

Targeting the NLRP3 inflammasome is a viable option for the treatment of pathogenic influenza virus infections

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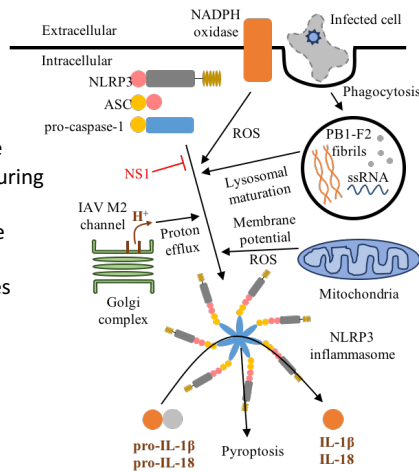
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Introduction

Fatal influenza A virus (IAV) infections in humans, such as those resulting from spill over of virus from birds, are associated with excessive production of cytokines, including IL-1 β . The emergence of novel avian IAV in humans has highlighted the need to identify the molecular mechanisms that drive these excessive immune responses and to identifying new therapeutic interventions.

The NLRP3 inflammasome is an innate immune signalling complex which becomes activated during IAV infection, resulting in the production of the potent pro-inflammatory cytokines IL-1 β and IL-18. Studies have demonstrated that mice lacking components of the NLRP3 inflammasome are more susceptible to IAV infection, suggesting that it plays a protective role by promoting inflammation. However, fatal IAV infections in humans are associated with high levels of IL-1 β in the lung which may drive hyperinflammation.

The NLRP3 inflammasome promotes inflammation during IAV infection. the NLRP3 complex can be activated by stimuli such as reactive oxygen species (ROS) or IAV proteins M2 and PB1-F2



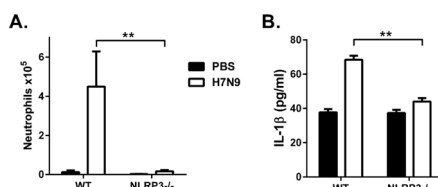
Methods

Mice were treated with the small molecule inhibitor of NLRP3, MCC950 via the intranasal route. We utilised the preclinical mouse model of IAV infection to examine the role of NLRP3. Mice were inoculated 50 PRU of PR8 (H1N1). Mice were monitored daily and those who lost $\geq 20\%$ of their original body weight were ethically euthanised. PR8 and H7N9 PB1-F2 peptides were also delivered into the lungs by intranasal inoculation. Cellular infiltrates and cytokines were measured in bronchoalveolar lavage (BAL).

H7N9 PB1-F2 Peptide Activates the NLRP3 Inflammasome *in vivo*

7 hrs following intranasal PB1-F2 peptide treatment

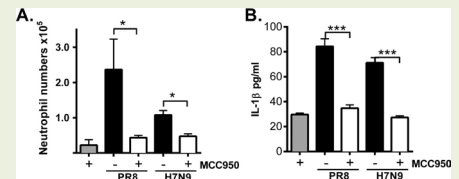
PB1-F2 peptide from H7N9 IAV activates the NLRP3 inflammasome in the airways



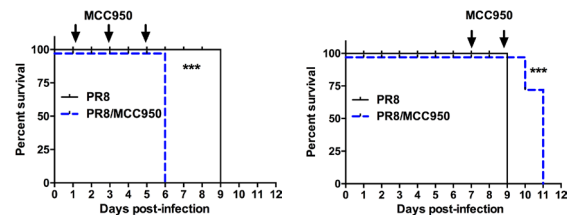
MCC950 Inhibits PR8 and H7N9 PB1-F2-mediated Activation of NLRP3

The NLRP3 inhibitor MCC950 reduces PB1-F2 mediated inflammation in the airways

7 hrs following intranasal PB1-F2 peptide treatment



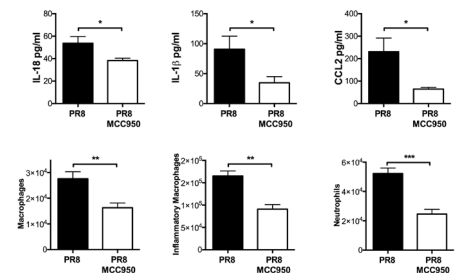
NLRP3 plays a temporal role during severe PR8 infection



Early inhibition of NLRP3 = detrimental

Late inhibition of NLRP3 = protective

A single treatment of MCC950 at day 7 reduces inflammation in the airways



Conclusions

Our findings significantly advance our understanding of the role of the NLRP3 inflammasome during IAV infection. We have identified H7N9 PB1-F2 as a novel inflammasome activator. Our data suggests the NLRP3 inflammasome plays a role in amplifying the production of local and systemic pro-inflammatory cytokines during severe IAV infections. These data provide the first evidence that temporally therapeutically targeting the NLRP3 inflammasome may be a clinical option for reducing inflammation associated with pandemic IAV infections.