Methods: HDM extracts with different biochemical properties were analyzed for their effects on airway/bronchial epithelial barrier function by measuring changes in transepithelial resistance and immunostaining of the junctional proteins ZO-1, occludin and E-cadherin. Furthermore, we examined the induction of a pro-inflammatory phenotype of human bronchial epithelium by these HDM extracts, as well as the epithelial remodeling and airway inflammation *in vivo* in a mouse model.
Results: We found that the different HDM extracts induced divergent responses. Importantly, the extract with lowest serine protease activity induced the most pronounced effects on barrier function *in vitro*, and induced an increased production of the pro-inflammatory chemokine CCL20. Remarkably, the same HDM extract induced HDM-specific IgE, a profound epithelial E-cadherin delocalization, goblet cell hyperplasia, cellular inflammation and increased levels of CCL17 and IL-5 *in vivo*.
Conclusion: Together, these results indicate that the disruption in epithelium barrier function is independent of serine protease activity, and is essential for allergic sensitization and airway remodeling *in vivo*.

1399

WITHDRAWN

151. Mechanisms of allergic inflammation of the airways

1398
LSC 2011 Abstract: Different biochemical properties of house dust mite induce divergent epithelial and inflammatory responses
S. Post, M.C. Nawijn, A.J.M. van Oosterhout, Irene Heijink. *Pathology & Medical Biology, Lab. Allergology & Pulmonary Diseases, University Medical Center Groningen, Groningen, NE*

Introduction: Allergic asthma is mainly caused by exposure to aeroallergens like house dust mite (HDM), when transepithelial delivery is facilitated by disruption of the epithelial barrier.
Objective: We aimed to gain more insight in which biochemical property of HDM is critical for the disruption of barrier function and initiates an inflammatory response.

1400
PGD_2 biosynthesis in several human mast cell models is catalyzed by cycloxygenase-1
Jeong-Hee Choi[1,2], Sven-Erik Dahlén[2], Gunnar Nilsson[1]. *[1]Clinical Immunology and Allergy Unit, Department of Medicine, Karolinska Institutet, Stockholm, Sweden; [2]Experimental Asthma and Allergy Research Unit, The National Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden*

Background: Mast cells and eicosanoid mediators play an important role in asthma. There are few studies comparing eicosanoid release from different human mast cell models.
Objective: We characterized release of eicosanoids from the LAD2 human mast cell line and primary cultured-human mast cells from cord blood (CBMC) and peripheral blood (PBMC).
Methods: Mast cells were stimulated with anti-human IgE via cross-linking of IgE antibodies for 30 minutes, and β-hexosaminidase, cysteinyl leukotrienes (CysLTs), LTB_4, prostaglandin D_2 (PGD_2) were measured, and expression of cyclooxygenase (COX)-1 and COX-2 were studied by western blot.
Results: LAD2 released β-hexosaminidase and PGD_2 after stimulation with anti-human IgE in a dose-dependent manner, but not CysLTs or LTB_4. CBMC and PBMC showed significant increase of cysLTs and LTB_4 in addition to β-hexosaminidase and PGD_2 after stimulation. IL-4 priming did not enhance CysLTs release in any of the mast cell models. IL-4 primed LAD2 however showed significant enhancement of PGD_2 release. The PGD_2 release from the three human mast cell models was consistently abolished by the selective COX-1 inhibitor, FR122047 ($p<0.001$), but not by the COX-2 inhibitor, etoricoxib. There was strong COX-1 expression in LAD2 which showed significant inhibition of PGD_2 release after treatment with COX-1 siRNA.
Conclusion: LAD2 is a good model for studies of PGD_2 release, but not for CysLTs. The PGD_2 release from the isolated human mast cells is dependent on COX-1, in line with recent *in vivo* findings in asthmatics (Daham K et al. Clin Exp Allergy 2011;41:36-45)
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1401

Alpha-melanocyte stimulating hormone potentially inhibits basophil activation, indicating a novel function of this neuropeptide in airway allergy

Ulrike Raap[1], Manuela Gehring[1], Thomas Luger[2], Alexander Kapp[1], Markus Böhm[2]. *[1]Department of Dermatology and Allergy, Hannover Medical School, Hannover, Germany; [2]Department of Dermatology, University of Muenster, Muenster, Germany*

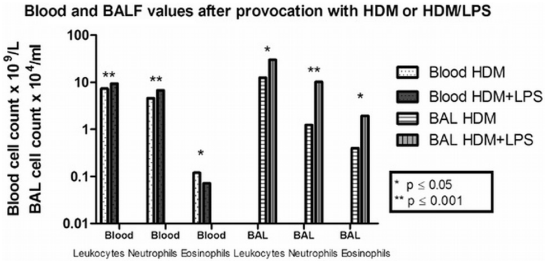
Background: It is well established that basophils play an important role in the initiation and control of airway allergy with potent effector functions during allergic responses. Using a mice model we could show that the neuropeptide alpha-melanocyte stimulating hormone (a-MSH) inhibits allergic airway inflammation. Thus we wanted to assess, whether basophils functionally respond to a-MSH in patients with upper airway allergy.
Methods: Human basophils of patients with allergic rhinitis to grass pollen only and nonatopic controls were purified with magnetic beads with a purity of 98-100%. Expression of Melanocortin-Receptor 1 (MC-1R) was analysed by FACS, RT-PCR and Western immunoblotting. Activation of basophils was studied by calcium 2+ mobilization assay and CD63 surface expression using the basophil activation test, release of cytokines was assessed with ELISA.
Results: Human peripheral blood basophils expressed MC-1Rs at protein and mRNA level. The MC-1R was functionally active in isolated basophils as shown by α-MSH-mediated intracellular increase of calcium^{2+}. α-MSH significantly inhibited anti-IgE, fMLP or PMA induced release of IL-4 and IL-6 ($p<0.05$-0.01). Further, α-MSH suppressed fMLP or grass pollen induced basophil activation assessed with CD63 surface expression ($p<0.001$). The effect of α-MSH on basophil activation was MC-1R-mediated as shown by blockade with a peptide analogue of agouti signaling protein.
Conclusion: Our data show that a-MSH inhibits the allergic immune response in human basophils, presenting a novel and promising anti-inflammatory function of this peptide in allergic airway diseases.

1402

Local and systemic inflammatory responses following bronchial instillation of house dust mite allergen (HDM) and HDM/lipopolysaccharide (LPS) in mild asthmatics

M. Berger[1], J.D. de Boer[1], P. Bresser[2], T. van der Poll[1], R. Lutter[1], P.J. Sterk[1], J.S. van der Zee[1]. *[1]Respiratory Medicine, Academic Medical Center, Amsterdam, Netherlands; [2]Respiratory Medicine, Onze Lieve Vrouwe Gasthuis, Amsterdam, Netherlands*

Rationale: Exposure to house dust, containing HDM and LPS, is associated with severity of allergic asthma. We hypothesized that adding LPS to bronchial provocation with HDM amplifies allergic inflammation in asthmatics on maintenance treatment with inhalation corticosteroids (ICS).
Aim: To assess the allergic inflammatory response in blood and bronchoalveolar lavage (BAL) fluid induced by provocation with HDM +/- LPS.
Methods: We included 39 mild asthmatic patients with HDM allergy. After 2 weeks run-in with fluticasone 100μg bid, blood was drawn and subjects underwent bronchoscopy for instillation of saline in one lung followed by instillation of HDM +/- LPS in the contralateral lung. Six hours later, blood was drawn and BAL was performed. Statistical comparisons were made by Univariate Analysis.
Results: Additional instillation of LPS to HDM resulted in a significant increase in peripheral blood leukocytes and neutrophils, and a decrease of eosinophils. Provocation with HDM+LPS significantly increased total cell numbers, neutrophils, and Eosinophil Cationic Protein (ECP) in BAL fluid (p=0.04), and showed a trend towards an increase in eosinophils.



Conclusion: Additional instillation of LPS to a provocation with HDM in mild asthma decreases circulating eosinophils and increases pulmonary eosinophil influx and ECP-release, despite treatment with ICS.

1403

Poly(I:C)-induced responses in nasal and bronchial epithelial cells of patients with asthma and healthy controls

A.H. Wagener[1], S. Luiten[2], L.N. Venekamp[1], P.W. Kunst[1], P.J. Sterk[1], C.M. van Drunen[2]. *[1]Dept. Pulmonology, Academic Medical Center, Amsterdam, Netherlands; [2]Dept. ENT, Academic Medical Center, Amsterdam, Netherlands*

Rationale: The majority of asthmatic exacerbations is associated with respiratory virus infection (Busse, Lancet 2010). Since airway epithelium is the primary site of a viral entry, we hypothesized that nasal and bronchial epithelium are equally responsive to respiratory viruses when measured as cytokine responses to poly(I:C).
Aim: We compared baseline cell activity and induced responses of primary human nasal and bronchial epithelial cells to poly(I:C) in allergic asthmatics and healthy controls.
Materials and method: This was a 2-group study in 8 allergic asthmatics (defined by GINA, $PC_{20}<8$ mg/ml) with rhinitis (defined by ARIA) and 8 healthy volunteers. Cultures were exposed for 24 hours to culture medium alone (baseline), or containing poly(I:C). Cytokine production was measured by Multiplex ELISA for 30 mediators and analysed by parametric analysis corrected for multiple testing.
Results: There were no significant between-group or between-upper and lower airway differences in baseline cell activity. Exposure to poly(I:C) induced up-regulation of several cytokines with no significant between-group differences. Asthmatics showed a higher activity for MIP-1b (p=0.009), MIP-1a (p=0.003), MCP-1 (p=0.038), and TNF-α (p=0.05) in nasal as compared to bronchial epithelial cells.
Conclusion: Expression is highly variable in individual subjects, and asthmatics demonstrate higher expression for MIP-1b, MIP-1a, MCP-1 and TNF-α in nasal *versus* bronchial epithelial cells after stimulation with poly(I:C). Our findings suggest that nasal and bronchial epithelium exhibit differential responses to double-stranded RNA, and thereby to viral infection.

1404

Alveolar mast cell expression of FcεRI differs between allergic asthma and rhinitis

Cecilia Andersson[1], Ellen Tufvesson[1], David Aronsson[1], Anders Bergqvist[1], Michiko Mori[2], Leif Bjermer[1], Jonas Erjefält[2]. *[1]Lund University, Respiratory Medicine & Allergology, Lund, Sweden; [2]Lund University, Experimental Medical Science/Airway Inflammation, Lund, Sweden*

Background: A significant proportion of patients with allergic rhinitis (AR) develop asthma. Our aim was to investigate expression of the high affinity IgE receptor (FcεRI) on alveolar mast cells in patients with AR with mild and uncontrolled asthma, AR with and without bronchial hyperactivity (BHR) and non-atopic controls.
Methods: Bronchial and transbronchial biopsies from controls, patients with AR and patients with AR with concurrent asthma were processed for immunohistochemical identification of MC_T and MC_{TC} and their expression of FcεRI and bound IgE.
Results: The alveolar parenchyma in uncontrolled asthmatics had an increase in densities of both MC_{TC} (p=0.05) and MC_T (p=0.003). In patients with AR with or without BHR and mild asthma no difference in tissue density of MC_T or MC_{TC} was observed in central airways and alveolar parenchyma compared to controls. Mast cell expression of FcεRI was high in all groups in central airways. The expression of FcεRI on alveolar mast cells was increased in AR patients with concurrent mild (p=0.01) and uncontrolled ($p<0.001$) asthma compared to healthy controls. The asthmatics also had increased numbers of alveolar mast cells that expressed surface-bound IgE. A similar increase in mast cell FcεRI expression and surface-bound IgE was not seen in patients with AR with or without BHR.
Conclusions: Our data suggest that patients with atopic asthma have increased alveolar mast cell expression of FcεRI and surface-bound IgE compared to healthy controls and patients with AR with or without BHR. This might reflect a peripheral involvement of mast cells in the allergic asthma response and underscores the need to target peripheral lung inflammation in this patient group.

1405

Nlrp3/caspase-1-independent IL-1β production mediates diesel exhaust particles-induced pulmonary inflammation

Sharen Provoost[1], Kurt Tournoy[1], Nele Pauwels[1], Tom Vanden Berghe[2], Peter Vandenabeele[2], Bart Lambrecht[3], Guy Joos[1], Tania Maes[1]. *[1]Department of Respiratory Medicine, Laboratory for Translational Research in Obstructive Pulmonary Diseases, Ghent University Hospital, Ghent, Belgium; [2]Department for Molecular Biomedical Research, Unit for Molecular Signaling and Cell Death, Flanders Institute for Biotechnology, Ghent, Belgium; [3]Department of Respiratory Medicine, Laboratory of Immunoregulation and Mucosal Immunology, Ghent University Hospital, Ghent, Belgium*

Inhalation of diesel exhaust particles (DEP) induces an inflammatory reaction in the lung; however, the mechanisms are largely unclear. Interleukin (IL)-1β/IL-1RI signaling is crucial in several lung inflammatory responses. Typically, caspase-1 is activated within the Nlrp3 inflammasome, that recognizes several damage associated molecular patterns, which results in cleavage of pro-IL-1β into mature IL-1β.
Here, we hypothesize that the Nlrp3/caspase-1/IL-1β pathway is critical in DEP-induced lung inflammation. Upon DEP exposure, IL-1RI KO mice showed reduced inflammation in the lung when compared to WT mice. In line, treatment with recombinant IL-1R antagonist (anakinra) and IL-1β neutralization impaired the DEP-induced lung inflammatory response. Upon DEP exposure, Nlrp3 and caspase-1 KO mice, however, showed similar IL-1β levels and comparable inflammation in the lung compared to WT mice.
In conclusion, these data demonstrate that the DEP-induced inflammation in the

lung acts through the IL-1β/IL-1RI axis. In addition, DEP initiates inflammation independent of the "classical" Nlrp3/caspase-1 pathway.

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