NETs drive immunopathology and severity during viral exacerbations of COPD. Targeting of NETs in COPD exacerbations could represent a future effective intervention to improve clinical outcomes.

Introduction The mechanisms driving the association between obesity and susceptibility to adverse clinical outcome from influenza infections, as highlighted during the 2009 H1N1 pandemic, are poorly understood. We hypothesized that dysregulated type I interferon would be a major mechanistic driver of obesity-related severity.

Methods 15 obese and 15 non-obese individuals were recruited to our study. Bronchial epithelial cells (BECs), bronchoalveolar lavage (BAL) cells and peripheral blood dendritic cells (pDCs) collected from these subjects were stimulated ex vivo with clinically relevant influenza viruses: A/Eng/195 (pandemic H1N1/09), A/Eng/691/10 (seasonal H3N2) and B/Florida (influenza B) followed by measurement of the anti-viral immune response at 8 and 24 hours post-infection. In vivo adipokine concentrations were profiled using multiplex ELISA. In subsequent studies, we administered exogenous leptin protein into the airways of BALB/c mice to model the obese pulmonary microenvironment and assess the effects imparted upon infection with influenza X31 (H3N2).

Results Ex vivo stimulation of BAL cells from obese individuals demonstrated impaired induction of interferons-α, -β and -λ compared to cells from non-obese controls when stimulated with each of the three influenza strains (figure 1A-D). Similar impairment was not observed in BECs or pDCs.

Concentrations of the adipokine leptin were augmented in BAL and bronchosorption from obese versus non-obese individuals (figure 1E&F) and correlated negatively with the magnitude of ex vivo BAL cell anti-viral response with greater leptin concentrations being significantly associated with weaker induction of IFNb; seen most strongly for B/Florida (r=-0.67, P=0.0008).

Exogenous pulmonary administration of leptin in mice directly impaired anti-viral type I IFN responses in vivo, reducing early induction of Ifnb, Pkr and Oas lung mRNA expression by influenza X31 (P<0.05) and subsequently leading to a later augmentation of neutrophilic inflammation (P<0.05) and pro-inflammatory cytokine (IL-6 and IL-1β) responses (P<0.01).

Conclusion Obese individuals have deficient pulmonary anti-viral type I and III IFN immune responses to influenza infection in BAL cells. Mechanistically, this occurs through increased airway leptin concentrations imparting suppressive effects upon anti-viral immune pathways. Leptin manipulation or IFN administration may provide novel strategies for conferring protection from severe viral infections in these susceptible individuals.
INFECTION EXPERIENCED LUNG STROMAL CELLS PROVIDE EARLY IMMUNE PROTECTION THAT IS INDEPENDENT OF THE ADAPTIVE IMMUNE RESPONSE

JC Worrell, GE Finney, KE Hargrave, C Hansell, J Singh-Nijjar, F Morton, JC o l e , MKL MacLeod.
1University of Glasgow, Glasgow, UK; 2University of Cambridge, Cambridge, UK
10.1136/thorax-2023-BTSabstracts.118

Introduction and Objectives Influenza A virus (IAV) infections contribute significantly to global mortality. Stromal cells may be permanently altered by inflammatory responses, a process known as trained immunity. We hypothesize that viral infection induces trained immunity in lung stromal cells, enhancing their protective responses to future lung injury via interactions with local immune cells.

Methods C57BL/6 mice were infected intranasally with IAV (WSN) and after 30 days re-challenged with IAV (X31). Mice were sacrificed at day 0, 2, 30 and 32 post-infection/re-infection. The transcriptional profiles and locations of infection experienced stromal cells were identified by RNA-scope, viral nucleoprotein (IAV-NP) was detected by immunofluorescence and flow cytometry. An in vitro co-culture assay measured T cell re-activation by naïve or IAV infected stromal cells. To assess the consequences of T cell depletion on viral load following IAV re-challenge, anti-CD4/CD8 blockade was performed during the memory phase of infection.

Results IAV infection led to formation of dense clusters of T and B cells. RNAscope revealed high expression of SpiB and Cxcl0 in lung epithelial cells and fibroblasts, specifically at sites near immune cell clusters. SpiB regulates genes involved in antigen processing/presentation, while Cxcl10 facilitates T cell communication, suggesting collaboration between these cell types could facilitate early viral control. However, depletion of CD4 and CD8 T cells prior to a re-challenge infection did not alter enhanced viral control in IAV-memory animals. To investigate whether IAV-memory stromal cells display enhanced intrinsic viral control, we infected stromal cells from naïve and IAV-memory mice with IAV in vitro. Less IAV-Nucleoprotein was found in stromal cells taken from memory than naïve animals, this was independent of type I interferon. Interestingly, the NP+epithelial cells in cultures of IAV-memory stromal cells expressed higher levels of MHCII than NP+epithelial cells from naïve animals. Both populations could present IAV antigens to CD4 and CD8 T cells, although infected IAV-memory stromal cells to a lesser extent than infected naïve-stromal cells, perhaps due to lower levels of antigen.

Conclusions IAV-experienced lung stromal cells can play dual roles in anti-viral control, early cell intrinsic control and a rapid ability to communicate with local T cells.

CELLULAR SENESCENCE AMELIORATES HUMAN RHINOVIRUS CLEARANCE

A Yang, LD a l y , YY a m a m o t o , JB a k e r , K Ito.
1Imperial College, London, UK; 2HiLung Inc., Kyoto, Japan
10.1136/thorax-2023-BTSabstracts.119

Abstract S111 Figure 1 In obesity, antiviral immune responses are impaired and airway metabolic milieu is altered. (A) Bronchoalveolar lavage (BAL) cells from 15 obese and 15 non-obese control subjects were infected ex vivo with H1N1.09 (H1N1), seasonal H3N2 and B/Florida (B/Flo) influenza strains. (B) IFN-α, (C) IFN-β and (D) IFN-λ antiviral immune proteins at 24 hour post-infection. Quantification of leptin in obese vs non-obese subjects in (E) bronchosorption and (F) BAL. *p<0.05; **p<0.01; ***p<0.001; ns=non-significant.