of routine care in an ambulatory HIV care service and is effective in identifying smokers and increasing referrals to smoking cessation services. Further work will evaluate the impact of this intervention in HIV positive subjects.

Lung infection mechanisms

S82 'THE KISS OF DEATH' – CALCINEURIN INHIBITORS PREVENT ACTIN-DEPENDENT LATERAL TRANSFER OF ASPERGILLUS FUMIGATUS IN NECROPTOTIC HUMAN MACROPHAGES

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Invasive fungal infections are a major cause of mortality in solid-organ transplantation where steroids and calcineurin inhibitors form the core of immunosuppression. Our group has previously shown in established hydrocortisone-based mouse models of invasive aspergillosis that calcineurin inhibitors increase mortality through effects on the innate immune response.[1] As alveolar macrophages present the primary resident innate immune cell in the airways responsible for fungal clearance, we perform a detailed study of the role of the calcineurin pathway in the human macrophage response to *A. fumigatus* (AF).

We show that the calcineurin-NFAT pathway is highly activated in the human lung transplant alveolar macrophage response to AF with inhibition resulting in impaired fungal clearance. Calcineurin inhibition leads to delayed phagocytosis, reduction in reactive-oxygen species production and an impairment of a novel actin-dependent process of lateral transfer of swollen AF conidia between human macrophages. Further characterisation reveals that transfer of AF occurs during macrophage necroptosis with subsequently around 50% control of germination in the receiving macrophage. To understand the calcineurin-dependent mechanism, next generation RNA sequencing was performed which confirms that calcineurin inhibition impairs the macrophage programmed cell death immune response. Utilising phosphoproteomics we additionally show that calcineurin inhibition impairs dephosphorylation of vasodilator-stimulated phosphoprotein (VASP), an important actin regulatory protein which promotes actin filament formation. High-resolution confocal microscopy confirms that VASP strongly co-localises to AF conidial phagocytosis and facilitates lateral transfer through tunnel-like structures. Lastly, we utilise a zebrafish model of invasive aspergillosis to confirm the in-vivo relevance of AF macrophage lateral transfer.

In conclusion our data shows the importance of the calcineurin pathway in the macrophage innate immune response to AF and highlights a novel calcineurin-actin dependent host defense mechanism which may have significant implications on persistence and dissemination within solid organ transplantation. To our knowledge this is the first report of a host-mediated cell-cell transfer mechanism for any pathogen.

REFERENCE

1 Herbst S, Shah A, Mazon Moya M, Marzola V, Jensen B, Reed A, *et al*. Phagocytosis-dependent activation of a TLR9-BTK-calcineurin-NFAT pathway co-ordinates innate immunity to Aspergillus fumigatus. *EMBO Mol Med*. 2015;**7**(3):240–58

S83 CALCINEURIN INHIBITION IMPAIRS PHENOTYPIC MATURATION OF DENDRITIC CELLS IN A *IN VITRO* MODEL OF INVASIVE ASPERGILLOSIS IN LUNG TRANSPLANT RECIPIENTS

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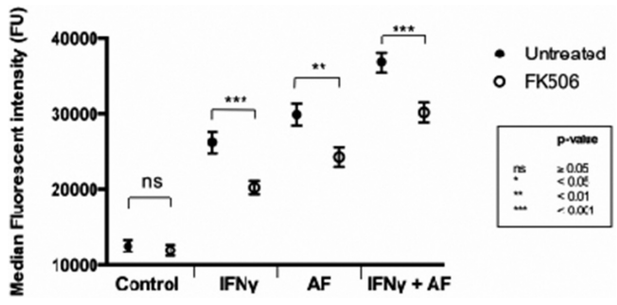
Introduction Invasive aspergillosis in lung transplant recipients on immunosuppression is associated with high morbidity and mortality. The calcineurin inhibitor tacrolimus (FK506) inhibits the calcineurin-NFAT axis, which impairs the innate response to fungal infection.[1] Dendritic cells (DC's) play a pivotal role in signalling to the adaptive immune system in infection – immature DC's phagocytose antigen, leading to maturation into DC's capable of stimulating T-cells. We investigated the effect of FK506 on DC function in invasive aspergillosis by assessing phenotypic maturation of DC's in response to *Aspergillus fumigatus* (AF) infection.

Methods Healthy volunteer PBMC's negatively isolated by Ficoll® gradient were differentiated into DC's with GM-CSF and IL-4. Day 5 cells were matured with IFN-γ. Day 7 cells were treated with FK506 and/or inoculated with swollen conidia of *A.fumigatus* (MOI 1:1). Cells were then stained with PE-bound anti-CD83 (a late maturation marker) and PerCP-Cy5.5-bound anti-CD-209 (a DC-specific marker) and analysed by the ImageStream® imaging flow cytometer. Statistical analysis was performed with Graphpad Prism v6.0, using unpaired t-tests with Welch correction.

Results 5000 cells/condition were analysed. DC's were subsetted by gating for CD-209 positivity. FK506 was not toxic to cells (similar cell viability between groups).

We demonstrated up-regulation of CD83 (measured by mean fluorescent intensity) with IFN-γ stimulation of DC's (12534 ± 799.3 vs. 26228 ± 1462, $p < 0.0001$; mean fluorescent units +/-SEM), AF infection of unstimulated DC's (12534 ± 799.3 vs. 29888 ± 1393, $p < 0.0001$) and for AF infection of IFN-γ-stimulated DC's (26228 ± 1462 vs. 36778 ± 1356, $p < 0.0001$).



Abstract S83 Figure 1

CD83 mean fluorescent intensity was reduced with FK506 treatment of IFN-γ-stimulated DC's (26228 ± 1462, vs. 20219 ± 846.0, $p = 0.0004$), AF-infected unstimulated DC's (29888 ± 1393 vs. 24289 ± 1253, $p = 0.0028$), and AF-infected, IFN-γ-stimulated DC's (36778 ± 1356 vs. 30159 ± 1279, $p = 0.0004$), but unchanged for unstimulated, un-infected DC's (12534 ± 799.3, vs. 11942 ± 762.5, $p = 0.5921$).

Conclusion Both *A.fumigatus* infection and IFN-γ stimulation promote phenotypic maturation of DC's *in vitro*, and treatment

with FK506 inhibits maturation in this context. This suggests an inhibitory effect of FK506 on innate antigen presentation to T-cells and may impair the adaptive immune response to invasive aspergillosis in lung transplants recipients.

REFERENCE

1 Herbst S, Shah A, Mazon Moya M, *et al*. *EMBO Mol Med*. 2015;**7**(3):240–58

S84 SPUTUM NEUTROPHILS BUT NOT INTERLEUKIN-8 (IL-8) OR INTERLEUKIN 17 (IL-17) CORRELATE WITH THE BRONCHIECTASIS SEVERITY INDEX (BSI)

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Background Bronchiectasis is a progressive neutrophilic inflammatory lung disease associated with abnormal local cytokine release with possible systemic overspill. Early data suggests that interleukin-17 (IL-17) could be involved in the enhanced infiltration of neutrophils in the lungs, via the induction of IL-8 release, and has emerged as a possible biomarker for other chronic neutrophilic lung diseases.

Aims

1. to investigate the potential use of IL-17 and IL-8 as biomarkers of disease severity in bronchiectasis by utilising a multidimensional clinical severity scoring system, the Bronchiectasis Severity Index (BSI).
2. correlate sputum neutrophils and pathogen status with serum or sputum IL-17 and IL-8 levels.

Methods Spontaneous sputa and sera were collected from stable adult bronchiectasis patients attending a specialist clinic. We quantified both IL-17 and IL -8 concentrations in the pulmonary compartment (sputum) and the systemic compartment (serum) of 119 stable bronchiectasis patients and 26 healthy volunteers. Sputum neutrophils were conducted using standard methods.

Results The mean patient age was 65 years, with 24% in mild BSI, 39% moderate BSI and 46% (43% idiopathic, 24% post infectious). IL-17 in the sputum of bronchiectasis patients was found to be two-fold greater than in serum suggesting "local" release (10 pg/ml vs 5 pg/ml). Statistical analysis revealed a significant correlation between these two variables, suggesting a "spillover" of cytokines from the lungs ($p < 0.001$).

However, there was no significant difference in serum IL-17 levels between bronchiectasis and healthy subjects (0 ± 2 pg/ml). In addition, no significant correlation was found between IL-8 and IL-17 levels in the sputum of patients. Sputum IL-17 levels were found to have a significant negative correlation with BSI severity scoring, but this was not reproduced when individual BSI parameters were analysed. IL-8 similarly performed poorly in correlating with BSI. In contrast more severe BSI scores were significantly associated with higher% neutrophils in sputa ($p = 0.045$).

Conclusions The clinical utility of IL-17 and IL-8 as biomarkers for the prediction of disease severity in bronchiectasis patients appears poor. These data may also suggest targeting these chemokines are of limited value. Focus in bronchiectasis may need shifted from neutrophil chemokines to factors that inhibit apoptosis and/or promote neutrophil persistence in the airway.

S85 PNEUMOLYSIN TRIGGERS THE PRODUCTION OF PLATELET-ACTIVATING FACTOR BY HUMAN NEUTROPHILS IN VITRO

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Introduction and objectives Pneumolysin (Ply), the major protein virulence factor of the pneumococcus, has been implicated in the pathogenesis of acute lung injury and acute coronary events, both of which are significant causes of mortality, in severe pneumococcal disease. However, the role of Ply in promoting neutrophil/platelet cross-talk, increasingly recognised as a key event in the immunopathogenesis of inflammation-mediated pulmonary and cardiovascular damage is unknown. This issue has been addressed in the current study, which is focused on the effects of exposure of isolated human blood neutrophils to Ply on the production of the platelet-targeted, pro-inflammatory lipids, platelet-activating factor (PAF) and thromboxane A_2 (TXA_2).

Methods Neutrophils, isolated from the blood of healthy, adult humans, were suspended at a concentration of 2×10^6/ml in Hanks balanced salt solution and preincubated for 10 min at 37°C followed by addition of recombinant Ply (5–80 ng/ml), or the pneumolysoid, delta6Ply (80 ng/ml, negative control), or the calcium ionophore, A23187, (2 μM, positive control). After 5 min of incubation, the reactions were terminated and PAF and TXA_2 assayed in the cell-free supernatants using sandwich ELISA procedures.

Results These are shown in the accompanying Table 1. Exposure of neutrophils to Ply resulted in dose-related enhancement of production of PAF, which achieved statistical significance at concentrations ≥20 ng/ml of the toxin, while delta6Ply was ineffective, and A23187 extremely potent. Similar, but less impressive effects were noted in the case of TXA_2.

Abstract S85 Table 1

Agent	PAF (pg/ml)	TXA_2 (pg/ml)
Control	4.5 ± 1.2	13.5 ± 1.4
Ply, 20 ng/ml	9.6 ± 2.4*	16.1 ± 2.0*
Ply, 40 ng/ml	12.1 ± 3.5*	17.7 ± 2.1*
Ply, 80 ng/ml	13.1 ± 4.0*	17.7 ± 2.3*
delta6Ply, 80 ng/ml	5.4 ± 1.0	12.3 ± 1.5
A23187, 2 μM	37.0 ± 5.3*	28.0 ± 1.4*

*$p < 0.05$-$p < 0.002$.

Conclusion Ply, via its pore-forming activity, activates the production of PAF and, to a lesser extent, TXA_2, by neutrophils, potentially augmenting pro-inflammatory cross-talk between these cells and platelets, an activity of the toxin which may contribute to the immunopathogenesis of lung and cardiac injury in severe pneumococcal disease.

S86 THE ANTI-INFLAMMATORY EFFECTS OF PNEUMOLYSIN

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