

WS17.5 Increased apoptosis and lack of invariant NKT cells in patients with cystic fibrosis leads to circulating antinuclear antibodies

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In patients with cystic fibrosis (CF) mutations in the epithelial chloride channel CFTR lead to increased ceramide and apoptosis. Because apoptotic bodies are recognized as autoantigens by the adaptive immune system we hypothesized that autoantibodies are present in blood of CF patients. Here we show that the majority of CF patients are persistently positive for autoantibodies to typical antinuclear antigens (ANA), also present in patients with systemic lupus erythematosus (SLE). Because invariant Natural Killer T (iNKT) cells may limit autoreactive B cells, we hypothesized that CF patients, similar to SLE patients, express low numbers of iNKT cells. Indeed, CF patients revealed significantly lower numbers of iNKT cells compared to healthy individuals. In contrast, CF KO mice revealed highly elevated iNKT cells in several organs and normal serum autoantibody levels, while gut corrected CF MHH mice, revealing less elevated iNKT cells, showed slightly increased autoantibody levels. iNKT cell numbers were also increased in brain tissues of CF MHH mice, excluding that accumulation of iNKT cell in CF mice is a consequence of microbial infection. iNKT cells recruited macrophages into lung tissues of CF MHH mice, probably eliminating apoptotic bodies. Genetic deletion of iNKT cells in CF MHH mice (CF MHH/J α 18) resulted in significantly elevated autoreactive B cell in spleen and lung and circulating autoantibody levels. This work established a novel link between CF and autoimmunity and demonstrates that iNKT cells in CF patients are not sufficient to control autoantibody production against apoptotic cell antigens in contrast to the CF KO mouse strain.

WS17.6 Cystic fibrosis airway epithelial cells interact with dendritic cells to produce immune tolerance

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Objectives: Despite ample evidence of dysregulated innate immunity in cystic fibrosis lung disease a role for adaptive immune impairment has been sparsely studied. The objective of this work was to use an *ex vivo* primary human model to investigate the interaction of airway epithelial cells with dendritic cells. We hypothesised that cystic fibrosis airway epithelial cells skew adaptive immunity to favour chronic infection and lung damage in people with cystic fibrosis.

Methods: We isolated cystic fibrosis primary human bronchial epithelial cells and assessed their modulation of monocyte-derived dendritic cells (moDC). Monocytes were cultured with conditioned medium from cystic fibrosis primary bronchial epithelial cells (n=6 individual patients) during moDC maturation with interleukin-4 and granulocyte macrophage-colony stimulating factor. Expression of typical dendritic cell and macrophage markers was measured by flow cytometry to assess resulting phenotype. Compared to control moDC, cystic fibrosis epithelial cell conditioned moDC were tolerogenic and macrophage-like (\downarrow CD1a, \downarrow CD86, \uparrow CD14 and \uparrow IL-10), inducing low T cell proliferation and interferon- γ production in an allogeneic mixed lymphocyte reaction.

Conclusions: We conclude that cystic fibrosis airway epithelial cells secrete factors that contribute to immune tolerance and may be permissive of chronic infection. This may represent an important novel mechanism that contributes to the pathogenesis of cystic fibrosis lung disease.

WS17.7 Itraconazole up-regulates the vitamin D receptor and reduces T-helper 2 responses in individuals with cystic fibrosis colonized with *Aspergillus fumigatus*

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Fungal colonization with *Aspergillus fumigatus* (*A. fumigatus*) in cystic fibrosis (CF) is increasingly recognized. While allergic bronchopulmonary aspergillosis (ABPA) leads to a deterioration of pulmonary function, the effect of *A. fumigatus* colonization in the absence of ABPA remains unexplored. To address this we examined a cohort of CF individuals with *A. fumigatus* who were ABPA-negative to identify the effects of itraconazole therapy on *Aspergillus*-induced lung inflammation.

We demonstrate using *in vitro* cell models that *A. fumigatus* downregulates the nuclear vitamin D receptor (VDR) in airway epithelial cells and that the secondary metabolite gliotoxin (Gt) is the main causative agent, resulting in a heightened Th2 response and increased interleukin (IL)-5 and IL-13 production.

In vivo, *A. fumigatus* positivity correlated with increased Gt in CF bronchoalveolar lavage fluid (BALF), mosaic pattern on high resolution computed tomography (HRCT) and increased BALF levels of IL-5 and IL-13. Following airway eradication of *A. fumigatus* with itraconazole, we observed decreased IL-5 and IL-13 in BALF, improved respiratory symptoms and reduced infective exacerbations that correlated with sustained pulmonary function.

This study provides rationale for the therapeutic effect of itraconazole and additionally highlights the therapeutic potential of vitamin D supplementation in preventing ABPA are only feasible with concurrent elimination of *A. fumigatus* with itraconazole to permit VDR expression and its positive functional consequences.

WS17.8 Inhibition of leukotriene B₄ signaling by alpha-1 antitrypsin: support for the use of aerosolized alpha-1 antitrypsin therapy in cystic fibrosis

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Cystic fibrosis (CF) is characterised by neutrophil-dominated airway inflammation, in part attributable to the potent chemotactic agent leukotriene B₄ (LTB₄). The aim of this study was to investigate the ability of exogenous alpha-1 antitrypsin (AAT) to inhibit LTB₄ signaling. The biological consequence of the described AAT induced inhibition was investigated at the level of neutrophil (PMN) released proteolytic enzymes including azurocidin, a potent activator of human monocytic cells.

PMNs isolated from healthy control volunteers (n=4) were stimulated with LTB₄ (100 nM/ 2 \times 10⁷) in the presence and absence of AAT (27.5 mM) for increasing increments of time (0, 5, 10 and 20 min). The level of degranulated proteins in surrounding supernatants was determined by western blot analysis. Proteins investigated included myeloperoxidase, hCAP-18 and matrix metalloproteinase-9 as markers for primary, secondary and tertiary granule release respectively. Levels of azurocidin released from primary granules and secretory vesicles was electrophoretically examined.

In vitro data has shown that levels of degranulated MPO, hCAP-18 and MMP-9 were significantly decreased in the presence of AAT (P<0.05). Densitometry of immuno-bands revealed that PMNs release azurocidin in response to LTB₄, an effect reversed by inclusion of AAT (P<0.05). The mechanism of inhibition involved direct binding of AAT to LTB₄ as reduced vibrational fine structure of the LTB₄/AAT UV absorbance spectrum indicated complexation of the two molecules in solution.

The results of this study indicate that AAT can inhibit LTB₄ signaling and further justifies the use of aerosolised AAT as an effective treatment for CF.