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| ***Ex vivo* viral infection of mouse precision cut lung slices** |
| Belinda J Thomas1,2, Julia G Chitty3, Callum Burnie3, Philip G Bardin1,2,4, Jane E Bourke1,3 |
| *1Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, VIC, Australia**2Molecular and Translational Science, Monash University, VIC, Australia* *3Phamacology, Biomedicine Discovery Institute, Monash University, VIC, Australia**4Monash Lung, Sleep, Allergy and Immunology, Monash Health, VIC, Australia*  |
| **Introduction/Aim:** Precision cut lung slices (PCLS) represent a unique organotypic modelling platform that allows investigation of mechanisms underlying respiratory diseases and responses to infection with all resident structural and inflammatory cells maintained, bridging the gap between *in vivo* and *in vitro* models. Multiple slices can be generated from a single mouse, providing a high-throughput screening tool for infection studies. We aimed to examine and compare *ex vivo* influenza A virus (IAV) infection to stimulation with the viral mimetic poly I:C. **Methods:** PCLS were prepared from naïve mice and infected with IAV (HKx31; 104-106 PFU) or stimulated with poly I:C (10 µg/ml). Responses were assessed over a 72 hour time period to determine PCLS viability, viral loads, and inflammatory mediator production. **Results:** LDH and MTT assays established PCLS remained viable throughout the 72 hour time-course, with minimal release of LDH into PCLS-conditioned media or decrease in PCLS mitochondrial activity. A dose-dependent and time-dependent increase in viral load was observed (10-fold increase 104 to 106 PFU at 24 hr, p<0.001; 10-fold increase 104 PFU from 0 to 72 hr, p<0.0001). This was accompanied by an increase in the inflammatory mediators IL-6, MCP-1, RANTES and KC. Similarly, stimulation with poly I:C had no effect of PCLS viability, while enhancing IL-6 and KC production. **Conclusion:** We have established PCLS can be infected with IAV *ex vivo* and remain viable, producing inflammatory mediators for up to 72 hours. Stimulation with poly I:C yields similar inflammatory responses. *Ex vivo* infection of PCLS may be a viable screening tool to reflect *in vivo* IAV infection in mice, and could be utilised in the future to allow for accelerated clinical translation of new therapeutics. **Grant Support:** This work is funded by grants from the NHMRC (APP2021687) and Monash Lung and Sleep Institute.**Declaration of Interest:** Nothing to declare |