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Animal models of SARS-CoV-2 and COVID-19 for the development of prophylactic and therapeutic interventions

Marcel Renn [a,b], Eva Bartok [a,c], Thomas Zillinger [a,d], Gunther Hartmann [a], Rayk Behrendt [e,*]

[a] Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, 53127 Bonn, Germany
[b] Mildred Scheel School of Oncology, University Hospital Bonn, Medical Faculty, 53127 Bonn, Germany
[c] Unit of Experimental Immunology, Department of Biomedical Sciences, Institute of Tropical Medicine, 2000 Antwerp, Belgium
[d] Institute of Immunology, School of Medicine, Philipps University Marburg, 35043 Marburg, Germany
[e] Institute for Immunology, Technische Universität Dresden, Medical Faculty Carl Gustav Carus, 01307 Dresden, Germany

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ABSTRACT

Infections of the Coronavirus SARS-CoV-2 continue to spread around the globe, causing Coronavirus Disease (COVID)-19. Infected people are at risk of developing acute interstitial pneumonia, which can result in lethal complications, particularly in patients with pre-existing co-morbidities. Novel prophylactic and therapeutic interventions are urgently needed to limit the infection-associated health risk for the population and to contain the pandemic. Animal models are indispensable to assessing the efficacy and safety of potential new antivirals, vaccines, and other innovative therapies, such as nucleic acid agonists of innate immune sensing receptors. In this review, we provide an overview of the commonly used animal models to study SARS-CoV-2 and COVID-19, including a summary of their susceptibility to infection, the spectrum of symptoms elicited, and the potential for drug development in each model. We hope that this review will help researchers to decide on the right model organism to quickly address their specific scientific questions.

Contents

Abbreviations: AAV, Adeno-associated virus; ACE2, Angiotensin-converting enzyme 2; Ad, Adenovirus; CAG, CMV enhancer chicken beta-actin promoter; HFH4, Hepatocyte nuclear factor-3/forkhead homologue 4; IFN, Interferon; IFNAR, Type I interferon receptor; ISG, Interferon-stimulated genes; K18, Keratin 18; NHP, Non-human primates; RBD, Receptor binding domain; SARS-CoV, Severe acute respiratory syndrome Coronavirus,; VOC, variants of concern.

* Corresponding author at: Institute for Immunology, Technische Universität Dresden, Medical Faculty Carl Gustav Carus, Fetscherstrasse 74, 01307 Dresden, Germany.
E-mail address: rayk.behrendt@tu-dresden.de (R. Behrendt).

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1. Introduction

In each of the last three decades, humanity has faced a different deadly zoonotic disease caused by the coronavirus family. While infections caused by the SARS Coronavirus (SARS-CoV) largely disappeared after 2002-2003, infections with the MERS coronavirus have been increasing since 2012. Up to 2020, the World Health Organization (WHO) reported around 8098 infections with SARS and around 2494 infections with MERS, which resulted in 774 and 858 infection-associated deaths, respectively. Now, the newly emerged SARS-Coronavirus 2 (SARS-CoV-2) has been shown to be the causative agent for Coronavirus disease 2019 (COVID-19) (Zhou et al., 2020; Zhu et al., 2020). As of May 6 2021, only 18 months after its discovery, highly effective human-to-human transmission had allowed the virus to infect around 156 million people worldwide, of which approximately 3.3 million have already succumbed to the disease (https://covid19.who.int). Due to its rapid spread, the WHO declared SARS-CoV-2 a pandemic virus in March 2020.

Within 10 days of exposure to SARS-CoV-2 up to 14% of infected humans experience severe symptoms, such as coughing, fever, and difficulties breathing. Around 5% of infections progress to a critical stage with interstitial pneumonia and become dependent on respirators (https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf). The disease is then lethal in about one third of these critically ill patients. Age and gender have been identified as major risk factors for COVID-19, with male patients above the age of 60 years displaying the highest infection fatality rate (Peckham et al., 2020). Besides the pulmonary disease, a number of extrapulmonary manifestations like thromboembolism, cardiac dysfunction and different neurological symptoms, most prominently a loss of smell and taste have been observed in COVID-19 patients (Gupta et al., 2020). Some of these impairments have been reported to persist after recovery (reviewed in Chen et al., 2020; Hu, Guo, Zhou, & Shi, 2020, p. 19).

After the initial identification of SARS-CoV-2 as a member of the betacoronaviridae family and the characterization of its similarity to SARS-CoV, it quickly became clear that SARS-CoV-2 uses the same cell-entry receptor as SARS-CoV: angiotensin-converting enzyme 2 (ACE2) (P. Zhou et al., 2020; Li et al., 2003; Hoffmann et al., 2020). ACE2 is located on the plasma membrane of cells in the lung, kidney, gastro-intestinal tract, liver, and testis, where it acts as a sensor of the renin-angiotensin-aldosterone system (RAAS), which regulates blood pressure and salt concentrations (F. Jiang et al., 2014). SARS-CoV-2 binds to ACE2 via the spike protein on the surface of the viral membrane, which leads to receptor internalization and subsequent infection of ACE2-positive cells through fusion of the viral and endosomal membranes (reviewed in Hofmann & Pöhlmann, 2004). The spike protein has only a low affinity for ACE2 orthologs in other species, including mice, rendering these species only weakly susceptible to SARS-CoV-2 infection (Bao et al., 2020; McCray et al., 2007) and complicating the use of these species as pre-clinical models. However, these difficulties can be overcome by genetic engineering, such as transgenic expression of the human ACE2 protein (see Section 2).

While ACE2 is clearly the most critical requirement for productive infection, SARS-CoV-2 also requires proteolytic processing of its spike protein for cellular entry, most importantly by transmembrane protease serine 2 (TMPRSS2) (Hoffmann et al., 2020), although other proteases such as cathepsins (Hoffmann et al., 2020) and furin (Cheng et al., 2020) can assist or substitute for this function in 293T and VeroE6 cell lines, respectively. Very recently, neuropilin-1 (NRP1) has been described as a possible co-receptor that augments the infection of murine olfactory epithelium via binding of the furin-cleaved SARS-CoV-2 S protein (Cantuti-Castelvetri et al., 2020). Species-specific differences in protein structure or tissue expression of these factors and their homologues have not been addressed in the context of SARS-CoV-2 infection and might also contribute to differences in the pathology SARS-CoV-2 observed in different animal host species.

With no vaccine immediately available, an unprecedented scientific response to the pandemic was initiated to better understand this dangerous new virus, the disease it causes, and to develop antiviral drugs as well as new therapeutic strategies to treat COVID-19 patients. To help achieve this goal as rapidly as possible, the systemic pathology, dynamics of the immune response, and efficacy tests for potential vaccines against SARS-CoV-2 needed to be studied in animal models of the disease. To this end, genetically engineered mice, mouse-adapted viruses, hamsters, ferrets and non-human primates have all been developed and employed to study infections by SARS viruses (Fig. 1). In this review, we briefly describe these animal models and how they have contributed to our current knowledge about SARS-CoV-2 and COVID-19. We then discuss findings in these models that have had implications for therapeutic interventions to manage COVID-19 associated inflammation and their relevance in drug development.

2. Mouse models

Mouse models continue to be valuable and attractive preclinical models to study systemic diseases, investigate viral infections, and evaluate therapeutic interventions. Genetically engineered mice can be generated as either somatic or germline models. The latter have the advantage that they stably pass down the genetic alteration to their progeny and can be used to establish a mouse colony with almost identical properties for long-term usage. Classically, germline mutations fall into one of two major categories: transgenic mice or gene-targeted mice. Briefly, transgenic mice harbor genetic information that is foreign to the mouse genome or an altered form of a mouse gene, which is stably integrated into the genome most commonly in a non-directed manner. The copy number and the integration sites of transgenes are fairly random, often resulting in different expression levels of the transgene among the individual founder animals derived from the same transgene. In addition, random insertion within regulatory or coding genomic regions of endogenous genes can potentially alter or abrogate their expression and function (Moreira et al., 2007). In contrast, in gene-targeted mice, the integration site as well as the number of alterations are clearly defined by use of a rationally designed targeting strategy. Gene targeting is commonly used to generate mouse models in which the expression of a mutant gene needs to be controlled by its endogenous promoter. The use of site-specific mutagenesis and a physiological expression pattern of the mutant gene results in lower overall variability in these models, which is why they are considered to be cleaner experimental systems, when compared with transgenic mice (Glaser, Anastassiadis, & Stewart, 2005). However, both systems have advantages for different applications, which have been reviewed elsewhere (Huijbers, 2017).

In contrast to editing the germline of mice, genome engineering can also be applied to somatic cells, to quickly introduce non-inheritable tissue-specific mutations. For short-term experiments, somatic mouse models have a considerable advantage over germline mouse models, because they avoid the laborious and, despite the recent advent of CRISPR/Cas editing, still technically challenging generation and subsequent breeding of specific mouse strains (reviewed in Mou, Kennedy, Anderson, Yin, & Xue, 2015). Delivery of nucleic-acid cargo to specific tissues can easily be achieved by viral transduction but also via *in vivo* electroporation of superficial tissues and organs. For instance, the wide-spread use of viral transfer systems based on retroviruses, adenoviruses, and adeno-associated viruses allows most researchers to generate somatic mouse models without the need for establishing a dedicated transgenic-mouse facility. In the following sections, we will explore these different approaches to studying SARS-CoV-2 infection in mice. An overview of these mouse models and their response to infection with SARS-CoV-2 is provided in Fig. 2. If available, we provide the official names of the mutant strains as deposited in the Mouse Genome Informatics (MGI) database (www. http://www.informatics.jax.org/).

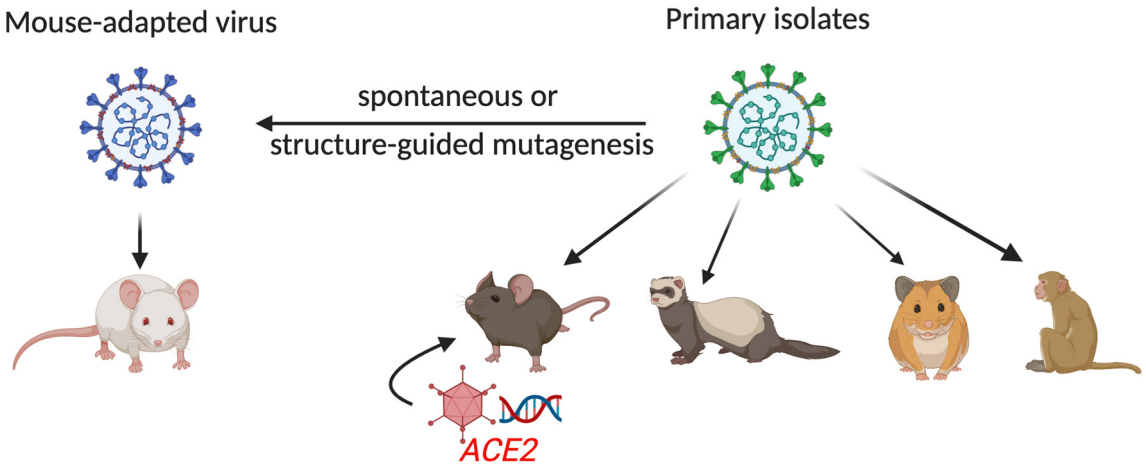

Fig. 1. Commonly used animal models to study SARS-CoV-2 and COVID-19. Non-human primates, ferrets, and hamsters are readily susceptible to infection with primary isolates of SARS-CoV-2. In order to study the virus or COVID-19 in the murine system, mice need to be genetically engineered to express the human *ACE2* gene. This can be achieved by introducing *ACE2* into the mouse germline or, more transiently, by inoculation of mice with ACE2-expressing viral vectors, such as adenoviruses. Alternatively, mouse-adapted SARS-CoV-2 variants have been generated through spontaneous mutagenesis following passaging of primary isolates in mice and by recombinant techniques, introducing site-specific mutations in the receptor-binding domain of the spike protein.

2.1. K18-hACE2 transgenic mice (MGI: Tg(K18-ACE2)2Prlmn)

This mouse line was generated in a mixed C57BL/6J x SJL/J F2 genetic background and then backcrossed to C57BL/6J mice. It expresses the human *ACE2* gene under the control of the mouse *keratin-18* (K18) promoter, which restricts expression mainly to epithelial tissues including airway epithelial cells (Chow et al., 1997). K18-hACE2 transgenic mice can be productively infected with SARS-CoV (Urbani), which leads to progressive weight loss and lethality within one week after intranasal infection. Viral RNA and infectious particles were detected in the lungs and brains of infected mice. In addition, both organs displayed inflammation that was characterized by immune-cell infiltrates and the increased expression of pro-inflammatory cytokines. Passive immunization with the spike-specific neutralizing antibody MAb21 protected these mice from disease (McCray et al., 2007). Several groups have employed K18-hACE2 mice to study SARS-CoV-2 and COVID-19, all of which reported weight loss and inflammation in the lungs within one week after intranasal infection (Moreau et al., 2020; Oladunni et al., 2020; Rathnasinghe et al., 2020; Winkler et al., 2020; Yinda et al., 2020; Zheng et al., 2021). Interestingly, lethality was only observed in mice in which the virus disseminated into the central nervous system (CNS) (Oladunni et al., 2020; Winkler et al., 2020; Yinda et al., 2020). A direct correlation between SARS-CoV-2 neuroinvasion and lethality has been confirmed recently by three independent studies in this model (Carossino et al., 2021; Kumari et al., 2021; Song et al., 2021).

2.2. HFH4-ACE2 transgene (MGI: Tg(FOXJ1-ACE2)1Rba)

In this transgenic mouse line on the mixed C3H × C57BL/6 genetic background, human *ACE2* is expressed under the control of the human *hepatocyte nuclear factor-3/forkhead homologue 4* (HFH4) promoter. This promoter is mainly active in ciliated cells of the lung epithelium, but expression of the transgene has also been detected in the brain, liver, kidney, and intestine. Infection of HFH4-ACE2 mice with SARS-CoV (Urbani) resulted in weight loss, pneumonia, and dissemination of the virus into the CNS (Menachery et al., 2016). Within one week after intranasal infection, all mice succumbed to the infection. As recently reported, SARS-CoV-2 can also infect HFH4-ACE2 mice (R.-D. Jiang et al., 2020). While the symptoms were comparable to the ones observed after infection with SARS-CoV, only mice in which the virus reached the CNS succumbed to the infection, as was also reported for K18-hACE2 transgenic mice. Viral loads in the infected mice did not significantly differ, suggesting that dissemination into the CNS did not only depend on the viral titer.

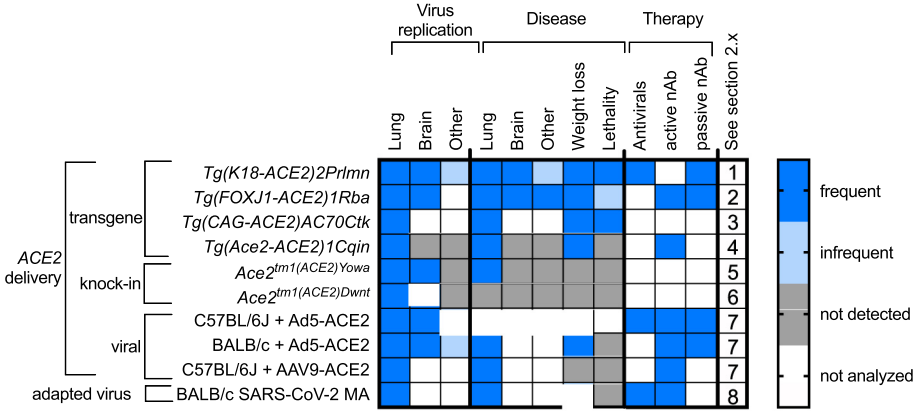

Fig. 2. Mouse models can be used to address specific aspects of SARS-CoV-2 infection. For all mouse models, sites of main viral replication, diseased organs as well as their use in therapeutic experiments are shown as a heatmap representing how frequently the depicted features have been observed in each model. "Active" and "passive" refer to the mode of immunization that conferred protection by neutralizing antibodies (nAb).

2.3. CAG-ACE2 transgene (MGI: Tg(CAG-ACE2)AC70Ctkt)

In another transgenic mouse model, the human *ACE2* gene is expressed under the control of the strong synthetic CAG promoter (Tseng et al., 2007). With this construct, several founders were generated in a mixed C3H/HeJ x C57BL/6J genetic background, and all displayed variable degrees of ACE2 expression. CAG-hACE2 mice with the highest expression (founder line "AC70") developed lethal pneumonia and CNS inflammation after SARS-CoV infection, as seen also in the K18-hACE2 mice. The other founders demonstrated lower transgene expression, no or only low viral antigen expression in the CNS, and were able to survive the infection (Tseng et al., 2007). No peer-reviewed data about SARS-CoV-2 using this model has been published to date.

2.4. mAce2-ACE2 transgene (MGI: Tg(Ace2-ACE2)1Cqin)

In order to mimic the expression profile of the endogenous *Ace2* gene, a transgenic mouse line expressing human *ACE2* under the control of the mouse *Ace2* promoter was generated. While all previously discussed *ACE2* transgenic mice were generated in inbred mouse strains, *mAce2-ACE2* mice were generated in the CD1 (ICR) outbred background. ACE2 expression was detected in the lung, heart, kidney, and intestine of these mice, and, when they were infected with SARS-CoV (PUMC01), they developed pneumonia, but the infection was not lethal. However, in the brain and other extrapulmonary organs, bleeding and mild inflammation were observed, although no infectious virus could be isolated (Yang et al., 2007). These differences in pathology might result from lower transgene expression under the endogenous *Ace2* promoter, positional effects resulting from transgene integration, or an altered antiviral immune response in the outbred strain compared to the immune response of inbred mouse strains. Infection of mAce2-hACE2 transgenic mice with SARS-CoV-2 (HB01) recapitulated the observations made with SARS-CoV, as described above (Bao et al., 2020).

2.5. ACE2-tdTomato knock-in (MGI: $Ace2^{tm1(ACE2)Yowa}$)

In order to more accurately reflect endogenous Ace2 expression, human *ACE2* cDNA was knocked into the endogenous mouse *Ace2* gene locus using CRISPR/Cas9-mediated homology-directed repair (Sun, Chen, et al., 2020). The knock-in destroyed the mouse *Ace2* coding sequence and, in addition to the human *ACE2* cDNA, introduced an IRES-td-Tomato fluorescent reporter cassette. ACE2-reporter expression was observed in the lung, kidney, spleen, and duodenum. Within the lung, reporter expression was found to be highest in club cells, providing evidence that these cells express high levels of ACE2 and could potentially serve as the primary target for SARS-CoV-2 entry. In line with this observation, high levels of viral protein were also found in club cells, suggesting that they serve as important sites of viral replication in the mouse lung. However, viral antigen was also detected in cells that were negative for the fluorescent reporter, raising the question of whether IRES-td-Tomato was faithfully reporting ACE2 expression or whether viral antigens were able to enter these cells by a so-far-unknown means. Nonetheless, intranasal infection of *ACE2* knock-in mice with SARS-CoV-2 resulted in interstitial pneumonia, but it was not associated with severe respiratory symptoms. Similar to what has been observed in COVID-19 patients, infection of older mice resulted in more severe lung pathology (Sun, Chen, et al., 2020). Interestingly, despite the fact that viral RNA was detected in the brains of the infected mice, no deaths were observed after infection with SARS-CoV-2. In order to investigate the possibility of fecal–oral transmission, *ACE2* knock-in mice were also infected with the virus via the intragastric route. Five days later, viral protein and inflammation were detected in the lungs, suggesting that, at least in this model, fecal–oral transmission of the virus was possible (Sun, Chen, et al., 2020).

2.6. Ace2 knock-in (MGI: $Ace2^{tm1(ACE2)Dwnt}$)

In this mouse line, the cDNA of human *ACE2* has been inserted into the endogenous mouse *Ace2* gene by classical gene-targeting in mouse embryonic stem (mES) cells. The mESc originated from the 129Sv/Pas genetic background, and chimeric founder mice were backcrossed to the C57BL/6J background. Zhou and colleagues used this new model to compare the fitness of the original SARS-CoV-2 S-614D with the emerging SARS-CoV-2 S-614G variant. The authors generated isogenic S-614G virus by mutating the spike protein gene in the genome of Wuhan-Hu-1 SARS-CoV-2 S-614D (Zhou et al., 2021). Both variants were used in a competitive setting to simultaneously infect *Ace2* knock-in mice intranasally. Viral genomic RNA was detected only in the nasal conchae, lungs, olfactory bulbs, and, at low levels, in the brain. As reported for other *Ace2* knock-in mice (see Section 2.5), infection did not cause any severe symptoms, but, in contrast to those reports, there was also no inflammation observed in the lungs of mice infected simultaneously with SARS-CoV-2 S-614D and SARS-CoV-2 S-614G. Although the experiments were terminated just four days after infection, it seems unlikely that the pathology would have worsened at later stages, because the viral burden had decreased significantly between days two and four post-infection. Finally, the authors quantified the frequencies of the SARS-CoV-2 S-614D and SARS-CoV-2 S-614G genomes by deep sequencing. Their data suggested that SARS-CoV-2 S-614G might have an advantage over SARS-CoV-2 S-614D *in vivo*, because on day four post-infection the number of reads from the S-614G genome were more prevalent in most of the organs tested (B. Zhou et al., 2021). Similar experiments in ferrets (Section 3) and hamsters (Section 4) support the notion that SARS-CoV-2 S-614G was able to outcompete SARS-CoV-2 S-614D *in vivo*.

2.7. Transient somatic expression of ACE2

In a recently published study, C57BL/6J and BALB/c mice were infected intranasally with an Ad5-ACE2 vector to increase their susceptibility to SARS-CoV-2. After inoculation with SARS-CoV-2, viral RNA was detected in the lungs, heart, spleen, and brain of these mice. All mice developed lung inflammation but could eventually clear the infection. Antibody-mediated blocking of the type I interferon (IFN) receptor (IFNAR) resulted in additional weight loss in infected mice. However, impaired IFN signaling had no effect on viral titers (Hassan et al., 2020). Sun and colleagues used the same approach but found, in addition, that a deficiency in Stat1, but not in Ifnγ signaling, increased the initial weight loss and tissue damage in the lungs and delayed clearance of the virus (Sun et al., 2020).

Another group used adeno-associated virus (AAV) 9 to express ACE2 in C57BL/6J mice. Although infectious particles could be recovered from mice that were infected with SARS-CoV-2, none of them showed any of the clinical symptoms of COVID-19. However, two weeks after infection, neutralizing antibodies to SARS-CoV-2 were detected in the sera of these mice. Analysis of the transcriptional changes in response to the infection showed a pro-inflammatory cytokine response that started at two days post-infection, with a dominant type I IFN transcriptional signature (75% of all induced transcripts were ISGs) in the lungs of the infected mice. Like in the Ad5-ACE2 model, IFNAR-deficiency had no influence on the viral titers (Israelow et al., 2020). In contrast to the mild pathology following AAV-mediated ACE2 expression in the lung and intranasal infection with SARS-CoV-2, intracisternal AAV-ACE2 delivery followed by intraventricular administration of SARS-CoV-2 resulted in weight loss and death of all infected mice (Song et al., 2021). Lethality was even observed when viral doses were reduced by 100-fold compared to the intranasal infection route (Song et al., 2021). The latter findings further support a direct link between CNS infection and lethality as seen in the K18-hACE2 (see Section 2.1) and HFH4-ACE2 (see Section 2.2) mouse models.

2.8. Mouse-adapted viruses

Instead of introducing genetic alterations into the host, the virus itself can be adapted by passaging it *in vivo* and selecting for desired biological features, such as increased pathology. By using this method, a mouse-pathogenic variant of SARS-CoV, called MA15, was generated from the Urbani strain and caused lethal pneumonia in BALB/c mice (Channappanavar et al., 2016; Roberts et al., 2007). As opposed to performing a phenotypic screen for virus mutants, the mouse pathogenic SARS-CoV-2 MA variant was created by introducing structure-guided mutations into the receptor-binding domain (RBD) of the spike protein, based on MA15 and other group 2B coronaviruses capable of infecting mice. SARS-CoV-2 MA caused non-lethal pneumonia in BALB/c mice, which was more severe in older mice (Dinnon et al., 2020). Subsequent passaging of SARS-CoV-2 MA in mouse lungs yielded the highly pathogenic SARS-CoV-2 MA10. SARS-CoV-2 MA10 caused acute lung injury, which was associated with 15% lethality in 10-week-old mice. One-year-old BALB/c mice showed significantly increased morbidity and mortality of up to 85% within one week post infection (Leist et al., 2020). In contrast, infection of C57BL/6 mice with SARS-CoV-2 MA10 caused only mild lung inflammation, around 10% weight loss and no mortality (Leist et al., 2020). Serial passaging of SARS-CoV-2 through the respiratory tract of BALB/c mice yielded a SARS-CoV-2 variant carrying the N501Y substitution that was suggested to allow for infection of non-genetically modified laboratory mice (Gu et al., 2020). Off note, also the VOC B.1.1.7 carries the N501Y substitution and thus can infect mice (Muruato et al., 2021). SARS-CoV-2 N501Y caused interstitial pneumonia in young and old BALB/c mice (Gu et al., 2020). The highly virulent, mouse-adapted SARS2-N501Y$_{MA30}$ has been generated by serial passaging of SARS2-N501Y in mouse lungs (Roy Wong et al., 2021). In the absence of significant immunologic pressure, mutations in the spike and in non-structural proteins progressively accumulated, some of which can also be found in VOC, such as B.1.351, initially found in South Africa. SARS2-N501Y$_{MA30}$ caused lethal disease in young BALB/c mice and middle-aged C57BL/6 mice by day 7 p.i. but only infected the lungs and not the brain (Roy Wong et al., 2021). Similar to observations with SARS-CoV-2 MA10, young C57BL/6 mice only showed moderate weight loss and no further pathology following infection with SARS2-N501Y$_{MA30}$ (Leist et al., 2020; Roy Wong et al., 2021). Strikingly, generation of highly virulent mouse-adapted viruses simply by passaging *in vivo*, closely resembles unrestricted SARS-CoV-2 spreading in the human population in the absence of safety measures. It illustrates that viral replication is sufficient to rapidly generate new VOC even in the absence of immunologic pressure imposed by vaccines and dramatically supports the implementation of strategies aiming at slowing down virus spread to reduce the risk of generating new highly pathogenic variants.

3. Ferrets

Ferrets (*Mustela putorius furo*) were derived by domestication from the European polecat. They are a common model for viral airway infections because they react similarly to humans to influenza A infections (fever, lethargy, signs of respiratory infection) and SARS-CoV (fever, nasal discharge, sneezing) (reviewed by Enkirch & von Messling, 2015; Wong, Layton, Wheatley, & Kent, 2019). Therefore, a large body of research on both influenza and SARS-CoV has already been performed in these animals. Following intranasal inoculation with wild-type strains of SARS-CoV-2 (NMC-nCoV02 or Muc-IMB-1), ferrets showed an increase in body temperature as well as occasional coughs. However, all of the animals survived the experimental infection and developed neutralizing antibodies after 8 to 12 days (Kim et al., 2020; Schlottau et al., 2020; Shi et al., 2020). Infectious viral particles were detectable in the nasal cavities and the trachea, and viral RNA was additionally found in the lungs, kidney, and intestine (Kim et al., 2020). Infected ferrets were found to spread the infection to animals that they were in direct contact with, who also subsequently lost weight. When animals were separated by an air permeable mesh, no weight loss was observed in the animals who had indirect contact through the mesh with infected animals. Moreover, viral RNA was only detectable in nasal washes and saliva from a proportion of these animals (Kim et al., 2020). Following the emergence of the SARS-CoV-2 S-614G variant, which harbors a mutation in the spike protein, its pathogenicity and transmission has been studied in ferrets. Ferrets were simultaneously infected intranasally with isogenic SARS-CoV-2 S-614D and SARS-CoV-2 S-614G viruses and cohoused with naïve ferrets. The transmission rate was about 66% at day five post-infection. Strikingly, transmission occurred only from intranasally infected animals in which SARS-CoV-2 S-614G had outcompeted SARS-CoV-2 S-614D. Together with the data obtained in mice (see Section 2.6) and hamsters (see Section 4), published in the same paper, this study helped to demonstrate that SARS-CoV-2 variants with increased viral fitness and contagiousness can arise naturally and demonstrates that ferrets are a powerful tool to experimentally investigate the different SARS-CoV2 variants currently arising throughout the world. Minks, like ferrets, belong to the family of mustelids. Hence, these data might provide an explanation for the selection and explosive spreading and of what is termed the SARS-CoV-2 “cluster 5” variant in Danish mink farms and its relevance in the frequently observed mink-to-human transmissions (Munnink et al., 2020). Although the cluster 5 variant does not seem to be more pathogenic compared to the predominant human SARS-CoV-2 variants, it will become more and more important to assess pathogenicity and transmission rates of naturally occurring variants throughout the course of the pandemic, because some emerging variants could potentially undermine antibody-based vaccination strategies. Such a scenario was suggested for the recently-identified N439K mutation in the spike protein of SARS-CoV-2 (Thomson et al., 2020), and close surveillance of mink and human populations is required.

4. Hamsters

Computer modelling of ACE2–SARS-CoV-2 spike RBD docking for a number of common laboratory mammalian species predicted that hamsters may also be susceptible to SARS-CoV-2 (Chan et al., 2020). Indeed, following intranasal inoculation with the virus, hamsters showed weight loss and the expression of viral antigens in the nose, bronchi, lungs, and intestine (Sia et al., 2020). Viral RNA and infectious viral particles could be found in nasal lavages, and a concomitant injury of the lung was detected by using CT as well as histopathology (Imai et al., 2020). The severity of the weight loss correlated with the age of the animals (Osterrieder et al., 2020), but no lethality was observed in this model. Transmission of the infection was found to be possible by direct contact or aerosols but not through bedding taken from the cages of infected animals (Sia et al., 2020). Hamsters were subsequently used to assess the efficacy of surgical masks as a protective measure against infectious droplets. When the masks were used as barriers between the cages, they provided only partial protection for the animals on the other side of the barrier (Chan et al., 2020). In cohousing experiments, hamsters were infected with equal amounts of isogenic SARS-CoV-2 S-614D and SARS-CoV-2 S-614G to compare the fitness of these two variants and their transmission rates over 12 days. At day two post-infection, the transmission rate to the naïve animals was already 100%, and SARS-CoV-2 S-614G became the dominant variant, with allele frequencies of the 614G mutation at around 90% in the exposed sentinel hamsters. These data indicate that SARS-CoV-2 S-614G had a replicative advantage over SARS-CoV-2 S-614D resulting in higher contagiousness (B. Zhou et al., 2021), which was independently confirmed by another group (Plante et al., 2020). Additionally, it was shown that human neutralizing antibodies protected hamsters from becoming infected with SARS-CoV-2 (Rogers et al., 2020).

5. Non-human primates (NHP)

Non-human primates are a very important model for drug and vaccine development due to their high genetic and also immunological similarity to humans. The macaque species cynomolgus monkey (*Macaca fasciularis*, crab-eating macaque) and rhesus macaque (*Macaca mulatta*) are the most commonly used species in this group of pre-clinical animal models (Cauvin, Peters, & Brennan, 2015). Single-cell RNAseq data have shown that the critical SARS-CoV-2 host factors, ACE2 and TMPRSS2, are expressed in the same cell types in macaques as in humans, namely type 2 pneumocytes and ciliated cells (Ziegler et al., 2020).

Primates are a highly valuable model for research on the pathogenesis of COVID-19 and the development of vaccines and antiviral drugs. It was previously discovered that cynomolgus monkeys can be infected with SARS-CoV and MERS-CoV, and now the same has also been shown for SARS-CoV-2 after intranasal inoculation (Rockx et al., 2020). While infected animals showed only weak or no symptoms, viral RNA could be detected by RT-PCR in nasal and pharyngeal swabs two to four days after infection, and infectious viral particles were also recovered from these sites by using a cell-culture system (Rockx et al., 2020). No general influence of age on the severity of the symptoms was observed, despite the fact that the viral titers were higher in older (15-20 years) animals. However, the relatively low number of experimentally infected monkeys compared to the high number of naturally infected humans must be kept in mind when interpreting these results. Moreover, during histopathological examination of the lungs, diffuse alveolar damage and immune-cell infiltration was seen in infected cynomolgus monkeys (Rockx et al., 2020), suggesting that the progression of disease is similar to that in humans.

In contrast to only 33% of the infected cynomolgus monkeys, 100% of infected rhesus macaques showed an elevation of body temperature (Lu et al., 2020). This was accompanied by an increase in inflammatory cytokines in the serum and more pronounced pathological changes in the lung tissue of the infected rhesus macaques (Lu et al., 2020). In other studies, rhesus macaques also lost weight after infection with SARS-CoV-2 (Munster et al., 2020). Viral RNA was detectable in all nasal, all pharyngeal, and in a portion of rectal swabs (13 out of 14 and 3 out of 4, depending on the study) taken from infected animals. Abnormalities in the lung that were similar to what has been seen in human patients were detectable in chest x-rays from both species (Lu et al., 2020; Munster et al., 2020). This observation has been corroborated by other studies in cynomolgus monkeys, which also describe an infection without symptoms but with characteristic abnormalities in chest CTs, such as ground-glass opacities, that are also typical of mild to moderate COVID-19 cases in humans (Finch et al., 2020). These abnormal regions, as well as the draining lymph nodes, also showed enhanced fluorodeoxyglucose (FDG) uptake in a PET/CT scan, which corresponds to increased immune-cell infiltration, as seen in the histopathology (Finch et al., 2020; Rockx et al., 2020). Because of their susceptibility to SARS-CoV-2 infection and their importance in the assessment of toxicity in drug development, several preclinical vaccine studies with macaques have already been published. The vaccines tested so far have included inactivated virus (Gao et al., 2020), recombinant adenoviruses expressing variants of the SARS-CoV-2 S proteins (Mercado et al., 2020), DNA vaccines encoding for the S-protein (Yu et al., 2020), vaccines made from recombinant RBD domains of SARS-CoV-2 in alum (J. Yang et al., 2020) and different mRNA vaccines (Corbett et al., 2020; Vogel et al., 2021). Because the infections in rhesus macaques are already mild without any intervention, quantification of the neutralizing antibody titers and reduced replication of SARS-CoV-2 were the main end points used for evaluating the efficacy of the vaccines in these studies. All vaccine candidates were found to fulfill their respective endpoints. Moreover, there were no adverse effects reported, such as antibody-dependent enhancement of the infection, which is of great importance for the development of human vaccines.

6. Other animals

In order to understand whether there are other significant sources for zoonotic transmission of SARS-CoV-2 and to screen for further potential *in vivo* models, a number of additional animals were experimentally infected with SARS-CoV-2.

Dogs and pigs showed no viral replication after intranasal infection (Schlottau et al., 2020; Shi et al., 2020). Fruit bats, which have been hypothesized to be the natural reservoir for SARS-CoV-2, were asymptomatically infected but could infect other fruit bats with the virus (Schlottau et al., 2020). Chickens and ducks showed no symptoms, viral particles, or seroconversion after intranasal inoculation, indicating that these avian species cannot be infected with SARS-CoV-2 (Schlottau et al., 2020; Shi et al., 2020). Following intranasal inoculation, domestic cats showed viral replication in the airways as well as the lungs, but they did not exhibit visible symptoms of disease. However, they were found to transmit the virus to other cats, making them at least theoretically a possible vector after contracting SARS-CoV-2 from infected humans (Halfmann et al., 2020), although no actual cases of cat–human transmission has been reported so far. Of note, for H7N2 influenza virus, cat-to-human caretaker transmission has been reported in animal shelters (Marinova-Petkova et al., 2017), a situation similar to the SARS-CoV-2 mink-to-human transmission at Danish fur farms that prompted the culling of millions of animals (Munnink et al., 2020). Despite the reassuring findings that most domestic and livestock species were not productively infected, it remains important to continue monitoring the susceptibility of these species to naturally occurring variants of the pandemic virus.

While research on these species and SARS-CoV-2 is important to better understand the risks of zoonotic transmission and to prevent illness in important pet and livestock species, they generally do not serve as appropriate model organisms for pharmaceutical development.

7. Which animal model to choose?

Aside from ethical considerations, the choice of the animal model to study SARS-CoV-2 and COVID-19 should ideally be dictated by the scientific question to be addressed. In reality, however, this decision is greatly impacted by the local infrastructure and available resources. For many researchers, small animal models like hamsters, ferrets or mice are the only feasible experimental approach (Table 1).

Naturally occurring viruses can be directly studied in ferrets or hamsters, because, unlike mice, they are susceptible to SARS-CoV-2 infection without needing additional genetic modification. Ferrets offer the advantage that their respiratory symptoms resemble human patients and that they can be employed to model human-to-human transmission of respiratory viruses. Consequently, ferrets have also been a model of choice for SARS-CoV-2 transmission studies (Zhou et al., 2021). In contrast to genetically engineered mice, ferrets and hamsters do not require additional “on site” breeding, allowing for large cohorts to be purchased and directly investigated in a single experiment. Hamsters have been the model of choice to investigate the efficacy of a variety SARS-CoV2 treatment approaches (see Section 9), but, due to the absence of lethality, the effects of severe infections cannot be studied in these models. Furthermore, the availability of reagents, such as monoclonal antibodies, is low for hamsters and even lower for ferrets. Housing and care costs for ferrets are also much higher than for mice or hamsters. However, “omics” approaches can be readily applied, and there are broader efforts to improve the availability and reliability of reagents for these models (Albrecht et al., 2018).

Mice expressing human ACE2 can be productively infected with different wild-type isolates of SARS-CoV-2. A major downside of ectopic ACE2 expression in mice is the potentially altered cell tropism of SARS-CoV-2 and higher ACE2 protein levels due to the use of synthetic promoters to drive ACE2 transcription (see Section 2). This makes it difficult to generate data on viral transmission between individuals that

Table 1
Choosing small animal models for SARS-CoV-2 research.

Aim of the study	Suggested models	Sections in this review
Biology of naturally occurring isolates	ACE2-transgenic mice	2
	Ferrets	3
	Hamsters	4
Systemic pathology including lethality	K18-hACE2	2.1
	HFH4-ACE2	2.2
Lung pathology	ACE2-transgenic mice	2 (except for 2.6)
	Ferrets	3
	Hamsters	4
Brain pathology	K18-hACE2	2.1
	HFH4-ACE2	2.2
	AAV-ACE2	2.7
Virus transmission	Ferrets	3
	Hamsters	4
Signaling pathways	Mouse-adapted viruses	2.8
Therapeutics	K18-hACE2	2.1
	HFH4-ACE2	2.2
Antivirals	All models	

would accurately reflect the situation in humans. Nevertheless, ACE2-expressing mice are well suited to identify antigenic determinants (e.g. in emerging variants of concern, VOC), characterize the mode of action and efficacy of specific antiviral agents, as well as to analyze correlates of protection against native SARS-CoV-2 viruses. K18-hACE2 (2.1) and HFH4-ACE2 (2.2) transgenic mice showed strong pathology in the lung and brain that was associated with lethality. Hence, these models currently represent the most comprehensive small animal models to investigate various features of SARS-CoV-2 biology, COVID-19 immune pathology and to rapidly test therapeutic as well as prophylactic interventions.

Except for *Ace2*$^{tm1(ACE2)Dwnt}$ knockin mice (see Section 2.6), also all other ACE2-expressing mice discussed in Section 2 developed interstitial lung disease and are useful to study infection-associated lung pathology. CNS-infection was only observed in some models (Fig. 2). The factors that determine whether the SARS-CoV(-2) spreads to the murine brain remain unclear, but this may also be associated with the level of ACE2 expression (Carossino et al., 2021; Kumari et al., 2021; Song et al., 2021). Hence the models in which ACE2 expression is driven by strong promoters (see Sections 2.1 and 2.2) or in which high concentrations of AAV-ACE2 were directly inoculated into the brain prior to infection (see Section 2.7) represent the best *in vivo* models to study infection-associated CNS pathology in SARS-CoV-2-infected mice.

Spontaneously or recombinantly-generated mouse-adapted SARS-CoV(-2) viruses have mostly been used to investigate the dynamics of the immune response during the course of infection, as demonstrated by the studies that have uncovered a dual role for the type I IFN response in the pathogenesis of SARS-CoV(-2) infections (Channappanavar et al., 2016; Dinnon et al., 2020). Moreover, because these mouse-adapted viruses can infect the vast spectrum of laboratory knockout mouse strains deficient in specific immune pathways, they can be rapidly employed to study the role of the immune system in both controlling viral replication and exacerbating tissue damage. Mouse-adapted viruses have also provided important insights into the emergence of new VOC, the control of which constitutes the major challenge in our future co-existence with SARS-CoV-2 (Roy Wong et al., 2021).

Obviously, NHP are considered to be the most relevant model for development of biopharmaceuticals against human infectious diseases like COVID-19 because they recapitulate the pharmacodynamics of biologics in the closest manner to humans (Cauvin et al., 2015). For instance, macaques can be infected by SARS-CoV-2 without any genetic modification, and they show core radiology and histopathology symptoms that are similar to those in humans (see Section 5). Moreover, their genetic similarity to humans frequently renders macaques the model of choice in toxicity studies. Additionally, the availability of research reagents, such as monoclonal antibodies, for NHP greatly surpasses most other species, with the exception of humans and mice, so that the immune responses induced by vaccines and antibodies can readily be studied in great detail. However, so far only mild courses of SARS-CoV-2 infection have been described in primates, making it harder to compare with the disease in humans. Nevertheless, vaccine candidates and drugs have been successfully tested in these models, showing the production of neutralizing antibodies and a reduction of viral replication. Their main disadvantage are the high costs and requirements for housing and care as well as the low throughput nature of primate research. Even though early stage research and development is more efficiently done in smaller animals like mice and hamsters, NHPs remain the most important model in late-stage preclinical development of new vaccines and drugs against SARS-CoV-2 (see Section 9.1).

8. Therapeutic insights from animal models

The release of type I interferons (IFN) is the dominant antiviral signaling pathway. They are produced by virus-infected cells after activation of innate nucleic acid sensors, and they are powerful alarmins that boost inflammation to rapidly clear infections. Under conditions of stress, these receptors are also activated in response to endogenous nucleic acids. Thus, when not properly controlled, the IFN-driven immune responses can inflict secondary damage to tissues that ultimately causes immune pathology in the context of viral infections. SARS-CoV-(2) is able to efficiently evade the type I IFN response, allowing for substantial viral replication to take place before the innate immune system detects the viral pathogen-associated (PAMPs) or damage-associated molecular patterns (DAMPs) released from infected dying cells (reviewed in Vabret et al., 2020). Thus, IFN-mediated innate antiviral immunity has been identified as a major factor influencing the susceptibility to SARS-CoV-2 infection and COVID-19 disease progression (Hadjadj et al., 2020; Israelow et al., 2020; Lee & Shin, 2020). Activation of IFN signaling in mice by injection of the viral RNA analogue poly I:C or by pre-treatment with recombinant IFN before infection prevented lethal disease caused by SARS-CoV and SARS-CoV-2, respectively (Channappanavar et al., 2016; Sun, Zhuang, et al., 2020). Infection of C57BL/6 mice lacking type I and type II (Ifnγ) IFN receptors (IFNR) with SARS-CoV-2 MA10 resulted in higher virus titers and weight-loss at day 4 p.i. compared with IFN receptor competent mice (Leist et al., 2020). However, SARS-CoV-2 evades and only weakly activates the type I IFN system while inducing pro-inflammatory cytokines including IFNγ (Blanco-Melo et al., 2020). Thus, it is unclear if uncontrolled replication followed by exacerbated pathology in SARS-CoV-2 MA10 infected IFNR-deficient mice results from a loss of type I or type II IFN signaling in C57BL/6 mice (Leist et al., 2020). Nevertheless, all observations to date suggest that an early IFN response is beneficial for the host and can limit SARS-CoV-(2) replication. Indeed, impaired type I IFN responses were associated with a severe pathology. For example, critically ill patients showed lower serum IFNα levels than mild or moderate cases (Hadjadj et al., 2020), and, in around 10% of severe COVID-19 cases, inhibitory anti-IFNα autoantibodies were identified which most likely depleted bioactive type I IFN from their serum (Bastard et al., 2020). Furthermore, inborn errors of the type I IFN system were highly associated with fatal SARS-CoV-2 infections (Zhang et al., 2020). These data strongly suggest that the IFN system has a central role in controlling primary infection and most likely virus spreading.

Deficiency of type I IFN signaling allowed for productive infection of BALB/c mice with SARS-CoV MA15 but did not result in detectable pathology and protected from lethality (Channappanavar et al., 2016). Although this appears to be in contrast to observations with SARS-CoV-2 MA10 described above (Leist et al., 2020), both studies cannot be directly compared due to differences in the viruses (SARS-CoV MA15 vs. SARS-CoV-2 MA10) and the genetic backgrounds and immune competence of the infected mice (*Ifnar1* knockout BALB/c vs. *Ifnar1*/*Ifngr1*

double-knockout C57BL/6). Similarly, AAV9-ACE2 transduced mice with a deficiency in the type I interferon pathway (either knockout of *Ifnar1* or *Irf3/7* double knockout) did not mount an inflammatory response upon infection with SARS-CoV-2, despite undiminished viral replication (Israelow et al., 2020). These observations suggest that, once unleashed, type I IFN might represent a major driver of the lung pathology associated with SARS-CoV-(2) infections. Notably, type III IFNs, which are often produced in epithelial tissues alongside with type I IFNs, have been implicated in lung damage in severe human COVID-19 cases (Broggi et al., 2020). Hence, targeted inhibition of type I IFN and possibly type III IFN signaling late in the infection could aid in limiting tissue damage while still not compromising the immune response. For late stages of severe COVID-19, inhibitors of IFN signaling, such as JAK inhibitors, are currently being tested (Lee & Shin, 2020). Alternatively, novel antagonists of innate nucleic acid sensors might help to mitigate an overly active inflammatory response at late stages of severe COVID-19 since these receptors may drive pathological cytokine expression in their function as principle sentinels of tissue integrity (Bartok & Hartmann, 2020). Pharmacologic inhibitors of intracellular nucleic acid sensors have been shown to limit sterile inflammation (Haag et al., 2018), also in response to tissue damage (Gong et al., 2021). Of course, it remains possible that the underlying mechanisms of the hyperinflammatory response are different between humans, and possibly other non-rodent animals, when compared to mice, and a benefit for the presence of type I IFN before or in the early stages of infection is highly likely (Monk et al., 2020). Thus, means to establish a transient, localized non-pathogenic antiviral state could be explored as a potent prophylactic strategy which might be universally applicable against a broad range of pathogenic viruses.

A major complication in hospitalized COVID-19 patients is a cardiac dysfunction that remains in around two thirds of patients even after recovery (Gupta et al., 2020). Although the heart is not primarily infected, pro-inflammatory cytokines produced in response to extensive SARS-CoV-2 replication in the lung act as major drivers of cardiac dysfunction (Mills et al., 2021). The FDA-approved bromodomain extraterminal inhibitor (BETi) INCB054329 reduced cytokine storm and restored cardiac function in human cardiac organoids after infection with SARS-CoV-2 (Mills et al., 2021). Mechanistically, BETi lead to internalization of ACE2 thereby protected cardiac organoids from infection. When administered to K18-hACE2 mice (see Section 2.1.) inflammation of the heart was significantly reduced, while weight loss was only mildly ameliorated. BETi had no effects in lung suggesting that targeting the BET pathway might be beneficial in reducing organ specific complications in COVID-19 patients but would require additional medication in parallel to manage systemic adverse effects of the infection (Mills et al., 2021).

Age is a significant risk factor for developing severe COVID-19. Infection of middle aged mice with mouse adapted SARS2-N501Y_{MA30} results in the activation of prostaglandin signaling and the production of eicosanoids like prostaglandin d2 (Roy Wong et al., 2021). Intriguingly, mice lacking prostaglandin d2 or its receptor were almost completely protected from lethality following infection with the highly virulent, mouse-adapted SARS-CoV-2 MA30 (Roy Wong et al., 2021). Most importantly, pharmacologic inhibition of PGD_2/DP1 signaling using the selective DP1-receptor antagonist asapiprant (BGE-175) ([2-(Oxazol-2-yl)-5-(4-{4- [(propan-2-yl)oxy]phenylsulfonyl} piperazine-1-yl)phenoxy] acetic acid) starting at day 2 post infection reduced the infection fatality rate to 10% of that in vehicle treated mice. Asapiprant has been evaluated as an anti-inflammatory drug for the treatment of allergic rhinitis and is now being evaluated in randomized, placebo-controlled clinical trial of hospitalized aged COVID-19 patients (NCT04705597).

9. Prophylactic SARS-CoV-2 antivirals in animal models

In the following section we will discuss three principle antiviral strategies against SARS-CoV-2 that have been developed and tested for efficacy in animal models. An overview is given in Table 2.

9.1. Blocking SARS-CoV-2 entry

Vaccine development has been the central, long-term approach to protecting the population and reopening society. Early on, there was accumulating evidence from mouse models that infection with the SARS-CoV-2 establishes protective immunity, supporting a central role of vaccination in controlling the pandemics. HFH4-hACE2 transgenic mice (see Section 2.2) which survived a first infection with SARS-CoV-2 developed neutralizing antibodies and were protected in a re-challenge experiment with the same SARS-CoV-2 isolate (R.-D. Jiang et al., 2020). An attenuated live vaccine completely protected K18-ACE2 transgenic mice (Section 2.1) from infection with SARS-CoV-2, while all unvaccinated control mice died (Seo & Jang, 2020). Interestingly, infection with the non-lethal mouse-adapted SARS-CoV-2 CMA3p20 completely protected BALB/c mice from a lethal challenge with SARS-CoV MA15 (Muruato et al., 2021). SARS-CoV replication was detected in the lungs of the challenged mice, suggesting that SARS-CoV-2 CMA3p20 did not induce sterilizing cross-immunity but sufficient protection from detrimental SARS-CoV infection (Muruato et al., 2021). An mRNA vaccine coding for a codon-optimized SARS-CoV-2 spike protein induced a protective Th1-dominated immune response and neutralizing antibodies in BALB/c and C57BL/6 mice. A single-dose vaccination with the same vaccine candidate completely protected K18-hACE2 transgenic mice from lethal infection with SARS-CoV-2 (BetaCoV/Singapore/2/2020) (de Alwis et al., 2021). Similarly, an mRNA vaccine (mRNA-1273) encoding for SARS-CoV-2 spike protein stabilized in a pre-fusion conformation elicited potent neutralizing humoral and Th1 cellular immune responses that protected young BALB/c mice from infection with SARS-CoV-2-MA for up to three months after vaccination (Corbett et al., 2020). The antibodies induced by mRNA-1273 also neutralized the SARS-CoV-2 D614G mutant in a cell culture system, suggesting that the strategy is sufficient to elicit cross-neutralizing antibodies against some VOC strains (Corbett, Edwards, et al., 2020). In a phase III clinical trial the drug showed 94.1 % efficacy at preventing COVID-19 illness similar to the mRNA vaccine BNT162b2 with 95% efficacy (Baden et al., 2021, p. 12; Polack et al., 2020). Vaccination of BALB/c mice with a vector vaccine expressing recombinant SARS-CoV-2 spike or nucleocapsid protein led to potent induction of antibodies. Only mice vaccinated with the spike vector developed neutralizing antibodies and were able to control infection with a mouse-adapted SARS-CoV-2 MA as well as the highly pathogenic SARS-CoV-2 MA10 (Dinnon et al., 2020; Leist et al., 2020). Moreover, nearly all the vaccines, which are currently approved in western countries, have been evaluated in NHP. The mRNA vaccines of Moderna (mRNA-1273) (Corbett, Flynn, et al., 2020) and Biontech (BNT-162b2) (Vogel et al., 2021) as well as the adenoviral vector vaccines of AstraZeneca (ChAdOx1 nCoV-19) (van Doremalen et al., 2020) and the Johnson and Johnson vaccine (Ad26.COV2.S) (Mercado et al., 2020) demonstrated protection against a SARS-CoV2 challenge in rhesus macaques before or while in parallel moving into clinical trials. Collectively, all these studies underscore the importance of macaques, especially rhesus macaques, as a gold standard in pharmacological and vaccine development.

Passive immunization with neutralizing antibodies protected HFH4-hACE2 mice from lethality after infection with SARS-CoV (Menachery et al., 2016). Injection of neutralizing antibodies or pretreatment with convalescent sera from COVID-19 patients reduced infection-associated pathology and lethality following SARS-CoV-2 infection in two different mouse models (Hassan et al., 2020; Sun, Zhuang, et al., 2020; Zheng et al., 2021). Neutralizing monoclonal antibodies against the spike protein also showed prophylactic and therapeutic activity in hamsters (Kreye et al., 2020). Similarly, recombinant antibodies like Bamlanivimab (Jones et al., 2021) or Casirivimab (Baum et al., 2020), which have been granted emergency authorization by the FDA have shown their efficacy in reducing viral load and lung pathology in macaques. These data suggest that passive immunization with monoclonal

Table 2
Prophylactic and therapeutic interventions against SARS-CoV-2 in animal models.

	Model					
Principle	Germline ACE2 transgenic mice	Somatic ACE2 expression in mice	Mouse-adapted viruses	Ferrets	Hamsters	NHP
Seroconversion	(Jiang et al., 2020)	(Sun, Chen, et al., 2020; Sun, Zhuang, et al., 2020)	(Muruato et al., 2021)			
Active immunization	(Corbett, Edwards, et al., 2020; de Alwis et al., 2021; Seo & Jang, 2020)		(Dinnon et al., 2020; Leist et al., 2020)			(Corbett, Flynn, et al., 2020; Mercado et al., 2020; van Doremalen et al., 2020; Vogel et al., 2021)
Passive immunization	(Hassan et al., 2020; Pymm et al., 2021; Zheng et al., 2021)	(Sun, Chen, et al., 2020; Sun, Zhuang, et al., 2020)			(Kreye et al., 2020)	(Baum et al., 2020; Jones et al., 2021)
ACE2 decoy	(Hassler et al., 2021)					
Fusion inhibitor				(de Vries et al., 2021)		
Anti-viral cytokines		(Sun, Chen, et al., 2020; Sun, Zhuang, et al., 2020)	(Dinnon et al., 2020)			
Anti-inflammatory drugs	(Mills et al., 2021; Roy Wong et al., 2021)					
Inhibitors of viral genome replication		(Sun, Chen, et al., 2020; Sun, Zhuang, et al., 2020; Wahl et al., 2021)		(Cox et al., 2021)	(Driouich et al., 2021; Kaptein et al., 2020; Rosenke et al., 2021; Yuan et al., 2021)	

antibodies can efficiently contribute to preventing SARS-CoV-2 transmission and many candidates are currently being evaluated in clinical trials (reviewed in Taylor et al., 2021)).

Nanobodies are small naturally occurring single-domain antibodies that can be isolated from alpacas, lamas and camels. Their lower molecular weight and higher stability compared with common immunoglobulins make them potent inhalable compounds for the treatment of respiratory diseases (Van Heeke et al., 2017). RBD-specific nanobodies have been reported to block infection with SARS-CoV-2 through interference with the membrane fusion process that is initiated upon binding of the spike to ACE2 (Koenig et al., 2021). Structure-guided design of multivalent nanobodies targeting different epitopes in the RBD or the nanobody single-chain fused to the FC fragment of human IgG1 greatly increased neutralizing activity against SARS-CoV-2 and the D614G N501Y variant, respectively. (Koenig et al., 2021; Pymm et al., 2021). Treatment with the FC-fused RBD-specific nanobodies protected C57BL/6 mice from infection with the SARS-CoV-2 strain hCoV-19/Australia/VIC2089/2020, which carries the N501Y D614G mutations that allow infection of unmodified laboratory mice (Pymm et al., 2021). In extension of these findings, mice have been genetically engineered to produce camelid nanobodies and multimeric nanobodies (Xu et al., 2021). The latter have been shown to neutralize escape mutants in cell culture and might represent a promising tool to quickly react against VOC (Xu et al., 2021).

Instead of antibodies blocking the interaction of the spike protein with ACE2, soluble ACE2 can neutralize SARS-CoV-2 virus particles (Monteil et al., 2020). A soluble stabilized human ACE2 protein exhibited enzymatic activity in plasma for up to three days after intraperitoneal (i.p.) injection and for 24 hours in lungs after intranasal (i.n.) administration (Hassler et al., 2021). Parallel i.p. and i.n. administration of the decoy receptor one hour before i.n. infection with SARS-CoV-2 protected K18-hACE2 transgenic mice (see Section 2.1) from lethality (Hassler et al., 2021). A major benefit of recombinant ACE2 is a considerably lower risk for selecting escape mutants compared with antibody based strategies, as mutations that reduced affinity of the spike protein to ACE2 would likely decrease viral fitness. Also, prolonged enzymatic activity of the recombinant ACE2 could restore ACE2 function that is lost due to excessive internalization of the receptor during the entry process and in this way aid in managing cardiovascular complications.

Fusion of the viral and endosomal membrane is a vulnerable step during SARS-CoV-2 infection and therefore a potential pharmacologic target to block viral entry. In a preclinical experiment in ferrets, intranasal treatment with fusion-inhibiting lipopetides fully prevented transmission of SARS-CoV-2 co-housed animals (de Vries et al., 2021).

9.2. Inhibitors of the viral polymerase

Nucleoside analogs that block the RNA-dependent RNA polymerase have a long standing history in antiviral therapy. Remdesivir (50mg/kg/day) protected Ad5-hACE2-sensitised mice from infection with SARS-CoV-2 (Sun, Zhuang, et al., 2020). Remdesivir also showed antiviral activity against SARS-CoV-2 in rhesus macaques (Williamson et al., 2020). However, the efficacy of remdesivir for the treatment of COVID-19 patients seems limited and remains controversial (Cohen & Kupferschmidt, 2020; Grein et al., 2020; Wang et al., 2020).

Molnupiravir (also known as EIDD-2801) is the orally available prodrug of the ribonucleoside analogue β-d-N4-hydroxycytidine (NHC) which inhibits the coronavirus RNA-dependent RNA-polymerase (Wahl et al., 2021). Human therapeutic doses of Molnupiravir significantly reduced viral titers in mice carrying xenotransplanted human lung tissue when administered orally by gavage starting as late as 48 hours post infection (Wahl et al., 2021). In a prophylactic setting given 12 hours before infection, Molnupiravir reduced viral titers in xenotransplanted human lung tissues by factor 10^5, demonstrating high efficacy of the drug in preventing and treating SARS-CoV-2 infections (Wahl et al., 2021). Oral administrations of Molnupiravir prevented the transmission of SARS-CoV2 between ferrets (Cox, Wolf, & Plemper, 2021) and reduced viral replication in hamsters (Rosenke et al., 2021). The efficacy of this compound in humans is currently evaluated in phase II/III clinical trials (NCT04405570 and NCT04405739). Likewise, the guanine analog Favipiravir showed efficient antiviral activity in hamsters (Driouich et al., 2021; Kaptein et al., 2020). In contrast, hydroxychloroquine, which has received a lot of media attention but ultimately failed clinical evaluation in humans (Mitjà et al., 2020) also showed no antiviral activity in hamsters (Kaptein et al., 2020), demonstrating the predictive value of this model at least in one case.

Furthermore, the FDA approved leprosy drug Clofazimine was identified in an *in vitro* screen for drugs with anti-SARS-CoV2 activity

displayed significant antiviral activity in hamsters (Yuan et al., 2021).

9.3. Boosting innate antiviral immunity against SARS-Co-V-2

In the presence of elevated IFN, SARS-CoV-2 infection was largely abrogated (see Section 8), suggesting that stimulation of IFN pathways could be an attractive prophylactive or therapeutic approach. Pre-treatment of BALB/c mice with recombinant IFNβ inhibited infection with SARS-CoV MA15. Furthermore, pre-treatment of BALB/C mice with PEG-IFNλ11 (type III IFN) prevented infection with SARS-CoV-2 MA (Dinnon et al., 2020) and PEG-IFNλ11 is also currently being evaluated for the treatment of COVID-19 (NCT04388709). In support of the data obtained in mice, promising results have been reported for the treatment of early hospitalized COVID-19 patients with nebulized recombinant IFNβ (Monk et al., 2020). A novel approach to induce a protective innate antiviral response could be the systemic or local stimulation of innate nucleic acid sensing receptors such as RIG-I with therapeutic oligonucleotide agonists to establish an antiviral state that hinders SARS-CoV-2 infection. This strategy has been shown to completely protect mice from a lethal challenge with influenza A virus (Coch et al., 2017). In line with this, pre-treatment of Ad5-ACE2 transduced mice with the viral dsRNA analogue poly I:C primed the innate immune response before intranasal inoculation with SARS-CoV-2 and significantly reduced viral titers and ameliorated the disease (Sun, Zhuang, et al., 2020).

Nevertheless, in light of the potentially damaging effect of uncontrolled IFN production and exposure, the translational aspects of these findings still need to be evaluated carefully for the following reasons: First, in mouse models, the IFN axis has been activated as a prophylactic or in a very early phase of the disease but not as a longitudinally applied therapeutic agent, as it is planned for PEG-IFNλ11 in COVID-19 patients. Second, despite the fact that type I and type III IFNs signal through distinct receptors, they induce an overlapping set of ISGs (Mesev, LeDesma, & Ploss, 2019), which most likely drive the pathology in SARS-infected tissues in the later stages of infection. Hence, in order to safely harness the power of the intertwined type I and III IFN responses to fight SARS-CoV2 and COVID-19, their dual role in the pathology of COVID-19 needs to be studied in more detail (Lee & Shin, 2020; Monk et al., 2020).

10. Conclusions

In less than a year, numerous different animal models have already yielded important insights into the biology of SARS-CoV-2 and the pathology of COVID-19 disease. These discoveries were driven equally by the use of animal models that were originally established in response to the SARS epidemic 20 years ago and by the rapid development of new *in vivo* models. The animal models were indispensable for the development of vaccines, antiviral treatments, and the establishment of new, safe immune-therapies that are tailored to specifically ameliorate the symptoms of COVID-19. Nevertheless, we have only recently begun to decipher the role of the immune response in the pathology of SARS-CoV-2 infections. As we establish our coexistence with the new virus, animal models will remain invaluable tools for rapidly and safely assessing the efficacy of pharmacologic and therapeutic countermeasures against SARS-CoV-2 and its naturally occurring variants.

Declaration of Competing Interest

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