



## REVIEW

## Insights into coronavirus immunity taught by the murine coronavirus

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Coronaviruses (CoVs) represent enveloped, ss RNA viruses with the ability to infect a range of vertebrates causing mainly lung, CNS, enteric, and hepatic disease. While the infection with human CoV is commonly associated with mild respiratory symptoms, the emergence of SARS-CoV, MERS-CoV, and SARS-CoV-2 highlights the potential for CoVs to cause severe respiratory and systemic disease. The devastating global health burden caused by SARS-CoV-2 has spawned countless studies seeking clinical correlates of disease severity and host susceptibility factors, revealing a complex network of antiviral immune circuits. The mouse hepatitis virus (MHV) is, like SARS-CoV-2, a beta-CoV and is endemic in wild mice. Laboratory MHV strains have been extensively studied to reveal coronavirus virulence factors and elucidate host mechanisms of antiviral immunity. These are reviewed here with the aim to identify translational insights for SARS-CoV-2 learned from murine CoVs.

**Keywords:** Animal models · Host/pathogen interaction · Immune responses · Mouse Hepatitis Virus · SARS-CoV-2

## Introduction

Coronaviridae are a family of enveloped, ss RNA viruses, consisting of species-restricted strains that can infect humans, mice, pigs, cows, chickens, cattle, cats, and bats [1]. Many of these viruses induce a respiratory and/or enteric infection, with murine strains additionally causing neurological and hepatic infections [1]. Human coronaviruses (CoVs) typically induce mild respiratory infections caused by the common cold coronaviruses (HCoV-229E, HCoV-OC43), however, highly virulent strains can cause severe acute respiratory syndromes and multiorgan involvement as seen by SARS-CoV [2], MERS-CoV [3], and SARS-CoV-2 [4, 5]. As of March 2021, SARS-CoV-2 has caused over 2.65 million deaths worldwide, a number expected to grow until vaccines will

be widely distributed. While inflammatory correlates help to identify pathways dysregulated in severe cases, preclinical models are needed to resolve causative mechanisms of viral spread, antiviral immunity, and multiorgan involvement. Here, we provide an overview of viral pathogenicity and host immune defence mechanisms that has been elucidated from studies using murine CoVs and discuss the suitability of these viruses as a preclinical model for SARS-CoV-2.

## Coronavirus genome and replication cycle

The CoV genome and replication cycle have been reviewed extensively elsewhere [1, 6] and are summarized briefly here. The ss RNA genome is stabilised by the nucleocapsid protein, and surrounded by a viral membrane envelope containing the spike, transmembrane, and envelope glycoproteins. The spike glycoprotein mediates viral attachment to host receptors and is cleaved by

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host proteases permitting fusion with the cell membrane. Following release of the viral genome into the host cytoplasm, two viral polypeptides encoding nonstructural genes are translated. The nonstructural proteins encoding the replicase–transcriptase complex — RNA polymerase, helicase, exoribonuclease, and methyltransferase are translated first as a polyprotein and subsequently cleaved creating the machinery for viral replication and translation of the structural genes. Encapsidated viral genomes and translated structural proteins are inserted into the ER–Golgi intermediate compartment, and mature virions are released via exocytosis. In addition to the species and tissue tropism determined by the spike protein, the requirement of the host cell's translation machinery generally restricts the efficient replication of CoVs across a species barrier.

## Mouse hepatitis virus (MHV) strains and mutants reveal coronavirus virulence factors

Murine CoVs, also referred to as mouse hepatitis viruses (MHV), consist of diverse strains that cause varying degrees of respiratory, gastrointestinal, hepatic, and neuronal symptoms [1, 7, 8]. All MHV strains belong to the genus of beta-CoVs, as do certain human CoVs (HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2) [9]. Distinct MHV strains exhibit differences in tropism and virulence, and studies of recombinant MHV variants have revealed host and viral factors that determine viral spread or circumvent immune recognition.

### The spike glycoprotein confers viral tropism

Studies comparing different MHV strains or recombinant variants illuminate the importance of the spike protein for tissue tropism. The MHV spike protein binds to the host cellular receptor carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1), which is expressed on epithelial cells in the liver, intestines, respiratory tract and pancreas, on proximal tubules of the kidneys, to a low extent on glial cells in the CNS, and broadly expressed on endothelial and hematopoietic cells [10, 11]. This host receptor–viral ligand pair is conserved across MHV strains, however, strain-specific differences in the spike protein confer differences in cellular tropism. While the MHV-A59 strain is neurotropic, hepatotropic, and mildly pneumotropic, the MHV-JHM and MHV-4 (an isolate of MHV-JHM) strains are highly neurotropic and weakly hepato- and pneumotropic, while MHV-2 and -3 strains are strongly hepatotropic, and the MHV-1 strain is strongly pneumotropic. A series of studies using chimeric MHV viruses identify the spike protein as the key determinant of tissue tropism. For instance, swapping the MHV-A59 spike protein with that of MHV-2 prevents the demyelinating consequences of oligodendrocyte infection in the CNS [12], while swapping in the spike protein of MHV-1 confers increased pneumotropism to MHV-A59 [8]. Similarly, replacement of the MHV-A59 spike glycoprotein with that of MHV-4, a neurotropic strain, attenuated

hepatotropism [13]. However, introduction of the MHV-A59 spike protein onto the MHV-JHM background failed to introduce a hepatotropic phenotype [14], suggesting that additional viral genes may influence tropism.

### Structural and nonstructural genes promote virulence

Recombinant MHV variants have also revealed the contribution of other structural and nonstructural genes in conferring virulence. Increased neurovirulence associated with MHV-JHM correlates with elevated cytokine production (IFN- $\beta$ , IL-1 $\beta$ , IL-6, CCL3, CCL4), but not CD8 T-cell responses compared to MHV-A59 infection [15]. Recombinant MHV-JHM viruses further reveal that the spike glycoprotein promotes CCL3/CCL4-driven macrophage immunopathology [16], while the magnitude of the T-cell response is orchestrated by other viral genes [17]. Perhaps the most relevant example in the context of SARS-CoV-2 infection, is the observation that MHV-1 structural proteins account for increased pneumovirulence associated with exacerbated interstitial pneumonia and elevated levels of the type I and II IFNs and TNE, but not viral titres [8]. A similar disconnect between viral titres and inflammatory cytokines and histopathology was reported by Thiel and colleagues, who showed that a single amino acid mutation in the MHV-A59 ADP-ribose-1"-phosphatase encoded by the nonstructural protein (Nsp) 3 resulted in reduced liver pathology and IL-6 production, despite having no effect on viral titres in the liver [18].

### Mechanisms of viral evasion

In addition to modulating host cytokine responses, CoVs have evolved mechanisms to evade host antiviral strategies including inhibition of type I IFN signalling cascades or circumventing recognition by pattern recognition receptors [19, 20]. For example, deletion of Nsp1 that encodes the 5' end of the viral replicase in the MHV-A59 genome resulted in attenuation of viral replication and impaired type I IFN responses in professional APCs [21]. Moreover, the coronavirus ribose 2'-O-methyltransferase encoded by Nsp16 serves to disguise viral mRNA as eukaryotic mRNA that is methylated at the 5' cap, thereby, avoiding recognition by the cytoplasmic PRR, melanoma differentiation-associated gene 5 (MDA5) and downstream type I IFN responses [19]. Notably, a similar Nsp16/10-dependent mechanism or RNA cap modification has been reported for SARS-CoV-2 [22]. Moreover, SARS-CoV-2 nonstructural proteins may inhibit splicing-, translational-, and protein trafficking processes involved in the host type I IFN response to viral infection [23, 24]. Although strain-specific differences exist among CoVs, recombinant MHV variants provide a platform for resolving host-viral interactions and virulence factors conferred by CoV structural and nonstructural genes.

## Mechanistic insight into antiviral mechanisms to Coronaviruses learned from MHV

### Host innate defence mechanisms contain early viral infection

Early control of MHV critically depends on IFN-mediated host defence. In vitro studies reveal that plasmacytoid DCs (pDCs) produce the first wave of type I IFNs within 12 h of exposure [25]. As a ss RNA virus that is temporarily in a ds form during viral genome replication, infected cells can sense the coronavirus via either the toll-like receptor 7 (TLR7) or MDA5, respectively [25, 26]. Studies using *Tlr7*-deficient pDCs [25] or *Mda5*-deficient macrophages [19] suggest that both sensors contribute to type I IFN-mediated attenuation of MHV titres in vitro. Similarly, a compensatory role for TLR7 in type I IFN production is observed in *Mda5*-deficient mice, although these mice exhibit reduced survival, higher viral titres and increased serum cytokines (IL-6, TNF, IFN- $\gamma$ ) compared to MDA5-sufficient counterparts [26]. Following early type I IFN production by pDCs, signalling via the type I IFN- $\alpha/\beta$  receptor (IFNAR) by macrophages, and to a lesser extent by CD11c-expressing cells, is required to control viral replication and secure host survival [27]. The inverse relationship between the magnitude of the type I IFN responses (or pDC number) and viral load has been mathematically modelled and reveals the importance for tissues bearing a high number of pDCs, such as the spleen, to act as a sink for viral replication, protecting peripheral organs [28] (Fig. 1A and B).

Type I IFN sensing leads to induction of an antiviral state and upregulation of chemokines and cytokines that activate sequential arms of the innate immune response. Studies elaborating the kinetics of early immune cell infiltration reveal that neutrophils are among the first cell type to accumulate in infected organs [29–31], promoting subsequent mononuclear cell infiltration and viral containment [32]. A recent study demonstrated that lung-infiltrating neutrophils upregulate the bacterial alarmins S100A8/A9 in response to CoVs, including MHV-A59, but not other viruses [33]. Pharmacological blockade of S100A9 reduced lung pathology and protected mice against fatal MHV infection in IFNAR-deficient mice, suggesting an important second line of innate immune defence by neutrophils following MHV-infection.

Proinflammatory cytokines are induced in response to CoVs, and genetic studies have begun to dissect the roles of inflammatory cytokines in MHV infection. Inflammasome-related cytokines IL-1 and IL-18 both contribute to controlling viral replication, and IL-18 signalling is additionally associated with proficient IFN- $\gamma$  production by T cells and host survival [34]. Earlier studies of IFN- $\gamma$ -deficient mice have highlighted the critical role for this cytokine to protect from fatal peritonitis following intraperitoneal MHV infection [35]. Thus, diverse innate immune cell types and inflammatory mediators orchestrate the early innate immune response to MHV (Fig. 1A).

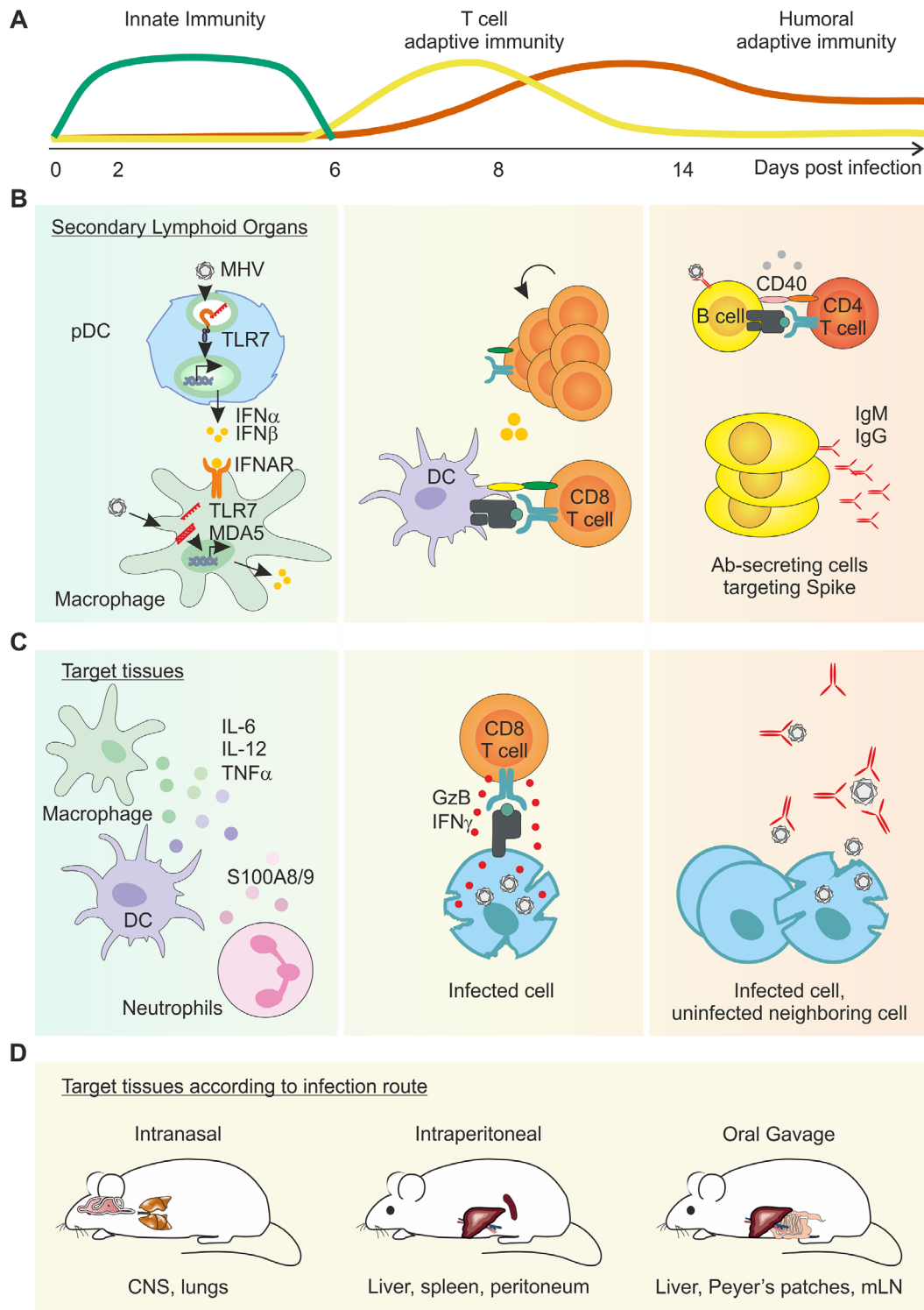
### Adaptive immunity mediates viral clearance and protective immunity to MHV

Following the first line of innate immune defence, MHV is critically controlled by cytotoxic T cells (Fig. 1B). Studies tracking virus-specific T cells, demonstrate that CD4<sup>+</sup> and CD8<sup>+</sup> T cells accumulate in the target organ from day 6 following infection [36], and that their CCR7-dependent cell recruitment is required to restrict viral replication and ensure host survival, at least in the CNS [37]. A series of adoptive transfer and T-cell depletion experiments in WT mice have shown that both CD8<sup>+</sup> and CD4<sup>+</sup> T cells are required to contain viral spread in the target tissue [38, 39]. While CD8 T cells eliminate virus-infected cells, CD4<sup>+</sup> T cells have been shown to enhance the peripheral activation and local cytotoxic activity of CD8<sup>+</sup> T cells [40], and promote T cell-dependent humoral immunity [41]. Notably, long-term control of MHV depends on neutralizing antibodies (Fig. 1C). B-cell deficient mice, or mice unable to secrete IgM experience viral recrudescence following initial viral clearance [41, 42], a manifestation that can be prevented by the transfer of convalescent serum [42] or spike-targeted neutralizing antibodies [43]. Longitudinal tracking of serum immunoglobulins following intranasal MHV infection demonstrates that neutralizing antibodies remain stable for at least 60 days following intranasal infection [41], however, further studies are warranted to explore later time points and functional protection following reinfection.

### Parallel immune phenotypes in MHV and SARS-CoV-2

Plasma levels of proinflammatory mediators, such as IL-2, IL-6, IL-7, IL-10, CXCL10, CCL3, and TNF, are significantly elevated in patients experiencing severe COVID-19 compared to a mild or moderate disease course [4]. Moreover, several reports suggest that type I IFN responses are attenuated in COVID-19 patients [44, 45], including observations of severe disease progression in individuals with loss of function *TLR7* variants (who exhibit further impaired type I IFN responses) [46], and genetic variants in components of the IFN-signalling pathway [47]. MHV infection also induces increased serum levels of proinflammatory cytokines, including TNF- $\alpha$ , IL-6, IL-1 $\beta$ , and IFN- $\gamma$  [29, 34, 48], which may be further elevated in virulent MHV variants [15, 16, 18, 19, 21], or older mice [49]. Furthermore, the critical role of type I IFNs in containing MHV replication has been dissected in genetic models as discussed above. Thus, MHV and SARS-CoV-2 induce similar soluble antiviral and proinflammatory mediators, which are correlated with virulence, age, and survival.

Further parallels between murine CoVs and SARS-CoV-2 are drawn in the nature of the humoral immune response. As in SARS-CoV-2, neutralizing antibodies are directed against the spike glycoprotein in MHV-infected mice. While IgM, IgG, and IgA antibody responses are generated following SARS-CoV-2 infection, near-germline antibodies directed at the receptor-binding domain



**Figure 1.** Schematic diagram of the overlapping layers of protective immunity against murine coronaviruses. (A) Schematic diagram of the timely overlap of innate and adaptive immune responses to MHV-A59. (B) Virus-infected cells rapidly produce type I IFN as the first line of innate immune defence. Plasmacytoid dendritic cells (pDC) residing in secondary lymphoid organs are a dominant source of type I IFNs, and signalling via the IFN- $\alpha/\beta$  receptor (IFNAR) on macrophages triggers downstream innate mechanisms to limit viral spread. Uptake of viral antigen and activation by dendritic cells (DC) instigates cytotoxic CD8 T-cell priming. Germinal centre B-cell responses also take place in secondary lymphoid organs, promoting the expansion and maturation of high affinity, antibody-secreting cells. (C) In the target tissue, cytokine-secreting, activated myeloid cells help contain viral spread until primed, cytotoxic CD8 T cells migrate to the site of infection and critically eliminate virus-infected cells. Long-term humoral immunity is mediated by neutralizing antibodies (Ab). (D) Schematic diagram of the known target organs following MHV-A59 infection according to distinct infection routes.

of the spike glycoprotein have been shown to exert potent neutralizing activity [50], akin to observed importance of germline IgM antibodies in MHV-A59-infected mice [41]. Moreover, the clinical benefit of convalescent serum transfer or spike-targeted antibody therapy in COVID-19 patients recapitulates earlier proof-of-principle experiments elaborated in MHV-infected mice [42, 43]. Thus, despite differences in tropism or receptor usage, CoVs induce potent, neutralizing antibodies against the spike glycoprotein in mice and men.

## Murine coronaviruses as a model for SARS-CoV-2

### Selection of MHV strains and infection routes for preclinical modelling of diverse facets of SARS-CoV-2 infection

Distinct MHV strains exhibit differences in virulence and tissue tropism, which should be taken into account when modelling SARS-CoV-2 infection. For instance, the MHV-JHM strain induces a high mortality in mice, superseding the virulence of SARS-CoV-2. The MHV-1 strain, although not widely used, is the most pneumotropic MHV strain and recapitulates moderate to severe pneumonia [31]. In turn, the prototypical laboratory variant is the MHV-A59 strain, which induces a lung, CNS, gastrointestinal, or hepatic infection according to the infection route and dose, and may be best suited to recapitulate the multiorgan involvement of SARS-CoV-2. Intracerebral and intranasal inoculation results in a CNS infection [51], and intranasal administration of MHV-A59 also induces an acute, self-resolving respiratory infection that recapitulates the acute pneumonia experienced by the majority of SARS-CoV-2-infected individuals. However, mice exposed to a sufficiently high dose [52], or aged [49], experience increased lung pathology, but succumb to the infection. Intraperitoneal or intravenous infection leads to liver disease [51], whereas oral application of the virus precipitates gastrointestinal symptoms and hepatitis at higher doses [53]. The known affected organs following different infection routes are recapitulated in Fig. 1D, however, further studies are needed to fully understand the dynamics of viral spread. In sum, MHV-A59 is well-suited as a preclinical model recapitulating an acute pulmonary and extrapulmonary coronavirus infection in a natural host.

### Comparison of MHV, humanized mice, and mouse-adapted viruses as SARS-CoV-2 models

As described above, several features of the antiviral immune response to MHV-A59 are shared with SARS-CoV-2 (early type I IFN responses, elevated serum cytokines, neutralizing antibodies against the spike glycoprotein). However, MHV is distinct to SARS-CoV-2 in its virulence factors and host cell entry receptors, and much effort has gone into generating humanized,

transgenic, or knock-in mice expressing *hACE2* [54–58] as well as mouse-adapted SARS-CoV-2 strains [59, 60]. These models recapitulate the pneumotropism of SARS-CoV-2 in human hosts, the neutralizing capacity of Spike-directed antibodies [58, 59] and requirement of type I IFN signalling and CD8 T cells to attenuate viral titres [57]. Nevertheless, it has been similarly difficult to recapitulate the spectrum of COVID-19 pathogenicity in *hACE2* or mouse-adapted SARS-CoV-2 mouse models. In many of these studies, a very high infection dose is needed, or very young or old mice are used for sufficient infection efficacy (Table 1). Aged *hACE2*-transgenic mice infected with SARS-CoV-2 [54] and aged WT mice infected with mouse-adapted SARS-CoV-2 [60] exhibit exacerbated lung pathology compared to young counterparts, but do not readily succumb to viral infection suggesting that these models may be suitable to examine long-term consequences of CoV infection. Nevertheless, none of these models recapitulate the prolonged clinical pneumonia exhibited in COVID-19 patients. Pulmonary viral titres are detected for up to 5 or 6 days [57, 58] and viral transcripts for 7–10 days [54–56] in *hACE2* models, while viral titres are cleared within 4 days of intranasal infection in mouse-adapted SARS-CoV-2 mice [59].

Additionally, marked differences exist between models in terms of recapitulating hypercytokinemia and multiorgan involvement. Similar to MHV infection [49, 52], Gu et al., report elevated serum proinflammatory cytokines (including IL-1 $\beta$ , TNF- $\alpha$ , IL-2, IL-4, IL-5, IL-6, IL-10, G-CSF, GM-CSF, and CCL2) in young and old mice infected with a mouse-adapted SARS-CoV-2 [60], and Sun et al., report elevated serum levels of IFN- $\gamma$ , IL-9, G-CSF, and Eotaxin in old but not young SARS-CoV-2-infected *hACE2*-transgenic mice [56]. In other models, serum cytokines were found to be unchanged compared to uninfected controls [59]. Finally, in terms of multiorgan involvement, with the exception of the heart [55], infection of *hACE2*-transgenic mice with SARS-CoV-2 does not recapitulate the multiorgan viral spread seen in COVID-19 [5]. Some studies, albeit greatly underpowered, report detectable viral titres in the brain [54, 56], and others report the absence of viral titres outside of the respiratory tract [58]. Moreover, intragastric SARS-CoV-2 infection of humanized mice did not result in detectable viral titres in the spleen, kidneys, liver, or intestine [56], in contrast to MHV-A59 [53]. In a mouse-adapted SARS-CoV-2 strain, detectable viral transcript was reported in the lung, heart, liver, and faeces, but not the intestines or kidneys [60], recapitulating only a limited spectrum of the multiorgan involvement in COVID-19 patients.

None of the available models recapitulate the prolonged detection of viral titres and pneumonia exhibited in severe COVID-19 patients. Considering the absence of a fully-adapted host replication machinery in *hACE2* mice, these models are advantageous for preclinical testing of therapeutic interventions at the level of Spike — *hACE2* interaction. MHV-A59 is best suited to elucidate CoV virulence mechanisms and multiorgan involvement, and mouse-adapted SARS-CoV-2 and MHV-A59 strains are most adept for the mechanistic dissection of the mediators of antiviral immunity in the upper and lower airways.



**Table 1.** Overview of preclinical models of SARS-CoV-2

Virus	Dose, Route	Host strain	Host age	Clinical features	Survival	Reported duration of viral pneumonia	Ref.
MHV-A59	5 × 10 <sup>4</sup> PFU, i.n. 1.5 × 10 <sup>4-5</sup> PFU, i.n. 5 × 10 <sup>3</sup> PFU, i.n.	C57Bl/6 C57Bl/6 A/J	6–9 weeks 4 weeks 6–8 weeks	Acute neuroinflammation Acute pneumonia Acute pneumonia	100% survival 97% survival 20% survival by day 10 0% survival in old mice by day 7	n.d. <11 days (infectious viral particles) 7 days (infectious viral particles) n.d.	[37] [48] [8] [49]
Mouse-adapted SARS-CoV-2 <sup>1</sup>	1.6 × 10 <sup>4</sup> PFU, i.n.	BALB/c	6 weeks (young), 9 months (old)	Pneumonia, weight loss, reduced O <sub>2</sub> saturation, and elevated serum proinflammatory cytokines in older mice. Acute pneumonia in young mice, accompanying lung pathology in older mice. No clinical symptoms or weight loss	60% survival	<4 days (infectious viral particles)	[59]
Mouse-adapted SARS-CoV-2 <sup>2</sup>	10 <sup>5</sup> PFU, i.n.	BALB/c	12 weeks (young), 12 months (old)	Acute pneumonia in young mice, accompanying lung pathology in older mice. Weight loss and impaired viral clearance in older mice.	100% survival	7 days (viral RNA)	[60]
SARS-CoV-2	3 × 10 <sup>4</sup> PFU, i.n.	hACE2 transgenic (HFH4 promoter) C3H and C57Bl/6 hACE2 transgenic (mACE2 promoter) ICR	8–10 weeks 6–11 months	Interstitial pneumonia, lymphopenia in mice with severe disease Lung pathology, weight loss, and viral titres in lungs	33–50% survival by day 7 100% survival	7 days (viral RNA) 7 days (viral RNA)	[54] [58]
	4 × 10 <sup>5</sup> PFU, i.n.	hACE2 (CRISPR/Casp9) C57Bl/6	4.5 weeks (young) 30 weeks (old)	Interstitial pneumonia and elevated cytokines in old mice, no clinical symptoms of weight loss in young mice Lung pathology, weight loss and viral titres in lungs	100% survival	6 days (viral RNA)	[56]
	10 <sup>5</sup> PFU, i.n./i.v.	AdV/hACE2 BALB/c	8–10 weeks	Lung pathology, weight loss and viral titres in lungs	100% survival	10 days (viral RNA)	[55]
	10 <sup>5</sup> PFU, i.n.	Ad5-hACE2 BALB/c and C57Bl/6	6–8 weeks	Lung pathology, weight loss and viral titres in lungs	100% survival	5 days C57Bl/6 6 days BALB/c (infectious viral particles)	[57]

Ad5, Adenovirus type 5; AdV, Adenovirus; HFH4, hepatocyte nuclear factor 3/forkhead homologue 4; i.n., intranasal; i.v., intravenous; n.d., not determined.

<sup>1</sup>Six passages in BALB/c mice (MASCP6).

<sup>2</sup> Spike Q498T/P499Y mutant binds mACE-2.

## Conclusions

MHV is a murine coronavirus that causes acute respiratory, CNS, gastrointestinal, and/or liver disease depending on the infection route and viral strain. The study of recombinant and natural MHV strains has elucidated the function of CoV structural and nonstructural genes, revealing determinants of tropism, virulence, and evasion of host immunity. Moreover, genetic and pharmacological studies in mice have helped to identify innate and adaptive mechanisms of antiviral immunity to CoVs. Immunological correlates of disease severity in COVID-19 patients underscore the cross-species conservation of antiviral mechanisms to CoVs. In addition to MHV, humanized ACE2-expressing mice and mouse-adapted SARS-CoV-2 strains have recently been developed. While none of the currently available models recapitulate the prolonged pneumonia exhibited in patients with severe COVID-19, the native murine CoV represents a well-suited model to study CoV virulence factors, multiorgan involvement, and antiviral immunity. Detailed histopathological studies following viral clearance are required to determine to what extent these models recapitulate long-term complications of CoV infection (“long COVID”), a condition facing many COVID-19 patients globally [61].

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**Abbreviations:** **IFNAR:** IFN- $\alpha/\beta$  receptor · **MDA5:** melanoma differentiation-associated gene 5 · **Nsp:** nonstructural protein · **pDCs:** plasmacytoid DCs

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