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/Jo18) 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 autointeraction production against apoptotic cell antigens in contrast to the CF KO mouse strain.

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

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Fungal colonization with *Aspergillus fumigatus* (A. fumigatus) in cystic fibrosis (CF) is increasingly recognized. While allergenic bronchoalveolar aspirategenerated by *A. fumigatus* 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.

**WS17.7 Itraconazole up-regulates the vitamin D receptor and reduces T-1 response in individuals with cystic fibrosis colonized with *Aspergillus fumigatus***

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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 (AA) 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 azurocin, a potent activator of human monocytic cells. PMNs isolated from healthy control volunteers (n = 4) were stimulated with LTB4 (100nM/2 x 10^7) 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, ICAP-18 and matrix metalloproteinase-9 as markers for primary, secondary and tertiary granule release respectively. Levels of azurocin released from primary granules and secretory vesicles was electromicroscopically examined. In vitro data has shown that levels of degranulated MPO, ICAP-18 and MMP-9 were significantly decreased in the presence of AAT (P < 0.05). Denaturation of immuno-bands revealed that PMNs release azurocin 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.