

Agenda of the 3rd EMBRN webinar, May 3rd 2021, 2.00-3.30 pm CEST

Introduction

Prof. Francesca Levi-Schaffer, EMBRN President

Pharmacology & Experimental Therapeutics Unit, Institute of Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

Keynote presentation (15 min)

Dr. Joana Vitte (Marseille, Montpellier, France): Tryptase, a mast cell marker in health and disease

Associate Professor, Aix-Marseille University and IDESP Montpellier, France

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

Simon P. Goldie (Southampton, Portsmouth, UK): *Staphylococcus aureus* within nasal mast cells as a driver of chronic rhinosinusitis: A role for simvastatin in disease management?

Anna-Karin Johnsson (Stockholm, Sweden): Characterization of lipid mediator release in IgE stimulated lung mast cells

Erika Haide Mendez Enriquez (Uppsala, Sweden): Mast cell-derived serotonin enhances methacholine-induced airway hyperresponsiveness in house dust mite-induced experimental asthma

Elin Rönnerberg (Stockholm, Sweden): Immunoprofiling reveals novel mast cell receptors and the continuous nature of human lung mast cell heterogeneity

Irit Shefler (Tel Aviv, Israel): Lung cancer-derived extracellular vesicles: a possible mediator of mast cell activation in the tumor microenvironment

Pratibha Gaur (Jerusalem, Israel): Dexamethasone and CD300a activation display additive inhibitory effect on human and murine mast cell functions

Nyssa B. Samanas (Seattle, USA): Characterization of the mast cell surface proteome leads to the identification of CD98hc as a critical surface molecule for optimal mast cell function

Brief summary, 4th webinar announcement, farewell

Short oral presentation #1

***Staphylococcus aureus* within nasal mast cells as a driver of chronic rhinosinusitis: A role for simvastatin in disease management?**

Simon P. Goldie^{1,2}, Stephen M. Hayes^{2,3}, Philip G. Harries¹, Rami J. Salib^{1,2}, Andrew F. Walls^{1,2}

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2. School of Clinical & Experimental Sciences, University of Southampton, United Kingdom
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Background: Chronic rhinosinusitis (CRS) is a debilitating condition, associated with high rates of airborne allergy and asthma. CRS can be divided into those with inflammation of the nasal epithelium without polyps and a more severe form of the condition associated with nasal polyps. *S. aureus* colonises the nasal cavity in 30% of the general population compared with 60% of patients with CRS with nasal polyps and are almost 5-fold more likely to demonstrate IgE towards *S. aureus* enterotoxin B. Recent work has identified *S. aureus* residing within mast cells where it appears to drive the inflammatory response, resisting destruction by the immune system and commonly used antibiotics. Intriguingly, patients taking statins have a significantly reduced odds ratio (0.53) of being diagnosed with CRS. Furthermore, simvastatin has been shown to demonstrate anti-staphylococcal effects and to cross the cell membrane. We have investigated the involvement of intracellular *S. aureus* in CRS with nasal polyps in a cohort of patients and tested if simvastatin has a role in reducing the intracellular burden of *S. aureus* in mast cells.

Methodology: Florescence *in situ* hybridisation (FISH) was used to determine the rate of colonisation of *S. aureus* in nasal polyp specimens from 9 CRS subjects with nasal polyps and 5 control subjects. Immunohistochemistry was used to co-localise *S. aureus* to mast cells using anti-*S. aureus* and anti-mast cell tryptase (AA1) antibodies. A *S. aureus* strain isolated from a CRS patient was co-cultured with 5×10^5 cells/ml of the LAD2 mast cell line to determine intracellular and extracellular survival in the presence of varying concentrations of simvastatin (100 $\mu\text{mol/ml}$ -0.1 $\mu\text{mol/ml}$). Lactate dehydrogenase (LDH) assays were performed to demonstrate LAD2 cell stress at varying concentrations of simvastatin both in the absence and presence of *S. aureus*.

Results: Our findings demonstrated the presence of subepithelial, intracellular bacteria in all 9 nasal polyp specimens but no control specimens. *S. aureus* was present in 78% of CRS with nasal polyp subjects in our series and demonstrated to reside within mast cells. Co-culture of a CRS related *S. aureus* strain with LAD2 cells was associated with a reduction in intracellular survival of more than 3 and 9 fold in the presence of simvastatin at 10 $\mu\text{mol/ml}$ and 100 $\mu\text{mol/ml}$ respectively ($P < 0.05$). LDH assays demonstrated a concentration dependant decrease in cell stress with increasing concentrations of simvastatin.

Conclusions: The observation of *S. aureus* within mast cells in the great majority of nasal polyp specimens investigated in CRS patients is consistent with the idea that this represents a means for evading bacterial clearance. Simvastatin may provide an inexpensive, safe treatment to manage the intracellular burden of *S. aureus* in CRS patients with nasal polyps.

Short oral presentation #2

Characterization of lipid mediator release in IgE stimulated lung mast cells

Anna-Karin Johnsson^{1,2}, Elin Rönnerberg¹, David Fuchs³, Johan Kolmert², Craig Wheelock³, Gunnar Nilsson¹ and Sven-Erik Dahlén²

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Lipid mediators, also termed oxylipins, are metabolites of ω -3 and ω -6 polyunsaturated fatty acids (PUFAs). Among them, the most well-known to play an important part of mast cell biology are the prostaglandins and leukotrienes, but in theory hundreds of different lipid mediators may be synthesized from arachidonic acid and other PUFAs. A lot of what is known about mast cell lipid mediator release has been gained from animal models, however, there are large variations between species. Here we will present results from a screen of lipid mediators (in total 115 included in the assay) secreted from human lung mast cells (HLMC) under steady state and after IgE-receptor activation. In order to examine further the enzymatic origin and interdependencies of lipid mediator production, we blocked the COX and 5-lipoxygenase pathways. This revealed that COX-1 is the predominant COX enzyme in HLMC prostanoid production, that shunting occurs within the COX-1 pathway and that the COX-1 and 5-LOX pathways are disconnected. In combination with chiral analysis, we also show that 15-HETE, often used as a marker for 15-lipoxygenase activity, is in HLMC in fact not generated by the 15-lipoxygenase pathway. Using this large and sensitive mass spectrometry panel, we have an unbeatable tool to look at the broad and detailed picture of lipid mediator production, which can detail the flow of lipid mediators and their metabolites along their biosynthetic routes.

Short oral presentation #3

Mast cell-derived serotonin enhances methacholine-induced airway hyperresponsiveness in house dust mite-induced experimental asthma

Erika Mendez-Enriquez¹, Perla Abigail Alvarado-Vazquez¹, Willem Abma², Oscar E. Simonson^{3,4}, Sergey Rodin⁴, Thorsten B. Feyerabend⁵, Hans-Reimer Rodewald⁵, Andrei Malinowski⁶, Christer Janson⁶, Mikael Adner², Jenny Hallgren^{1*}

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Asthma is a common chronic lung disease with recurrent breathing difficulties as main symptom. In allergic asthma, the airways are sensitive to allergens and hyperreactive to stimuli e.g. methacholine, causing extensive airway narrowing, a characteristic defined as airway hyperresponsiveness (AHR). Objective: To determine the role of mast cells in a mouse model of house dust mite-induced experimental asthma. Methods: Mast cell deficient and wild type mice from the CPA3^{cre} strain and a model of experimental asthma involving repeated intranasal injections of house dust mite for three weeks were used. AHR was assessed *in vivo* and *ex vivo*, and antigen-induced airway contraction was assessed *ex vivo*. Lung inflammation, smooth muscle cells and presence of mast cells and basophils were also assessed. Results: Allergic sensitization induced mast cells localizing around the mouse airways. Mast cells were critical for allergen-induced trachea contraction *ex vivo*, via activation of Fc-gamma chain-dependent and serotonin (5-HT_{2A}) receptors. Mast cells and 5-HT_{2A} receptors contributed to AHR *in vivo*. In the absence of mast cells, lung-associated 5-HT was reduced. Primary mouse and human lung mast cells expressed the M3 muscarinic acetylcholine receptor, and methacholine induced Ca²⁺ flux in human LAD-2 mast cells and 5-HT release in mice lung-derived mast cells. Conclusion: Altogether, mast cells contribute to methacholine-induced airway contraction and AHR by the release of 5-HT, implicating mast cells in asthma-related airway hyperreactivity. Thus, inhibition of muscarinic acetylcholine receptors by tiotropium (used in the clinic) may also target human mast cells.

Short oral presentation #4

Immunoprofiling reveals novel mast cell receptors and the continuous nature of human lung mast cell heterogeneity

Elin Rönnberg^{1,2}, Daryl Zhong Hao Boey^{1,2}, Avinash Ravindran^{1,2}, Jesper Säfholm^{2,3}, Ann-Charlotte Orre⁴, Mamdoh Al-Ameri⁴, Mikael Adner^{2,3}, Sven-Erik Dahlén^{2,3}, Joakim S. Dahlin^{1,2}, Gunnar Nilsson^{1,2,5}.

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Abstract

Background: Immunohistochemical analysis of granule-associated proteases has revealed that human lung mast cells constitute a heterogeneous population of cells, with distinct subpopulations identified. However, a systematic and comprehensive analysis of cell-surface markers to study human lung mast cell heterogeneity has yet to be performed.

Methods: Human lung mast cells were obtained from lung lobectomies, and the expression of 332 cell-surface markers was analyzed using flow cytometry and the LEGENDScreen™ kit. Markers that exhibited high variance were selected for additional analyses to reveal whether they were correlated and whether discrete mast cell subpopulations were discernable.

Results: We identified the expression of 102 surface markers on human lung mast cells. Several markers showed high continuous variation in expression within the mast cell population. Six of these markers were correlated: SUSD2, CD49a, CD326, CD34, CD66 and HLA-DR. The expression of these markers was also correlated with the size and granularity of mast cells. However, no marker produced an expression profile consistent with a bi- or multimodal distribution.

Conclusions: LEGENDScreen analysis identified more than 100 cell-surface markers on mast cells, including 23 that, to the best of our knowledge, have not been previously described on human mast cells. Several of the newly described markers are known to be involved in sensing the microenvironment, and their identification can shed new light on mast cell functions. The exhaustive expression profiling of the 332 surface markers failed to detect distinct mast cell subpopulations. Instead, we demonstrate the continuous nature of human lung mast cell heterogeneity.

Short oral presentation #5

Lung cancer-derived extracellular vesicles: a possible mediator of mast cell activation in the tumor microenvironment

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Background: Activated mast cells are often found in the tumor microenvironment. They have both pro- and anti-tumorigenic roles, depending on the tumor type. In non-small cell lung cancer (NSCLC), mast cell numbers correlate with tumor angiogenesis and poor prognosis. Several lines of evidence suggest that the tumor microenvironment contains multiple soluble factors that can drive mast cell recruitment and activation. However, it is not yet clear how mast cells are activated by tumor cells. In the present study, we explored whether tumor-derived microvesicles (TMV) from non-small cell lung cancer (NSCLC) cells are able to activate mast cells and promote angiogenesis in the tumor microenvironment.

Methods: TMV were isolated from NSCLC cell lines or from surgical tissue specimens. Migration of mast cells was conducted by transwell assay and cytokines release was first measured by cytokine array assay and then verified by specific ELISAs. For angiogenesis analysis, we examined angiogenic response in human umbilical cord vein endothelial cells (HUVECs) by measuring Matrigel tube formation and migration by scratch assay.

Results: Activation of mast cells with NSCLC-TMV resulted in ERK phosphorylation, enhanced mast cell migratory ability and increased release of several cytokines and chemokines, such as TNF- α , MCP-1 and CCL18. The chemokine CCL18 was previously reported to induce angiogenesis both in vivo and in vitro. In the present work, we have found that CCL18 secreted from mast cell activated by NSCLC-TMV increased HUVECs migration and tube formation thus promoting angiogenesis.

Conclusion: Our data are thus, consistent with the conclusion that TMV have the potential to influence mast cell activity and thereby affect angiogenesis in the tumor microenvironment.

Short oral presentation #6

Dexamethasone and CD300a activation display additive inhibitory effect on human and murine mast cell functions

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Background : Glucocorticosteroids (GCs) are the most common agents used to control inflammation in allergic diseases. GCs elicit a series of side effects especially in long term treatment. Therefore, alternative treatments are needed. CD300a is an inhibitory receptor functionally displayed on both human and mouse mast cells (MCs). The possible interplay between GCs and inhibitory receptors such as CD300a has not yet been investigated on MCs.

Aim : We aimed to evaluate the combined effect of dexamethasone (Dex) and CD300a on human and murine MCs functions and in anti-IgE-induced peritonitis in mice.

Methods : Cord blood derived MCs (CBMCs)/ bone marrow derived MCs (BMMCs) were incubated with Dex and anti-CD300a (activated by an anti-CD300a mAbs) followed by activation via IgE-mediated activation. MCs mediator and cytokine release were assessed. Mice were injected subcutaneously with vehicle/Dex for 1 hr followed by i.p. injection with anti-CD300a/IgG2b. After 40 mins, anti-IgE Abs were injected i.p. for activation of peritoneal MCs. Mice were euthanized after 30 mins and inflammation parameters were analysed.

Results : In CBMCs/BMMCs, the combined treatment of Dex with anti-CD300a significantly reduced release of pro-inflammatory cytokines compared to their mono-treatments. Additionally, in IgE-sensitized and challenged BMMCs, the combined treatment led to a slight but significant inhibition of β -hexosaminidase release. Further, we demonstrated a significant decrease in tryptase release in the anti-IgE induced peritonitis model. TNF- α levels were reduced, although not significantly. Finally, Giemsa staining confirmed the biochemical analysis, showing a significant reduction in numbers of de-granulated MCs in the combined treated group.

Conclusion : Our results suggest that Dex and anti-CD300a combined treatment might have clinical relevance in terms of GCs sparing.

Short oral presentation #7

Characterization of the mast cell surface proteome leads to the identification of CD98hc as a critical surface molecule for optimal mast cell function

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Mast cells are known for their involvement in many distinct pathological conditions, suggesting that mast cells recognize and respond to various stimuli and thus require a rich cell surface protein repertoire. These mast cell surface proteomes have not been comprehensively characterized. In this study, we aimed to further characterize the mast cell surface proteome to obtain a better understanding of how mast cells function in health and disease.

We enriched for glycosylated surface proteins expressed in murine bone marrow-derived mast cells (BMCMCs) and identified them using mass spectrometry analysis. This approach resulted in the identification of 1270 proteins in BMCMCs, 403 of which were localized to the plasma membrane. The most common protein classes among plasma membrane proteins are represented by small GTPases, receptors and transporters. Novel surface proteins in mast cells were validated by qPCR and flow cytometry analysis in BMCMCs and peritoneal mast cells (PMCs).

Among the novel surface proteins, we found that CD98 heavy chain (CD98hc) encoded by the *Slc3a2* gene was highly expressed in mast cells. *Slc3a2* gene disruption by CRISPR/Cas9 gene editing resulted in a significant reduction in CD98hc expression, mast cell degranulation, adhesion and proliferation. Our study indicates that we can use glycoprotein enrichment coupled with mass spectrometry to identify novel surface molecules in mast cells. Moreover, we found that CD98hc plays an important role in mast cell function.