attenuation of the IL-1 $\beta$  response. In conclusion, NF $\kappa$ B-dependent production of proinflammatory cytokines in MCs is differentially controlled by PI3K-activating ligand/receptor systems.

#### Activation induced apoptosis and cell death in purified human basophils.

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#### Background:

We have previously reported activation-related changes in FACS scatter of basophils in whole blood. With purified basophils this was followed by a loss of cells. Here we investigate activation-induced apoptosis and cell death of highly purified basophils and compare with basophile activation markers, mediators and scatter profile. In addition, the influence by IL-3 and the activation buffer was investigated.

#### Methods:

Basophils were purified from human blood (allergic/non allergic donors) and incubated with the relevant allergen in dilution series or  $\alpha$ -IgE. Following 1h the release of histamine and expression of cell surface markers CD63 and CD203c were measured. Following overnight incubation supernatants were analysed for cytokines and the cells were stained for FACS analysis with the early apoptosis marker Annexin combined with 7AAD (dead cells).

#### Results

Allergen induced activation of basophils was followed by increased apoptosis and cell death. Allergen induced activation in the presence of IL-3 was followed by increased early apoptosis compared to activation without IL-3 however the level of cell death (7AAD) was less than without IL-3. Spontaneous apoptosis was less pronounced with IL-3 added to the cultures. Activation dose response curves of apoptosis, CD63, histamine and scatter changes was comparable. IL-13 and IL-4 was induced by activation in a dose dependent manner and in highly apoptotic cells as well.

#### Conclusion

These data indicate that basophil apoptosis and cell death is induced by IgE dependent activation accompanied with a change in cell shape/FACS scatter. Future investigations are needed to investigate if this is also the case in-vivo and how this influences the role of basophils in allergic inflammation.

#### Mast cells are sensitive to apoptosis induced via the lysosomal pathway, by a mechanism involving serglycin proteoglycan

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Apoptosis induced by the lysosomal pathway involves the release of lysosomal proteases into the cytosol, leading to downstream proteolytic activation of pro-apoptotic compounds. We have previously shown that serglycin, a sulfated proteoglycan, is essential for storage of a number of proteases in mast cell secretory lysosomes (granules). We hypothesized that mast cell apoptosis induced by the lysosomal pathway is accompanied by the release of serglycin and serglycin-associated proteases into the cytosol and that these compounds may have the ability to activate pro-apoptotic components. Indeed, we show that wild-type bone marrow-derived mast cells (BMMCs) are highly susceptible to apoptosis induced by lysosomal membrane permeabilization (LMP) caused by lysosomotropic compounds as LeuLeuOMe and siramesine, whereas serglycin<sup>-/-</sup> BMMCs are largely resistant. LMP-induced apoptosis was cysteine cathepsin- and caspase-dependent, and the decreased susceptibility of serglycin<sup>-/-</sup> cells to LMP-mediated apoptosis was accompanied by defective caspase-3 activation. The apoptosis-promoting effect of serglycin appeared to involve proteases that are stored in complex with serglycin, as shown by reduced LMP-mediated apoptosis in BMMCs lacking mouse mast cell protease 4 (mMCP-4), mMCP-6 or mast cell carboxypeptidase A. Further, using different human cell lines, we found that mast cell lines are particularly susceptible to LMP-induced apoptosis and high levels of serglycin expression lead to a higher LMP-mediated apoptosis susceptibility. Together, these findings implicate serglycin as a new player in apoptosis and suggest that the mechanism by which serglycin promotes apoptosis involves effects on lysosomal/granule integrity and effects related to proteases that are stored in complex with serglycin.

#### Evidence for a hyper-responsive mast cell phenotype

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Background: Activation of mast cells via antigen-specific IgE with the subsequent release of inflammatory mediators is a critical step in the initiation of the allergic inflammatory response. However, the intensity of the allergic inflammation does not seem to be explained solely on the amount of antigen-specific IgE. Therefore we hypothesize that mast cells generated from certain mast cell related disorders such as allergen-driven atopy, may be intrinsically hyper-responsive.

Methods: Human progenitor cells from patients with atopy and from normal control donors were isolated from a small volume (100 ml) of peripheral blood and cultured in the presence of IL-3 (first week only), stem cell factor (SCF) and IL-6 for 7-8 weeks. Seven to eight weeks old mature mast cells were triggered via Fc $\epsilon$ R1 for antigen-mediated degranulation and monitored by the release of  $\beta$ -hexosaminidase.

Results: Antigen-mediated degranulation was enhanced in the mast cells generated from the atopic patients compared to control derived mast cells. Unlike in the mast cells from control individuals, SCF modestly enhance this response. In addition, mast cells obtained from atopic patients showed an increase in rate of growth and survival compared to mast cells from control donors.

Conclusion: This study illustrates that the reactivity of mast cells generated from the blood of specific patient populations may vary in their reactivity, possibly influencing the severity of mast cell-dependent diseases. Furthermore, our protocol has great potential in studies evaluating mast cell related functions in different patient populations.

#### Rab GTPases and Mast Cell Exocytosis

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Regulated exocytosis is a central step in mast cell function effecting the release of a variety of pro-inflammatory and immunomodulatory substances. The latter mediate the physiological functions of mast cells in innate and adaptive immunological responses as well as their pathological functions in allergic and inflammatory reactions. However, the precise mechanisms that govern and coordinate mast cell exocytosis are largely unresolved. We analyzed the role of Rab GTPases in this process. Seventeen Rabs were found to localize to the secretory granules, either permanently, or transiently in response to activation of exocytosis. Four Rab GTPases were identified as coordinators of the spatial location of the secretory granules. Finally, twenty-two Rab GTPases were identified as modulators of regulated exocytosis, the contribution of eighteen of which was hereto forth unrecognized. This group of Rabs includes GTPases that are implicated in endocytic recycling through the pericentriolar recycling endosome, therefore marking this compartment as an essential site for mast cell exocytosis. Direct quantitative monitoring of individual cells revealed heterogeneity both in cells responsiveness to external signals and their robustness to experimental perturbations. Taken together, our results indicate the tight connections that link the exocytic and endocytic systems in mast cells and provide significant insights into the mechanisms that govern the biogenesis and acquisition of secretion competence of the secretory granules of mast cells.

#### **Mast cells are critical for the limitation of thrombin-induced inflammation**

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The serine protease thrombin is a major player in the coagulation cascade and is known to act via proteinase-activated receptors (PARs). Lately, it was shown that thrombin can have proinflammatory effects on different cell types and that PARs are expressed by many cells including mast cells (MCs), highly inflammatory cells that are located around blood vessels and critically contribute to skin inflammation. We, therefore, investigated the effects of thrombin on MCs. *In vitro* we examined murine bone marrow-derived mast cells (BMMCs), peritoneal cultured MCs (PCMCs), and freshly isolated peritoneal mast cell (PMC). Thrombin stimulation resulted in a dose-dependent degranulation of PCMCs and PMCs but not BMMCs. Furthermore, we used specific PAR1 and PAR4 agonistic peptides and found that PCMC but not BMMCs degranulate in response to these peptides. Quantitative PCR analyses of BMMCs and PCMCs displayed expression of all three thrombin receptors, PAR 1, 3, and 4 with the highest expression rate for PAR1. The intracutaneous injection of thrombin into ears of C57BL/6 mice resulted in a strong degranulation of MC assessed by quantitative histomorphometry. Thrombin injection also induced significant immediate inflammatory skin reactions in C57BL/6 mice. Surprisingly, this ear swelling was more pronounced in MC-deficient C57BL/6 KitW-sh/W-sh mice, and reconstitution of C57BL/6 KitW-sh/W-sh mice with C57BL/6 BMMCs normalized this effect. This suggests that MCs are necessary for the termination of thrombin-induced inflammatory responses. Interestingly, we also found that mast cell supernatant can degrade thrombin *ex vivo*. In summary, our data show that thrombin-induced immediate inflammatory skin reactions are controlled by cutaneous MC. Therefore, the regulation of mast cell function may be a promising target for treating injury-associated inflammation.

Functional implication of the Lnk adaptor protein in Stem Cell Factor-dependent mast cells migration.

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The Lnk adaptor protein is mainly expressed in the haematopoietic system. It possesses a potential dimerization (DD), a PH and SH2 functional domains and a conserved C-terminal tyrosine phosphorylation site that allow its interaction with different signalling effectors. Mice deficient for this protein have demonstrated its role as a negative regulator of signalling pathways controlling the proliferation of haematopoietic stem cells (HSC), myeloid and B-lymphoid progenitors. This phenotype is partly due to hypersensitivity to several cytokines and growth factors, notably Stem Cell Factor (SCF). Our previous studies have implicated for the first time Lnk as an important inhibitor of SCF-dependent migration of primary mast cells. However, the molecular mechanism underlying this regulation has not been identified.

To analyze the functional contribution of the different Lnk domains to Kit-dependent cell migration, we used Lnk-deficient bone marrow-derived mast cells (BMMCs) expressing wild-type or Lnk mutated forms as our cellular system. First, we examined the effect of Lnk on the actin reorganization and cell spreading by immunofluorescence. Our results showed that the Lnk SH2 domain is important for inhibiting SCF-mediated actin polymerization and cell spreading of BMMC.

In order to dissect at the molecular level the mechanism by which Lnk negatively regulates Kit-dependent cytoskeleton reorganization and cell migration, we examined the activation of two signalling effectors involved in these processes, the SHP-2 phosphatase and the Vav1 protein. Our results showed that Lnk down-regulates SCF-mediated activation of both molecules. Interestingly, our preliminary data suggest that Vav1 and Lnk interact in a SCF-independent fashion; we are currently examining which domains are involved in this association.

Altogether, our findings suggest that Lnk down-regulates specific SCF-dependent signalling pathways, implicated in cytoskeleton rearrangement and migration of primary mast cells.

#### **The role of the CCL2/CCR2 axis in mouse mast cell migration *in vitro* and *in vivo*.**

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Tissue-resident mast cells (MCs) are important in allergic diseases. In a mouse model of allergic airways inflammation, an increase in peribronchiolar MCs was associated with increased concentrations of the chemokine CCL2 in lung lavage. MC progenitors (MCps) arising in bone marrow (BM) are recruited to tissues by transendothelial migration, and we found that CCL2 is chemotactic for MCps in freshly isolated BM *in vitro*. Immature, but not mature, BM-derived MCs migrated in response to CCL2 when cultured in IL-3+stem cell factor (SCF) but not when cultured in IL-3 alone. However, the cells under both culture conditions expressed mRNA for CCR2, the receptor for CCL2, and bound the radiolabeled chemokine with similar affinities, highlighting SCF as a key mediator in coupling CCR2 to downstream events, culminating in chemotaxis. A cytoplasmic protein, FROUNT, which has previously been shown to have an essential role in CCR2-mediated chemotaxis of human cells, was significantly up-regulated in IL-3 and SCF cultured BMMC compared to IL-3 cultured BMMC. Immature BM-derived MCs from IL-3 +SCF cultures, when administered *i.v.*, accumulated at skin sites injected with CCL2 *in vivo*. MCP recruitment to the allergen-sensitized/challenged lung was significantly reduced in CCR2(-/-) and CCL2(-/-) mouse strains. However, reconstitution studies of sublethally irradiated and BM-reconstituted mice indicated that BM cells and stromal elements could provide CCL2, whereas the CCR2 function resided with stromal elements rather than BM cells. These experiments revealed a new function of SCF in chemokine receptor coupling, but they suggest a complex role of the CCL2/CCR2 axis in recruiting MCps during pulmonary inflammation.

#### **Mast Cells influence Lewis Lung Cancer growth in a mast cell deficient $W^{sh/sh}$ mouse model.**

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Mast cells are known to accumulate around tumors and are in many cases associated with poor outcome of the disease. But there are also studies that claim the opposite implying that the mechanisms behind the mast cell influence on tumor growth and progression, both stimulating and inhibiting effects, are not yet completely illuminated.

To study the mechanisms behind mast cell and tumor interaction *in vitro* we have been using the murine Lewis lung carcinoma (LLC) cell line and bone marrow derived mast cell (BMMCs). BMMCs were seeded in Transwell® migration

chambers and their migration towards LLC conditioned medium was monitored with Calcein AM. The BMCCs showed an increased ability to migrate towards LLC conditioned medium compared to control medium.

In order to study the mast cell effect on LLC growth we have established an *in vivo* model where 50 000 LLC cells were s.c. injected on the flank of either C57/B6 mice or mast cell deficient *W<sup>sh/sh</sup>* mice. The resulting tumors showed a difference in tumor weight between the groups, with the tumors from *W<sup>sh/sh</sup>* mice being significantly smaller.

Our *in vitro* data show that LLC cells secrete factors that attract mast cells and our *in vivo* findings indicate that mast cells supply a favorable environment for LLC tumor growth. Both models will provide an opportunity to further study mechanisms behind mast cell and tumor interaction.

Poster presentation is preferred. Suggested Category: 4. Mast cell and basophil migration, proliferation and apoptosis

#### **Menstrual cycling affects disease activity in chronic spontaneous urticaria**

Markus Magerl, Katharina Cierpinsky, Dina Pisarevskaja, Martin Metz, Marcus Maurer

**Introduction:** Menstrual cycling is widely held to modulate disease activity in patients with chronic spontaneous urticaria. In our clinical experience, many women report aggravation of urticaria symptoms shortly before or during menstruation. However, evidence backing this assumption is very limited. In this study we monitored urticaria activity in 20 women (aged 21-52, median 37.5) with chronic spontaneous urticaria during their menstrual cycle. To avoid bias, the subjects were asked about several aspects of their condition and they were not informed about the aim of the observation. **Results:** Urticaria activity showed significant menstruation cycle-dependent changes as assessed by use of the urticaria activity score (UAS). UAS values showed a maximum during menstruation (week 1, mean UAS 2.79 +/- 1.70), and a minimum in week 3 (usually after ovulation, mean UAS 1.94 +/- 1.42,  $p < 0.001$ ). Also, combined UAS values of weeks 4 and 1 (before and during menstruation, mean UAS 2.7 +/- 1.64) were higher than the combined UAS values of weeks 2 and 3 (before, during and after ovulation, mean UAS 2.17 +/- 1.39,  $p < 0.002$ ).

**Conclusion:** Disease activity in patients with chronic spontaneous urticaria changes significantly with menstrual cycling and is highest during menstruation. Further investigations are needed to characterize on the underlying mechanism and management implications.

#### **Pediatric mastocytosis is a clonal disease associated with c-kit extracellular domain mutations that have different functional and signalling properties compared with kit-phototransferase domain mutations**

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Adult mastocytosis is a non curable clonal disease associated with c-KIT mutations, mostly a point mutation in exon 17 (at codon 816), located in the phosphotransferase domain (PTD) of the receptor. In contrast, pediatric mastocytosis often spontaneously regresses and is considered as a reactive disease. Previous studies on childhood mastocytosis assessed only few patients and mostly focused on codon 816 mutations, with various results. Here, we analyzed the entire c-KIT sequence from cutaneous biopsies of 50 children with mastocytosis (age 0 to 16 years). Mutation of codon 816 (exon 17) was found in 42% of the cases, while mutations outside exon 17 were observed in 44%. Unexpectedly, these mutations were located in the fifth immunoglobulin loop of c-KIT's extracellular domain (ECD), which is encoded by exons 8 and 9.

In a second time, KIT-ECD versus KIT-PTD mutants were introduced into rodent Ba/F3, EML, Rat2, and human TF1 cells to investigate their biologic effect. Both ECD and PTD mutations induced constitutive receptor autophosphorylation and ligand-independent proliferation of the three hematopoietic cells. Unlike ECD mutants, PTD mutants enhanced cluster formation and up-regulated several mast cell-related antigens in Ba/F3 cells. PTD mutants failed to support colony formation and erythropoietin-mediated erythroid differentiation. ECD and PTD mutants also displayed distinct whole-genome transcriptional profiles in EML cells. We observed differences in their signaling properties: they both activated STAT, whereas AKT was only activated by ECD mutants. Consistently, AKT inhibitor suppressed ECD mutant-dependent proliferation, clonogenicity, and erythroid differentiation. Expression of myristoylated AKT restored erythroid differentiation in EML-PTD cells, suggesting the differential role of AKT in those

mutants. These findings strongly support the idea that, although pediatric mastocytosis can spontaneously regress, it is a clonal disease most commonly associated with activating mutations in c-KIT but differences observed in signalling might explain their diverse phenotypes.

#### **MAST CELL AND BASOPHIL DRIVEN DISEASES Clinical presentation of clonal and non-clonal Systemic Mast Cell Activation Disorders**

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**Funding:** supported by grants from the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (FIS060529 and PS09/00032); Junta de Comunidades de Castilla La Mancha (FISCAM 2007/36) Systemic Mast Cell Activation Disorders in the absence of mastocytosis in the skin are characterized by severe, even life-threatening, and recurrent symptoms such as generalized pruritus, hives, flushing, tachycardia, abdominal pain, diarrhea, syncope or near-syncope episodes; with increased or normal serum tryptase, with or without trigger (s), with or without specific IgE against the suspected trigger (s). The presence of clonal mast cells in a subset of patients with idiopathic recurrent anaphylaxis has been reported and named monoclonal mast cell activation syndrome.

The prospective study of large series of SMCAD by our group<sup>5</sup> allow us the possibility to clearly identify two different molecular subgroups among Systemic Mast Cell Activation Disorders; i) clonal cases identified by the presence of activating KIT mutations ii) non-clonal cases. The vast majority of the clonal cases fulfilled the WHO diagnostic criteria for SM and thus, they were classified as indolent systemic mastocytosis in the absence of skin lesions; while a small percentage carry KIT mutation in the absence of other criteria for SM. Our experience, in a large series of cases confirms an association between the presence of anaphylaxis (or near anaphylaxis) with both urticaria and angioedema usually in the absence of cardiovascular symptoms and nc-SMCAD, while indolent systemic mastocytosis should be suspected in male patients with recurrent anaphylaxis who present with syncope or near-syncope episodes usually without associated hives or angioedema.

The aim of our presentation is to provide prospective data on the clinical, biological, immunophenotypic and molecular characteristics of Systemic Mast Cell Activation Disorders in the absence of skin lesions as well as to present a clinical score and a diagnostic algorithm to separate clonal vs. non-clonal cases on the basis of demographic and clinical data together with serum tryptase levels. (for bibliography, see: Alvarez-Twose et al., J.Allergy Clin Immunol. 2010 Jun;125(6):1269-1278)

#### **BAT-on-a-chip: Optimisation of conditions for the use of a cell line in a live basophil allergen array.**

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We have previously demonstrated as proof-of-principle that purified human peripheral blood basophils stripped and re-sensitised with an allergic donor's serum can bind to a protein array containing appropriate allergens, and that activation can be detected using an antibody to CD63. Our aim is to develop a high-throughput diagnostic device which combines the advantages of basophil activation tests (e.g. clinical relevance) with the numerical power of microarrays. Such a device would be useful for pre-screening a large number of candidate allergens, using either appropriate extracts or recombinant molecules.

In order to avoid the costs (reagents for purification), logistics (large blood donations, long experimental procedure) and intrinsic difficulties (use of anti-allergic medication, non-responder status of donors) associated with the use of peripheral blood basophils, we sought to replace the human cells with a human or humanised basophil or mast cell line.

Four different clones of the human KU-812 basophil-like cell line, grown in the presence of various combinations of cytokines (IL-3, IL-3+IL-4, IL-3+IL-4+GM-CSF) for up to 21 days, showed no significant morphological changes (granulation), no detectable FcεRI expression at the protein level and did not bind to the array. Binding and incubation parameters (buffer, incubation time, temperature, cell density, washing steps, non-specific binding blocking reagents, addition of extracellular matrix components) were experimentally optimised for human LAD-2 mast cells and the humanised rat cell line RBL703/21 (obtained from the Paul Ehrlich Institute in Langen, Germany). This has resulted in a reproducible protocol

for basophil binding achieving a good compromise between low background binding and retention on the array during the washing steps.

LAD-2 demonstrated very low FcεRI occupancy and inconsistent cell activation, as well as the lack of binding to the extracellular matrix components tested. Activation of RBL703/21 measured via CD63 upregulation, in contrast to peripheral blood basophils, showed unfavourable signal-to-noise ratio. Alternative ways for detection of activation were assessed, such as Annexin V binding, Calcium influx monitoring using OregonGreen and fluorescent inducible reporter systems (NFAT-EGFP and RnIL-4p-DsRed). The on-going progress in the development of the in vitro test will be discussed

### Psychic Co-morbidity in adult Patients with Mastocytosis

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In mastocytosis, several medical conditions like risk of anaphylaxis, chronic pruritus, diarrhoea, osteoporosis and others can alter physical but also mental health. In this study anxiety, quality of life, and general burden was examined in comparison with patients with anaphylaxis due to insect venom allergy. For each group, 54 patients, matched by sex and age were enrolled. Psychic symptoms were recorded by questionnaires STAI, PHQ-D, FKB-20, and SF-36. Patients with mastocytosis show higher psychological burden compared with patients with insect venom allergy. Mastocytosis patients demonstrate significantly higher anxiety levels in the STATE (p=0.009) and TRAIT scale (p=0.003) of the STAI questionnaire and their perception of stress was also significantly elevated as detected by the PHQ stress scale (p=0.011). Quality of life is significantly reduced in mastocytosis patients as detected by lower values for vitality (p=0.039) in SF-36 and vital body dynamic (p=0.035) in FKB-20. Mastocytosis patients seem to be exposed to increased distress and have a lower quality of life. Health care of mastocytosis patients should focus more detailed on these aspects of the disease.

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### Mast cells play distinct roles in the pathogenesis of allergic airway inflammation and hyperresponsiveness

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Mast cells are main effector cells of allergic responses, e.g. in allergic asthma. However, their role in the pathogenesis and their contribution to hallmarks of chronic allergic airway diseases is less well known. We investigated mast cell-deficient C57BL/6-Kit<sup>W-sh/W-sh</sup> mice, bone marrow-derived mast cell-reconstituted C57BL/6-Kit<sup>W-sh/W-sh</sup> mice and wild-type controls in a model of chronic allergic airway inflammation, in which mice were sensitised towards the model allergen ovalbumin together with adjuvant intraperitoneally, and inflammation in the airways was induced by repetitive intranasal allergen instillations during a period of 91 days. Sensitisation measured as plasma levels of allergen-specific IgE was successful in each experimental group. Induction of allergic inflammation increased IgE levels in a mast cell independent manner. However, mast cell-reconstitution further amplified IgE production. Cellular allergic airway inflammation, measured as total cell counts in bronchoalveolar lavage fluid, as well as eosinophil and neutrophil counts, was most pronounced in mast cell-deficient mice, suggesting a protective role of mast cells in the manifestation of chronic inflammation.

Interestingly, measurement of airway hyperresponsiveness using forced-oscillation technique revealed an increased resistance of the central airways (Rn) and tissue damping (G) in mast cell-reconstituted animals compared to wild-type controls. At the same time, tissue resistance (H) was strongest in wild-type mice, and reduced to the negative control levels in mast cell-deficient mice. This suggests that mast cells may modulate processes leading to airway hyperresponsiveness by their mere number, their tissue distribution, and possible also their phenotype. Further investigations will answer these questions in more detail.

### A descriptive analysis of oral drug challenges in Non Steroidal Anti-Inflammatory Drug (NSAID)- induced urticaria, angioedema and anaphylaxis

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**Background:** NSAID-induced urticaria, angioedema and anaphylaxis are common but not well-characterized clinical syndromes

**Objective:** To study the clinical characteristics of NSAID-induced acute cutaneous and anaphylactic reactions through a structured approach to oral drug challenges, with particular emphasis on selective NSAID hypersensitivity.

**Methods:** Retrospective analysis of the drug challenge results of all patients with NSAID-induced urticaria, angioedema or anaphylaxis presenting to the Royal Adelaide hospital between February 2006 and June 2010. Oral drug challenges were either with homologous NSAID to confirm diagnosis or with heterologous NSAID to explore cross-reactivity or safe therapeutic options. Aspirin exacerbated respiratory disease was excluded.

**Results:** 68 patients (53 females, 15 males, mean age 48.3) reported a total of 77 instances of defined NSAID-induced reactions of which 62% were purely cutaneous and 35% were anaphylaxes. Ibuprofen was most commonly incriminated (35%) whereas most cases of anaphylaxis were related to diclofenac (48%). 40 patients underwent a homologous challenge of which 17 were positive. Presentation with anaphylaxis, shorter reaction-to-challenge time and challenges with diclofenac were main predictors of a positive challenge. 28 patients had a challenge with heterologous NSAID, including 21 aspirin challenges (7 positive). Overall structured challenges enabled us to identify 23 (34%) selective Reactors (SR), 19 (28%) Cross Reactors (CR) and 23 (34%) NSAID-tolerant patients. SR presented most often with anaphylaxis and were otherwise healthy except for some with a background of B-lactam allergy. CR in contrast often had a background of chronic urticaria or asthma and rather than anaphylaxis, presented with milder cutaneous reactions especially isolated periorbital angioedema.

**Conclusion:** A structured approach to drug challenges allows identification of selective and multiple NSAID hypersensitivity syndromes and establishes a safe therapeutic alternative. Most cases of NSAID-induced anaphylaxis represent selective hypersensitivity and hence do not require avoiding other NSAID.

### A role for mast cell-derived IL1B in autoinflammatory diseases

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Within the last decade the spectrum of autoinflammatory diseases has been widely extended. In addition to the rare hereditary periodic fever syndromes, autoinflammatory mechanisms have been identified in various complex inflammatory disorders such as gout or Crohn's disease. In many of these diseases IL1B functions as the key mediator of disease activity, and blocking IL1B leads to effective symptom control. Recently, the source of IL1B production has been linked to skin mast cells in patients with Cryopyrin-associated periodic syndromes (CAPS). Urticarial exanthema in CAPS is unresponsive to antihistamines but may be treated successfully with anti-IL1B neutralising drugs.

Another rare autoinflammatory disorder, the Schnitzler syndrome (SchS) is also characterized by urticarial skin lesions. Additionally, these patients present with monoclonal gammopathy, arthralgias, bone pain, lymphadenopathy and episodes of fever. We hypothesized that similar to CAPS, IL1B is responsible for the pathology in SchS. Therefore, we treated 8 patients with SchS formerly unresponsive to antihistamines, NSAIDs and immunosuppressives with the anti-IL1B receptor antagonist anakinra. Anakinra was well tolerated and highly effective in reducing the clinical symptoms and inflammation markers in the majority of patients (6/8) on long-term treatment.

Here we provide evidence of very effective disease control by blocking IL1B. The potent control of symptom development by anakinra points to a major role of IL1B in the pathogenesis of SchS. It can be speculated that the source of IL1B production in SchS are skin mast cells, further investigations are however needed to verify this hypothesis.

## The immunophenotypical criterion for systemic mastocytosis revisited: the role of CD2

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The term mastocytosis denotes a heterogeneous group of disorders characterized by abnormal proliferation and/or accumulation of pathological mast cells (MC) in one or multiple organs such as the skin or bone marrow (BM). Since diagnostic criteria for skin involvement have not been described yet, BM biopsy and aspirate are mandatory for diagnosis and classification of the patients.

In recent years, major methodological-related advances have been made regarding the diagnosis of tissue involvement in mastocytosis such as the immunohistochemical assessment of MC tryptase, the immunophenotypical characterization of BMCC using flow cytometry (FCM) and the molecular studies to detect activating (and other) c-kit mutations. Despite these advances, WHO diagnostic criteria have not been modified and it includes as a minor criterion the detection of CD25 and/or CD2 positive MC in BM or blood or other extracutaneous organs.

First studies on the immunophenotype of BMCC supported the notion that co-expression of CD2 (LFA-2) and CD25 antigens represent an aberrant hallmark of BMCC from adult mastocytosis since they were co-expressed in MC from patients with indolent systemic mastocytosis (SM) but not in normal BMCC. However, further studies in larger series of SM patients revealed that CD2 is not positive in all cases and, when positive, may present different patterns of expression. The pattern of CD2 expression, in association with other aberrant immunophenotypic features and mutational patterns correlates with different disease outcomes.

In conclusion the immunophenotypical criterion for SM should be: "expression of CD25 with or without expression of CD2".

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## Mast cells foster adenocarcinoma development while contrasting anaplastic variants in prostate carcinogenesis

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Prostate carcinoma is most often a multifocal disease, with areas of localized, well-differentiated adenocarcinoma coexisting with poorly differentiated lesions within the same tumor. Mast cells (MC), classically known as the primary responders in allergic reactions, have recently come to the limelight as important players either in cancer promotion or inhibition, depending on cancer type. We have evidence of such dual role in prostate cancer. Within the same human tumor, MC are specifically enriched in areas of adenocarcinoma, whereas lacking around anaplastic foci. This observation has been confirmed in spontaneous tumors from TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) mice and in two novel tumor cells lines, derived from them, phenocopying well- and poorly differentiated adenocarcinoma: T1525 and T23, respectively. In line with the observations on human and murine spontaneous tumors, MC do not impact the anaplastic T23 tumor growth in vivo, but they do promote the progression of the well-differentiated adenocarcinoma T1525, that does not develop when transplanted into MC-deficient mice, or when MC are pharmacologically stabilized with sodium cromoglycate. MMP-9 protease is necessary for MC pro-tumorigenic role: accordingly, tumors can develop in MC-deficient mice reconstituted with wt MC, but not in those reconstituted with MC from MMP-9ko donors. Strikingly, pharmacological inhibition or genetic ablation of MC in TRAMP mice result in early development of undifferentiated tumors characterized by marked anaplasia and epithelial-to-mesenchymal transition, suggesting that the growth of anaplastic clones may be even antagonized, and not just unaffected, by MC.

Our data demonstrate that MC can influence the histotype of tumors arising from the prostate and that their targeting may change tumor outcome. A possible therapeutic corollary is that MC depletion may favor the growth of undifferentiated and aggressive tumor variants.

## Mast cell reconstitution in mast cell deficient C57BL/6-Kit<sup>W-sh/W-sh</sup> mice alters lung mast cell distribution in a model of chronic airway inflammation

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Mast cells play an important role in chronic inflammatory diseases such as allergic asthma. On the other hand, little is known about the number, tissue distribution and phenotype of mast cells in healthy and inflamed conditions, and how the heterogeneity of mast cells could influence symptoms and severity of the disease.

We addressed this question applying a mouse model of chronic allergic airway inflammation. Wild-type C57BL/6, mast cell deficient C57BL/6-Kit<sup>W-sh/W-sh</sup> and C57BL/6-Kit<sup>W-sh/W-sh</sup> mice reconstituted with bone marrow-derived mast cells were sensitized and challenged with ovalbumin following a 91-day protocol. Lung sections were studied using histological methods to detect and characterise mast cells, inflammation and remodelling.

Inflammatory cell infiltration in the lungs was significantly increased in sensitized and challenged mice in all the groups compared to controls. Interestingly, there was also a significant increase in inflammatory cell infiltration in control C57BL/6-Kit<sup>W-sh/W-sh</sup> mice compared with wild-type controls. This suggests a protective role of mast cells in the airways during chronic inflammation. The number of mast cells in the lungs was significantly increased in reconstituted mice compared to wild-type mice. Mast cells in wild-type mice were located in central airways, while in reconstituted mice they were prominently sited in parenchyma and also in smaller airways, pointing to a different homing of mast cells to the lung tissue. Consequently, this homing of mast cells in the lungs might alter immune responses. Importantly, this finding suggests that mast cell reconstitution causes a completely new condition, which might not be comparable to the wild-type and therefore not serve as an adequate control. Further investigations on the phenotype of the lung mast cells in reconstituted mice are currently in progress.

## Peripheral histamine 1-receptor antagonists effectively reverse stress-induced visceral hypersensitivity in a rat model of maternal separation.

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BACKGROUND: Irritable bowel syndrome (IBS) is in part characterized by increased sensitivity to colorectal distension. We previously showed that the mast cell stabilizer ketotifen reduces IBS symptoms and increases the threshold of discomfort (to rectal distension) in hypersensitive IBS-patients. However, as ketotifen also has histamine (H)1-receptor antagonistic properties and is known to penetrate the blood-brain barrier, its beneficial effect observed in this trial could also result from H1 receptor blockade, either at the peripheral or central level.

AIM: To establish whether fexofenadine and ebastine, two peripherally restricted H1-receptor antagonists, are able to reverse stress-induced, mast cell dependent visceral hypersensitivity in a rat model of maternal separation (MS). The mast cell stabilizer doxantrazole was used a positive control.

METHODS: Adult MS and nonhandled rats (n=9 in all groups) were subjected to acute stress (1 hour water avoidance, WA). The visceromotor response (VMR; abdominal contractions induced by 1, 1½, 2ml colorectal distensions and quantified by EMG) was established pre- and 24 hours post-WA and expressed as area-under-curve (AUC, volume-vs-response, significant difference when P<0.05\*: Wilcoxon). Rats were then treated, for 24 hours, with fexofenadine (1,8 mg/kg), ebastine (1 mg/kg), doxantrazole (10 mg/kg) or vehicle alone (all i.p.) and re-evaluated for colonic sensitivity changes.

RESULTS: WA did not induce increased sensitivity to distension in nonhandled rats (AUC pre- vs post-WA; 66±3 vs 70±5) but induced increased VMR in all MS-groups. Subsequent treatment with fexofenadine, ebastine and doxantrazole reversed established visceral hypersensitivity (pre-WA vs post-WA vs post-WA+treatment): (64±4 vs 98±6\* vs 69±5\*), (69±5 vs 120±8\* vs 67±3\*) and (70±2 vs 103±8\* vs 80±5\*) respectively. Vehicle alone was unable to reverse post-WA hypersensitivity to distension.

CONCLUSION: Our data confirm that mast cell activation is a key event in stress-induced visceral hypersensitivity and suggest that histamine is the most important mast cell mediator in this process. Since low dose fexofenadine and ebastine (as used here) do not cross the blood brain barrier and will, therefore, not induce unwanted central side-effects,

these data justify future IBS patient trials with 2<sup>nd</sup> generation H1-receptor antagonists.

### **MN8001, a dendritic polyglycerol, inhibits type I allergic responses**

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

### **Dietary n-3 polyunsaturated fatty acids suppress the allergic immune response but do not affect mast cell degranulation in a mouse model for cow's milk allergy**

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Cow's milk allergy is the most common food allergy in children and no effective treatment is available. N-3 polyunsaturated fatty acids (PUFA) may prevent allergic disease. Aim of this study was to assess the contribution of mast cells to the whey-specific acute allergic skin response and to study the effects of dietary supplementation with n-3 PUFA on prevention of food allergy. Mice were fed a 4% soy oil/6% tuna oil diet rich in n-3 PUFA or a control diet (10% soy oil) before and during oral sensitization with whey. Serum immunoglobulins and mouse mast cell protease-1 (mMCP-1), percentages (%) splenic Th1, Th2 and regulatory T cells (Treg) and the acute allergic skin response (ear swelling) were measured. Administration of the mast cell stabilizer cromolyn prior to whey challenge, was found to reduce the acute skin response and anaphylaxis score, demonstrating the involvement of mast cells in the allergic effector response. No effect of n-3 PUFA on mast cell degranulation was observed as assessed by ear swelling and mMCP-1 measurements. However, a strong reduction in whey-specific IgG<sub>1</sub> levels in serum was observed in the n-3 diet group, and IgE showed the same tendency. Hence, the induction of the Th2 type humoral response was suppressed. Furthermore, only mice fed the n-3 diet had a higher % Treg in spleen after sensitization as compared to sham mice. In addition, both % Th1 and Th2 cells were reduced; this was most pronounced in sham mice and resulted in an increased Th1/Th2 ratio. In conclusion, humoral and T-cell responses suggest that dietary n-3 PUFA skew away from the allergic phenotype in a murine model for food allergy; however the allergic effector response was not suppressed.

### **Stress-induced colonic afferent activation in rat depends on mast cell degranulation which is triggered by peripheral CRF and free immunoglobulin light chains**

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**Introduction:** Irritable bowel syndrome is in part characterized by increased sensitivity to colorectal distension. In our maternal separation (MS) model we showed that this visceral hypersensitivity is triggered by stress-induced degranulation of mucosal mast cells. Peripheral corticotrophin releasing factor (CRF) was implicated in their activation but CRF receptor antagonists were ineffective in clinical trials. Here we investigate the contribution of CRF and free Immunoglobulin Light Chains (IgLcs) in (acute & chronic) stress-induced visceral hypersensitivity.

**Methods:** Adult MS and nonhandled (NH) rats were subjected to 1 hr water avoidance stress (WA). The visceromotor response (VMR) to colorectal distension was established pre- and post-WA and expressed as area-under-curve (AUC, volume-vs-response, significant P<0.05: Wilcoxon). Receptor-antagonist  $\alpha$ -helical CRF was administered immediately before- or 15 days post-WA and compared with post-WA administration of the mast cell stabilizer doxantrazole and peri-WA application of the IgLC antagonist F991 (all administrations i.p. & all groups n=9).

**Results:** WA lead to increased VMR to distension in MS vehicle-treated rats (pre-WA vs post-WA AUC; 65 vs 93, P\*) but not in NH rats. Doxantrazole reversed post-WA hypersensitivity (101 vs 80, P\*) whereas vehicle treated rats remained hypersensitive. Established WA-induced hypersensitivity (70 vs 98, P\*) could not be reversed by  $\alpha$ -helical CRF treatment (98 vs 100, ns) but pre-WA  $\alpha$ -helical CRF (68 vs 71, ns) and peri-WA F991 (65 vs 69, ns) both prevented WA-induced visceral hypersensitivity.

**Conclusion:** Our results indicate that mast cell degranulation in acute but not in chronic phase depends on CRF. Instead, and although IgLC-data are far from conclusive, our results may suggest that humoral antigen-specific mechanisms are involved in chronic post-stress mast cell activation. This may explain why recent patient trials with CRF-receptor antagonists were unsuccessful.

### **Co-factor dependent anaphylaxis driven by innate immune signals is mediated by basophils**

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It is well known that some forms of anaphylaxis depend on co- or augmentation-factors, best documented for wheat-dependent exercise-induced anaphylaxis. Other well documented co-factors are alcohol consumption or infections. However, how infections trigger anaphylaxis is still enigmatic. To analyze how innate immune signals may augment systemic anaphylaxis, mice were actively sensitized with Ovalbumine (OVA) and challenged with titrated doses of OVA to determine the threshold dose of OVA eliciting systemic anaphylaxis. Anaphylaxis was measured by detecting core body temperature decrease, decrease in blood pressure and serum histamine levels. Challenge with the OVA dose just below threshold resulted in a weak response (-1,92±0,26°C), while pretreatment of mice with different pathogen-associated-molecular-pattern (PAMPs) triggered full-blown anaphylaxis with fast decline in body temperature and significantly increased temperature drop of around -4°C. PAMP pretreatment significantly reduced blood pressure (-25,3±8,8mmHg versus +1,8±6,1mmHg following PBS pretreatment) and increased serum histamine levels (174±0,2ng/ml versus 98,7±27,2ng/ml). To identify underlying mechanisms, in vitro studies were carried out. However, PAMPs failed to augment mediator release from mast cells (MC) generated from murine bone marrow or fetal skin as well as from peritoneal-MCs. Moreover, investigating MC-deficient Kitw-sash mice (sash), we observed unaltered co-factor dependent anaphylaxis: Sash mice showed the same PAMP-dependent reaction (-4,7±0,87°C) as WT mice (-4,06±0,59°C) in contrast to PBS pretreatment (sash: -1,52±0,39°C; WT: -1,38±0,44°C). In addition, co-factor dependent anaphylaxis was completely abrogated following basophil depletion. In conclusion, we present for the first time a model, which allows investigation of co-factor dependent anaphylaxis. In this model innate immune signals were sufficient to elicit full-blown anaphylaxis in response to low doses of antigen. Surprisingly, co-factor dependent anaphylaxis was independent of mast cells but dependent on basophils. Our results for the first time show a mechanism how infections trigger anaphylaxis. This is of major clinical importance and may lead to new therapeutic strategies.

### **Mast cells as target in cancer therapy**

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**Purpose:** Cancer growth and inflammation are closely related processes. At present it is believed that infiltration of immune cells strongly contributes to tumor cell proliferation and metastasis. Recently mast cells have been identified as an important cell type in growth of pancreatic carcinoma in a murine model. We have investigated human cancer biopsy specimens for the presence of mast cells as a target for therapeutic intervention.

**Methods:** On tissue microarrays of cancer biopsies, also known as tissue chips, the clinical relevance of potential biological targets for diagnosis and therapy can be evaluated. The tissue chips(Biomax, USA) we have used are made from cores (range 12-210 cores/slide), of 5µm thickness and diameters of 1.5mm or 1mm. Each core represents one specimen providing an overview of influence of different ages (range 20-90), comparison of normal, malignant, metastasized, benign, adjacent, and inflamed tissues. Tissue arrays were immunostained for mast cells. Positive cells were quantified under a microscope.

**Results:** Mast cells were especially prominent in breast cancer, lung cancer, skin cancer and pancreatic cancer.

**Conclusion:** Breasts, lung, skin and pancreatic cancer types seem especially promising for mast-cell directed anticancer therapy.

**Keywords:** Mast cells, Tissue microarrays, cancer

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#### Protective role of mast cells and mouse mast cell protease-4 in renal fibrosis

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Chronic kidney disease (CKD) involves an inflammatory process characterized by interstitial leukocyte infiltration, tubular atrophy and tubulointerstitial fibrosis with the final consequence of organ failure. Unilateral ureteral obstruction (UUO) is a well established inflammatory model of kidney disease resulting in accelerated tubulointerstitial fibrosis with deposition of interstitial collagen I, III, IV and fibronectin, interstitial infiltration and activation of inflammatory cells including monocytes/macrophages and T-cells. However, the mechanisms involved in maintaining the homeostatic renal integrity and the role of mast cells (MCs) and mast cell mediators in this model remain unclear. Current treatments for fibrotic diseases such as progressive kidney disease typically target the inflammatory response. In this study, we demonstrate that MC-deficient  $W^{sh}/W^{sh}$  mice have increased UUO-induced interstitial fibrosis when compared to wild-type mice. Moreover, mice deficient in mouse mast cell protease-4 (mMCP-4), the functional counterpart of the unique human chymase, also showed aggravated UUO-induced interstitial fibrosis. This suggests that the protective effects of MCs involved mMCP-4. Both deficient strains had higher levels of renal tubular damage, more interstitial fibrosis and increased E-cadherin/decreased  $\alpha$ -smooth muscle actin expression, indicating epithelial-mesenchymal transition and thus a destruction of renal epithelial cells. In addition, they had higher numbers of MAC1<sup>+</sup> cells and CD3<sup>+</sup> T cells. They also produced elevated levels of profibrotic TGF- $\beta$  and MCP-1. Reconstitution of  $W^{sh}/W^{sh}$  mice with either wild-type or mMCP4-deficient mast cells confirmed the protective effect of mMCP-4. These findings suggest a crucial role of MCs in protection against UUO-induced fibrosis by the release of mMCP4, which decreases inflammatory cell infiltration. Our study should help to better identify potential anti-fibrotic strategies in obstructive nephropathy.

#### Basophil activation test – a useful tool in food allergy?

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#### Background:

The diagnostic work up of food allergy requires several steps (history, SPT, sIgE), but it can only be proven by double-blind, placebo-controlled food challenges (DBPCFC) which bear the risk of severe allergic reactions. Simple and reliable *in vitro* assays are preferred and a promising option might be the basophil activation test (BAT).

**Aim:** We investigated the usefulness of the BAT to differentiate between tomato cultivars and its relation to the clinical response.

**Methods:** Subjects (18-60 years) with a positive history of tomato allergy and an accordingly positive skin prick test (SPT) were studied. Tomato allergy was confirmed by DBPCFC. Basophil activation from 6 identified tomato allergic subjects was assessed by flow cytometry. Cells were stimulated with extracts from different tomato cultivars at different allergen concentrations, stained with anti-CD203c-PE and analysed.

**Results:** The cultivar used for organic farming (Matina) exhibited a higher skin (SPT) and clinical (DBPCFC) reactivity compared to an old land race cultivar (Reisetomate). The results of the BAT revealed a dose-dependent increase in basophil activation for both cultivars. However, Matina caused basophil activation at lower concentration and less amount of proteins were needed to activate 30% of all basophils (40 mg) compared to Reisetomate (87 mg). No basophil activation was observed if healthy donors were studied.

#### Conclusion

Our results suggest that the BAT is useful to determine sensitisation towards food allergen extracts like tomato and suggests differences regarding clinical response.

More studies of sensitised vs. allergic subjects are needed to determine the value of BAT in the diagnostic work-up of food allergy.

#### Expression and Regulation of Antiviral Response Genes in Mast Cells

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Several studies have shown different roles of mast cells (MC) in innate and adaptive immune responses. In fact, crosstalk between CD8<sup>+</sup> T cells and MC has shown to induce multiple genes implicated in immune responses such as Type I IFN. Two novel genes, Receptor Transporter Protein (RTP4) and Virus Inhibitory Protein, Endoplasmic Reticulum-associated, Interferon-inducible (Viperin) are IFN inducible and were found to be over-expressed in arrays. The aim of this study is to characterize the expression and protein production of RTP4 and Viperin in mast cells. Bone marrow derived mast cells (BMMC) from WT, IFN $\alpha$ , MyD88<sup>-/-</sup> and TRIF<sup>-/-</sup> mice, were exposed to TLR ligands (LPS, pIC, CpG, P(dA-dT) and New Castle Disease Virus (NDV)) during 8 and 48 h. mRNA and protein extraction were performed for further qRT-PCR and SDS PAGE analysis for RTP4 and Viperin. Intracellular stimulation of TLR was performed combining nucleic acids and Lipofectamine. Stimulation of WT cells with pIC, PdA-dT and NDV lead to expression of Viperin and RTP4 in comparison to untreated cells. The same trend was observed in TRIF and MyD88 deficient MC. In contrast, in the IFN $\alpha$  deficient cells, expression of genes and protein production was abrogated to the same levels of WT cells. Direct stimulation of the well recognized viral sensors TLR 3 and 9, as well as, infection of mast cells with NDV induce the expression of RTP4 and Viperin. The findings suggest that activation of MC with the ulterior expression of genes is Type I IFN dependent. In contrast, the adaptor proteins MyD88 and TRIF and the pathways that they represent are not relevant in the expression of RTP4 and Viperin. These findings provide bases for performing further studies focused to elucidate the functions of these proteins and show an alternative role of MC in innate immune responses.

#### Cultured human mast cells are heterogeneous for expression of the high affinity IgE receptor Fc $\epsilon$ R

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**Background:** Mast cells expressing the high affinity IgE receptor (FcεRI) are a cornerstone of the type I allergic reaction. We present data from cultured human mast cells suggesting that this is only one subtype of mast cells.

**Objective:** We studied the response of mast cells through FcεRI and effect of stabilization of surface expression of FcεRI by IgE. **Methods:** Mast cells were cultured from CD133+ progenitors from peripheral (PBMC) or cord blood. FcεRI was stabilized with 2μg/ml IgE for 2 days. IgE density was measured with QiFiKit. Cells were activated by addition of anti-FcεRI antibody in log dilutions from 1ng-10μg/ml, and labeled with anti-CD63 and anti-CD203c. Maximal activation, sensitivity and cooperativity were determined from non-linear curves fitted to activation data.

**Results:** All cultures were homogeneous for tryptase and metachromasy. Only cells expressing FcεRI, but all cells expressing FcεRI, were activated. PBMC bind 203 000 molecules IgE/cell. All cells binding IgE can be activated by crosslinking of FcεRI to upregulate CD63. Expression of CD203c and histamine release correlates with expression of CD63. Stabilization of FcεRI with IgE doubled the number of CD63+ activatable cells (p=0.0001), increased sensitivity and slope factor of PBMC six-fold. Anti-IgE reversed this effect (p=0.0002), but did not reduce activation levels below that of cell lines not stabilized with IgE. **Conclusion:** The fraction of PBMC that binds high levels of IgE can be activated through FcεRI. Baseline expression of FcεRI is independent of anti-IgE. The fraction of PBMC that does not express FcεRI has a different phenotype.

### **The Antimicrobial Activity of Mast Cells against Staphylococcus aureus and Pathogen Countermeasures**

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Staphylococcus aureus is an important human pathogen that can cause a broad spectrum of serious community-acquired and nosocomial infections. Treatment of S. aureus infections is complicated due to the prevalent of methicillin-resistant S. aureus (MRSA), which are resistant to multiple antibiotics. The substantial challenge posed by MRSA makes it imperative to develop more efficient therapeutic strategies to eliminate the pathogen. For this reason, a better understanding of the antimicrobial mechanisms mobilized by the host immune defenses to defeat S. aureus will facilitate the design of such therapeutic options. In this study we have investigated the interactions of S. aureus with mast cells, which are multifunctional highly effective sentinel cells that line the surfaces of the body. We found that mast cells exert a phagocytosis-independent antimicrobial activity against S. aureus that is largely dependent on mast cells degranulation. This was demonstrated by the failure of mast cells that were either inhibited in their capacity to degranulate by treatment with cromolyn or pre-degranulated by treatment with ionomycin to kill S. aureus. This pathogen, in turn, subverts the extracellular antimicrobial activity of mast cells by internalizing within these eukaryotic cells. The ability of S. aureus to gain access into mast cells was also demonstrated during in vivo infection using a mouse model of skin infection. Intracellular S. aureus survives for long periods in the intracellular milieu in a low-replicating status by reprogramming the metabolic activities including the upregulation for S. aureus intracellular persistence. Gaining access into an intracellular niche is not only a strategy used by S. aureus to subvert the antimicrobial mechanisms of mast cells but it also might serve as a bacterial reservoir for chronic or recurrent infections.

### **PEST Domain-Enriched Tyrosine Phosphatase (PEP) positively regulates mast cell antigen-mediated signalling and anaphylaxis**

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PEST Domain-Enriched Tyrosine Phosphatase (PEP) suppresses the activity of Src family protein tyrosine kinases

and thereby serves as a potent inhibitor of T-cell receptor signalling. PEP is also expressed in bone marrow-derived mast cells (BMMC) and we have previously reported that its expression is up-regulated by the anti-inflammatory glucocorticoid, dexamethasone. However, the function of PEP in mast cells is not known. We have therefore investigated the role of PEP in mast cell action making use of mice deficient in PEP gene expression. Here we show that in the absence of PEP, IgE-mediated systemic anaphylaxis is strongly reduced and the glucocorticoid-mediated inhibitions in serum histamine levels and percentage number of degranulated mast cells are impaired in PEP<sup>-/-</sup> mice on treatment with dexamethasone. The surface expression of the IgE and c-Kit receptors and mast cell responses such as cell survival or apoptosis, proliferation and cell cycle profile are however, unaffected in BMMC from the PEP<sup>-/-</sup> mice. Relative to the PEP<sup>+/+</sup> BMMC, cross-linking of the high affinity IgE receptor produced a sustained phosphorylation of MKK4 and a subsequent hyperphosphorylation of the JNK signalling pathway in the PEP<sup>-/-</sup> BMMC. There was also impairment in PLC $\gamma$ 1 phosphorylation which correlated with a decreased mobilization of calcium and an impaired attenuation of this effect by glucocorticoids in the BMMC from the PEP<sup>-/-</sup> mice. These effects demonstrate that PEP plays an important role in anaphylaxis and the impaired glucocorticoid response is most likely a consequence of the sustained JNK signalling on the glucocorticoid receptor.

### **Infections affect mast cell reactivity – possible implications for asthma exacerbations**

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Mast cells play a central role in allergic asthma, where they contribute to both airway hyperresponsiveness and airway inflammation. Asthma exacerbations induced by infections is a serious condition. However, the contribution of mast cells to this state is not clear. To explore the effect of bacterial and viral infections on mast cell reactivity to IgE-mediated activation we have used in vitro differentiated mast cells of different phenotypes, i.e., connective tissue like mast cells (CTLMC) and mucosal like mast cells (MLMC). These cells have been treated with TLR-ligands before sensitization with IgE and subsequent aggregation of FcεRI. The release of mast cell mediators were measured with ELISA. We first investigated the expression of TLRs in the different mast cell phenotypes. Real-time PCR analysis indicated higher expression of TLR 1, 2, 3, 5, 7, 8 and 9 in CTLMC as compared to MLMC. We then cultured CTLMC and MLMC in the presence of specific ligands for TLR1/2 (PamOct2Cys-(VPGVG)4VPGKG); TLR2/6 (FSL-1), TLR3 (Poly I:C) and TLR4 (LPS) for short (24 h) or long term (96 h). The cells were then washed, sensitized with IgE, and activated by adding antigen. Cells that had been exposed to TLR-ligands for 96h exhibit an increased reactivity after IgE-receptor aggregation assessed as increased release of β-hexosaminidase, cysteinyl leukotriens and LTB4. MLMC

### **The induction of psoriatic lesion by the tape-stripping technique is associated with mast cell IL-6 and changes in IL-33 expression in the epidermis and dermis**

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Degranulation of mast cells is among the earliest changes during the development of psoriatic lesion, and activated mast cells can release a wide range of proinflammatory cytokines, e.g. IL-6 and TNF-α. To study expression of IL-6 in mast cells and IL-33 (a known inducer of IL-6 in mast cells), the nonlesional skin of 18 psoriatic patients was tape-stripped, the Köbner reaction was induced in 8 patients as judged at the 2-3 week follow-up, and skin biopsies were collected before the tape-stripping and at time points 2h and 3d or at 1d and 7d for immunohistochemical and double-staining analyses. The PASI score and total mast cell numbers did not differ between Köbner-positive and -negative groups. In contrast, the percentage of mast cells showing IL-6 was about 1.5-fold higher in all time-point biopsies in the Köbner positive than in the Köbner-negative subjects, even in the untreated skin. Similarly, dermal IL-33+ cells increased in number in the Köbner-positive group at 3-7 days, but did not so in the Köbner-negative group. In the epidermis, the staining of IL-33 was mostly nuclear and predominantly in suprabasal layers. There was no marked change in the Köbner-negative group.

Instead, the immunostaining decreased much in the Köbner-positive group at 2h, 1d and 3d biopsies suggesting rapid release from keratinocytes in response to the injury, but then it increased again at 7d biopsies. In summary, mast cell IL-6 is associated with the lesion induction already in the untreated nonlesional skin, IL-33 is rapidly released in the epidermis at 2h-1d, and thereafter IL-33 expression is increased in the dermis and epidermis at 3-7 days. This suggests a new "vicious circle" in the early psoriatic pathogenesis.

#### **PGE2 limits aeroallergen-induced airway reactivity and prevents lung mast cell activity in mice**

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Bronchial asthma is a respiratory disease caused by obstruction of the bronchi that is sustained over a chronic inflammation of the lower airways. Current treatments of allergic asthma do not halt the underlying process and sometimes may even be unable to control the symptoms of the disease. Thus, there is a need to find new targets for preclinical development of novel therapeutic strategies. New asthma drugs focus on counteracting the pro-inflammatory mediators that drive acute and chronic inflammatory responses. This approach contrasts with the limited interest in the use of endogenous molecules with therapeutical potential in asthma, despite the positive experience with synthetic glucocorticoids, which are based on chemical modifications of a potent natural endogenous anti-inflammatory hormone. One such substance is prostaglandin (PG) E<sub>2</sub>. Indeed, numerous and varied clinical observations suggest that PGE<sub>2</sub> limits pulmonary inflammation and controls tissue repair. We showed in vivo that selective inhibition of COX-2 (i.e. reduced PGE<sub>2</sub> production) reduced protection to Ag and was paralleled by increased lung mast cell activity. These results suggested that the protective role of endogenous PGE<sub>2</sub> could be mediated by restrained MC activity. We then assessed the effect of local PGE<sub>2</sub> administration in HDM-induced airway reactivity in mice. Airway function, inflammation and mast cell activity were evaluated. Locally administered exogenous PGE<sub>2</sub>, decreased AHR, eosinophilia and Th<sub>2</sub> cytokines production accompanied by prevention of mast cells activity. A time-course experiment suggested that immunosuppressive mechanisms might partly explain such beneficial role of PGE<sub>2</sub>. We indeed observed that lung dendritic cells switched to a tolerogenic profile to aeroallergens of HDM. We have also shown in vivo that the PGE<sub>2</sub> receptor specifically mediating its protective effect is EP<sub>2</sub> receptor, and have uncovered in vitro the ability of PGE<sub>2</sub> to directly inhibit mast cells activity.

#### **Falsely elevated serum tryptase due to interfering heterophilic antibodies in patients with suspected mastocytosis**

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A baseline serum tryptase level of more than 20 µg/ml is a minor diagnostic criterion for systemic mastocytosis and is used as a simple and practical tool for mastocytosis screening. The remaining diagnostic criteria necessitate biopsies of bone marrow and/or skin and a mutational analysis of the cKIT protein (D816V). Serum tryptase is quantified using anti-tryptase antibodies in a commercially available ELISA sandwich

technique (ImmunoCAP, Phadia, Uppsala, SWE). Unfortunately those diagnostic antibodies may also be bridged by naturally occurring heterophilic antibodies present in the sera of certain patients. We investigated the frequency of heterophilic antibodies in patients with baseline tryptase levels of >20 µg/ml in two tertiary care referral centres for Allergy in Switzerland.

We included sera of 100 consecutive patients with an elevated baseline tryptase level (median 29.6 µg/ml) collected during the last 3 years, which have been stored at -20°C. All patients were referred for diagnostic work-up of anaphylaxis, mainly hymenoptera venom allergy. After incubation of all collected sera with commercially available blocking tubes for heterophilic antibodies (Scantibodies Laboratory Inc, Santee, USA) tryptase levels dropped in 4/100 patients (4%) to an extent of -87% to

-98% into the low normal range (median 3.5 µg/ml). The median inter-assay variability (IV) of the remaining tests of 6.2% was comparable to the range reported by the manufacturer (3-5% IV). All of those 4 patients with falsely elevated serum tryptase have had a complete but negative diagnostic work-up for mastocytosis including bone marrow biopsy.

We recommend to include this simple and inexpensive blocking test in clinical laboratory routine to prevent unnecessary, potentially unpleasant and expensive diagnostic procedures. In hymenoptera venom allergy where raised tryptase levels alone are an important risk factor for further severe sting reactions, it is of additional importance to detect falsely raised tryptase levels.

#### **Flow cytometric basophile activation test and specific immunoglobulin E in patients with atopic dermatitis**

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Allergy is a great problem in modern society. Generally allergists use anamnesis data and skin tests to diagnose allergic. Laboratory methods for allergodiagnosics are not uncommon. Recently a new flow cytometric basophile activation test (BAT) has been developed in a few companies. We investigated 51 (4 m. – 9 y.) patients with severe and light atopic dermatitis (AD). We identified the level of specific IgE to allergens of cow milk, house dust and wheat (Immulate, Siemens, USA) in blood serum and detected spontaneous and specific activation of basophiles (CD3-CRTh2+CD203c+) induced by these allergens and level of Th<sub>2</sub> (CD3+CRTh2+) in peripheral blood with Allergenkit (FC500, Beckman-Coulter, USA).

Percent of patients with high spontaneous activation of basophiles (>6%) and high level of Th<sub>2</sub> (>0,6%) were not different from the results we got for severe and light AD (74 and 64%, 44 and 32%), but the percent of patients with very high spontaneous activation of basophiles (>30%) in group with severe AD was two times higher than in group with light AD (30 and 14%).

In the groups with normal and high level IgE the percent of patients with high and very high spontaneous activation of basophiles were practically equal (75 and 65%, 20 and 19%). Inversely, in the group with high level IgE the percent of patients with high level Th<sub>2</sub> was 5 times higher than in the group of patients with normal level IgE (55 and 10%). It is a fact that Th<sub>2</sub> induces synthesis of IgE by B cells mediated by interleukin 4.

In our study the results of specific IgE tests matched those of BAT in 54% of cases of food allergy and in 65% of cases of house dust. The specific IgE was not defined for approximately 1/3 of patients with positive BAT. The specific IgE was detected in 15% of patients with food allergy and in 6% of patients with allergy to house dust, but BAT for these cases was negative.

#### **Pharmacological modulation of LAD2 cells activation by using different anti-inflammatory and anti-allergic mechanism of action.**

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Introduction: Mast cells have a central role in the pathogenesis of allergic diseases and may constitute a new therapeutic target for asthma. Studies with human mast cells are limited by the difficulties in obtaining them and by the absence of suitable long term cultures. LAD2 cells are a human mast cell line recently established at the *National Institutes of Health*. The aim of this study was to evaluate the effect of several reference compounds with different mechanism of action on LAD2 cells degranulation.

Methods: LAD2 cells were sensitized with 100 ng/ml of biotin-labelled IgE O.N. Cells were activated with 125 ng/ml of

streptavidin for 30 min. After the activation supernatants were collected for  $\beta$ -hexosaminidase release determination. Cells were lysed and both, supernatants and lysates, were incubated for 90 min (37°C) with 1mM  $\beta$ -hexosaminidase substrate (p-nitrophenyl N-acetyl-D-glucosamide). The inhibitory effect of the following mechanisms of action were evaluated: corticosteroids (fluticasone and dexamethasone), PDE4 (roflumilast), CRAC channel (YM-58483), PI3K (wortmannin), JAK3 (CP-690550), Syk (R406 and Astellas 9a) and cromoglycate. In addition, the effect of Syk inhibition on histamine release and cytokine production was also evaluated. Results: Among all tested compounds, Syk, PI3K and CRAC inhibitors, were active on mast cell degranulation (IC<sub>50</sub>: 69, 18 and 357nM, respectively), consistently with their mechanism of action. The Syk inhibitor showed same potency blocking  $\beta$ -hexosaminase and histamine release and MIP-1 $\beta$  production. Conclusion: IgE-mediated LAD2 cells activation is inhibited by compounds acting through Fc $\epsilon$ R1 signalling pathway, such as Syk, PI3K and CRAC. Therefore, LAD2 cells constitute a new useful tool for *in vitro* testing of anti-inflammatory and anti-allergic compounds.

**Phosphoregulation in Janus kinase/STAT-pathway of human Thelper2-lymphocytes and HumanMastCell-1-line – application in the histamine liberation test**

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Allergic reactions are associated with excessive histamine (His) production and shifts toward Th2 responses. Signal transduction (ST) within Th2 cells is specifically correlated to STAT6 and the question arises as to whether His influences the JAK/STAT-pathway and mast cells as main His-secreting cells. The aim of this study is to demonstrate the influences of both His and IL-4 on HMC-1 and PBMC cell cultures, and to monitor the *ex vivo* expression of Th2-specific STAT6 in atopic subjects. HMC-1 cells and PBMC were stimulated 1 – 3 days with different stimulators e.g. PHA and T-cell selective PMA. PBMC were prepared from 6 atopics (adult IgE > 500 IU) and 6 sex- and age-matched non-atopics (IgE < 50 IU). His (0.1 – 20  $\mu$ g/ml), rIL-4 (0.1 – 1  $\mu$ M) and methoxy-progesterone acetate (MPA 0.1 – 100  $\mu$ g/ml) were added separately or in combination 4 h post-plating. Western blots were performed to determine latent (STAT6) and phosphorylated (p)STAT6. His was measured using fluorimetry after HPLC separation. Recombinant IL-4 (rIL-4) induced cell proliferation. His and MPA inhibited the proliferation of stimulated HMC-1 and PBMC in both groups. These effects were significantly enhanced even in the presence of the combined agents, but only in "non-atopic" cell proliferation experiments (p < 0.01). Western blot analyses revealed that the rIL4 response correlated with latent STAT6 expression in the non-atopic group. By applying anti-activated STAT6, both His and rIL-4 induced pSTAT6 in the atopic and ST in HMC-1 cell cultures. In the non-atopic samples, His and rIL-4 did not significantly alter STAT6 phosphorylation. It can be deduced that His modulates the IL-4-induced JAK/STAT-phosphoregulation in only the atopic and HMC-1 cell cultures. This may explain the up-regulation of the autocrine stimulated Th2 cells that seems to be predominantly aggravated in atopy. Nevertheless, HMC-1 and PBMC incubates can be a profitable tool in allergo-toxicological His-liberation test (HLT).

**Effects of antihistamines on innate immune responses to severe bacterial infection in mice**

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Sedating and non-sedating H1R antihistamines and H2R-blockers are widely used for the treatment of allergies, insomnia and motion sickness, or the prevention and treatment of gastric ulcers, respectively. However it has been shown that histamine receptors are also involved in innate immune responses to bacteria and that pharmacological blockade in patients can negatively affect the outcome of severe bacterial infections. To characterize the contribution of the individual histamine receptors in such infections in more detail, we induced septic peritonitis in H1R -/- or WT mice, and in C57BL/6 mice treated with 1<sup>st</sup> generation H1R antihistamine, H2R antagonist, H3R/H4R antagonist, second generation H1R antihistamine, or vehicle. While H1R-/- mice as well as C57BL/6 mice receiving the second generation H1R antihistamine desloratadine did not show any differences in morbidity and mortality in this model as compared to WT or C57BL/6 mice treated with vehicle, significant increases in morbidity were observed for all other treatment groups as

compared with vehicle. These findings indicate that histamine contributes to some extent to optimal host responses in mice with septic peritonitis by receptors other than H1R and that pharmacological blockade of histamine receptors may impair innate immune responses. In the model used here, which mimics perforated appendicitis in humans, modern second generation antihistamines can be considered as safe medications without any relevant effects on morbidity and mortality, probably because of their higher H1R specificity.

**Newly acquainted: Interaction of Mast Cells with invariant Natural Killer T cells.**

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The paradigm of antigen presentation dictates that peptides are loaded and displayed on MHC molecules to T cells, most likely by dendritic cells as the antigen presenting cells (APCs). In recent years the spectrum of antigens has expanded to glycolipids. These are presented by the family of CD1 molecules, of which CD1d is conserved among species. Glycolipids loaded on CD1d are specifically recognized by a subset of innate-like lymphocytes called invariant Natural Killer T cells (iNKT), which upon activation skew immune responses to Th1 and Th2 phenotypes through the production of large amounts of cytokines. iNKT are located mainly in the liver and spleen but are also present at mucosal surfaces, where they can potentially interact with APCs other than dendritic cells during immune responses against pathogens and other inflammatory processes. Mast cells (MCs) are a key cell type that arises from bone marrow precursors. They are present in most tissues but are strategically located at the host's interfaces with the environment, such as the skin and mucosa. Their functions beyond their role in allergic reactions remained elusive until recently, where increasing evidence shows that apart from the immediate production and release of mediators, MCs can work as APCs inducing the activation of T cells and modulating immune responses.

Our study challenges the antigen presentation paradigm. We propose MCs as APCs that activates iNKT cells *via* CD1d. We show that mouse peritoneal MCs and bone marrow-derived MCs, express surface CD1d and present the prototypical glycolipid  $\alpha$ -Galactosylceramide ( $\alpha$ GalCer) to iNKT cells, inducing their proliferation and the secretion of IFN $\gamma$  and IL-4. Moreover, intraperitoneal administration of  $\alpha$ GalCer in mice induces up-regulation of CD1d in peritoneal MCs, suggesting the potential interaction of MCs with iNKT *in vivo*. Our data reveal a novel immunomodulatory function of MCs and expand the possibilities of antigen presentation.

**Estradiol and progesterone stimulate the production of angiogenic factors and molecules involved in tissue remodelling by mast cells**

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Mast cells (MCs) have long been suspected to be important players on embryo implantation because their degranulation causes the release of pivotal factors e.g. histamine, MMPs, tryptase and VEGF, all known to be involved in the attachment and posterior invasion of the embryo into the uterus. Moreover, MC degranulation correlates with angiogenesis during pregnancy in a rat model. We could previously show that estradiol and progesterone regulate the *in vivo* migration of MCs from the periphery to the uterus as well as their *in situ* maturation and degranulation. Here, we studied the ability of estradiol and progesterone to induce the expression of the pro-angiogenic factors, tissue remodelling molecules and of molecules involved in the degradation of the extra-cellular matrix, a crucial process for proper embryo implantation. The effect of both hormones was tested *in vitro* in a human mast cell line (HMC-1) as well as *in vivo* by using ovariectomized mouse with hormonal replacement. We observed that both, estradiol and progesterone induce an up-regulation of VEGF and uPA in MCs both *in vitro* and *in vivo* as well as of MMP9 activity. We propose that female hormones may not only modulate the migration of MCs from the periphery to the uterus but also promote their maturation which seems to induce the secretion of key factors for a proper embryo implantation.

**Anti-apoptotic Bfl-1 is the major effector in activation-induced human mast cell survival**

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Allergy and asthma are typical examples of diseases where the number of mast cells in the affected tissue increases and a correlation between the number of mast cells and the severity of the symptoms has been reported. In allergy, mast cells are known for their ability to mediate IgE-dependent responses, where aggregation of Fc $\epsilon$ RI leads to the release of mast cell mediators causing allergic symptoms. Following activation mast cells have the ability to survive, regranulate and be re-stimulated and thereby continue to mediate the allergic symptoms. A fundamental question in mast cell biology is how this survival is mediated. We have previously shown that mouse mast cells deficient in the anti-apoptotic bcl-2 family member A1 do not exhibit activation-induced survival upon Fc $\epsilon$ RI crosslinking. Here we describe that Fc $\epsilon$ RI crosslinking promotes activation-induced survival in human mast cells and this is associated with an upregulation of the anti-apoptotic Bcl-2 family member Bfl-1, the human homologue of A1. By staining for Bfl-1 in skin biopsies from allergen challenged skin we observed a clear upregulation of Bfl-1 in skin residing mast cells in comparison with control biopsies. Major inhibition of activation-induced mast cell survival was obtained using *bfl-1* siRNA compared to a non-targeting siRNA pool transfected into cord-blood derived mast cells. Activation-induced survival was conserved when the anti-apoptotic proteins Bcl-XL, Bcl-2, Bcl-w and Mcl-1 were inhibited by the inhibitors ABT-737 and roscovitine, indicating a minor role for these anti-apoptotic Bcl-2 family members in activation-induced mast cell survival. Taken together, our results highlight Bfl-1 as a major effector in activation-induced human mast cell survival. We therefore suggest that it would be of great interest to identify potential binding-partners of Bfl-1 in mast cells and to evaluate Bfl-1 as a target for treatment of allergic diseases.

#### **Mast cells again: a possible role in pathology of cardio-esophageal region**

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**Background:** Mast cells reside within the connective tissue of a variety of tissues and all vascularized organs. Since 1996, few studies have been performed on mast cell density in gastrointestinal biopsies, mainly in adult age group. We recently studied mast cell density in gastric biopsies in pediatric age group. Mast cell density was  $12.6 \pm 0.87$  in  $0.25 \text{ mm}^2$  (range: 0–81) in our study. Mast cell density has not been studied in cardio-esophageal region to best of our knowledge. In this study we wanted to obtain an estimate of mast cell density in this region and compare it with mast cell density in antrum.

**Methods:** From April 2007 till March 2010, we chose children (<14 years old) who underwent upper endoscopy and from whom taken biopsy was stated to be from lower third of esophagus, but in microscopic examination either cardio-esophageal mucosa or only cardiac mucosa was seen. The specimens were fixed in 10% buffered formalin, processed, embedded in paraffin and cut in sequential 3 micrometer sections. Superficial and deep sections were stained by Hematoxylin-eosin (two slides) and one slide was stained by Giemsa stain. Mast cells were counted by Giemsa stain at  $\times 1000$  magnification in 10 fields with a Zeiss standard 20 light microscope and the sum was calculated for each case (measuring  $0.25 \text{ mm}^2$ ). Biopsy specimens of the antrum of these children were also evaluated. The statistical analysis was performed using SPSS, version 17 (SPSS Inc, Chicago, IL, USA).

**Results:** 71 children (<14 years old) were included in this study of which, 63.4% (n=45) were female and 36.6% (n=26) were male. The mean age of patients was  $7.20 \pm 4.21$  years (range: 0.2–14 years). The most common clinical manifestations were recurrent abdominal pain (64.8%) and vomiting (23.9%).

The mean mast cell density in the cardiac mucosa was  $33.41 \pm 32.75$  in  $0.25 \text{ mm}^2$  (range: 0–155), which was two times of

that in antral mucosa. We found a significant correlation at the 0.05 level between mast cell density of cardiac mucosa and the antrum.

**Discussion:** It is for the first time that mast cell density is evaluated in the cardio-esophageal junction mucosa. Higher counts were seen more in cardiac mucosa in this study (mean density of mast cell in cardiac mucosa was two times that in the antrum, also it was higher than the mean in antrum in our previous study which was 12 per  $0.25 \text{ mm}^2$ ). We found a significant correlation between mast cell density of cardiac mucosa and the antrum that could hint to a single underlying etiology for the inflammatory process in gastro-esophageal junction and gastric mucosa.

In conclusion, we believe that mast cells may also have a role in pathology of lower esophagus and they should be counted and reported if they are more than 30 per  $0.25 \text{ mm}^2$ . Clinicopathological correlation will further clarify role of these cells in the future.

**Keywords:** Mast cells, Gastroesophageal junction, Gastroesophageal reflux disease

#### **Omalizumab treatment in patients with therapy resistant urticaria**

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Chronic urticaria is a severe skin disease characterized by itchy wheals and/or angioedema with an estimated lifetime prevalence of 3% to 5% in the general population. The current treatment guidelines recommend non-sedating H1-antihistamines as first-line treatment, increasing their dosage as second-line treatment, and switching to another such antihistamine or adding a leukotriene receptor antagonist as the third-line treatment. Thereafter, among several other fourth-line options including cyclosporine A, the guidelines suggest the possibility of using omalizumab, a humanized mAb that blocks the IgE receptor.

Since September 2008, our specialized university clinic has used omalizumab to treat 24 patients with chronic spontaneous urticaria or inducible urticarias, including cholinergic urticaria, solar urticaria, urticaria factitia/symptomatic dermographism, cold urticaria, delayed pressure urticaria and localized heat urticaria. All patients suffered from their urticaria for years and had numerous unsuccessful therapies. After anti-IgE treatment, more than 80% (20 of 24) of the patients showed a dramatic improvement of their urticaria symptoms. The overall excellent responses to omalizumab treatment reported here indicate that anti-IgE is a safe and effective treatment for recalcitrant urticarias.

#### **Cigarette smoke induces release of TGF- $\beta$ in airways and modulates tryptase expression and mast cell maturation**

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Chronic obstructive pulmonary disease (COPD) is a multicomponent disease characterized by emphysema and/or chronic bronchitis. COPD is mostly associated with cigarette smoking. Many inflammatory cells are present in the airways of patients with COPD. Mediators released by these cells like reactive oxygen species, pro-inflammatory cytokines and tissue-degrading enzymes, could cause tissue destruction and the induction of emphysema and chronic inflammation. The involvement of mast cells in the pathogenesis of lung emphysema is not well described. In the current study, the effect of cigarette smoke on mast cells was investigated *in vitro* and *in vivo*. We studied the distribution of mast cells during development of lung emphysema in an animal model for cigarette smoke-induced emphysema. We show that cigarette smoke induced the expression of tryptase (mouse mast cell protease-6) and suppressed surface expression of Fc $\epsilon$ RI and *c-kit*, and inhibited IgE-mediated degranulation and cytokine release. In addition, smoke exposure increased the number of tryptase-positive mast cells in the airways. Cigarette smoke induced *in vitro* TGF- $\beta$  production by mast cells. Besides, TGF- $\beta$  was increased in BALF of smoke-exposed mice. In turn, TGF- $\beta$  expression may induce an increased tryptase expression in mast cells and it affects mast cell development, which could be of functional importance in the pathogenesis of lung emphysema.

## CD300a and wasp venom immunotherapy

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**Background:** Venom immunotherapy (VIT) provides an effective treatment for venom allergy. However effects on basophils and mast cells of VIT remain unknown. CD300a is a membrane molecule with an inhibitory function in other cells types.

**Objective:** To evaluate the expression of the inhibitory molecule CD300a on basophils and compare its expression before and after the 3 days of the build up phase of VIT.

**Methods:** Aliquots of 100µL blood were stimulated with different concentrations of wasp venom, with anti-IgE or with buffer during 20 minutes. After stopping the reaction, cells were stained with anti-IgE, anti-CD123 for selection, with CD63 and CD203c as activation markers and with CD300a. Cells were lysed and fixed with Facslysis and analysed with the Facs Canto. Experiments

were carried out before starting VIT and on day 3 short after the last injection with wasp antigen.

**Results:** A high CD300a expression was found on basophils of wasp allergic patients. Although CD203c and CD63 did not change during immunotherapy, an increase in the CD300a expression was found immediately after the build up phase of VIT.

**Conclusion:** The increase of CD300a during might contribute to the induction of clinical tolerance during the build up phase of VIT.

## Updosing of antihistamines in urticaria treatment – the patients' perspective

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**Background:** The first line treatment of chronic spontaneous urticaria are non-sedating H<sub>1</sub>-antihistamines. However, many patients do not respond sufficiently to approved doses. In these cases, the current guidelines recommend to updose H<sub>1</sub>-antihistamines up to fourfold. As of yet, it is largely unclear how chronic spontaneous urticaria patients perceive treatment with H<sub>1</sub>-antihistamines in standard and higher than standard doses. **Methods:** In a nationwide survey, patients with chronic spontaneous urticaria who had received H<sub>1</sub>-antihistamine treatment were asked about their experience. In total, 319 completed surveys of patients from all over Germany were available for analyses. **Results:** 75% of all participants had experience with up dosing of H<sub>1</sub>-antihistamines. Absence of efficacy of the standard dose was the most commonly reported reason for increasing the dose. Around half of the patients (51%) reported that they had concerns regarding the step of up dosing. These included fear of adverse drug effects (26%), side effects of long term use (23%), loss of efficacy over time (19%) and drug addiction (9%). In 45% of reported events of H<sub>1</sub>-antihistamine up dosing, this treatment was rated to be solely effective or clearly more effective than standard dosed H<sub>1</sub>-antihistamine therapy. While the reported frequency of adverse effects did not differ considerably between high and standard H<sub>1</sub>-antihistamine doses (34% and 33%), the magnitude of side effects (most commonly sedation) might increase during up dosing: Of 102 reported events of side effects during up dosing, 35 were rated to be clearly (38%) and 39 to be slightly more intense (34%) as compared to the side effects that had appeared during treatment with standard doses of the same drug. **Conclusions:** Taken together, these data show that patients with chronic spontaneous urticaria who receive higher than regular H<sub>1</sub>-antihistamine doses commonly experience better control of symptoms and that 'side effects' are perceived to be stronger by some patients. Up dosing of H<sub>1</sub>-antihistamines in chronic spontaneous urticaria should be preceded by indepth patient information, addressing all relevant aspects including the adverse effects profile of the H<sub>1</sub>-antihistamine to be used.

## Uterine mast cells peak during the fertile phase of the estrous cycle in mice and remain at high levels during early pregnancy stages

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Mast cells (MCs) are best known for their key effector functions in allergic diseases. Besides their "allergic activation" numerous other signals can lead to the activation of MCs. Recent studies have shown that MCs are able to produce a variety of both, pro- and anti-inflammatory mediators upon activation. Little is known about the function and relevance of MCs during reproductive processes although their presence in non-pregnant and pregnant uteri was confirmed in several species including humans, rodents and goats. Here, we aimed to quantify MCs in uterine tissue during the different phases of the estrous cycle and at early pregnancy stages in mice and to elucidate their possible participation in implantation.

Each phase of the estrous cycle was confirmed by characterizing the cell content in HE-stained vaginal lavage of virgin females, from which uterine samples were collected. Pregnant females were sacrificed on day 2, 5 or 10 of gestation and their uterine horns were obtained. MC detection was performed by Toluidine Blue in paraffin-embedded samples while MC-related molecules as well as molecules implied in tissue remodelling were quantified at mRNA level by real time PCR.

We observed that MCs accumulate in uterus from virgin females, peaking at their sexual receptivity. MCs numbers remain high if fecundation takes place but decline shortly thereafter if it does not. Interestingly, we observed a statistically significant positive correlation between the mRNA of the MC-proteases Mcpt-5 and Mcpt-8 and the tissue remodelling molecule urokinase plasminogen activator uPA. Our data indicate that uterine MCs expand and become activated with the onset of pregnancy and further suggest that MC may play a pivotal role on implantation and establishment of pregnancy by positively influencing tissue remodelling of the uterus.

## Identification and characterization of mast cell functions in *Sporothrix schenckii* infections

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Sporotrichosis, the most common of the deep mycoses, presents clinically with subacute or chronic cutaneous nodules and plaques and more rarely as systemic disease. The causative organism, *Sporothrix schenckii* (SC), is a dimorphic fungus that is ubiquitously found in the environment. Mast cells (MCs) have recently been shown to importantly contribute to host defence responses against bacteria and parasites. In contrast, the role of MCs in host defence responses against SC or other fungal pathogens remains to be characterized.

We, therefore, used a murine model to test the role of MCs in infections with SC. Specifically, we assessed and compared SC infections both clinically as well as by histopathological analyses in genetically mast cell-deficient mice and their normal wild type littermates. Interestingly, we found decreased immune responses against SC in MC-deficient mice with weaker post-infection inflammatory responses both in histology and clinical manifestation. Further studies are needed to identify relevant pathways of MC functions in SC infections and characterize the MC receptors and mediators involved.

## Characterization of Cytoplasmic Lipid Bodies in Human Mast Cells as an Arachidonic Acid Storage Site

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Mast cells (MCs) play a crucial role in host-defense mechanisms of innate immunity and are involved in various inflammatory diseases such as atherosclerosis. Activation of MCs triggers acute exocytosis of cytoplasmic secretory granules, and a late-onset sustained release of potent biologically active lipid mediators derived from arachidonic acid (AA). Human lung MCs have been found to harbor intracellular lipid bodies (LBs), which contain AA. To learn whether the LBs could serve as a source of eicosanoids in activated MCs, we have initiated their molecular characterization.

We have devised a protocol for generating mature and functional MCs from human peripheral blood-derived CD34+ progenitors. In such MCs, we identified cytoplasmic lipid bodies by Oil-Red O staining. Flow cytometric analysis revealed a time-dependent increase in the number and size of LBs, as the cells matured in culture. Proteins of the PAT family, typical of lipid droplets, were identified at the mRNA level and visualized by immunofluorescence microscopy on the surface of the LBs. Lipid analysis revealed that triacylglycerides (TGs) are the major class of lipid in the LBs.

Importantly, addition of AA to the MCs led to incorporation of the AA into the TG pool of the LBs.

Identification of PAT family members in the LBs of MCs demonstrates their close relationship with lipid droplets. We propose that the LBs of MCs represent a metabolically active large intracytoplasmic storage pool for AA, and so may be critically important for sustained generation of eicosanoids by activated MCs in chronically inflamed tissue sites, such as atherosclerotic plaques.

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### **In colorectal cancer mast cells contribute to systemic regulatory T-cell dysfunction.**

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T-regulatory cells (Treg) and mast cells (MC) are abundant in colorectal cancer (CRC) tumors. Interaction between the two is known to promote immune suppression or loss of Treg functions and autoimmunity. Here, we demonstrate that in both human CRC and murine polyposis the outcome of this interaction is the generation of potentially immune suppressive but proinflammatory Treg (DeltaTreg). These Treg shut down IL10, gain potential to express IL17, and switch from suppressing to promoting MC expansion and degranulation. This change is also brought about by direct coculture of MC and Treg, or culture of Treg in medium containing IL6 and IL2. IL6 deficiency in the bone marrow of mice susceptible to polyposis eliminated IL17 production by the polyp infiltrating Treg, but did not significantly affect the growth of polyps or the generation of proinflammatory Treg. IL6-deficient MC could generate proinflammatory Treg. Thus, MC induce Treg to switch function and escalate inflammation in CRC without losing T-cell-suppressive properties. IL6 and IL17 are not needed in this process.

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### **A descriptive analysis of oral drug challenges in Non Steroidal Anti-Inflammatory Drug (NSAID)- induced urticaria, angioedema and anaphylaxis**

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Background: NSAID-induced urticaria, angioedema and anaphylaxis are common but not well-characterized clinical syndromes

Objective: To study the clinical characteristics of NSAID-induced acute cutaneous and anaphylactic reactions through a structured approach to oral drug challenges, with particular emphasis on selective NSAID hypersensitivity.

Methods: Retrospective analysis of the drug challenge results of all patients with NSAID-induced urticaria, angioedema or anaphylaxis presenting to the Royal Adelaide hospital between February 2006 and June 2010. Oral drug challenges were either with homologous NSAID to confirm diagnosis or with heterologous NSAID to explore cross-reactivity or safe therapeutic options. Aspirin exacerbated respiratory disease was excluded.

Results: 68 patients (53 females, 15 males, mean age 48.3) reported a total of 77 instances of defined NSAID-induced reactions of which 62% were purely cutaneous and 35% were anaphylaxes. Ibuprofen was most commonly incriminated (35%) whereas most cases of anaphylaxis were related to diclofenac (48%). 40 patients underwent a homologous challenge of which 17 were positive. Presentation with anaphylaxis, shorter reaction-to-challenge time and challenges with diclofenac were main predictors of a positive challenge. 28 patients had a challenge with heterologous NSAID, including 21 aspirin challenges (7 positive). Overall structured challenges enabled us to identify 23 (34%)

selective Reactors (SR), 19 (28%) Cross Reactors (CR) and 23 (34%) NSAID-tolerant patients. SR presented most often with anaphylaxis and were otherwise healthy except for some with a background of B-lactam allergy. CR in contrast often had a background of chronic urticaria or asthma and rather than anaphylaxis, presented with milder cutaneous reactions especially isolated periorbital angioedema.

Conclusion: A structured approach to drug challenges allows identification of selective and multiple NSAID hypersensitivity syndromes and establishes a safe therapeutic alternative. Most cases of NSAID-induced anaphylaxis represent selective hypersensitivity and hence do not require avoiding other NSAID.

### **Novel Molecular and Therapeutic Concepts in Mast Cell Disorders**

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Mastocytosis is a myeloid neoplasm characterized by abnormal accumulation and sometimes activation of tissue mast cells (MC) in various organs. Clinical systems result from effects of mast cell derived mediators and/or pathologic infiltration of various organs by MC with consecutive organ destruction, which is seen primarily in advanced systemic mastocytosis (SM). In a substantial number of patients the skin is affected. Mastocytosis can be divided into cutaneous mastocytosis (CM) and SM as well as the rare localized MC tumors, i.e. mastocytomas and MC sarcoma. CM is usually diagnosed in (early) childhood. By contrast, most adult patients are suffering from SM. The WHO classification discriminates between indolent SM (ISM), SM with an associated clonal hematologic non-MC-lineage disease (SM-AHNMD), aggressive SM (ASM), and mast cell leukemia (MCL). Organ systems frequently involved in SM are the bone marrow, skin, liver, and the gastrointestinal tract. In a vast majority of all patients with SM, the KIT mutation D816V is detectable, independent of the category of SM. Since in all SM patients the course of disease ranges from completely asymptomatic with normal life expectancy (ISM) to highly aggressive with short (<1y) survival times (MCL), other (additional) oncogenic factors are considered to lead to transformation in advanced SM. In these patients but not in patients with ISM, MC express CD30 and are triggered by additional KIT-independent signalling pathways involving Btk and/or Lyn. Other factors, such as a co-existing allergy, may lead to MC activation with consecutive anaphylactic reactions which can be severe or even life-threatening in SM. Treatment of ISM is usually focusing on symptom relieve by histamine receptor antagonists and other supportive therapy. However, in aggressive and leukemic variants, cytoreductive and targeted drugs need to be applied. Unfortunately, the prognosis in these patients remains poor even when treated with novel KIT-targeting agents, polychemotherapy, or stem cell transplantation.

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