349. Immunologic mechanisms in COPD and asthma

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The profile of dendritic cell and T cell response is related to the viral trigger in children with severe asthma exacerbation

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Viral infection are associated with asthma exacerbations (AE). Activation of dendritic cells (DC) plays a key role in the response to virus and drives the activation and polarization of T cells. Mobilization of Pattern Recognition Receptor (PRR): Toll Like Receptor (TLR3), RNA helicases (RIG-I, MDA-5) are involved. Our purpose was to analyze expression and function of the PRR in DC and to corroborate this with the presence of virus and the T cell response.

Methods: 54 allergic asthmatic children (6-15 y) included during hospitalization for severe AE. Virus identified on nasal secretions by RT-PCR. T cell response determined in blood and induced sputum at the inclusion and in the stable state, 8 weeks later. Mononuclear cells (MNC) stimulated *in vitro* with poly(IC) and liposomes containing poly(IC) and levels of IL-4, IFN-g, IL-17A measured. Expression of markers of maturation (CD80, CD86) and PRR studied in circulating DC by flow cytometry.

Results: Virus were indentified in 60% (Rhinovirus: 82%). A Th1 and Th17 (IFNg, IL17A) response was observed in the airways and the blood from the infected patients (V+) during exacerbation whereas a Th2 (IL-4) response prevailed in non-infected patients (V-). The stimulation of MNC induced a Th2 and Th17 response for V+ at inclusion, but Th1 in V-. A defect in RNA helicases expression by blood DC was observed in V+ at inclusion, while the expression of the markers of maturation did not differ among both groups.

Conclusion: Viral infection modifies the T cell response during AE and is associated with a defect of RNA-helicase expression in DC. This could contribute to describe new mechanisms in the virus induced AE.

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Lung dendritic cells from chronic obstructive pulmonary disease patients induce type 1 T regulatory cells

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The high mortality rate and health care costs associated with Chronic Obstructive Pulmonary Disease (COPD) are due to a great extend to recurrent infectious exacerbations. Impaired T cell immunity might explain this susceptibility to infections. Mature dendritic cells (DCs) are crucial players in the induction of T cell responses against infectious agents. By contrast, immature DCs induce tolerance by promoting the differentiation of regulatory T cells (Tregs). We have previously shown

that lung DCs of COPD patients express low levels of co-stimulatory molecules, respond poorly to stimulation and display low ability to prime autologous lung T cells and allogeneic naive T cells. Importantly, naïve T cells primed with lung DCs from patients with COPD inhibit T cell proliferation. Here, we have characterized the gene and protein expression profile of these regulatory cells and investigated the mechanism of their suppressive function. Naïve CD4+ T cells primed with lung DCs from patients with COPD showed increased gene expression for Foxp3, Ahr and GATA3 (assessed by qRT-PCR) compared to T cells primed with lung DCs from smokers without COPD. Accordingly, flow cytometry analysis showed higher IL-10 and Foxp3 intracellular protein expression. These findings suggest that the induced regulatory cells are Tregs type 1. Type 1 Tregs suppress immune responses primarily through IL-10. Indeed, naïve T cells that had been primed with COPD lung DCs failed to inhibit T cell proliferation in the presence of blocking IL-10 receptor antibody. Our findings show that lung DCs from patients with COPD induce type 1 Tregs.

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Lung B cell-derived CXCL13 is critical for lymphoid follicle formation in chronic obstructive pulmonary disease

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Lymphoid follicles (LFs) that have a similar organization to lymph nodes are found in small airways and alveoli in Chronic Obstructive Pulmonary Disease (COPD), but the mechanism of their development is unclear. During lymph node ontogeny lymphotoxin (LT)-expressing lymphoid-tissue inducer cells induce lymphokine production (mainly CXCL13) to LT-receptor-expressing stromal cells. Lymphokines attract haemopoietic cells leading to lymphoid organ development. We examined peripheral lung tissue from COPD patients with LFs (COPD-LF$^+$), without LFs (COPD-LF$^-$) and never-smokers. Lung CXCL13 was significantly increased in COPD-LF$^+$ compared to COPD-LF$^-$ and never-smokers and positively correlated to surface area of LFs. Immunostaining showed CXCL13 expression in B cell areas of LFs. Flow cytometry indicated that among lung cells, B cells have the highest expression for LT receptors. Ex-vivo, lipopolysaccharide (LPS) and a LT-receptor agonist induced CXCL13 production to whole lung cell cultures from COPD-LF$^+$. The LPS induction of CXCL13 was decreased by neutralizing LT. Depletion of B cells from the cultures significantly decreased CXCL13 and LT. Isolated lung B cells showed high migration towards lung tissue homogenates from COPD-LF$^+$ that was significantly decreased by CXCL13 neutralization. When isolated lung B cells were exposed to CXCL13, LT was significantly increased, indicating a positive feedback loop between LT and CXCL13. We propose that the initiating event to LF formation in CODP is B cell stimulation, leading to LT expression and CXCL13 production. CXCL13 positively feeds back LT, amplifying its levels and attracting more B cells that organize themselves into LFs.

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Dendritic cells-nerve interaction in allergic airway inflammation

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Introduction: Dendritic cells (DC) play as antigen-presenting cells a decisive role within the allergic airway inflammation. The colocalisation of DC in sensory airway ganglia has been not explored so far.
The aim of the present study is to evaluate possible interactions of DC in sensory ganglia concerning calcitonin gene-related-peptides (CGRP)-expression during allergic airway inflammation.
Methods: The BALB/c mice were treated with intranasal house dust mite (HDM) extract (25µg/50µl) for 5 days a week within a total period of 7 weeks. The jugular-nodose ganglion complex was removed 24 hours after final allergen challenge and histological slices were prepared. Immunohistology was performed to detect the colocalisation of DC by MHC-II and CD11c and neurons by neuronal marker PGP 9.5.
Results: Under physiological conditions dendritic cells are found in the vagal sensory airway ganglia of the mouse and that they were significantly increased during an allergic airway inflammation (DCs/neurons: control 23.48±7.613% vs. HDM 49.75±4.194%, p = 0.0003). Additionally, an increased number of CGRP positive neurons in vagal sensory airway ganglia during allergic airway inflammation was found (CGRP positive neurons/total neurons: HDM 52.07±3.040% vs. control 21.63±3.799%, p = 0.0001).
Conclusion: The finding of the presence of DC in the airway jugular-nodose ganglion indicates a role of the DC in these ganglia under physiological conditions. The increased numbers of DC and CGRP-positive neurons in these ganglia suggest the involvement of these cells the pathogenesis of allergic airway inflammation. However, the exact functions of DC and CGRP in allergic airway inflammation remain to be explored in future studies.

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LSC 2012 Abstract – The protective role of Pim1 in cigarette smoke induced damage of airway epithelium

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Rationale: The main risk factor of developing COPD is exposure to cigarette smoke. CS exposure induces airway epithelial cell damage, release of DAMPs and an innate inflammatory response. We previously observed increased expression of Pim1 in vivo after sub-chronic CS exposure in mice. Pim1 is a serine/threonine kinase involved in cell growth and survival by preventing apoptosis induction through the mitochondrial pathway. We hypothesize that Pim1 plays a protective role in the airway epithelium after CS exposure by phosphorylating BAD and enhancing cell survival.
Methods: Pim1-KO mice were exposed to CS twice a day for 4 days. Inflammatory cells and KC levels in BAL were determined. Beas-2b cells were treated with CS extract (CSE) for 4 hours with(out) Pim-inhibitor. Mitochondrial membrane potential (ΨM) and apoptosis/necrosis induction were measured by flowcytometry. BAD phosphorylation was determined by Western Blotting.
Results: CS exposure induces neutrophilic airway inflammation and increases KC levels in Pim1-KO mice, but not in WT controls. CSE induces a dose-dependent loss of BAD phosphorylation, loss of ΨM and necrotic cell death in Beas-2b cells. All of these CSE-induced effects are aggravated by inhibition of Pim1.
Conclusion: Pim1 protects airway epithelial cells from CS-induced damage and cell death by phosphorylating BAD and increasing the threshold for apoptosis. In vivo, this protective effect suffices to prevent CS-induced neutrophilic airway inflammation.

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LSC 2012 Abstract – Role of ADAM19 and neuregulin-1 in Muc5ac expression in lungs of cigarette smoke-exposed mice

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Mucus hypersecretion is an important feature of COPD, resulting in chronic cough and contributing to dyspnea by obstructing the airway lumen. Signalling through the epidermal growth factor receptor (EGFR) plays an ubiquitous role in the production of mucins. We hypothesize that A Disintegrin And Metalloproteinase 19 (ADAM19) stimulates mucin production by shedding of the EGFR-ligand neuregulin-1.
C57BL/6 mice were exposed to air or cigarette smoke (CS) for 4 or 24 weeks. IHC for ADAM19 on lung tissue sections showed intense staining in bronchial and vascular smooth muscle cells, as well as in endothelium, and a faint staining in bronchial and alveolar epithelial cells. Quantification of ADAM19 protein expression in the airway wall showed a significant increase upon 4 weeks of CS-exposure, but not upon 24 weeks. Accordingly, protein levels of neuregulin-1 were significantly elevated in BAL fluid of mice exposed to CS for 4 weeks, but not for 24 weeks. Finally, pulmonary Muc5ac mRNA expression was significantly increased upon both 4 and 24 weeks of CS-exposure, while we found no differences in the mRNA expression of Muc5b.
These data demonstrate that 4 weeks of CS-exposure leads to increased expression of ADAM19 and enhanced shedding of neuregulin-1. Binding of neuregulin-1 to EGFR may contribute to the increased expression of Muc5ac. However, especially upon chronic CS-exposure, other EGFR-ligands or alternative mechanisms may be involved in mucin production.

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The IL-2-dependent Th1 response to bacterial infections is suppressed in COPD

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Susceptibility to bacterial infections is enhanced in COPD, which promotes exacerbations. IL-2 triggers proliferation of Th1 cells important for infection defence. We elucidated modulation of IL-2 release from Th1 cells by LPS and in COPD.
Peripheral blood CD4+ cells of n=10 age-/gender-matched never-smokers (NS), smokers without (S) and with COPD were ex vivo activated towards Th1 by αCD3/αCD28 antibodies and IL-12. IL-2 release (ELISA) and cell count/death was analyzed after 24-96h of cultivation.
Activation towards Th1 increased IL-2 release and cell count. IL-2 release was increased in COPD and negatively correlated to FEV_1 [% pred.]. Nonetypeable H. influenzae extract suppressed IL-2 release from Th1 cells of NS, which was abolished by Polymyxin B or CLI095 (LPS/TLR4 inhibitors). LPS reduced IL-2

release and cell count of Th1 cells, these effects were enhanced in COPD. LPS effect on IL-2 negatively correlated with FEV_1 [% pred.] and was abolished by CLI095. In the presence of LPS, blocking MyD88/IRAK was more efficient in restoring IL-2 release in NS vs. COPD, whereas blocking TRIF/IKKε was more efficient in COPD, and moxifloxacin (MXF) increased IL-2 release and cell count of Th1 cells. MXF effect on IL-2 was enhanced in COPD and correlated to the IL-2-inducing effect of p38MAPK inhibitor SB203580. All effects were $p<0.05$. LPS and MXF did not induce Th1 cell death.
Th1 response to bacterial infections is impaired in COPD due to a shift from MyD88/IRAK to TRIF/IKKε signalling, which enhances suppression of IL-2 and Th1 growth by LPS. MXF might reinforce IL-2 expression and Th1 growth by blocking p38MAPK signalling. Targeting TLR4 signalling combined with MXF might reduce exacerbation rates.

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LSC 2012 Abstract – FOXO transcription factors regulate innate immune mechanisms in respiratory epithelial cells during bacterial infection

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Bacterial pathogens are a leading cause of lung infections and contribute to acute exacerbations in patients with respiratory tract diseases. The innate immune system of the lung controls and prevents colonization of the respiratory tract with bacterial pathogens. Here, we show that FOXO transcription factors regulate innate immune mechanism of respiratory epithelial cells in response to bacterial pathogens such as Haemophilus influenzae and Pseudomoas aeruginosa. Infection with bacterial pathogens led to the activation of FOXO transcription factors in respiratory epithelial cells in vivo and in vitro. siRNA mediated knock down of FOXO3 in bronchial epithelial cells resulted in reduced expression of factors of the innate immune system such as antimicrobial peptides and factors involved in a proinflammatory response. In addition, FOXO3 plays a role in the internalization of bacterial pathogens. These data show that FOXO transcription factors are involved in the cellular response to bacterial stimuli and have a central role in regulating innate immune functions of respiratory epithelial cells.