Conclusions This study demonstrates a latent class modelling approach for IA diagnosis in LTR with a combination of culture, composite biomarker testing, and radiology required for optimal IA diagnosis.

S18 MECHANISMS REGULATING COLLAGENOLYTIC AND ELASTOLYTIC ACTIVITY IN *M. AVIUM* INFECTION

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Background The incidence of pulmonary non-tuberculous mycobacterial (NTM) infection is increasing. In the UK mycobacterium avium complex (MAC), is the commonest NTM infection outside of CF lung disease. Patients with pulmonary MAC infection develop cavitating lung disease or nodular bronchiectasis, but the mechanisms of tissue destruction are not well-characterised, unlike *M. tuberculosis* infection. We have previously shown that clinical isolates of *M. avium* surprisingly do not drive secretion of MMP-9 by infected macrophages. Instead, *M. avium* drives functionally unopposed MMP-1, previously thought to be an *M. tuberculosis*-specific response, and MMP-7. We investigated the mechanisms regulating MAC-induced MMP-1 and -7 secretion.

Methods Monocytes were isolated from healthy human volunteer blood by density centrifugation and adherence, before incubation in GM-CSF for 7 days to generate monocyte-derived macrophages (MDMs). MDMs were stimulated with four different clinical isolates of *M. avium* at MOI 100 for up to 72 hours. Whole cell lysates, and cytoplasmic and nuclear extracts were collected 15 mins – 4 hours after infection, and analysed by western blot for protein phosphorylation or TransAm assay for NF- κ B activation. Supernatants collected at 72 hours were analysed by ELISA for MMP-1 and 7.

Results Infection with *M. avium* caused activation of all 3 MAPK (p38, JNK, ERK) pathways as early as 15 min post exposure with maximal phosphorylation at 30 min. *M. avium* infection drove maximal nuclear translocation of NF- κ B and degradation of cytosolic I κ B α at 30 min, returning to baseline by 4 hours. *M. avium*-induced MMP-1 secretion from MDMs is ERK and JNK, but not p38- dependant (figure 1). Treatment with caffeic acid phenethyl ester (CAPE), an NF- κ B inhibitor, reduced *M. avium*-induced MMP-1 secretion by 30%. Both MMP-1 and -7 upregulation were suppressed by PI3 kinase inhibitor LY294002. *M. avium*-induced MMP-7 upregulation was not inhibited by indomethacin.

Conclusions MMP-1 and -7 may drive the destructive pulmonary pathophysiology that characterises MAC infection. However, regulation of the host macrophage response to *M. avium* is divergent to that *M. tuberculosis*, with p38- independent MMP-1 secretion. This divergence in intracellular signalling may necessitate deviation in potential adjunctive patient therapies for *M. tuberculosis* and *M. avium*.

Abstract S18 Figure 1 MMP-1 and -7 secretion from *M. avium* stimulated MDMs, pre-incubated with p38, ERK or JNK inhibitor prior to infection. Supernatants were harvested at 72 hours and MMP concentration analysed by ELISA.

S19 THE ROLE OF LYMPH NODE-RESIDENT NEUTROPHILS IN ADAPTIVE IMMUNITY

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Introduction Neutrophils play a key role in the early response to a diverse range of infectious and inflammatory stimuli. However, persistent neutrophilic inflammation can result in collateral tissue damage, as evident in a number of chronic respiratory diseases. In addition to their role in innate immunity, neutrophils can also shape the adaptive immune response, in part through antigen presentation. Whilst there is accumulating evidence that neutrophils can migrate to draining lymph

Abstract S19 Figure 1 Surface MHCII expression of tissue-resident neutrophils in (A) mice and (B) humans. Lines represent mean $\pm$ S.D.

nodes following infectious challenges, the role of tissue-resident neutrophils in physiological settings is less clear. We hypothesise that neutrophils are present within lymph nodes and can influence adaptive immunity under physiological conditions.

Methods Lymph nodes from unchallenged C57BL/6 and LysM-GFP mice were harvested; single cell suspensions were generated for flow cytometric analysis, and frozen sections stained for confocal microscopy. Two-photon intravital imaging of popliteal lymph nodes was performed to examine neutrophil dynamic behaviour *in vivo*. Human lymph nodes were harvested from organ donors and analysed by flow cytometry and mass cytometry.

Results Neutrophils were present in lymph nodes in mice without prior inflammatory or infectious challenge. Whilst some neutrophils were within blood vessels (11% in inguinal lymph node, 10% in popliteal lymph node, 12% in mesenteric lymph node) or lymphatic vessels (15% in inguinal lymph node, 21% in popliteal lymph node, 18% in mesenteric lymph node), the majority were located in lymph node tissues. Lymph node neutrophils showed higher surface expression of major histocompatibility complex II (MHCII) compared to blood, bone marrow and splenic neutrophils (figure 1A). *In vivo*, neutrophils were capable of immune complex uptake, and their dynamic behaviour differed according to their location within the lymph node. Neutrophils were also present in human lymph nodes, and expressed surface MHCII (figure 1B). Immune cell profiles of matched lymph nodes and spleen were compared using mass cytometry. Isolated human blood neutrophils upregulated surface MHCII upon *ex vivo* immune complex stimulation.

Conclusion We have demonstrated the presence of tissue-resident neutrophils within murine and human lymph nodes, and their capacity to express MHCII, potentially influencing the adaptive immune response via antigen presentation.

Introduction and Objectives Chronic Lung Allograft Dysfunction (CLAD) is a major limiting factor to survival post-lung transplant (LTx), restricting 5 year survival to approximately 55%. The mechanism by which CLAD and its sub-types occur are not fully understood and changes in the microbiota may play a role in the development of this condition. Moreover, azithromycin prolongs CLAD-free post-transplantation. This study aims to determine the effect of azithromycin over time on the airway microbiota post-LTx and how the microbiota changes with the development of CLAD.

Methods As part of a double-blind RCT in UZ Gasthuisberg, Leuven, Belgium,¹ patients undergoing LTx were previously randomised to receive either azithromycin (n=43; 250 mg three times per week) or placebo (n=40) treatment following discharge post-transplant. Regular routine bronchoscopy was carried out on all patients and bronchoalveolar lavage (BAL) samples from discharge, 12, 24 months and at diagnosis of suspected rejection were processed for microbiota analysis using 16S Illumina sequencing and 16S quantitative PCR.

Results To date, 42 azithromycin treated (n=17 patients) and 52 (n=22 patients) placebo samples have been analysed. Microbiota diversity was significantly higher (p=0.0467) in the azithromycin group compared to placebo. Furthermore, a trend for reduced dominance by *Pseudomonas*, with re-emergence of taxa considered to constitute a 'healthy' microbiota (e.g., *Prevotella*, *Veillonella*, *Streptococcus*) was observed. There were no significant differences in 16S copies per mL BAL between the two groups. Eight samples (n=5 azithromycin, n=3 placebo) at suspected rejection have also been analysed. The azithromycin group exhibited low relative abundances of *Pseudomonas* (mean 7.7%), while the placebo group showed dominance by this taxa (mean 84.87%).

Conclusions Restoration of a diverse microbiota, while preventing dominance by *Pseudomonas*, may be a factor contributing towards the prophylactic effects of azithromycin observed in LTx patients¹. Further analysis of microbiota data alongside clinical data e.g., development of CLAD, CLAD-free survival time, etc. is ongoing.

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S20 IMPACT OF AZITHROMYCIN ON THE POST-LUNG TRANSPLANT MICROBIOTA

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