**INFECTION EXPERIENCED LUNG STROMAL CELLS PROVIDE EARLY IMMUNE PROTECTION THAT IS INDEPENDENT OF THE ADAPTIVE IMMUNE RESPONSE**

1JC Worrell, 1GE Finney, 1KE Hargrave, 1C Hansell, 2J Singh-Nijjar, 1F Morton, 1JC Cole, 1MKL MacLeod. 1University of Glasgow, Glasgow, UK; 2University of Cambridge, Cambridge, UK

**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. RNA-scope revealed high expression of SpiB and Cxcl10 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**

1A Yang, 1LD Daly, 2YY Yamamoto, 1J Baker, 1K Ito. 1Imperial College, London, UK; 2HiLung Inc., Kyoto, Japan

**Introduction and Objectives** 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

---

*S112 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