Animal models in SARS-CoV-2 research

This Comment discusses the main animal models that have had a key role in our understanding of the immune and viral dynamics of SARS-CoV-2.

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19), the seventh coronavirus known to cause disease in humans. The virus continues to emerge since its first report in late 2019, rapidly acquiring mutations that can modulate pathogenicity, transmission and antibody evasion. As of February 2022, SARS-CoV-2 and its variants have infected more than 420 million people with over 5.8 million deaths. Owing to the global impact of COVID-19, research into this disease has been proceeding at an unprecedented pace. Nevertheless, a large number of questions remain to be addressed, including the origin of the virus, the reason for its high transmissibility and the underlying mechanisms of its broad spectrum of clinical manifestations. In addition, highly effective vaccines and antiviral agent that can provide optimal protection against rapidly evolving variants, such as the recently emerged Omicron variants (BA.1 and BA.2), are still in need.

Animal models represent an indispensable component of COVID-19 research. Although in vitro, ex vivo and organoid models have revealed key virological features of SARS-CoV-2, animal models that recapitulate the clinical and pathological characteristics of COVID-19 in humans are essential for studies on viral pathogenesis, transmission, therapeutic agents and vaccines. Insights into pathogenesis and virus–host interactions based on in vitro evidence require validation in animal models to confirm their physiological relevance. Novel antiviral agents must be evaluated in animal models as properties such bioavailability, serum concentration and half-life, and tissue accessibility can be assessed only in vivo. Similarly, transmission and vaccine studies can be evaluated only through physiologically relevant animal models. In this Comment, we highlight key animal models that were promptly established during the COVID-19 pandemic to facilitate in vivo research on SARS-CoV-2. We also mention a number of representative studies that have advanced our knowledge on COVID-19 using each animal model.

Golden hamster model

Golden, or Syrian, hamsters (*Mesocricetus auratus*) are naturally susceptible to SARS-CoV-2 infection, owing to the high degree of similarity between hamster ACE2 and human ACE2 (hACE2). SARS-CoV-2 infection in golden hamsters does not result in a lethal outcome[1–3]. The infected hamsters progressively lose up to 10% of their body weight within the first week after infection, before gradually returning to their original weight by about 10 days after infection. They develop ruffled fur, hunched posture, lethargy and labored breathing during the acute phase of infection, with gradual resolution by about day 7 after infection. Infectious virus titers can be retrieved from the respiratory tract of infected hamsters and are approximately 1–2 logs higher in the nasal turbinate than in the lung, peaking at 2–4 days after infection. The infected hamsters develop lung pathologies, including alveolar destruction, proteinaceous exudation, hyaline membrane formation, marked mononuclear cell infiltration, cell debris-filled bronchiolar lumen, alveolar collapse, lung consolidation and pulmonary hemorrhage. These lung pathologies are largely resolved by day 14 after infection, with air-exchange structures being restored to normal. In addition to SARS-CoV-2-induced pathologies, the transmission of SARS-CoV-2 is highly efficient among hamsters by direct contact and aerosols, whereas transmission via fomites was not as efficient[2,4]. Using this golden hamster model, Chan et al.[4] demonstrated early in the COVID-19 pandemic that surgical mask partition could significantly reduce the probability of noncontact transmission of SARS-CoV-2 from index to naive hamsters. Zhang et al.[5] and Sia et al.[2] demonstrated that SARS-CoV-2 could infect and damage mature and immature olfactory sensory neurons at the nasal mucosa of hamsters, which may explain the anosmia reported in patients with COVID-19.

The hamster model has also been highly useful for studies focusing on the virology and pathogenicity of SARS-CoV-2, such as those investigating the role of key residues and motifs of emerging virus variants. For example, Zhou et al.[6] demonstrated that the D614G substitution in spike significantly increased virus transmissibility, and Liu et al.[7] showed that the N501Y substitution in spike resulted in consistent fitness gains for replication in the upper respiratory tract and enhanced viral transmission. Similarly, Saito et al.[8] showed that the P681R substitution results in higher pathogenicity compared with parental virus, and Wu et al.[9] showed that the R203K/G204R double substitution in nucleocapsid protein rendered higher viral fitness than the parental virus. Overall, the golden hamster model mimics the clinical, virological, histopathological and immunological features of mild-to-moderate COVID-19 in humans. The relative availability and ease of handling allows the hamster model to be used as an important platform for studies on the pathogenesis of, transmission of, treatment of and vaccination against SARS-CoV-2.

Mouse model

Mice (*Mus musculus*) are the most frequently used experimental animals because of their low cost, high accessibility, rapid breeding speed, ease of manipulation and the high availability of reagents. However, mice are not naturally susceptible to prototypical SARS-CoV-2 infection, owing to amino acid substitutions that are present on mouse ACE2 compared with hACE2 in the ACE2–spike binding interface. To allow in vivo SARS-CoV-2 mouse studies, a number of strategies have been adopted to circumvent this issue.

Mice can be sensitized to SARS-CoV-2 infection by introducing hACE2 expression through transgenic, knock-in, viral-vector transduction or virus-adaptation strategies[10–22]. Among these models, the K18-hACE2 model — which was originally constructed for in vivo evaluation of SARS-CoV — is currently one of the most commonly used mouse models for SARS-CoV-2 investigations because of its availability[23]. In the K18-hACE2 mice, expression of hACE2 is driven by the human cytokeratin 18 gene (*KRT18*; here abbreviated K18) for high-level expression of hACE2 in epithelial cells[23]. Infection of K18-hACE2 mice with SARS-CoV-2 causes dose-dependent respiratory manifestations and lethality[16,24]. Viral load in the lungs

Table 1 | Characteristics of different animal models for in vivo SARS-CoV-2 studies

Animal model	Respiratory infection	Clinical disease	Transmission	Ease of handling	Cost
Hamsters	Robust virus replication in the upper and lower respiratory tracts	Mild to moderate	Efficient	Easy	Low
Transgenic mice	Robust virus replication in the upper and lower respiratory tracts	Mild to lethal	Not efficient	Easy	Low
Wild-type mice	Robust virus replication in the upper and lower respiratory tracts; rapid virus clearance	Mild	No transmission	Easy	Low
Ferrets	Robust virus replication in the upper respiratory tract	Mild	Efficient	Moderate	Moderate
Nonhuman primates	Robust virus replication in the upper and lower respiratory tracts	Mild to moderate	Efficient	Difficult	High

of SARS-CoV-2-infected K18-hACE2 transgenic mice generally peaks between 2 and 4 days after infection. Lung pathology of the K18-hACE2 mice starts early upon infection, becomes mild-to-moderate by day 4 after infection and progresses to moderate-to-severe by day 6 after infection. Similar to SARS-CoV infection, a subset of SARS-CoV-2-infected K18-hACE2 mice develop central nervous system infection. Viral burden and pathology in the brain are undetectable at early stages, but progress rapidly within 4–5 days after infection until the mice succumb to the infection. It is important to note that SARS-CoV-2-induced mortality in K18-hACE2 mice can be due to central nervous system and/or respiratory tract infection, enabling examination of neurological involvement in COVID-19 (ref. [24]).

In addition, SARS-CoV-2 can be adapted to bind to mouse ACE2 to allow SARS-CoV-2 infection in wild-type mice, as described by Gu et al.[15], who serially passaged infected lung homogenates in BALB/c mice and obtained the mouse-adapted N501Y substitution in the receptor-binding domain (RBD) of SARS-CoV-2 spike after a single passage. Interestingly, the N501Y substitution was later identified in several emerging SARS-CoV-2 variants, including Alpha, Beta, Gamma and Omicron. To this end, the infection of wild-type mice with these N501Y-carrying SARS-CoV-2 variants represents a natural mouse model of SARS-CoV-2 infection without the need of prior genetic modification of the mice or the virus[25].

Depending on the strategies for granting virus entry in mice, clinical manifestations of SARS-CoV-2 challenge in mice can range from mild to lethal infection and thus can be used for a broad array of evaluations. For example, Johnson et al.[26] demonstrated that removing the PRRA motif in the furin-like cleavage site of SARS-CoV-2 spike reduced virus replication in both the upper and lower respiratory tracts, and attenuated its pathogenicity, in the K18-hACE2 transgenic mice. In addition, mouse models have frequently been used as first-line animal models to evaluate the effect of therapeutic agents, owing to their availability and ease of manipulation[27]. Importantly, K18-hACE2 and other hACE2-transgenic mice are also used to compare the pathogenicity of different SARS-CoV-2 variants. In addition to assessing viral replication and histopathological changes in the lung, infection outcome supports measurements of survival rate as a key quantitative indicator of pathogenicity[28]. Notably, transmission of SARS-CoV-2 is not efficient between mice. Although hACE2-transgenic mice support moderate levels (about 50%) of close contact transmission, the efficiency of respiratory droplet transmission was low (30%)[29]. In wild-type mice challenged with N501Y-carrying Alpha variant, transmission of SARS-CoV-2 was not detected among contacted mice[25]. Importantly, humanized mouse models have also substantially contributed to COVID-19 research[30–35]. One example of a humanized mouse model is the VelocImmune mouse, engineered by replacing mouse immunoglobulin heavy and kappa light variable region germ-line gene segments with their human counterparts; this model has been used to screen for antibodies against the RBD region of SARS-CoV-2 spike[30,31].

Currently there is a rich repertoire of mouse models, each of which has strengths and limitations, that is available for SARS-CoV-2 studies. A number of transgenic mouse models can support survival measurements, but the ectopic expression of hACE2 results in altered receptor expression level and location[25]. Mouse-adapted viruses are compatible with wild-type mice, but the virus may acquire non-naturally occurring substitutions that alter viral characteristics. It is also labor-intensive to incorporate the mouse-adapted substitutions for each emerging SARS-CoV-2 variant under evaluation. The use of wild-type mice together with N501Y-carrying variants represents a mouse model that does not require genetic modification of mice or viruses. However, this model only supports infection of SARS-CoV-2 variants that carry the N501Y substitution and with relatively mild infection outcomes, thus limiting its widespread use for in vivo studies

Ferret model

Ferrets (*Mustela putorius furo*) are naturally susceptible to SARS-CoV-2 infection. Infected ferrets develop elevated body temperatures within 2 days after infection, which peaks on day 4 after infection. Viral replication is largely limited to the upper respiratory tract and is most robust in the nasal turbinate. In the lung, infected ferrets show increased immune cell infiltration and accumulation of cell debris along the alveolar and bronchial epithelium. An overall mild disease is observed in SARS-CoV-2-infected ferrets. Reduced activity is observed between 2 and 6 days after infection, with occasional coughs and no detectable body weight loss or fatalities[36]. Despite the mild disease outcome, SARS-CoV-2 is efficiently spread among ferrets through both direct and indirect contact, which makes them a robust model for studies of SARS-CoV-2 transmission. Using the ferret model, Peacock et al.[20] demonstrated the critical contribution of the furin cleavage site in SARS-CoV-2 spike to the transmissibility of the virus. Cox et al.[37] showed that therapeutic treatment of SARS-CoV-2-infected ferrets with molnupiravir (EIDD-2801) orally twice a day significantly reduced SARS-CoV-2 replication in the upper respiratory tract, and completely inhibited virus transmission to untreated contact animals. In addition to transmission studies, Blanco-Melo et al.[38] demonstrated early in the pandemic that the imbalanced host response characterized by dampened innate antiviral defense contributed to the mild clinical manifestations in ferrets, revealing a key pathogenic feature of the disease.

Nonhuman primate models

Species of nonhuman primate — including rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), common marmosets

(*Callithrix jacchus*) and baboons (*Papio hamadryas*) — are susceptible to SARS-CoV-2 infection. Infection of nonhuman primates by SARS-CoV-2 generally results in mild-to-moderate respiratory manifestations. Although the required expertise and biosafety level 3 facilities to handle non-human primates are relatively scarce, these models are indispensable and are important platforms for the evaluation of therapeutic agents and vaccines for COVID-19 before they enter clinical trials. The Pfizer–BioNTech vaccine candidates were evaluated in rhesus macaques and were shown to protect the lower respiratory tract of the vaccinated animals against the presence of viral RNA and disease development, providing key scientific support that green-lit the clinical trials[39]. Williamson et al.[40] demonstrated that remdesivir treatment in SARS-CoV-2-infected rhesus macaques reduced virus titers in bronchoalveolar lavage, reduced pulmonary infiltrates on radiographs and reduced signs of respiratory disease. This finding confirmed remdesivir as the first antiviral treatment with proven efficacy against SARS-CoV-2 in a nonhuman primate model of COVID-19 (ref. [40]). In addition, Chandrashekar et al.[41] and Deng et al.[42] showed that SARS-CoV-2 infection in rhesus macaques induced humoral and cellular immune responses that provided protection against SARS-CoV-2 rechallenge.

Conclusion

Studies using animal models — including hamsters, mice, ferrets and nonhuman primates — have facilitated SARS-CoV-2 in vivo research and generated critical knowledge on the pathogenesis and transmission dynamics of the virus, which in turn has led to the rapid development of therapeutic agents and vaccines. As each animal model has its strengths and limitations, we recommend selecting the optimal animal model with respect to the research questions being addressed (Table 1). Investigations using two, or even three, animal models may often be necessary to help to draw definitive conclusions, as has recently been done to evaluate the pathogenesis of Omicron[28,43,44].

Over the past two years, a large number of animal models have been established and have provided key information on the COVID-19 pandemic. SARS-CoV-2 studies can also be conveniently conducted in animal models that are already available[45] to generate important knowledge on the pathogenesis, as well as the long-term sequelae, of COVID-19. However, we think that future animal studies should aim to further optimize our current repertoire of animal models for better studies on the dynamics of viral infection. For example, as hamsters represent mild-to-moderate SARS-CoV-2 infection, hACE2-transgenic hamsters or hamster-adapted SARS-CoV-2 can be developed to recapitulate severe COVID-19 infection while supporting robust animal-to-animal transmission. These future methods and technological advances to improve animal models will enable us to investigate future pathogenic threats swiftly and efficiently. ❐

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Competing interests

The authors declare no competing interests.