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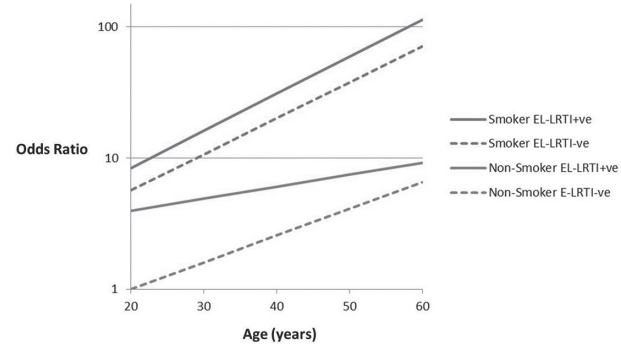
Introduction Smoking commonly triggers Chronic Mucus Hypersecretion (CMH) indicating both accelerated FEV_1 decline and arguably early-phase COPD development. Early-life respiratory infections are proposed as another cause of adult CMH and also lead to impaired adult lung function. We investigated how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

(EL-LRTI+ve versus EL-LRTI–ve) was greater in non-smokers (OR = 4.0 (95% confidence interval (CI): 1.9 to 8.2; P < 0.001) than in smokers (OR = 1.48 CI: 0.8 to 2.6; P = 0.17). In non-smokers only the association between EL-LRTI and adult CMH lessened with ageing. Thus by age 60, the effect of EL-LRTI on the odds of having CMH (EL-LRTI+ve versus EL-LRTI–ve) amongst non-smokers (OR = 1.4 (CI: 0.8 to 2.4; P = 0.21)) and smokers (OR = 1.6 CI: 0.8 to 3.1; P = 0.15) was similar.
Conclusion Infants with respiratory infections become adults predisposed to developing CMH potentially reflecting pulmonary damage sustained during early-life or an overarching altered susceptibility to respiratory insults. This relationship is modulated by smoking and ageing.

T3 HUMAN RHINOVIRUS IMPAIRS THE INNATE IMMUNE RESPONSE TO BACTERIA IN MONOCYTE DERIVED MACROPHAGES FROM PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Introduction Exacerbations of Chronic Obstructive Pulmonary Disease (COPD) are associated with accelerated disease progression, hospitalisation and death. Respiratory viruses are identified in approximately half of all exacerbations. We have previously found that human rhinovirus infection leads to a secondary outgrowth of bacteria which is associated increased exacerbation severity. The mechanisms of how HRV increases risk of secondary bacterial outgrowth are unknown.
Hypothesis We hypothesised that HRV infection impairs phagocytosis of bacteria by monocyte derived macrophages (MDM) which may lead to an increased risk of secondary bacterial outgrowth during COPD exacerbations.
Methods Participants were recruited from the London COPD Cohort. MDM were generated by culture in GM-CSF or M-CSF for 12 days. MDM were incubated with HRV 16 for 24 hours at increasing multiplicity of infection (MOI) 0.5–10 for 24 hours or poly-IC at increasing concentrations 0–300 μg/ml.

Phagocytic capacity was then assessed by incubating MDMs with fluorescently labelled heat killed *Haemophilus influenzae* or *Streptococcus pneumoniae* for 4 hours and uptake measured by fluorimetry.

The pro-inflammatory cytokine CXCL-8 and anti-inflammatory cytokine IL-10 were measured by ELISA according to the manufacturer's instructions.
Results HRV16 impaired phagocytosis of *H. influenzae* (HRV (MOI of 5) 2.84 ± 0.92 *vs* media control 4.36 ± 1.06 RFU × 10^3 n = 8, p = 0.01) and *S. pneumoniae* (p < 0.01) by MDM in a virus-dose- dependent manner without impairing cell viability. HRV16 alone induced CXCL-8 and IL-10 release from MDM compared to media alone. HRV16 (MOI 5) significantly impaired IL-10 response to *H. influenzae* compared to media alone (0.59 (0.33–0.96) ng/ml *vs* 1.83 (1.11–3.00) ng/ml respectively, n = 6, p = 0.03) and impaired CXCL-8 response to *H influenzae* compared to media alone 4.41 (3.45–5.85) ng/ml *vs* 24.65 (11.63–29.77) ng/ml respectively, n = 5, p = 0.01).

Poly-IC impaired phagocytosis of *H. influenzae* in a concentration-dependent manner without significantly impairing cell viability. Poly IC alone also induced IL-8 release from MDM.
Conclusions HRV impairs phagocytosis of bacteria by MDM in COPD and impairs cytokine response to bacteria which may inhibit neutrophil influx and prevent resolution of inflammation. This may lead to an outgrowth of bacteria and prolonged exacerbations in COPD.

T4 GLOBAL SPREAD OF MYCOBACTERIUM ABSCESSUS CLONES AMONGST CYSTIC FIBROSIS PATIENT

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Introduction Lung infections with *Mycobacterium abscessus,* a species of multidrug resistant nontuberculous mycobacteria, have increased in frequency worldwide, emerging as an important global threat to individuals with cystic fibrosis (CF) where they cause accelerated inflammatory lung damage and death. *M. abscessus* was previously thought to be independently acquired by susceptible individuals from the environment. However, using whole genome sequencing and detailed epidemiological analysis of a cohort of patients attending the CF centre at Papworth Hospital, we found strong evidence for transmission between patients. We therefore sought to examine the mechanism of acquisition of *M. abscessus* in CF individuals across the world.
Methods We undertook whole genome sequencing on 1080 isolates from 517 patients from the UK, US, the Republic of Ireland, mainland Europe and Australia. This was then correlated with clinical metadata and phenotypic functional analysis.
Results Our genomic analysis revealed that the majority of infections are from densely clustered *M. abscessus* genotypes with low levels of diversity, indicating a high level of human associated spread. Moreover, the phylogeny reveals the presence of three recently emerged dominant circulating clones that have globally spread. We found that these clones are associated with worse clinical outcomes and show increased virulence in both cell-based and mouse infection models. Within patients we found evidence of genetic diversity and evolutionary adaptation through the processes of convergent evolution and hypermutation.
Conclusions The majority of *M. abscessus* infections in patients with Cystic Fibrosis are caused by genetically related clusters, indicating recent patient-to-patient transmission despite conventional infection control measures. Transmission appears to have facilitated evolution of *M. abscessus* from an environmental organism into a transmissible human pathogen.

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Correction

Allinson JP, Hardy R, Donaldson GC, *et al*. T2 Early-life respiratory tract infection and adult susceptibility to chronic mucus hypersecretion – a prospective 64 year national birth cohort study. *Thorax* 2016;71:A1–A2. doi:10.1136/thoraxjnl-2016-209333.2

Text within the figure legend has been corrected.

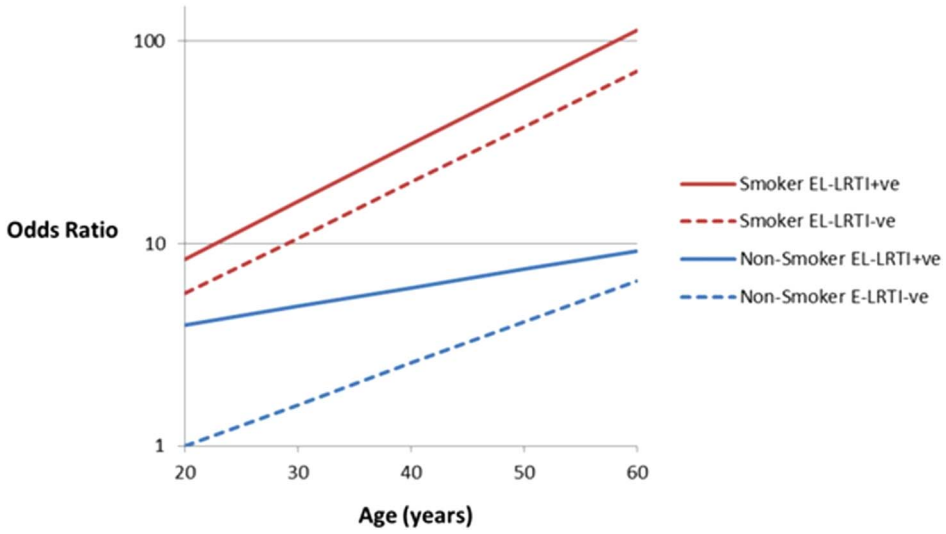

Figure 1 Odds ratios of Chronic Mucus Hypersecretion (CMH) across adult life according to concurrent smoking and history of lower respiratory tract infection between ages 0 and 2 years (relative to CMH presence at age 20 years in the baseline group of non-smokers without early-life lower respiratory tract infection).
EL-LRTI+ve, Early-Life Respiratory Tract infection(s)present; EL-LRTI−ve, No Early-Life Respiratory Tract infection(s).

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