TOWARDS THE RAT AS A MODEL FOR COVID-19 LUNG DISEASE

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Background:
Laboratory models of COVID-19 are difficult due to logistics, the inability of initial SARS-CoV-2 strains to infect rodents; and the need to reproduce disease reflecting post-viral inflammatory damage. Here we have defined the biology of the SARS-CoV-2 Spike (S) protein interaction with ACE2 in the rat to investigate the rat as a model of SARS-CoV-2-mediated inflammatory lung injury.

Methods:
Immunoflorescent staining for SARS-CoV-2 receptors was performed in aged, male rat lungs and bronchial alveolar lavage (BAL) cells. S-protein pseudotyped GFP reporter lentivirus was generated and used to infect HEK-ACE2, primary human or rat macrophages. Respiratory mechanics was measured in rats treated with S-lenti using a flexiVent mechanical ventilator.

Results:
Staining of ACE2 and TMPRSS2 demonstrates the presence of SARS-CoV-2 receptors throughout the rat lung and on cells in BAL. Computational comparison suggests that rat ACE2 is similar to mouse and Wuhan (W) S will not bind efficiently. This was confirmed using ^{W}S-lenti *in vitro*. A mouse adapted mutation ($Q^{498}P^{499}$-YT) S-mutation was generated that was infectious *in vitro*. Administration of ^{W}S or $^{QP\text{-}YT}$S-lenti to rats, however did not induce a significant inflammatory response or change in respiratory function. Beta and omicron S-variants have been cloned to generate variants expected to bind better to rat ACE2 and ACE2 lenti has been generated to increase ACE2 in the lung, with results are on-going.

Conclusion:
There is a need for flexible laboratory models to study post-viral COVID-19 inflammatory lung disease. The rat offers a manipulatable model to assess lung respiratory mechanics, and now (i) we know the rat lung contains appropriate SARS-CoV-2 receptors, (ii) we have a number of S-protein variants that can bind rat receptors and (iii) are now investigating the kinds of SARS-CoV-2 and host stimuli that can contribute to lung inflammation and dysfunction *in vivo*.

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