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LETTER TO THE EDITOR

Update on animal models for COVID-19 research

Researchers around the world have rapidly mobilised to work on coronavirus disease 2019 (COVID-19) research. In April, we contributed to a review of early studies using animal models for research into the mechanistic basis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and COVID-19, largely working from preliminary reports published on preprint servers (Cleary et al., 2020). Many further studies using small animal models of SARS-CoV-2 infection have been peer reviewed and published since we wrote our review. Here, we summarise key findings of these studies.

1 | MOUSE STUDIES

Mice cannot cough or sneeze, and mice infected with influenza A virus do not transmit infections to co-housed naïve mice. Therefore, mouse models are not generally considered useful for preclinical testing of interventions targeting the transmission of respiratory viruses. However, results from a mouse SARS-CoV-2 infection model suggest that mice may be useful as a model species for studying SARS-CoV-2 transmission:

- Bao, Gao et al. (2020) inoculated mice expressing human **ACE2** (hACE2) driven by the mouse *Ace2* promoter (*Ace2*-hACE2 mice) with 10^5 of 50% tissue culture infectious dose ($TCID_{50}$) of SARS-CoV-2 and, 1 day later, co-housed these infected mice either in direct contact with naïve mice or with a barrier that prevented direct contact but allowed airflow from infected to naïve mice. A subset of both directly and indirectly exposed naïve mice became infected, demonstrating the highly transmissive nature of SARS-CoV-2. Separate studies using aerosolised delivery of SARS-CoV-2 at 36 $TCID_{50}{\cdot}min^{-1}$ suggested that virus in aerosol had to be delivered at this rate for over 25 min in order to provoke lung pathology, supportive of the concept that duration of infected aerosol exposure increases COVID-19 risk.

Other strains of mice expressing human hACE2 have been created for studies of coronavirus pathogenesis—mice with hACE2 expression driven by the human *KRT18* promoter (K18-hACE2 mice) and mice with hACE2 expression driven by the human *FOXJ1* promoter (HFH4-hACE2 mice) (McCray et al., 2007; Menachery et al., 2016). Studies using these strains are suggestive that SARS-CoV-2 infection might produce worse pathology than has previously been described in *Ace2*-hACE2 mice (Bao, Deng, et al., 2020), indicating that mouse models may be useful for the evaluation of potential therapeutic agents for severe COVID-19:

- Jiang et al. (2020) infected HFH4-hACE2 mice with 3×10^4 or 7×10^5 plaque-forming units (PFU) of SARS-CoV-2. At the lower dose of virus, infection was lethal in a subset of mice, accompanied by lymphopenia and severe pneumonia with pulmonary oedema, congestion, and hyaline membrane formation. A study of three mice that survived infection indicated that previous infection was protective against mortality when mice were rechallenged with the higher dose of SARS-CoV-2.
- Winkler et al. (2020) infected K18-hACE2 mice with 2.5×10^4 PFU of SARS-CoV-2. Infectious virus persisted in the lungs at 7 days after infection, and mice lost weight and showed severe lung inflammation together with lung function abnormalities. Other readouts with relevance to human COVID-19 included signs of coagulopathy (prolonged prothrombin times preceded by elevations in serum D-dimer) and lymphopenia. This study also reports a dataset of transcriptional changes in lung cells over time following SARS-CoV-2 infection.

Availability of hACE2 mice has been limited, with demand greatly increased. Once hACE2 mice are obtained, crossing them with knockout strains for modelling the effects of genetic risk factors in susceptible mice can take 6 months or longer. Several groups have found innovative ways around these problems, with some able to make important contributions to our understanding of the complex role of **IFN** signalling in SARS-CoV-2 infection (Grajales-Reyes & Colonna, 2020):

- Mouse-adapted SARS-CoV-2 would avoid the need for hACE2 mice and permit the use of more readily available strains. Clinical isolates of SARS-CoV-2 were therefore passaged six times through the lungs of BALB/c mice (Gu et al., 2020). The resultant strain (MASCp6) delivered at 1.6×10^4 PFU infected both young mice (6 weeks) and aged mice (9 months). Lung inflammation and injury were more pronounced in the aged mice, and young mice were successfully used to demonstrate efficacy of a candidate vaccine.
- Sun, Chen et al. (2020) have inserted *hACE2* into the endogenous mouse *Ace2* locus using CRISPR/Cas9. In these mice, hACE2 would be expected to have the physiological expression levels, distribution, and regulation of mouse ACE2. These mice were susceptible to SARS-CoV-2 infection at 4×10^5 PFU and when

Abbreviations: COVID-19, coronavirus disease 2019; FFU, focus-forming units; PFU, plaque-forming units; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; $TCID_{50}$, 50% tissue culture infectious dose.

aged (30 weeks old) showed greater lung neutrophil infiltration than young mice (4.5 weeks old). Infection also occurred via the intragastric route.

- Transfection of *hACE2* in lung cells through intratracheal instillations of adenoviral vectors containing *hACE2* is another approach for rapidly producing susceptible mice. Sun, Zhuang et al. (2020) used this approach to study knockout strains and lymphocyte-depleting antibodies with an inoculation of 10^5 PFU of SARS-CoV-2. Viral clearance was impaired with depletion of $CD4^+/CD8^+$ lymphocytes or knockout of *Ifnar1* or *Stat1*. Weight loss and lung inflammation were more pronounced in the *Stat1* knockouts suggesting a role for STAT1 in mediating immunopathology. Prophylactic treatment of these mice with either convalescent plasma from COVID-19 patients or **remdesivir** reduced weight loss and viral load in the lungs following inoculation with SARS-CoV-2.
- Hassan et al. (2020) also used an adenoviral hACE2 transfection method in various mouse strains. BALB/c and C57BL/6 mice were infected with 10^5 focus-forming units (FFU) of SARS-CoV-2 via intranasal and intravenous routes. Antibody blockade of **Ifnar1** worsened weight loss and inflammation, and an anti-**Spike protein**-neutralising monoclonal antibody was protective.
- Adeno-associated virus (AAV) vectors have been developed, which may produce lower levels of vector-associated immunogenicity than adenoviruses (Jooss & Chirmule, 2003). Israelow et al. (2020) used AAV vectors to express hACE2 in the lungs of wild-type C57BL/6 mice, as well as *Ifnar1* knockouts and *Irf3/7* double knockouts. These mice were infected with 10^6 PFU of SARS-CoV-2 and produced strong lung type I IFN-stimulated gene responses. Viral clearance was unaltered in the knockout strains. In contrast, inflammatory monocyte, monocyte-derived macrophage, and T-cell recruitment to the lungs was decreased in both *Ifnar1* and *Irf3/7* knockout strains, with activated NK cell recruitment preserved in *Irf3/7* double knockouts.

2 | HAMSTER STUDIES

Hamsters can also be used to study aerosol transmission of SARS-CoV-2 (Sia et al., 2020). Observational studies strongly support the use of face coverings in reducing SARS-CoV-2 transmission. However, the novelty of SARS-CoV-2, limited personal protective equipment resources, and scepticism around the efficacy of mandatory face covering policies have however led to calls for interventional studies into whether surgical masks prevent SARS-CoV-2 transmission:

- Chan et al. (2020) used a Syrian hamster model to study the effect of surgical mask partitions on the transmission of SARS-CoV-2 from hamsters infected with 10^5 PFU of SARS-CoV-2 to naïve hamsters. Surgical mask partitions successfully reduced the incidence of transmission, but not completely. We interpret these findings as supportive of the use of simple face coverings to reduce risk of viral transmission and the use of masks with higher filtration efficiency by workers with high occupational risk of SARS-CoV-2 exposure.

Although homologous recombination has permitted the creation of many knockout strains of mice and other animals, this technology has not been successfully used to delete genes in hamsters. CRISPR/Cas9 technology has recently overcome this major limitation of hamster models, and we eagerly await peer review of mechanistic studies, which are ongoing in hamsters (Park, 2020).

We applaud researchers for their rapid characterisation and publication of novel animal models for COVID-19 research during the pandemic. Careful reporting and considerations of animal welfare and model validity are important for the reproducibility and translation of preclinical studies. The streamlined ARRIVE guidelines checklist for reporting animal experiments (Percie du Sert et al., 2020), and a recent perspective on improving the use of preclinical models in sepsis research (Nandi et al., 2020), might be useful resources for researchers working in the fast-moving and challenging field of COVID-19 research.

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Many further small animal COVID-19 research studies have been reported through preprint servers. A change in editorial policy at the *British Journal of Pharmacology* has meant that we were not able to cite these preprints. The authors would like to acknowledge the important work of these groups and their rapid dissemination of useful preclinical research findings in difficult circumstances.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Simon J. Cleary[1]
Mélia Magnen[1]
Mark R. Looney[1]
Clive P. Page[2]

[1]*Department of Medicine, UCSF, San Francisco, CA, USA*
[2]*Sackler Institute of Pulmonary Pharmacology, King's College London, London, UK*

Correspondence
Clive P. Page, Sackler Institute of Pulmonary Pharmacology, King's College London, London, UK.
Email: clive.page@kcl.ac.uk

ORCID

Simon J. Cleary https://orcid.org/0000-0001-5573-6363
Mark R. Looney https://orcid.org/0000-0003-0241-9190

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