cell immune-profiling (10X genomics). Bronchoalveolar lavage (BAL) and nasal samples were stored for metagenomic sequencing with Nanopore and immunoassays.

**Results** Eleven patients (mean age ±SD, 51.8 ±16.8 years; 63% male) with severe asthma and high symptom burden (mean ACQ-5 ±SD, 2.9 ±1.1) underwent pre-/post-azithromycin bronchoscopy. *H. influenzae* was isolated in 36% on BAL culture pre-azithromycin. No pathogenic organisms were isolated post-azithromycin therapy. Analyses performed on 55,962 airways cells (n=6) revealed significant downregulation of gene ontology categories related to innate immune defence responses and cytokine mediated signalling pathways, with upregulation of genes encoding extracellular matrix components (p adj <1x10^-7) post-azithromycin therapy (table 1). This corresponded to reduced expression of CXCL10 and S100A8, with upregulation of CST1 and mast cell CPA3. CD8 T cells underwent the greatest transcriptional reprogramming, downregulating heat shock proteins and pathways implicated in T cell activation and cytokine responses.

**Conclusions** In addition to known antimicrobial effects, single cell transcriptomics reveals azithromycin suppresses Th1 and neutrophilic airways inflammation, modulates T cell functions and upregulates corticosteroid responsiveness markers (CST1, CPA3). In ongoing work, transcriptomic data from the full study population (n=11) will be analysed and integrated with airway immune-profiling (10X genomics). Bronchoalveolar lavage (BAL) and nasal samples were stored for metagenomic sequencing with Nanopore and immunoassays.

**References**

2.  http://dx.doi.org/10.2139/ssrn.4473145

**Introduction**

The existence of resident fungal communities ('mycobiome') within the lungs has only recently been discovered, and their role in health and disease remains unknown. Mycobiota are altered in chronic obstructive pulmonary disease (COPD), but the consequences of these perturbations have not been characterised. Given that fungi can promote type 2 inflammation and mucus hypersecretion, features of COPD that are augmented during exacerbations, we hypothesised that the mycobiome could be a key determinant of exacerbation pathogenesis.

**Methods** We quantified total sputum fungal burden by qPCR at baseline and during infection in separate cohorts of experimentally induced and naturally occurring viral exacerbations of COPD. Fungal burden was correlated with measures of immunopathology and clinical exacerbation severity. Subsequently, we modelled fungal dysbiosis in mice through administration of low dose *Aspergillus Fumigatus* (a major constituent of the COPD airway mycobiome) prior to infection with rhinovirus A1 to investigate effects upon pulmonary immune responses.

**Results** Individuals with COPD across the two studies (n=52) had increased sputum fungal burden compared to healthy control subjects (n=19) at stable state (figure 1A). Experimental rhinovirus infection led to increased sputum fungal loads from baseline in COPD subjects but not healthy controls with significant differences between these groups at day 9 and 12 post-infection (figure 1B). Similarly, sputum fungal burden increased during virus-associated naturally occurring exacerbations (figure 1C). During experimental challenge, sputum fungal loads positively correlated with viral loads, type 2 inflammatory responses including eotaxin 1/3, IL-4, IL-5 and Muc5ac (figure 1D) and clinical exacerbation severity (lower respiratory tract symptom scores) (figure 1D).

In mice, administration of low dose *Aspergillus* to model airway fungal dysbiosis enhanced rhinovirus replication at 24 hours post-infection. *Aspergillus* administration also elevated recruitment of lung neutrophils, eosinophils and induction of IL-13 and Muc5ac proteins by rhinovirus (all P<0.05).

**Conclusion** The airway mycobiome is altered in COPD and further perturbed by respiratory viral infections. Mycobiota may be an important driver of type 2 immunopathology and severity during virus-induced COPD exacerbations. Our

**THE RESPIRATORY MYCOBIOME IS PERTURBED DURING VIRAL INFECTION IN COPD AND DRIVES TYPE 2 IMMUNOPATHOLOGY AND EXACERBATION SEVERITY**

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Abstract T4 Figure 1 Viral infection perturbs the airway mycobiome in COPD. (A) sputum fungal burden in individuals with COPD vs healthy subjects across the two studies. (B) sputum fungal burden following experimental rhinovirus A1 challenge in COPD and healthy subjects (* COPD vs healthy, † COPD baseline vs COPD day 9/12). (C) sputum fungal burden during naturally occurring exacerbations. (D) correlation analysis between sputum fungal burden and markers of immunopathology and clinical severity during experimentally-induced exacerbation. LRT: lower respiratory tract...
ongoing profiling of fungal community composition within these studies will shed further light on the key mycobiont that may be amenable to future therapeutic targeting.

**T5 UNDERSTANDING THE EXTRACELLULAR IMMUNOPROTEASOME IN THE ACUTE RESPIRATORY DISTRESS SYNDROME**

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The constitutive proteasome and its inflammation-driven derivative, the immunoproteasome (IP), perform important intracellular proteolytic functions, including terminal protein degradation and antigen processing. However, the IP is increasingly recognised as being present in the extracellular space during pathology, though its mechanisms of release and functions are unknown. The lungs of patients with acute respiratory distress syndrome (ARDS) are flooded with complex oedema fluid and characterising the pathogenic components within this extracellular environment may help identify therapeutic targets. We hypothesised that extracellular IP is a feature of and plays a role in ARDS.

We show that the levels and activity of IP are elevated in bronchoalveolar lavage fluid from patients with ARDS, the human healthy volunteer LPS model and the murine inhaled LPS model. Furthermore, in a series of in vitro experiments, we demonstrate that IP is released constitutively from macrophage-like cells and primary human macrophages. Importantly, this release can be augmented by activation of the NLRP3 and AIM2 inflammasomes. Using both pharmacological and genetic strategies we show that targeting the inflammasome pathway abrogates IP release from macrophages, confirming the importance of this pathway in IP release. We next sought to identify extracellular substrates of IP, the cleavage of which might contribute to the inflamed environment of the lung in ARDS. We report that IP is able to cleave several anti-inflammatory proteins that are present in the ARDS lung, including anti-protérases and the phospholipid-binding protein Annexin A1.

In conclusion, extracellular IP is a feature in human ARDS and models of ARDS. We have identified a potential mechanism of release of IP, which is closely linked to inflammasome activation, and postulate that extracellular IP may play a pro-inflammatory role in the acutely inflamed lung.

**T6 GENOME-WIDE MUTAGENESIS SCREENS IDENTIFY REGULATORS OF CELLULAR IRON METABOLISM AND FERROPTOSIS**

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Introduction and Objectives Regulation of iron metabolism is essential for lung health, with mutations in the gene encoding iron regulatory protein 2 (IRP2, which helps maintain cellular iron homeostasis), being associated with the development of COPD. Increased IRP2 protein levels are found in the lungs of patients with COPD and increased iron in BAL fluid from these patients is associated with exacerbation risk. Ferroptosis (iron-dependent cell death), can be induced by cigarette smoke and may promote COPD pathogenesis. Dysregulated iron metabolism is also found in non-small cell lung cancer and pulmonary fibrosis. Better understanding how cells regulate iron levels therefore represents a promising avenue for lung research.

Methods A549 lung adenocarcinoma and HeLa cells were modified by CRISPR/Cas9 to add a fluorescent tag to IRP2, creating dynamic and responsive iron-reporter cell lines. These were used in genome-wide forward genetic screens to identify genes that regulate cellular iron homeostasis.

Results 122 unique genes were classed as significant hits (FDR <0.25), of which 64 can be mapped to known iron-regulatory pathways, including transferrin-dependent iron uptake, iron-sulfur cluster synthesis and ferritin breakdown. SETD2, a histone methyltransferase which can be mutated in lung adenocarcinoma and mesothelioma, was identified as a novel top hit in A549 cells, providing the first example of a chromatin modifier as a mediator of iron levels. SETD2 depletion leads increased levels of the cargo receptor NCOA4 (responsible for ferritin breakdown), intracellular iron depletion and activation of the IRP2 response to promote iron uptake. As a regulator of alternative splicing, SETD2 loss is further associated with the differential expression of NCOA4 isoforms. Finally, certain SETD2 mutant cancer cell lines are more resistant to ferroptosis, whilst knockdown of SETD2 promotes resistance in wild type lines.

Conclusions We establish a robust reporting system for intracellular iron levels in patient-derived lung cancer cell lines, definitively map the cellular pathways of iron metabolism, identify novel regulators of iron homeostasis, and determine their impact on the induction of ferroptosis. SETD2 loss of function mutations, which are common in cancer, may promote resistance to ferroptosis, with potentially important implications for future therapies targeting this cell death pathway.