Treatment-naïve biopsies were analysed from seven TB patients, ten sarcoidosis patients and seven control samples. Bioinformatic analysis of RNAseq data identified one third of differentially expressed genes were communal to TB and sarcoidosis relative to control samples (absolute log₂ fold change ≥ 1.5, adjusted P value < 0.05), with overlap of numerous pathways, including the extracellular matrix and cytokine signalling. Importantly, a TB unique cluster demonstrates TB results from a dysregulated inflammatory immune response, whereas a sarcoidosis predominant cluster relates to elevated lysosomal activity.

To translate these insights, we compared three primary human cell culture models: classical 2D; 3D alginate; and 3D collagen model. We demonstrated the Mtb-infected 3D collagen model most closely reflected clinical TB biopsies. We investigated signalling pathways shared between human disease and the 3D model, and used a systematic selection process to identify twelve intracellular enzymes as potential therapeutic targets. Sphingosine kinase 1 (SphK1) inhibition controlled Mtb growth in a dose-dependent manner, concurrently lowering intracellular pH in infected monocytes and suppressing inflammatory mediator secretion. Immunohistochemical staining confirmed that SphK1 is expressed in human lung TB granulomas, and therefore represents a potential novel host-directed therapeutic target to improve TB outcomes.

T4 REINFECTION WITH INFLUENZA A VIRUS LEADS TO RAPID CHANGES IN IMMUNOMODULATORY MOLECULES AND INFLAMMATORY SUBTYPES OF LUNG FIBROBLASTS AND EPITHELIAL CELLS

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Introduction and Objectives Influenza A virus (IAV) induces respiratory infections resulting in significant global mortality. Stromal cells are essential sources of chemokines and growth factors that promote immune cell survival and anti-viral responses. The concept that stromal cells are permanently altered by inflammatory responses is referred to as trained immunity. We hypothesise that IAV infections influence future immune responses *via* changes to lung stromal cells, enabling rapid communication with immune cells following subsequent infection.

Methods C57BL/6 mice were infected intranasally with IAV (WSN, 150PFU) for 30 days and subsequently re-challenged with IAV (X31, 200PFU) for 2 days. Mice were sacrificed at day 0, 2, 30 and 32 post infection. A Nanostring assay was used to examine the transcriptional profiles of FACS sorted lung fibroblasts and epithelial cells. Differentially expressed genes were validated by qPCR and flow cytometry. The expression of upstream transcriptional drivers of these changes were compared with publicly available RNA-seq and ChIP-seq datasets. The potential impact of these changes on stromal-immune cell communication were assessed using immunohistochemistry and immunofluorescence.

Results Genes involved in T cell communication were significantly upregulated in lung fibroblasts (Cd274, Cxcl10) and epithelial cells (Tnfsf10, Icam2) following secondary IAV infection (d32), compared to d30. These changes were accompanied by elevated IFN-response genes (Bst2, Ifi47, Irf7).

Fibroblasts displayed enrichment in genes involved in biological processes regulating T cell activation, while epithelial cells were enriched for genes that regulate cytokines (IFNα/β, TNFα). After secondary IAV infection, stromal cells rapidly upregulated genes involved in antigen processing and presentation (Tap1, Tapbp), compared to d30. The transcription factor SpiB was identified as a shared upstream regulator of these genes. Interestingly, in contrast to d2 post primary infection, MHCII+ epithelial cells were elevated following secondary infection, while the frequency of IFN-responsive fibroblasts was decreased. T and B cells were located near airway epithelial cells at d30 and were retained following secondary infection, indicating ongoing communication between these cells.

Conclusions Our data, show that lung fibroblasts and epithelial cells can display overlapping and functionally discrete responses to IAV infection, enabling them to rapidly communicate with lung T cells following a subsequent infection.

Please refer to page A188 for declarations of interest related to this abstract.

T5 RESPIRATORY PARTICLE AND DROPLET EMISSION DURING SPEECH AND EXERCISE

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Background The coronavirus disease-19 (COVID-19) pandemic has profoundly impacted sports and exercise, disrupting a plethora of events worldwide. Aerosol transmission is increasingly recognised as an important route for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with systematic evaluation of particulate matter release during exercise required to understand and mitigate transmission risk.

Methods Healthy participants (n=25) performed a two stepped, flat-wave cardiopulmonary exercise test (CPET) on a cycle ergometer to replicate vigorous exercise (80% of anaerobic workload) and high intensity exercise (anaerobic workload + 30% of the difference between anaerobic workload and peak workload), as determined by a maximally exhaustive CPET performed one hour previously. Concurrent measurements of aerosol and ventilatory data were recorded via a sampling line connected to an aerodynamic particle sizer (APS). Further synchronous data were collected at rest and when speaking at 70-80dBA. Droplet data were collected at rest and during high intensity exercise, using water sensitive paper.

Findings Median aerosol number concentration during speaking (at 70-80dBA), 0.26cm⁻³, was greater than during vigorous exercise, 0.12 cm⁻³ (p<0.001) but not different to high intensity exercise, 0.24cm⁻³ (p=0.92). Median aerosol mass concentration during speaking, 0.40µg/m⁻³, was greater than during vigorous exercise (0.17µg/m⁻³, p<0.001), but not different to

Abstract T5 Figure 1 Box and whisker plots showing (a) Aerosol number emission rates (aerosol number concentration normalised for ventilation) (b) Aerosol mass emission rates (aerosol mass normalised for ventilation) across the same series of activities, for all 25 participants

high intensity exercise ($0.42\mu\text{g}/\text{m}^3$, $p=0.083$). Rest and exercise demonstrated similar aerosol size distributions, however speaking emitted an additional, larger mode at $2\text{--}3\mu\text{m}$. Mean minute ventilation was 11L/min, 15L/min, 63L/min and 114L/min at rest, speaking, vigorous exercise and high intensity exercise, respectively. Median mass emission rate (aerosol mass concentration normalised for ventilation) produced by speaking, $0.092\text{ng}/\text{s}^{-1}$, was not different to vigorous exercise, $0.207\text{ng}/\text{s}^{-1}$, ($p=0.726$) but was lower than high intensity exercise $0.682\text{ng}/\text{s}^{-1}$, $p<0.001$).

Interpretation The size distribution of airborne particles emitted during exercise, match that of breathing at rest, with increased minute ventilation a primary driver of the increased aerosol mass emissions that occur during exertion. Aerosol mass emission rates during vigorous exercise are not different to speaking at a conversational, to loud conversational volume. These findings enhance our understanding of particle release during the fundamental physiological process of exercise, enabling appropriate mitigations for airborne pathogens, including SARS-CoV-2.

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T6 THERAPEUTICALLY TARGETING PTBP1/PKM2-DRIVEN GLYCOLYSIS IN ENDOTHELIAL CELLS: A NOVEL APPROACH TO TREAT PULMONARY ARTERIAL HYPERTENSION

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Background Pulmonary Arterial Hypertension (PAH) is an often-fatal disease, characterised by the development of apoptosis-resistant, hyperproliferative, and hyper-glycolytic endothelial cells (ECs). Loss-of-function *BMPR2* mutations and metabolic dysfunction are pivotal to PAH EC dysfunction. Enhanced expression of glycolysis enzyme pyruvate kinase M2 isoform (PKM2) and upstream splicing factor poly-pyrimidine

tract binding protein (PTBP1) have been observed regardless of genetic background,¹ suggesting that targeting the PTBP1/PKM2 axis could be a potent therapeutic strategy in PAH. Apigenin has been recognised for its low toxicity and ability to suppress PTBP1 and PKM2-driven Warburg glycolysis and tumorigenesis.² This work investigates apigenin's effects on the PKM2/PTBP1 axis in ECs; whether treatment could correct hallmark characteristics of PAH EC dysfunction; and the influence of *BMPR2* mutations.

Methods Here, we examined the proteomic, metabolic and functional profiles of blood outgrowth endothelial cells (BOECs) obtained from healthy control volunteers and PAH patients with *BMPR2* mutations, following apigenin treatment or *PTBP1/PKM2* knockdown. We hypothesised that, apigenin treatment would reduce glycolysis by inhibiting PTBP1 driven splicing of *PKM* into *PKM2*, and PKM2-dependent expression of glycolytic pathway components, such as lactate-dehydrogenase A (LDHA) and lactate production.

Results In control and *BMPR2* mutant BOECs, apigenin inhibited expression of PTBP1 and tetrameric PKM2. In control and *BMPR2* mutant BOECs, apigenin also suppressed LDHA protein expression and lactate production. Apigenin inhibited cell cycle progression and caspase 3/7 activity in control and *BMPR2* mutant BOECs, suggesting treatment may reduce EC susceptibility to proliferation and apoptosis, hallmarks of PAH ECs.

Conclusions Apigenin treatment may suppress EC glycolysis, possibly through suppression of PKM2-driven glycolytic gene expression and tetrameric activity. This encouraging data suggests apigenin may correct endothelial cell dysfunction observed in PAH, including susceptibility to apoptosis and proliferation.

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