Immune responses to human respiratory coronaviruses infection in mouse models

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Human respiratory coronaviruses (HCoVs), including the recently emerged SARS-CoV-2, the causative agent of the coronavirus disease 2019 (COVID-19) pandemic, potentially cause severe lung infections and multiple organ damages, emphasizing the urgent need for antiviral therapeutics and vaccines against HCoVs. Small animal models, especially mice, are ideal tools for deciphering the pathogenesis of HCoV infections as well as virus-induced immune responses, which is critical for antiviral drug development and vaccine design. In this review, we focus on the antiviral innate immune response, antibody response and T cell response in HCoV infected mouse models, and discuss the potential implications for understanding the anti-HCoV immunity and fighting the COVID-19 pandemic.

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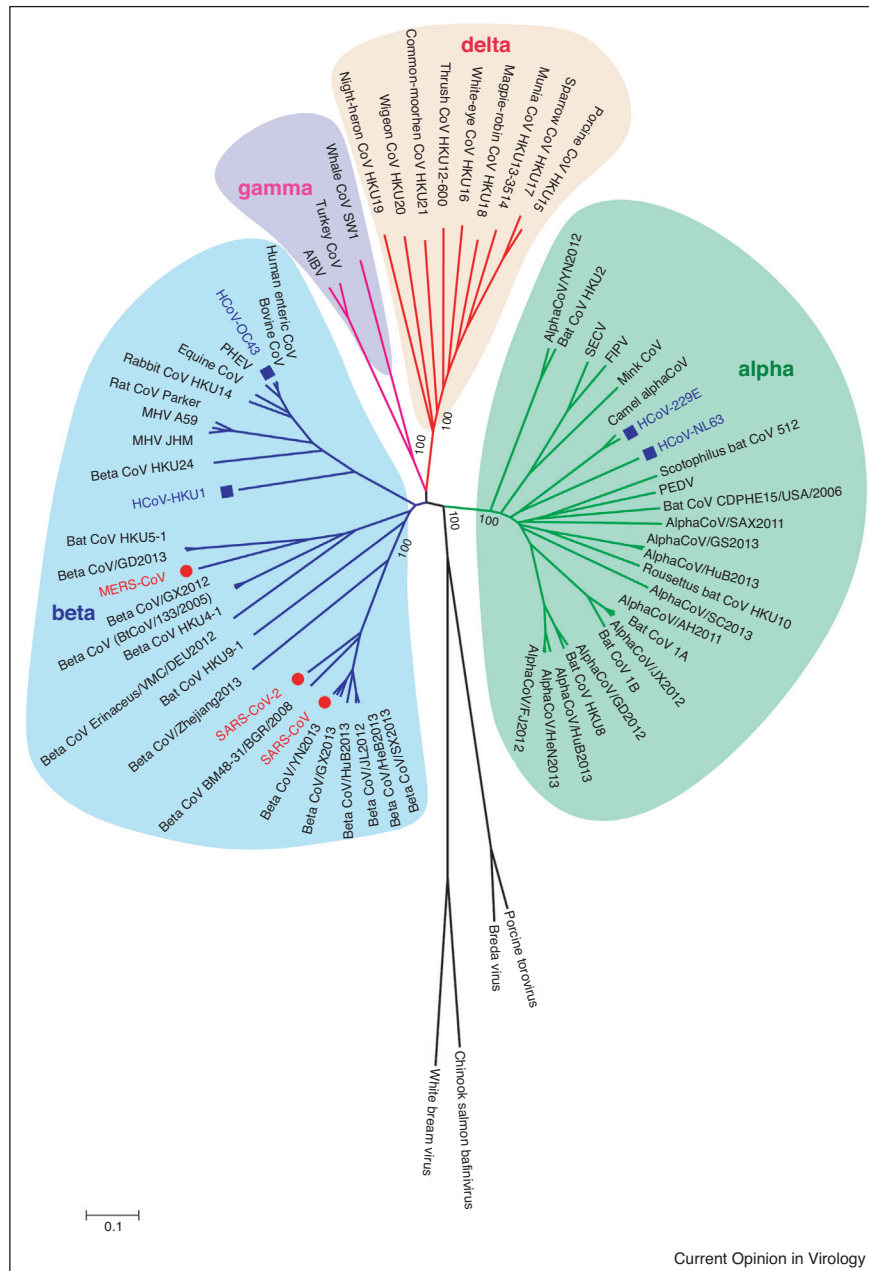[3] These authors contributed equally.

Introduction

The human respiratory coronaviruses (HCoVs) belong to the Coronaviridae family, the Coronavirinae subfamily, and the Coronavirus genera, with a ∼30 kb long positive-sense RNA genome, encoding four structural proteins (spike (S), envelope (E), membrane (M) and nucleocapsid (N) protein) and several accessory proteins. There are seven HCoVs including three highly pathogenic CoVs and four low pathogenic CoVs (Figure 1), causing a wide spectrum of symptoms and diseases from a flu-like to severe acute respiratory infection in humans. The severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are highly pathogenic HCoVs that can cause severe pneumonia, multiple organ damages and result in global pandemics. Low pathogenic HCoVs (HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1) are believed to cause only mild and self-limiting respiratory infections in humans. The SARS-CoV outbreak started in the winter of 2002 in China, which infected 8098 patients with 774 total deaths (9.6%) [1•,2], MERS-CoV was first identified in 2012 in Saudi Arabia and caused more than two thousand cases with around 35% fatality, which is still circulating in Middle East countries and perhaps in Africa [3,4,5••], SARS-CoV-2 was first discovered in December 2019 in China, and it is the causative agent for the current coronavirus disease 2019 (COVID-19) pandemic. As of October 29, 2021, more than 245 million confirmed cases with near 5 million deaths have been reported to WHO [6•,7]. HCoV-229E and HCoV-OC43 were isolated in the 1960s from nasopharyngeal samples of individuals experiencing common colds [8,9]. HCoV-NL63 and HCoV-HKU1 were discovered right after the SARS-CoV epidemic [10,11]. Low pathogenic HCoVs represent nearly 15–30% of the common cold respiratory tract infections in humans each year, and more than 90% of adults are serologically positive against these low pathogenic HCoVs [12]. Occasionally, in the elderly, child and immunocompromised patients, these four HCoVs cause life-threatening pneumonia and bronchiolitis as well as fetal encephalitis [13–17].

The emergence of SARS-CoV, MERS-CoV and SARS-CoV-2, three strains of animal coronaviruses that crossed the species barrier to infect human and caused severe respiratory infections in humans within the last 20 years, have emphasized that coronaviruses represent a major public health threat that are not restrained by international borders. There is an urgent need to understand the pathogenesis of HCoV infection and the regulatory mechanisms for anti-HCoV immune responses, which are critical for vaccine design and drug development. However, antiviral immune responses are also a double-edge sword. It is of great importance to distinguish protective versus pathogenic immune response and restore the balance of immune regulation. These studies require animal models, especially mice which are cost-effective, easily available and handling and provide multiple approaches to validate therapies and vaccines *in vivo*. In this review, we focus on antiviral innate immune responses, antibody responses and T cell responses in HCoV-infected mouse models, and discuss the potential

Figure 1

Phylogenetic analyses of Coronaviridae family.
All available complete genomes of Coronaviridae family from GenBank were collected and used for the evolutional analysis using MEGA
7.0. Three highly pathogenic (red) and four low pathogenic (blue) human coronaviruses are highlighted in the phylogenetic tree.

implications for understanding the anti-HCoV immunity and fighting the COVID-19 pandemic.

Application of mouse models in HCOV research

Animal models are essential tools in infectious disease research, including HCoV infections. Several animals have therefore been evaluated as models for HCoV

studies, including mice, hamsters, ferrets, and non-human primates (NHPs). Promising animal models that can reproduce manifestations of clinical patients have been broadly applied not only for elucidating disease pathogenesis and host immune response of infections but also for the evaluation of antiviral therapies and vaccine candidates. However, each model has its limitations. For example, as the gold standard animal model, non-

Table 1

Rceptors and mouse models of HCoVs

HCoVs	Receptor	Receptor transgenic mice	Receptor knock-in mice	Delivery receptor by exogenous vector	Genetically adapted virus
SARS-CoV	hACE2 [18•]	mAce2-hACE2 ICR mice [25]; K18-hACE2 C57BL/6 mice [24]	N/A	N/A	MA15 in young BALB/c mice [43]; v2163 in young BALB/c mice [46]
MERS-CoV	hDPP4 [19•]	CAG-hCD26 C57BL/6 J and/or B6C3F1/J mice [27]; codon-optimized CAG-hDPP4 C57BL/6 mice [31]; hDPP4 Tg C57BL/6 mice [32]	hDPP4 whole humanized using the VelociGene technology [33]; mDPP4 (A288 L/T330R) combined with MERS-15 [28]; hDPP4 KI combined with MERS$_{MA}$ [26]; CRISPR/Cas9 KI [34];	Ad5-hDPP4 [39••]	MERS-15 in CRISPR–Cas9 genetically engineered C57BL/6 mice [28]; MERS$_{MA30}$ in hDPP4-KI C57BL/6 mice [26]
SARS-CoV-2	hACE2 [20]	mAce2 hACE2 ICR mice [29]; HFH4-hACE2 C3B6 mice [30]; K18-hACE2 [35–37]	CRISPR/Cas9 KI [38]	Ad5-hACE2 [40]; AAV-hACE2 [41]; VEEV-VRP-hACE2 [42]	SARS-CoV-2 MA10 in BALB/c mice [47]; SARS-CoV-2 MA in BALB/c mice [44]; MASCp6 in aged BALB/c mice [45]
HCoV-HKU1	9-O-acetylatedsialic acids [23]	N/A	N/A	N/A	N/A
HCoV-OC43	9-O-acetylatedsialic acids [23]	Mice are susceptible to OC43			
HCoV-NL63	hACE2 [22]	N/A	N/A	N/A	N/A
HCoV-229E	hAPN [21]	hAPN$^{+/+}$ Stat1$^{-/-}$ ICR mice [84]	N/A	N/A	N/A

human primates (NHPs) are closely related to humans, but their application is limited due to the cost and availability. In SARS-CoV, MERS-CoV and SARS-CoV-2 infections, NHPs only reproduce mild diseases observed in human [2]. On the contrary, mice are ideal small animals because they are cost-effective, easily available and handling and of clear genetic background. However, laboratory mice also have some disadvantages in certain infections. For examples, they do not support the infections by several HCoVs at the receptor level, including MERS-CoV, some variants of SARS-CoV-2, 229E, NL63 and HKU1 [18•,19•,20–23]. To solve this problem, several strategies have been developed, such as application of HCoV receptor transgenic or knock-in mice [24–38], *in vivo* transduction of replication-defective vectors expressing HCoV receptors [39••,40–42], modifying viruses by serial passages in the respiratory tract of mice for generation of mouse-adapted (MA) strains or by reverse genetics to remodel the interaction between the S proteins and the receptors [43–47]. However, there is still no available mouse model for HCoV-HKU1 and HCoV-NL63. Table 1 summarizes most of the available mouse models for HCoV infections. These mouse models can permit HCoV entry, but they have pros and cons in various aspects. Some transgenic mouse models permit efficient viral entry but may cause severe diseases and even death due to brain infection which is not ideal for the study of respiratory tract infection. The mouse models generated by exogenous receptor transduction can successfully mimic human lung infection and be used in the study of host immune responses. However, these models often induce mild disease and are hard to recapitulate severe pneumonia. Mouse-adapted viruses generated by serial passaging the virus in the mouse respiratory tract can efficiently replicate in the mouse lungs and induce severe pneumonia, which are used to study the immune responses and pathogenesis. However, these viruses are different from the parental virus and containing distinct adaptive mutations. Mouse hepatitis coronavirus (MHV) also has served as models for dissecting the viral and immunologic determinants of coronavirus disease [48–50]. Distinct MHV strains exhibit differences in tropism and virulence. For example, MHV-1, but not the other strains of MHV infected mice produces clinical and pathological SARS-like disease with high mortality, which also could be used as a model for human respiratory coronavirus infections [51]. Researchers need to select proper mouse models based on their study objectives.

Innate immune response

The innate immune system acts as the first line of defense against pathogens, including HCoVs. The innate immune system recognizes invaded pathogens by sensing their pathogen-associated molecular patterns (PAMPs) with various pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), the nucleotide-binding oligomerization domain (NOD)-like receptor family

proteins (NLRs), and so on [52]. Following downstream signaling pathway activation, cells produce interferon (IFN) and inflammatory molecules and then promote the body to form an 'antiviral state' against virus infections by producing hundreds of interferon-stimulated genes (ISGs) [53[••]]. However, activation of the innate immune system must be tightly regulated, because excessive activation may lead to systemic inflammation and tissue damage, which are detrimental to the host [54[•]].

The innate immune responses to SARS-CoV, MERS-CoV and SARS-CoV-2 are frequently characterized by high-level production of pro-inflammatory chemokines and cytokines in patients, animal models as well as in tissue cultures *in vitro* [40,55–60]. Innate viral recognition leads to both nuclear factor-κB (NF-κB)-mediated induction of pro-inflammatory cytokines and interferon regulatory factor 3 (IRF3) and IRF7-mediated induction of type I and type III IFNs. TLR7 is a membrane-bound PRR on endosomes, where it can recognize ssRNA motifs from invading SARS-CoV, MERS-CoV, and MHV, then TLR7 dimerizes and recruits the myeloid differentiation primary response 88 (MyD88) adaptor protein triggering IRF7 and NF-κB, which stimulates the production of type I IFNs and proinflammatory cytokines, respectively, for host defense [61[••],62]. Melanoma differentiation-associated gene 5 (MDA5) is a cytosolic PRRs in the RLR family that can sense double-stranded long RNAs in MHV infection and then lead to the production of type I IFNs and proinflammatory cytokines via the activation and nuclear translocation of IRF3 and NF-κB [63,64]. RIG-I is another cytosolic RLR mediating type I IFN response in MHV and influenza virus infections [63].

Regulated type I IFN (IFN-I) production is protective in SARS-CoV, MERS-CoV, SARS-CoV-2, and mouse hepatitis coronavirus (MHV) infections by enhancing viral clearance [54[•],61[••],65[••],66,67]. Dysregulation of the innate immune response are pathogenic in SARS-CoV-infected and MERS-CoV-infected mice. Channappanavar *et al.* found that early treatment with recombinant IFN-β or poly(I:C) to induce IFN-I production in the lung resulted in complete protection from lethal infection. However, administration of IFN-β at the peak of SARS-CoV replication led to delayed viral clearance and enhanced lethality rather than protection [65[••]]. This delayed-IFN-I enhanced disease is characterized by apoptosis of T cells and elevated inflammatory monocyte and macrophage (IMMs) accumulation in the lungs. Antibody-mediated depletion of IMMs is fully protective. These data suggest that regulated IFN-I production is crucial for viral clearance as well as reduced immunopathology induced by inflammation [61[••],68].

Despite this innate host antiviral strategy, some HCoVs remain highly pathogenic, at least in part due to the

various viral mechanisms to evade and suppress the IFN response. HCoV immune escape strategies include encoding viral proteins dedicated to evading innate recognition by PRRs [69–74], inhibiting IFN-I and IFN-III production [75–80], blocking IFNAR and IFNAR signaling [77,81,82], and directly suppressing ISG effector functions [83].

Comparing with SARS-CoV, MERS-CoV and SARS-CoV-2, innate immune responses against human common cold coronaviruses remain largely unclear. Caroline Lassnig *et al.* has generated double transgenic hAPN$^{+/+}$ STAT1$^{-/-}$ mice that are susceptible to HCoV-229E infection indicating the protective role of IFN-I and STAT1 pathway [84]. Intranasal administration of recombinant IFN-α before infection protected against experimental HCoV-229E infection, resulting in reduced viral loads, diminished incidence and severity of symptoms in volunteers [85]. Structural (M and N) and accessory (NS2a and NS5a) proteins of HCoV-OC43 are able to inhibit antiviral response elements (ISRE, IFN-β promoter, and NF-κB-RE) to block the activation of IFN-I and NF-κB signaling pathways *in vitro* [86]. However, another study showed that OC43 N protein potentiates NF-κB activation following cytokine or TLR ligand stimulation by binding RNA, specifically microRNA 9 [87].

Innate immune cells recognize coronaviruses by cytosolic and endosomal RNA sensors and produce pro-inflammatory cytokines, chemokines and IFNs to fight infections. However, overwhelming chemokines and cytokines or dysregulated innate response could increase lung pathology, and contribute to the lethal SARS-CoV and MERS-CoV infections. All of these evidences suggest that tightly regulated host innate immunity is crucial to protect the infected mice.

Humoral immune response
Humoral immune responses to HCoVs are mediated by antibodies that are directly against viral structural proteins, mainly the spike glycoprotein and the nucleocapsid protein. A large amount of relevant information on antibody responses to HCoVs focused on serological tests in patients, few studies were performed in mouse models [88–93]. Seroconversion in SARS were within 10–16 days post onset of disease (d.p.o.) in patients [88,89], while 2–3 weeks after MERS-CoV and SARS-CoV-2 infections [90–93]. A low level of neutralizing antibodies could be detected in the mouse models infected with SARS-CoV-2 by 10 d.p.i. and wane afterwards [30,40]. However, in MHV infected mice, the T cell-dependent humoral immunity remained stable for at least 60 days. Further, B cells or IgM deficient mice experience viral recrudescence following initial viral clearance indicating humoral immunity mediates protective immunity in MHV infection [94–96]. Passive transfer of neutralizing antibodies

was found to protect SARS-CoV, MERS-CoV and SARS-CoV-2 infections in mice [40,97–100]. Antibody responses to SARS-CoV also waned over time in SARS convalescents at 2–3 years after the symptom onset [101•]. Large serological surveys of natural infections with HCoV-229E and HCoV-OC43 has revealed similar patterns of antibody waning that were associated with reinfections [102,103]. One study found a two-way cross-reactive binding but not neutralizing antibody against spike protein between SARS-CoV-2 and HCoV-OC43. However, high HCoV-OC43 S-IgG levels were found to relate with systemic inflammatory responses in COVID-19 patients, which might be a risk factor for clinical outcomes of COVID-19 [104•].

Humoral immunity is required for controlling CoV infections. Several studies have indicated that convalescent plasma transfusion could elicit protective role in infected patients and mice [40,105,106]. However, antibody-dependent enhancement (ADE) is still of concern and require further studies.

T cell response

Humoral and T cell immune responses are the major components of the adaptive immune system. In contrast to the innate immune response, the adaptive immune response has an immunological memory that are highly specific against reinfections. While antibody response is not long lasting, memory T cells were reported to have significant longer longevity in HCoV patients [100,107••].

T cell responses against HCoV infections also have been characterized in mice [39••,40,108•,109–112]. Depletion of CD4+ T cells delayed virus clearance and further enhanced interstitial pneumonitis in 12–14 month-old BALB/c mice infected with SARS-CoV intranasally (i. n.) [109]. MERS-CoV could be cleared in mice lacking B cells, while there was a delayed viral clearance in mice lacking T cells, indicating that T cells are required to clear MERS-CoV [39••]. However, another study showed that depletion of CD8+ T cells in a sublethal MERS mouse model resulted in diminished lung pathology and clinical disease without impacting the viral titers, suggesting that these cells may also play a role in immunopathogenesis [110]. Both CD4+ and CD8+ T cells contributed to SARS-CoV-2 clearance in C57BL/6 and BALB/c mice [40,108•]. The role of CD4+ and CD8+ T cells in low pathogenic HCoV infections have not yet been addressed.

In the above-mentioned studies, the role of bulk CD4+ or CD8+ T cell populations were studied. However, only viral antigen-specific T cells can respond efficiently against a viral infection. These T cells recognize virus-derived peptides (epitopes) presented by MHC class I or class II molecules on virus-infected or antigen presenting cells. Usually, HCoV-specific T cell epitopes in the model mouse will be mapped and then the function of specific T cells can be further investigated [39••,108• ,111,113–116]. Several studies showed that virus-specific CD4+ and CD8+ T cells contributed to rapid viral clearance and amelioration of the clinical disease in HCoV infected mice, demonstrated by adoptive transfer of virus-specific CD4+ and CD8+ T cells or immunization of mice with Venezuelan equine encephalitis replicon particles (VRPs) encoding a single dominant T cell epitope [108•,112,117••]. A study focusing on COVID-19 convalescent donors and vaccinees showed that SARS-CoV-2 variants of concern (VOC) partially escaped from humoral but not T cell response [118•], indicating that T cell response is more conserved and could mediate cross protection against virus containing homologous epitopes. Coronaviruses are relatively conservative in structural proteins and cross-reactive T cell epitopes among different CoVs have been reported. One of HCoV-OC43-specific CD4+ T cell epitopes located in the membrane (M) protein (M133-147) is identical with a MHV-specific CD4+ T cell epitope (M133-147) in C57BL/6 mice [119]. However, the OC43-specific CD8+ T cell response and other low pathogenic HCoV-specific T cell responses are still unknown. Immunization of mice with a VRP encoding a CD4+ T cell epitope from SARS-CoV N protein resulted in reduced viral load of MERS-CoV, proved that SARS-CoV-specific CD4+ T cells are cross-protective against MERS-CoV in some degree [117••]. Another research showed that there are cross-reactive T cell responses between SARS-CoV and SARS-CoV-2 in BALB/c mice [108•]. The role of virus-specific and cross-reactive T cell responses against HCoVs need to be further studied in mice.

Optimal virus-specific T cell responses play an essential role in viral clearance. However, SARS-CoV specific T cell responses can be impaired by the defects in respiratory dendritic cells (rDC) and alveolar macrophages (AM). Depletion of AM or pretreatment of mice with poly I:C could correct these defects and improve T cell responses in SARS-CoV infected mice [120•]. Zhao et al. also found that suboptimal T cell responses resulted from an impairment of rDC migration from the lungs to the lymph nodes, which was governed by elevated prostaglandin D2 (PGD2) and phospholipase (PLA2G2D) expression in aged mice after SARS-CoV infection [121•]. In addition, Zhuang et al. showed that IFN-I signaling was required for optimal SARS-CoV-2–specific T cell development and functionality.

These studies indicate that both virus-specific CD4+ and CD8+ T cells are protective in coronavirus infections, and restoring impaired T cell response may improve the outcome in some patients. In addition, T cell response is required for optimal protective effect for a CoV vaccine.

Perspective for COVID-19 immunity from other HCoV infections

Today, it is well-known that HCoVs are undergoing rapid evolution due to high nucleotide substitution and recombination rate and linked with major outbreaks worldwide of human fatal pneumonia since the beginning of the 21st century [122–124]. Here, we review the immune responses against HCoVs in mice and discuss key scientific issues that need attention in SARS-CoV-2 infection immunity.

Identifying factors that are protective versus pathogenic in patients, especially in severe disease, could inform the selection of therapeutic options. Delayed and dysregulated IFN responses contribute to the pathogenesis of SARS-CoV and MERS-CoV rather than protection. It suggests that early and timely treatment of type I interferon may help prevent from severe disease.

Vaccine immunization is the best expedient path to herd immunity. Whether anti-SARS-CoV-2 neutralizing antibodies or specific T cells alone can effectively prevent SARS-CoV-2 pandemic remain unclear. How much antibodies or T cell response are sufficient? How to avoid ADE? What are the roles of pre-existing cross-reactive immune responses in the SARS-CoV-2 infection? All of these questions need to be answered. Neutralizing antibodies against HCoVs wane following infection or immunization, potentially allowing for SARS-CoV-2 reinfection which is similar to the common cold coronaviruses. Therefore, vaccine design and immunization strategies need to be improved. Introduction of T cell epitopes in the vaccines probably could prolong the immunization durability, and intranasal immunization may enhance the generation of protective mucosal immunity [117[••],125,126].

Author contributions

All authors contributed to writing and critical revision of the manuscript. All authors approved the final version.

Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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