## WS22.1 Basophil activation is a reliable biomarker of allergic bronchopulmonary aspergillosis (ABPA) in CF: Interim results of a longitudinal cohort study

R.B. Moss[1], J. Waters[2], C. Dunn[1], Z. Davies[1], C. Everson[1], R. Tirouvanziam[3], J. Tameresis[1], L.A. Herzenberg[2], Y. Gernez[2,4]. *[1]Stanford University, Pediatrics, Palo Alto, United States; [2]Stanford University School of Medicine, Genetics, Stanford, United States; [3]Emory University, Pediatrics, Atlanta, United States; [4]Highland Alameda County Hospital, Internal Medicine, Oakland, United States*

We previously described upregulation of blood basophil CD203c as a novel feature of CF-ABPA in a cross-sectional study (Gernez, JCF 2012;11:502). Here we present interim 1 year results of a longitudinal cohort study comparing CF-ABPA patients ($n = 18$) with two control groups, CF patients colonized with *A. fumigatus* (Af) but without ABPA (AC; $n = 17$) and CF patients without Af colonization or ABPA (CF; $n = 16$). Patients are tested every six months and when ill with pulmonary exacerbation. Basophil CD203c surface expression reliably and robustly discriminated CF-ABPA from AC and CF over time (repeated measures ANOVA $p = 0.0003$). *Ex vivo* stimulation with Af extract (optimal at 10 minutes exposure) or recombinant Asp f1 (optimal at 30 min) produced similar results without significant difference in categorization. CD203c tended to increase slightly over time in CF, be stable in AC, and remain elevated but quite variable in CF-ABPA. Total IgE level, presence or severity (level of specific IgE antibody) of common respiratory aeroallergen sensitization, genotype, lung function (FEV1 % predicted) and exacerbations did not affect CD203c responses to *ex vivo* Af stimulation. CD203c responses correlated with Af-specific IgE levels ($p = 0.01$). Colonization with Af was more frequent when co-infected with mucoid or nonmucoid *P. aeruginosa* ($p = 0.02$, $p = 0.04$ respectively) and less frequent with *S. aureus* ($p < 0.0001$). Inhaled corticosteroid use, co-infection with *S. maltophilia*, diabetes and overall pulmonary exacerbations were similar in CF-ABPA and control groups. We conclude that the blood basophil CD203c assay is suitable for evaluation as a diagnostic and monitoring biomarker of ABPA in CF.

## WS22.2 *Pseudomonas aeruginosa*-induced apoptosis in airway epithelial cells is mediated by gap junctional communication in a CFTR-dependent manner

D. Losa[1], T. Köhler[2], J. Bellec[3], T. Dudez[1], S. Crespin[1], M. Bacchetta[1], T.H. Nguyen[3], C. Van Delden[2], M. Chanson[1]. *[1]Geneva University Hospitals and University of Geneva, Department of Pediatrics, Geneva, Switzerland; [2]University of Geneva, Department of Microbiology, Geneva, Switzerland; [3]INSERM UMR 1064, Nantes, France*

Gap junctions (GJs) mediate horizontal communication between cells and are thought to contribute to a coordinated response of the airway epithelial cells (AECs) to infection. Here, we examined innate immune recognition and epithelial responses at the level of the GJ protein connexin43 (Cx43) using a model of *P. aeruginosa* (*Pa*) infection of the polarized AEC line Calu-3.

PAO1, which is not internalized by AECs, induced functional expression of Cx43 by a mechanism involving flagellin binding to TLR5. The latter increased was not observed in cells infected with a *fliC* PAO1 mutant strain. Expression of Cx43 by the TLR5 signaling cascades involved an opposite regulation exerted by JNK and p38 MAPKs. PAO1 triggered pro-inflammatory (evaluated by IL-8 release) and pro-apoptotic (evaluated by cleaved caspase-3 and annexin V detection) responses. In the presence of a JNK inhibitor, PAO1-induced AEC apoptosis was increased. Interestingly, the latter effect was prevented by lentiviral expression of a Cx43-specific shRNA and by pharmacological GJ blockade. However, GJ blockade did not change PAO1-evoked IL-8 release. Thus, PAO1-induced apoptosis in AECs is mediated by GJs, and JNK signaling appears to act as a negative regulator of Cx43 expression. Moreover, we found that JNK activity was up-regulated by pharmacological inhibition of CFTR. Decreased CFTR activity was associated with reduced Cx43 expression and apoptosis.

These results indicate that Cx43 expression is a component of the response of AECs to innate immune activation by regulating the inflammation/apoptosis balance. Defective CFTR could alter this equilibrium with deleterious consequences on the CF AEC response to *Pa*.

## WS22.3 Role of macrophage proteases in the killing of intracellular bacteria

N. Cifani[1], E.P. Reeves[2], N.G. McElvaney[2], B. Pompili[1], P. Del Porto[1], F. Ascenzioni[1]. *[1]Sapienza University, Department of Biology and Biotechnology, Rome, Italy; [2]Respiratory Research Division, Department of Medicine, Education and Research Centre, Beaumont Hospital, Dublin, Ireland*

Macrophages have a vast arsenal of microbicidal mechanisms among which cathepsins, that consist of serine proteases, aspartate proteases, and cysteine proteases contribute, together with other lysosomal hydrolases, to completely destroy the engulfed pathogens. Although matrix metalloproteinases (MMPs) activities have been thought to occur extracellularly or at the cell surface, it has been reported that macrophage MMP-12 displays direct intracellular antimicrobial activity. To dissect the contribution of proteases to bactericidal activity of macrophages, THP-1-derived macrophages were pre-treated with protease inhibitors (E-64 for cysteine proteases, pepstatin A for aspartate proteases, pefabloc for serine proteases and EDTA for MMPs) and infected with *Pseudomonas aeruginosa*. The intracellular *P. aeruginosa* survival was evaluated in un-treated *vs* treated cells and was significantly reduced in cells pre-treated with PepstatinA, EDTA or a cocktail containing all protease inhibitors. Since EDTA does not inhibit exclusively MMPs, two additional specific MMP inhibitors were used, GM6001 and phosporamidon, confirming the data obtained with EDTA. These results demonstrated that MMP and aspartate protease inhibitors improve *P. aeruginosa* killing by THP-1 macrophages, suggesting that protease inhibitors may improve macrophage bactericidal ability. Although other experiments are required to highlight the molecular mechanism/s underlying this effect, it can be hypothesized that MMP and aspartate proteases directly modulate important antimicrobial mechanisms or it may affect the bacterial proteases, thus decreasing the ability of the bacteria to survive inside the cells.

## WS22.4 Biofilm formation by cystic fibrosis-relevant *Burkholderia cepacia* complex (Bcc) bacteria allows them to evade neutrophil anti-microbial activities

M. Murphy[1], M. Callaghan[1], E. Caraher[1]. *[1]Centre for Microbial-Host Interactions (CMHI), Institute of Technology Tallaght, Dublin, Ireland*

Pathogenic *Burkholderia cepacia* complex (Bcc) species chronically-colonise cystic fibrosis patients' lungs, despite continuous pulmonary recruitment of neutrophils. There, they may induce '*cepacia* syndrome', an acute deterioration of lung function with associated septicaemia which is often fatal. Most Bcc species are multi-drug-resistant and form biofilms, further reducing antibiotic susceptibility.

In order to ascertain whether Bcc biofilm formation confers an additional advantage on the pathogens by evading neutrophil antimicrobial responses, we examined the interaction of neutrophils with Bcc biofilms *in vitro*.

Confocal microscopy demonstrated that the mature biofilm phenotype ($\geqslant 72$ hr) can protect Bcc bacteria from phagocytosis, illustrating that differentiated, neutrophil-like cells (dHL60s) remain at the biofilm surface, impeding phagocytosis. Their presence reduced initial biofilm formation of *B. multivorans* LMG 13010, but significantly increased biomass of established biofilms of *B. multivorans* and *B. dolosa* LMG 18941 ($p < 0.01$). Experiments repeated using whole-cell lysates of dHL60s confirmed increased biofilm biomass was formed in the presence of lysate ($p < 0.001$). Bcc biofilms induced dHL60s to secrete IL-8, but secretion did not vary with respect to the age of biofilm (24–72 hr) despite greater numbers of bacteria, suggesting the biofilm masks their detection.

Hence, improving clearance of colonising Bcc requires approaches to abrogate biofilm formation and overcome biofilm-mediated resistance to killing by neutrophils.

This work is funded by Science Foundation Ireland (SFI).