present in the blood and lung during respiratory diseases including bacterial pneumonia and ARDS. However, the precise pathogenic role of immune complexes in these conditions is unclear.

Binding of immune complexes to the uniquely human stimulatory IgG receptor FcγRIIA is constitutively suppressed on neutrophils and macrophages. Elucidating the mechanism behind this suppression will help us understand how the body regulates its response immune complexes.

The erythroleukemia cell line K562 that expresses the FcγRIIA inherently and exhibits FcγRIIA suppression was used as a model. The effects of microbial neuraminidases on immune complex binding by the FcγRIIA were tested by flow cytometry. We also investigated whether there is a protein in close proximity to FcγRIIA that could potentially block its IgG binding site by protein protein-cross-linking. Lastly, the stimulatory character of the receptor was explored by calcium signalling. Although the receptor exhibits suppressed immune complex binding, microbial neuraminidases can significantly augment the ability of the FcγRIIA to bind immune complexes. The cross-linking experiments reveal that there is a protein in close proximity with FcγRIIA that could be blocking its IgG binding site under basal conditions. Finally, while FcγRIIA exhibits limited binding of immune complexes in its native state, the stimulatory signals produced by the receptor on contact with immune complexes are significantly strong. Understanding how the body regulates immune complexes could lead to identification of novel methods for both activating and inhibiting the progression of immune complex mediated inflammation.

P3869 Anti-inflammatory activity of macrooids in peripheral blood mononuclear cells and the identification of potential biomarkers of azithromycin administration
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Many studies in the recent past have determined that macrooid antibiotics have anti-inflammatory and immunomodulatory activity in addition to their efficacy in treating bacterial infection. Macrooids have been successfully used in the treatment of severe chronic inflammatory respiratory disorders, including diffuse panbronchiolitis (DBP), cystic fibrosis (CF) and bronchiolitis obliterans (BO). We investigated the ability of azithromycin to attenuate the effects of a lymphocyte directed pro-inflammatory stimulus in PBMCs from healthy volunteers. Our results demonstrate that azithromycin significantly inhibited the induction of proliferation and the release of IL17 in these cells. Treatment with azithromycin in the absence of a pro-inflammatory stimulus in PBMCs induced the release of IL10 in a dose dependent manner 24 h after challenge. In the same model, azithromycin caused a dose dependent, bell-shaped release of GMCSF 24 h after treatment. In conclusion, azithromycin exhibits significant anti-inflammatory and immunomodulatory properties and IL10 and GMCSF may prove to be useful biomarkers following macrooid therapy in respiratory disorders.

P3870 A novel macrooid/flurboraketide, solithromycin (CEM-101), reverses corticosteroid insensitivity via activation of phosphatase-P2A
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Introduction: Activation of PI3 kinase causes oxidative stress-induced corticosteroid (CS) insensitivity via HDAC2 reduction. We have recently demonstrated that a novel macrooid/flurboraketide, solithromycin (Soli, CEM-101) restores CS sensitivity via HDAC up-regulation due to PI3K signaling inhibition (ATS2010). However, the mechanism of this effect has not been elucidated

Aims: To investigate the role of a serine/threonine phosphatase P2A on regulation of the PI3K pathway as the target of Soli.
Methods: CS sensitivity was determined by IC50 of dexamethasone (Des) on TNFa-induced IL-8 production in U937 monocytic cells. Activities of HDAC2 and P2A were measured by fluorescence-based activity assay. Phosphorylation levels of Akt as a marker of PI3K activation were determined by Western blotting. Okadaic acid (OA) was used to inhibit P2A as needed.
Results: OA enhanced H2O2-induced Akt phosphorylation and HDAC2 reduction in U937 cells, and recombinant P2A reduced Akt phosphorylation levels. Soli restored Dex sensitivity under H2O2 exposure, but pretreatment with OA abrogated Soli-mediated restoration of Dex sensitivity, inhibition of Akt phosphorylation, and HDAC2 activation. In addition, P2A immunoprecipitates from the membrane fraction and recombinant P2A were directly activated by Soli.
Conclusions: P2A might be a negative regulator of PI3K signalling. Soli activates P2A directly, inhibits Akt phosphorylation, then restores HDAC2 activity, resulting in CS sensitivity under oxidative stress. Thus, Soli might be a potential treatment for steroid insensitive diseases such as COPD and severe asthma.

P3871 Exposure to cigarette smoke affects the response of dendritic cells to pneumococci
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Development of chronic obstructive pulmonary disease (COPD) is linked to flavivirus. Acute exacerbation potentially related to infection by streptococcus pneumoniae is responsible for the progression of the disease. In vitro association with dendritic cells (DC) mobilization is involved in the pathophysiology of the disease. We hypothesize that cigarette smoke impairs the response of DC to pathogens, a mechanism that may be involved in COPD progression.

Dendritic cell-derived DC of healthy donors were exposed to cigarette smoke extract (CSE) and next to pneumococci (serotype 1). First, endocytosis and bactericidal of S. pneumoniae was analyzed. DC phenotype (costimulatory molecules and endocytosis receptors) and production of immunoregulatory cytokines was measured as well as the capacity of DC to activate autologous T-cells.

Our data showed that CSE exposure inhibited the pneumococcus-dependent expression of CD40, CBDS and CD86 costimulatory molecules. Similarly, immunofluorescence cytokine production (IL-12 and TNF-α) was inhibited by CSE exposure. In DC/T coculture, this was associated with a decrease secretion of IL-17 and IFN-γ by T cells. Unexpectedly, this exposure to CSE increased the endocytosis of pneumococcus, whereas CD206 and CD36 expression was affected in an opposite manner. The effect of CSE was mostly related to oxidative stress since it was inhibited by addition of N-acetyl cysteine.

In summary, CSE exposure impairs the capacity of DC to activate antigen-specific T-cells against pneumococcus, although it does not alter their endocytosis. These data will be confirmed by the ex vivo analysis of DC phenotype in COPD patients with exacerbation.

P3872 OM-85 shapes dendritic cell activation into a “pre-alert” phenotype
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Background: OM-85 (Broncho-Vaxom®) is a bovine bacillus haptens produced by Bacillus subtilis and it has proven efficacy in the prevention of recurrent infections of the airways and acute exacerbations in COPD. OM-85 is a haptoallergenic bacterial extract comprising fractions of 21 different inactivated bacterial strains. Previous work has shown that OM-85 acts as an immunomodulator, and that it may act on human dendritic cells (DC). However, the molecular mechanisms through which OM-85 activates DC remained largely unknown.

Aims: Investigate the impact of OM-85 stimulation on DC and identify the macrophage receptors implicated in OM-85 responses.
Methods: Primary and in vitro derived hDC were used to determine the precise OM-85 effects on DC biology. Stably transfected cell lines expressing human receptors were used to characterize OM-85 receptor-dependent activity.
Results: In hDC, OM-85 induced the secretion of IL-6 and of several chemokines (i.e. CXCL1, CXCL6, CCL3, CCL20, CCL23) with a potency comparable to the prototypical activating stimulus LPS. OM-85 potentiated the effect of IFNγ in terms of IL-6, IL-12 and IL-10 release. The induction of selected chemokines by suboptimal doses of classical pro-inflammatory stimuli were boosted by the presence of OM-85. In addition, it was identified that OM-85 activates TLR2, NOD1 and NOD2 receptors in a significant, dose-response manner.
Conclusions: OM-85 induces a mild and well shaped hDC activation through selected pattern recognition receptors. This activation may contribute to the generation of a “pre-alert” state” resulting in an early protection towards incoming infections. These results provide new insights in the understanding of the mode of action of OM-85, opening new venues for further investigation.

P3873 Altered phenotype of blood dendritic cells in patients with acute pneumonia
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Background: Dendritic cells (DCs) play a key role in the host defense against pathogens. However, the phenotype of blood DCs in patients with acute respiratory infections is unknown.

Objective: To investigate the number and the expression of function-associated molecules of blood DCs in patients with acute infections pneumonia.
Methods: Sixteen patients with an acute pneumonia and 19 controls without pneumonia were included in the study. The number as well as the expression of function-associated molecules of myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) were analysed in peripheral blood using four-colour flow cytometry.
Results: Elevated concentrations of procalcitonin (median: 0.55 ng/ml) and the