THE COVID-19 RACE

how mouse models will help researchers cross the finish line

The emergence of SARS-CoV-2 virus and the disease it causes, COVID-19, have fuelled significant shifts in preclinical research across the globe. Amid the worldwide health emergency created by high infection rates, rapid transmission spread and relatively high mortality rates, researchers are under pressure to understand the disease's pathogenesis, develop and test investigational therapeutics and explore viable vaccine approaches.

By Dr Megan MacBride

Although there is not a deep history of preclinical studies on this novel coronavirus to draw on, the preclinical work carried out on the closely-related virus SARS-CoV (which caused the SARS pandemic in 2003) provides a foundation that may yield useful insights into animal models for COVID-19 studies. The two coronaviruses share important characteristics; for instance, both are zoonotic coronaviruses that crossed the species barrier and, for both viruses, entry into human cells requires binding of viral spike (S) proteins to human cell receptors and the priming of S proteins by host cell proteases. Given such similarities, the animal models developed for SARS-CoV research may serve as a viable starting point for SARS-CoV-2 studies.

The race is on to stem the tide of the current pandemic by identifying effective approaches to treat and prevent COVID-19. Can mouse models help researchers cross the finish line? A review of the challenges associated with virus research and the effective use of mouse models in SARS-CoV research following the 2003 SARS pandemic, reveals the potential utility of both genetically-engineered mouse models as well as standard laboratory mice in COVID-19 studies.

The virus dilemma

The nature of viruses creates challenges for preclinical investigators in search of relevant animal models for COVID-19 studies. Many viruses can only establish infection in a narrow range of species; as such, a virus that is able to infect humans may not have the same capacity to infect mice, and *vice versa*. This species specificity is often a function of the protein-protein interactions between the surface of the virus and that of the host cell. The virus relies on interactions with receptors (or 'docking stations') for entering the host cell and establishing infection. If the host cell does not carry a protein on its surface to which the virus can bind, then it is not susceptible to infection by that strain of virus. This is precisely the case with both SARS-CoV and SARS-CoV-2; viral spike protein binds the mouse and rat angiotensin I converting enzyme 2 (ACE2) receptor versions with lower affinity compared to human ACE2, and thus lab animals have limited viral replication and spontaneously clear infection soon after exposure[1-3].

Though humans and mice share many of the same genes, many challenges exist when attempting to develop a mouse model that mimics the

human response to a virus, some of which are related to the unique characteristics of the virus.

- The mouse model may not possess the same receptor protein as found in humans.
- The mouse protein may not be expressed on the same cells in the same patterns as seen in humans.
- The virus may be able to use the human version of the protein as its docking station but may be unable to use the mouse version of the same protein as effectively due to sequence differences which reduce binding affinity.
- Differences between the mouse and human immune system can make modelling the human immune response to infection challenging.

Models developed for the study of SARS-CoV after the SARS pandemic and to study the MERS virus (MERS-CoV) sought to overcome limitations like these, and a number of those models appear to have utility in COVID-19 studies: ACE2 models (both knockout and humanised), humanised dipeptidyl peptidase 4 (DPP4) mice and transmembrane serine protease 2 (TMPRSS2) knockout mice. Of particular relevance, multiple recent publications have noted that SARS-CoV-2 uses the SARS-CoV receptor ACE2 for cellular entry and the serine protease TMPRSS2 for S protein priming[4-6] and a recent paper postulates that the MERS receptor DPP4 may serve as a co-receptor for SARS-CoV-2 viral entry based on projected high binding affinity to the spike protein[7].

Other transgenic and standard strains may help elucidate the connections between the higher mortality rate seen in older COVID-19 patients as well as an intriguing potential connection between APOE genotype and disease severity.

The ACE2 connection

Research on SARS and acute respiratory distress syndrome (ARDS) – a severe complication of COVID-19, particularly among those fatally infected[8] – has revealed a connection between these conditions and the ACE2 receptor. Both SARS-CoV and SARS-CoV-2 possess spike (S) proteins on the surface of their outer coat, which bind with high affinity to the human ACE2 protein. The ACE2 protein is expressed on the surface of cells in the human respiratory tract and gut[9], and binding of the viral spike to ACE2 facilitates entry into human cells for both viruses. This finding forms the basis, in part, for applying ACE2 mouse models to studies of both SARS and COVID-19.

Following the SARS-CoV 2003 outbreak, several genetically-engineered mouse models were developed and employed to study SARS and ARDS, including transgenic models in which the human version of the ACE2 receptor protein is present and knockout models deficient for ACE2. The findings of these studies show the utility of ACE2 mouse models in investigating the role of ACE2 in SARS CoV infections and the possibility that this protein may be a suitable therapeutic target for the COVID-19 virus.

Experiments in ACE2 knockout mice provided crucial evidence that ACE2 was the key receptor for SARS-CoV. Following SARS-CoV exposure, wild type mice supported viral replication and demonstrated mild lung pathology, whereas ACE2 knockout mice displayed reduced lung pathology and generated only very low levels of infectious virus in the lungs[10]. The lower affinity of SARS-CoV for mouse ACE2 limited the severity of infection in wild type mice and spurred generations of

Young BALB/c (top), C57BL/6 (above) and 129S6 mice have been demonstrated to support replication of the SARS-CoV virus and, in combination with mouse-adapted SARS-CoV-2 viruses, may be useful to study disease pathogenesis as well as in the development of COVID-19 therapeutics or vaccines

References

1 Li, W, Greenough, TC, Moore, MJ et al. Efficient replication of severe acute respiratory syndrome coronavirus in mouse cells is limited by murine angiotensin-converting enzyme 2. J Virol. 2004;78(20):11429 11433. doi:10.1128/JVI.78.20.11429-11433.2004.

2 Wan, Y, Shang, J, Graham, R, Baric, RS, Li, F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. J Virol. 2020;94(7): e00127-20. Published 2020 Mar 17. doi:10.1128/JVI.00127-20.

3 Bao, L, Deng, W, Huang, B et al. The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice [published online ahead of print, 2020 May 7]. Nature. 2020;10.1038/s41586-020-2312-y. doi:10.1038/s41586-020-2312-y.

4 Hoffmann, M, Kleine-Weber, H, Schroeder, S et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181(2):271 280.e8. doi:10.1016/j.cell.2020.02.052.

5 Shang, J, Ye, G, Shi, K et al. Structural basis of receptor recognition by SARS-CoV-2. Nature. 2020;581(7807): 221 224. doi:10.1038/s41586-020-2179-y.

6 Yan, R, Zhang ,Y, Li, Y, Xia, L, Guo, Y, Zhou, Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science. 2020;367(6485):1444 1448. doi:10.1126/science.abb2762.

7 Li, Y, Zhang, Z, Yang, L et al. The MERS-CoV Receptor DPP4 as a Candidate Binding Target of the SARS-CoV-2 Spike [published online ahead of print, 2020 May 13]. iScience. 2020;23(6):101160. doi:10.1016/j.isci.2020.101160.

Continued on page 47

transgenic mice expressing human ACE2 to serve as hosts permissive for infection. Such hACE2 mice supported high viral replication and developed significant clinical illness and mortality after infection with SARS-CoV, in contrast to wild type mice[11].

Beyond serving as a receptor for SARS-CoV, ACE2 is an essential protective function in acute lung injury. ACE2 deficient mice were susceptible to more severe lung injuries compared to wild type mice in several different acute lung injury models. Treatment with catalytically-active recombinant ACE2 protein decreased the level of acute lung injury in both the wild type and ACE2 knockout mice[12]. Binding of SARS-CoV viral spike protein to ACE2 resulted in downregulation of surface ACE2 expression, directly impacting the development of lung failure following viral infection[9].

Despite the encouraging findings of studies conducted soon after the 2003 SARS outbreak, once the immediate danger had passed, interest in studying the virus waned. Subsequently, humanised ACE2 mice (hACE2) were no longer actively used and they were moved into a cryopreserved state where they remained until quite recently. As soon as it became apparent that COVID-19 posed a significant global health threat, many labs recognised the potential application of these hACE2 models in COVID-19 research and began recovering these lines in preparation to establish live breeding colonies and produce study cohorts of hACE2 mice.

Though investigatory work on COVID-19 is in the early stages, one recent study published in *Nature* in May 2020 found that transgenic mice expressing human ACE2 developed weight loss and interstitial pneumonia following SARS-CoV-2 infection, with the same results not observed in wild type mice infected with the virus[3]. Findings such as these demonstrate that ACE2 is an exciting target of research for COVID-19 and ARDS. In particular, transgenic mice expressing human ACE2 will play an essential role in basic research on COVID-19 as well as evaluation of therapeutics and vaccines.

The role of TMPRSS2

Once the SARS S protein binds to the ACE2 receptor, it is further primed by TMPRSS2. TMPRSS2 is expressed in human epithelial tissues, including those found in the lining of the lungs, bronchi and upper respiratory airways[13]. In SARS research, TMPRSS2 knockout mice have demonstrated that TMPRSS2 plays a role in SARS-CoV entry into cells, suggesting that the inhibition of TMPRSS2 may be an effective therapeutic against SARS-CoV-2 infections.

In one study, TMPRSS2 knockout mice infected with a mouse-adapted SARS-CoV strain exhibited only weak pro-inflammatory responses and no weight loss, whereas wild type mice developed signs of acute pneumonia and weight loss. In the knockout model, the loss of TMPRSS2 inhibited viral infection in several ways: it restricted the primary sites of viral infection within the lungs, diminished viral replication and dissemination, and was associated with less severe immunopathology compared to the wild type mice. Such results demonstrate the role of TMPRSS2 in the spread of SARS-CoV within the airways of infected mice and the subsequent pulmonary pathology and systemic immunopathology[14].

hDPP4 is the MERS receptor – does it also play a role in COVID-19?

Middle Eastern Respiratory Syndrome (MERS) is caused by a coronavirus called MERS-CoV which is related to SARS-CoV-2. Like the viruses which cause SARS and COVID-19, MERS-CoV spike protein binds to a human cell surface receptor (in this case DPP4) to facilitate entry. Until recently, it was not thought that DPP4 played a role in COVID-19, but modelling using information from crystal structures identified DPP4 as a potential co-receptor for SARS-CoV-2[7]. DPP4 may help facilitate entry into cells upon binding by the viral spike protein. Mouse DPP4 is not permissive for MERS infection, so mice expressing the human version were developed to facilitate studies of that disease[15]. Those mice may now assist investigations into what role DPP4 plays in SARS-CoV-2 infection.

Does APOE genotype impact COVID-19 severity?

Apolipoprotein (APOE) is a protein involved in cholesterol transport. The human population has three variants of this gene: E2, E3 and E4, the latter associated with atherosclerosis and Alzheimer's Disease. APOE also plays an important immunomodulatory role and is implicated in some viral infections. APOE*4 is associated with heightened susceptibility to inflammation compared to APOE*2 and APOE*3 in both mice and humans. A recent publication identified that the E4 allele may be associated with risk of more severe COVID-19 infection and clinical outcomes compared to the E3 allele[16]. With information emerging on the host inflammatory response to COVID-19 and vascular involvement, this connection is intriguing and needs more study. Luckily, mice which carry each of the three human alleles in

place of the mouse gene are readily available and may contribute to this line of inquiry.

Standard mouse strains also relevant

While various transgenic models will play a central role in COVID-19 studies, several inbred mouse strains previously used in SARS research may also have utility in studying the novel coronavirus. Young BALB/c, C57BL/6 and 129S6 mice have been demonstrated to support replication of the SARS-CoV virus[17] and, in combination with mouse-adapted SARS-CoV-2 viruses, may be useful to study disease pathogenesis as well as in the development of COVID-19 therapeutics or vaccines. Following infection with mouse-adapted SARS-CoV-2, more severe disease was seen in aged BALB/c mice (aged 9-12 months) compared to young mice[18,19]. Inbred mouse strains are widely available, and these experimental systems could serve a role in modelling the age-related differences in mortality seen in human COVID-19 patients. Additionally, since C57BL/6 mice have been used in various models of acute lung injury, they may provide utility in studies on potential therapies for ARDS, a severe complication of COVID-19.

Modeling human immune responses in a mouse

Availability of a COVID-19 vaccine is viewed as critical to avoiding a resurgence that would jeopardise human health, overwhelm healthcare systems and further impact global economies. Many COVID-19 vaccine candidates are under investigation, some already in clinical trials. Yet a significant challenge in vaccine development is the fact that the antigens presented to the immune system by major histocompatibility complex (MHC) proteins – called human leukocyte antigen (HLA) in humans – differ greatly between species. Mice that carry human HLA genes model human response to epitope-based vaccines more faithfully than wild type mice or even non-human primates, so these models may aid in COVID-19 vaccine discovery work. Additionally, mice engrafted with human immune cells may be useful in studying the immune system's response to both infection as well as COVID-19 vaccine candidates. In fact, immunodeficient NOG mice engrafted with human peripheral blood mononuclear cells already have been used to study SARS vaccine response.

What's next?

While mouse models previously used to study SARS-CoV can assist in jump-starting COVID-19 research, before these models can be applied to SARS-CoV-2 they must be appropriately validated and adequately scaled. Simply inserting a relevant human gene into the genome of a mouse does not ensure the resulting line will be a model of viral infection that provides value to investigators. It is possible the human gene inserted into the mouse genome is insufficiently expressed or not expressed in the prime target cells accessible to the virus (such as the epithelium of the respiratory airways in the case of COVID-19). Even if gene expression is targeted and sufficient, the human protein is nevertheless inserted into the cellular environment of a mouse, which poses limitations.

The validation process ensures that the genetic modification produces the desired physiological results in the mouse. Validation experiments can be rigorous to carry out; in the case of a highly contagious and virulent virus like COVID-19, many such experiments must be conducted using specialised lab infrastructure and procedures implemented by highly-trained staff, which may not be accessible on a widespread basis. While these steps impact the timeline for research deemed highly urgent, they are nonetheless vital to establishing that the model is relevant and suitable for the research objective.

Whether the model recapitulates the disease pathology faithfully is another consideration. While this is always a critical factor, it is particularly so in diseases for which symptoms may be caused or exacerbated by the host's immune response, as is the case with some of the severe manifestations of COVID-19. Even if the human ACE2 protein facilitates entry of the coronavirus into the mouse cells, hACE2 mice may not manifest the full range of disease as seen in humans, since some SARS-CoV-2 complications seem to be elicited by the host immune response to the virus. Thus, individual mouse models may only have utility for study of specific disease aspects, and a wide range of models may be required to truly understand COVID-19.

While many useful models exist today, undoubtedly new models are still needed to advance COVID-19 research. A variety of genetic engineering technologies are at the disposal of model generation companies, each with different considerations and timelines. A relatively simple model in which a human gene is randomly inserted into the mouse genome might require a four- to five-month timeline for model design and generation. A similar timeline would apply in using CRISPR/Cas-9 to make targeted modifications to the murine version of the gene, for instance, to make the ACE2 protein more human-like. More complex modifications,

Continued from page 46

8 Matthay, MA, Aldrich, JM, Gotts, JE. Treatment for severe acute respiratory distress syndrome from COVID-19. Lancet Respir Med. 2020;8(5):433 434. doi:10.1016/S2213-2600(20)30127-2.
9 Hamming, I, Timens, W, Bulthuis, ML, Lely, AT, Navis, G, van Goor, H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004;203(2):631 637. doi:10.1002/path.1570.
10 Kuba, K, Imai, Y, Rao, S et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med. 2005;11(8):875 879. doi:10.1038/nm1267.
11 Tseng, CT, Huang, C, Newman, P et al. Severe acute respiratory syndrome coronavirus infection of mice transgenic for the human Angiotensin-converting enzyme 2 virus receptor. J Virol. 2007;81(3):1162 1173. doi:10.1128/JVI.01702-06.
12 Imai, Y, Kuba, K, Rao, S et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature. 2005;436(7047): 112 116. doi:10.1038/nature03712.
13 Bugge, TH, Antalis, TM, Wu, Q. Type II transmembrane serine proteases. J Biol Chem. 2009;284(35):23177 23181. doi:10.1074/jbc.R109.021006.
14 Iwata-Yoshikawa, N, Okamura, T, Shimizu, Y, Hasegawa, H, Takeda, M, Nagata, N. TMPRSS2 Contributes to Virus Spread and Immunopathology in the Airways of Murine Models after Coronavirus Infection. J Virol. 2019;93(6):e01815-18. Published 2019 Mar 5. doi:10.1128/JVI.01815-18.

Continued on page 48

Continued from page 47

such as inserting either an entire human gene into a specific target in the mouse genome, would require upwards of 15-18 months for model design and generation, plus additional time for breeding scale-up.

Mouse models have played a vital role in infectious disease research as a whole and in advancing investigators' understanding of the SARS-CoV virus following the 2003 outbreak. Preclinical studies conducted with humanised ACE2 mice and knockouts of ACE2 and TMPRSS2 have helped elucidate the pathogenesis of SARS-CoV, to which the virus that causes COVID-19 is closely related. Additionally, several inbred mouse strains have been used in SARS research and may have great utility for modelling SARS-CoV-2 now that mouse-adapted viruses have been generated. As investigators work rapidly to develop and test COVID-19 therapeutics and vaccines, mouse models will be critical tools in this race. DDW

15 Agrawal, AS, Garron, T, Tao, X et al. Generation of a transgenic mouse model of Middle East respiratory syndrome coronavirus infection and disease. J Virol. 2015;89(7):3659 3670. doi:10.1128/JVI.03427-14.
16 Kuo, CL, Pilling, LC, Atkins, JL et al. APOE e4 genotype predicts severe COVID-19 in the UK Biobank community cohort [published online ahead of print, 2020 May 26]. J Gerontol A Biol Sci Med Sci. 2020;glaa131. doi:10.1093/gerona/glaa131.
17 Subbarao, K, Roberts, A. Is there an ideal animal model for SARS?. Trends Microbiol. 2006;14(7):299 303. doi:10.1016/j.tim.2006.05.007.
18 Gu, H, Chen, Q, Yang, G, He, L, Fan, H, Deng, Y-Q, Wang, Y, Teng, Y, Zhao, Z, Cui, Y, Li, Y, Li, X-F, Li, J, Zhang, N, Yang, X, Chen, S, Zhao, G, Wang, X, Luo, D, Wang, H, Yang, X, Li, Y, Han, G, He, Y, Zhou, X, Geng, S, Sheng, X, Jiang, S, Sun, S, Qin, C-F, Zhou, Y. Rapid Adaptation of SARS-CoV-2 in BALB/c Mice: Novel Mouse Model for Vaccine Efficacy. 2020. https://doi.org/10.1101/2020.05.02.073411.
19 Dinnon, KH, Leist, SR, Schäfer, A, Edwards, CE, Martinez, DR, Montgomery, SA, West, A, Yount, BL, Hou, YJ, Adams, LE, Gully, KL, Brown, AJ, Huang, E, Bryant, MD, Choong, IC, Glenn, JS, Gralinski, LE, Sheahan, TP, Baric, RS. A Mouse-Adapted SARS-CoV-2 Model for the Evaluation of COVID-19 Medical Countermeasures. 2020. https://doi.org/10.1101/2020.05.06.081497.

Dr Megan MacBride *is Director, Product Marketing at Taconic Biosciences. She holds a PhD in chemistry from the Pennsylvania State University and has 14 years of experience in commercialisation of animal models.*