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

[1]A Shah, [1]S Kannambath, [1]S Herbst, [2]A Rogers, [2]M Carby, [2]A Reed, [1]S Mostowy, [1]S Shaunak, [1]D Armstrong-James. *[1]Imperial College, London, UK; [2]Royal Brompton and Harefield NHS Foundation Trust, London, UK*

10.1136/thoraxjnl-2015-207770.88

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

[1]AG Adlakha, [2]DPH Armstrong-James, [1]B Lenhard. *[1]MRC Clinical Sciences Centre, Imperial College, London, UK; [2]National Heart and Lung Institute, Imperial College, London, UK*

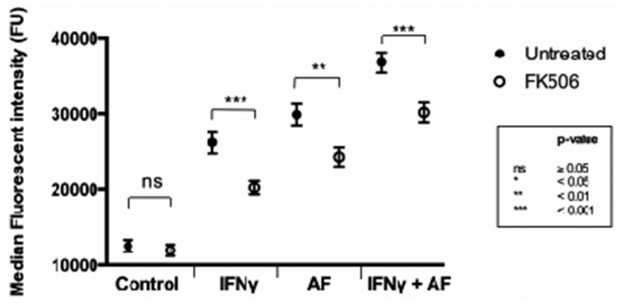
10.1136/thoraxjnl-2015-207770.89

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