4th EMBRN webinar: BASOPHILS AND MAST CELLS IN HEALTH AND DISEASE

September 27th 2021, 9.00-10.30 am CEST (Amsterdam, Berlin, Rome, Stockholm, Vienna)

Introduction (5 min)

Prof. Bernhard F. Gibbs, EMBRN Vice-President

Carl von Ossietzky University Oldenburg, Faculty for Medicine and Health Sciences, University Clinics for Dermatology, Oldenburg, Germany

Keynote presentation (20-25 min)

Prof. Didier G. Ebo (Antwerp and Ghent, Belgium): Flow assisted cellular allergy diagnostics: can mast cells beat basophils?

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Selected oral presentations (6 min presentation and 4 min Q&A and discussion each)

John Tchen (Paris, France)* Mcpt8-CRE-tdT or "CTM8" mice: A new mouse model demonstrates that basophils have a non-redundant role in pristane-induced lupus-like disease development

William Worrall (Toulouse, France)* The anti-FcεRI antibody MAR-1 depletes basophils and cross-reacts with myeloid cells through its Fc portion.

Manuela Bratti (Paris, France)* Intracellular recycling compartment localized Insulin-Regulated Aminopeptidase (IRAP) controls FcR-mediated inflammatory diseases

Jessy Elst (Antwerp, Belgium)* The mast cell activation test in chlorhexidine allergy: a proof of concept

Yu-Wen Yeh (Hong Kong, China)* Wild mice and naturalized laboratory mice express lung parenchymal mast cells

Hadas Pahima (Jerusalem, Israel)* CD48 contributes to the IgE-dependent activation of murine mast cells

Brief summary, announcement, farewell

***Abstract included below (speakers highlighted)**

Mcpt8-CRE-tdT or “CTM8” mice: A new mouse model demonstrates that basophils have a non-redundant role in pristane-induced lupus-like disease development

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Tissue-specific mouse models are essential tools to decipher the role of each cell compartment and/or their expressed genes in the pathophysiology of diseases. Here, we describe a new knock-in mouse model allowing expressions of both the fluorescent protein tdTomato and the CRE recombinase specifically in the basophil compartment, under the control of the Mcpt8 gene. These “CTM8” mice did not show any abnormalities in their immune cell distribution nor in their basophil function. CTM8 mice allowed the identification of basophils by immunofluorescence and flow cytometry, and the basophil-specific CRE-mediated floxed gene deletion. Breeding of our CTM8 mice with the ROSA26flox-stop-DTA mice led to the generation of basophil-deficient mice with no detectable abnormalities in other cell compartments.
Basophils are involved in systemic lupus erythematous (SLE) pathophysiology by supporting and amplifying autoantibody production. Transient depletion of basophils through DT injection in *Mcpt8DTR* mice showed promising therapeutic effects on two distinct lupus-like mouse models (Lyn–/– and pristane-induced) and demonstrated the disease-amplifying role of basophils in these models. Here, constitutive basophil deficiency prevented pristane-induced lupus-like disease development by limiting autoantibody titers and renal damages, strongly suggesting that basophils have a non-redundant role in pristane-induced lupus-like disease induction and amplification. Moreover, we describe a new mouse model that will help to further decipher the role of basophils and their expressed genes in health and disease.

The anti-FcεRI antibody MAR-1 depletes basophils and cross-reacts with myeloid cells through its Fc portion

William Worrall[a, #], Jasper Kamphuis[a, #], Julien Stackowicz[b, c], Aurélie Mougel[a], Emilie Mauré[a], Pierre Bruhns[b], Friederike Jönsson[b], Laurent Guilleminault[a, d], Laurent L. Reber[a, b]

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MAR-1 is a monoclonal antibody used to stain mouse high-affinity IgE receptor FcεRI on basophils and mast cells. This clone is also used *in vivo* to deplete basophils and induce anaphylaxis. However, the use of MAR-1 has been controversial because this mAb can also bind the IgG receptors FcγRI and FcγRIV. We have investigated the mechanisms of MAR-1's interaction with FcγRs, depletion of basophils and anaphylaxis. We found that MAR-1 stains mast cells and basophils and induces anaphylaxis through its interaction with FcεRI, independently of FcγRs. However, MAR-1 recognizes myeloid cells such as monocytes and macrophages in a FcεRI-independent manner, by engaging FcγRs through its Fc portion. Indeed, an "Fc silent" recombinant version of MAR-1 only recognizes basophils and mast cells. Strikingly, basophil depletion was also mediated by the Fc portion of MAR-1, since the Fc silent MAR-1 was unable to deplete basophils. We propose that Fc silent MAR-1 should be preferred to assess FcεRI expression. However, this Fc silent format cannot be used for basophil depletion experiments.

Intracellular recycling compartment localized Insulin-Regulated Aminopeptidase (IRAP) controls FcR-mediated inflammatory diseases

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We investigated the role of Insulin-Regulated Amino Peptidase (IRAP) in IgE receptor (Fc RI) signaling by mast cells (MC). We confirmed that IRAP locates to an intracellular recycling compartment that rapidly recruits to the plasma membrane upon FcεRI stimulation. IRAP-/- versus WT MC exhibited a reduced ability to degranulate and release various chemokines and cytokines. Tyrosine-phosphorylation of several signaling effectors was diminished in IRAP-/- MC. Importantly, we identified Syk, a central tyrosine kinase downstream of FcεRI, as being less phosphorylated at the plasma membrane in IRAP-/- MC. In addition, pSHP-1S591, which positively regulates ITAM signaling, was decreased as well as further downstream events such as intracellular calcium increases. *In vivo*, IRAP-/- versus WT mice exhibited a less severe IgE-dependent passive systemic anaphylaxis. IgG-triggered active systemic anaphylaxis in humanized FcγRIIA transgenic mice was also reduced in the absence of IRAP and *in vivo* profiling confirmed decreased phosphorylation of Syk and SHP-1S591 in FcγRIIA-expressing neutrophils and monocytes. In a chronic model of autoimmune arthritis, disease development was reduced in IRAP-/- mice both in a FcγRIIA transgenic or WT background. Our findings support a role of IRAP-positive recycling compartments in FcR signaling, enhancing phosphorylation events at the plasma membrane and FcR-mediated inflammatory diseases.

The mast cell activation test in chlorhexidine allergy: a proof of concept

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Background:. Conventional confirmatory testing for immediate drug hypersensitivity reactions (IDHRs) includes skin tests and quantification of drug-specific IgE antibodies (sIgE). However, these tests are not absolutely predictive for the clinical outcome and can yield false negative and false positive results.

Aim: To assess whether the mast cell activation test (MAT) could benefit diagnosis of chlorhexidine (CHX) IgE-mediated hypersensitivity.

Methods: Human mast cells (dMCs) were generated from CD34+ progenitor cells and sensitized with patients' sera to become dMCIgE+ and subsequently incubated with CHX. We compared the outcome of the MAT with serum from patients with and without positive skin test and basophil activation test (BAT) to CHX.

Results: dMC sensitized with sera from patients with a positive skin test and BAT to CHX showed a dose-dependent degranulation upon stimulation with chlorhexidine, determined by upregulation of the degranulation marker CD63. In contrast, dMC sensitized with sera from patients with a negative skin test and BAT to chlorhexidine were unresponsive in the MAT.

Conclusion: The MAT can be used to diagnose IgE-dependent IDHRs. Besides, it shows potential to assess the clinical relevance of drug-sIgE antibodies in their ability to elicit MC degranulation and therefore discriminate between allergy, and merely sensitization.

Wild mice and naturalized laboratory mice express lung parenchymal mast cells

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Background
Conventional laboratory strains of mice profoundly lack lung parenchymal mast cells, which stands in clear contrast to the abundant presence of mast cells in human lungs. Ultra-hygienic specific pathogen-free (SPF) laboratory mice display reduced richness and complexity of microbiota. Accumulating evidence suggests that wild mice may be a more relevant model to mimic human immune responses. This project aimed to investigate the lung distribution of mast cells in free-living wild mice.
Results
Wild mice were trapped at Oslo, Norway and Hemtabad, India, and their mouse (Mus musculus) identity was confirmed by genotyping. Mast cells were identified at a substantial density in the lung parenchymal tissues of wild mice from both habitats using toluidine blue, tryptase and cKit staining. In contrast, lung mast cells were indeed absent in the conventional C57BL/6 or BALB/c strains of laboratory mice examined in parallel. Consistently, wild mice also expressed higher pulmonary levels of stem cell factor, a critical mast cell growth factor. Higher levels of histamine were recorded in the lung tissues of the wild mice. Having speculated that gut microbiota could have impacted the lung tissue residency of mast cells, we next bred C57BL/6 laboratory mice in a purposefully built, closed environment with bedding material obtained from the natural environment with wild rodent infestation in order to normalize the gut microbiota of these super clean SPF laboratory mice to that of the dirty wild mice. Interestingly, C57BL/6 mice born and spent their entire life in this environment developed lung mast cells at an appreciable density.
Conclusions
As wild populations of mice present various problems as a research tool (e.g., unreliable supply, large individual variation, etc.), our “naturalization” or “re-wilding” approach may provide a practical solution to the establishment of mouse models with constitutive lung mast cells, which may more closely mimic human asthma.

CD48 contributes to the IgE-dependent activation of murine mast cells

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Background: CD48 is a glycosylphosphatidylinositol (GpI) receptor belonging to the SLAM family expressed on all the hematopoietic cells, including mast cells (MCs) in both mouse and human. Being a GpI anchored receptor it lacks an intracellular domain molecule. Therefore, CD48 is considered to be a co-activating receptor rather than a *bona fide* activating receptor. We and others have previously shown its role in bacteria and bacterial toxins-driven MC activation.

Aim: To investigate the role of CD48 in IgE-dependent MC activation systems *in vitro* and *in vivo.*

Results: *In vitro*, IgE-dependent activation (IgE+anti-IgE or IgEanti-DNP+DNP) of CD48-/- bone marrow derived mast cells (BMMCs) resulted in reduced release of both beta-hexosaminidase and TNFα in comparison to WT BMMCs. As expected, a MC-dependent allergic peritonitis model (OVA / *S.aureus* enterotoxin B(SEB)) induced in Sash-MC deficient mice after i.p. reconstitution with CD48-/- BMMCs, displayed a decreased level of inflammation as measured by total cells and eosinophil numbers in the peritoneum, in comparison to Sash mice reconstituted with WT BMMCs. However in CD48-/- mice OVA/SEB allergic peritonitis induced a significant increase in the level of inflammation as measured in total peritoneal but not in eosinophil numbers.

Conclusion: CD48 has a clear activating/co-activating role on IgE- dependent activation as displayed specifically on MCs. This role is less evident if other cells participating in allergic inflammation are also deficient for this receptor possibly because of arising compensatory factors.