BTS/BALR/BLF Early Career Investigators Symposium

T1 CALCINEURIN INHIBITION IMPAIRS THE DENDRITIC CELL TRANSCRIPTIONAL RESPONSE TO ASPERGILLUS FUMIGATUS INFECTION IN LUNG TRANSPLANT RECIPIENTS

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Background Lung transplant recipients on calcineurin inhibitor immunosuppression have increased susceptibility to, and increased mortality from, invasive aspergillosis. Tacrolimus (FK506) diminishes the innate immune response to *Aspergillus fumigatus* infection partly by inhibition of the calcineurin-NFAT axis. We investigated the effects of FK506 on transcriptional regulation in dendritic cells (DC's), and assessed interferon-gamma as a treatment, with a combination of RNA-Seq and histone modification ChIP-seq.

Methods Healthy volunteer monocytes were negatively isolated from gradient-centrifugation-selected PBMC's and differentiated into DC's with GM-CSF and IL-4. DC's were treated with FK506, interferon-gamma and/or inoculated with swollen conidia of *A.fumigatus* (MOI 1:1). For RNA-Seq, extracted mRNA was poly-A purified and reverse-transcribed to ds-DNA, and for ChIP-seq, DNA was cross-linked, sonicated, then immunoprecipitated with antibodies against histone marks H3k4me1 and H3k27ac. Resultant DNA was PCR-amplified to generate libraries for next generation sequencing on the Illumina HiSeq 2500. Computational sequencing analysis pipelines used open-source C++ and R-based packages (Bowtie, Kallisto, edgeR and MACS).

Results *A.fumigatus* infection in DC's elicited upregulation of genes belonging to two key groups of early-phase response transcription factors – the early growth response family (EGR1 – log fold-change 4.90, FDR p-value = 0.0003) and the nuclear receptor family (NR4A2 – logFC 6.96, $p = 1.56 \times 10^{-6}$). FK506 treatment ablated significant differential expression of these genes whilst subsequent interferon-gamma treatment restored their upregulation (EGR1 – logFC 4.43, p = 0.00093; NR4A2 – logFC 5.56, p = 0.00034).

Active gene enhancers regions were identified by presence of significant peaks of H3k4me1 and H3k27ac antibody binding. Motif analysis of enhancers within regulatory domains around differentially-expressed genes identified enrichment of core binding motifs of NFAT ($p = 7.8 \times 10^{-9}$) and FOXF2 ($p = 8.6 \times 10^{-10}$) transcription factors, which was lost after FK506 treatment.

Conclusions Transcriptome analysis has revealed the key genes involved in early dendritic cell responses to *A.fumigatus* infection, and their ablation by FK-506 treatment suggests a deleterious genome-level effect of calcineurin inhibitors in this context. Furthermore, interferon-gamma treatment restores a more favourable transcriptomic response to infection in FK506-treated DC's. The condition-dependent differential enrichment of enhancer motifs suggests a role for both suspected (NFAT) and previously unidentified (FOXF2) transcription factors in the DC response to *A.fumigatus* infection.

T2 EARLY-LIFE RESPIRATORY TRACT INFECTION AND ADULT SUSCEPTIBILITY TO CHRONIC MUCUS HYPERSECRETION – A PROSPECTIVE 64 YEAR NATIONAL BIRTH COHORT STUDY

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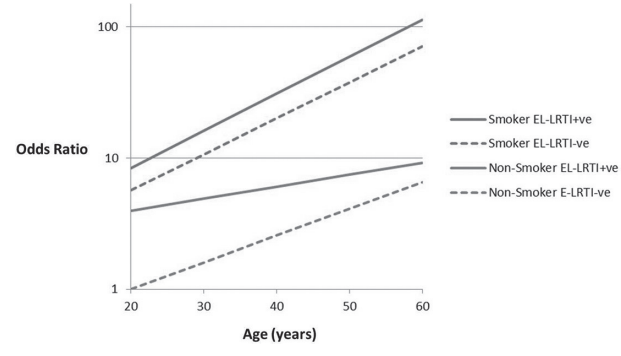
Introduction Smoking commonly triggers Chronic Mucus Hypersecretion (CMH) indicating both accelerated FEV_1 decline and arguably early-phase COPD development. Early-life respiratory infections are proposed as another cause of adult CMH and also lead to impaired adult lung function. We investigated how smoking may modify the relationship between early-life infection and CMH across adult life.

Methods The MRC National Survey of Health and Development has prospectively studied a nationally representative sample of men and women since their birth during one week in March 1946 within England, Scotland and Wales. During early-life (ages 0 to 2 years) lower respiratory tract infection presence (EL-LRTI), father's occupational social class and estimated local pollution exposure were recorded for each study member. Smoking status and MRC questionnaire defined CMH were recorded six times between age 20 and 60–64 years. Random-effects logistic regression models for repeated measures were used to describe CMH trajectories across adult life by smoking status and EL-LRTI adjusting for sex, birth weight, early-life pollution exposure (high vs low) and social class (manual vs non-manual).

Results Amongst the 3617 individuals included (52% male; 63% ever-smokers) 25% experienced an EL-LRTI. CMH prevalence increased during adulthood (cumulative prevalence = 12%). Smokers had higher odds of CMH at all ages compared to non-smokers (Figure 1). For smokers and non-smokers, those with EL-LRTI had higher odds of CMH than those without EL-LRTI. There was evidence of an interaction between smoking status and EL-LRTI (Figure 1) such that at age 20 the effect of EL-LRTI



Abstract T2 Figure 1 Odds rations of chronis Mucus Hypersecretion (CMH) across adult life according to concurret smoking and history of lower respiratory tracts infection between ages 0 and 2 years (relative to CMH presence at age 20 yeards in the baseline group of non-smokers without early-life lower repiratory tract infections)
EL-LRTI+ve = Early-Life Respiratory Tract Infections(s) present
EL-LRTI-ve = No Early-Life Respiratory Tract Infections(s)

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