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Immunobiology of COVID-19: Mechanistic and therapeutic insights from animal models

Hong-Yi Zheng[1], Tian-Zhang Song[1], Yong-Tang Zheng[1,2,*]

[1] *State Key Laboratory of Genetic Evolution & Animal Models*, *KIZ-CUHK Joint Laboratory of Bioresources and Molecular Research in Common Diseases*, *Center for Biosafety Mega-Science*, *Kunming Institute of Zoology*, *Chinese Academy of Sciences*, *Kunming*, *Yunnan* 650223, *China*

[2] *National Resource Center for Non-Human Primates*, *National Research Facility for Phenotypic & Genetic Analysis of Model Animals (Primate Facility)*, *Kunming Institute of Zoology*, *Chinese Academy of Sciences*, *Kunming*, *Yunnan* 650107, *China*

ABSTRACT

The distribution of the immune system throughout the body complicates *in vitro* assessments of coronavirus disease 2019 (COVID-19) immunobiology, often resulting in a lack of reproducibility when extrapolated to the whole organism. Consequently, developing animal models is imperative for a comprehensive understanding of the pathology and immunology of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. This review summarizes current progress related to COVID-19 animal models, including non-human primates (NHPs), mice, and hamsters, with a focus on their roles in exploring the mechanisms of immunopathology, immune protection, and long-term effects of SARS-CoV-2 infection, as well as their application in immunoprevention and immunotherapy of SARS-CoV-2 infection. Differences among these animal models and their specific applications are also highlighted, as no single model can fully encapsulate all aspects of COVID-19. To effectively address the challenges posed by COVID-19, it is essential to select appropriate animal models that can accurately replicate both fatal and non-fatal infections with varying courses and severities. Optimizing animal model libraries and associated research tools is key to resolving the global COVID-19 pandemic, serving as a robust resource for future emerging infectious diseases.

Keywords: SARS-CoV-2; COVID-19; Animal models; Infection immunology; Immunotherapy

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the pathogen responsible for coronavirus disease 2019 (COVID-19), which can manifest as mild to severe respiratory illness and can cause death in humans (Riggioni et al., 2020). Since its emergence in late 2019, the highly transmissible and variable nature of this virus has posed a significant challenge to the global medical community. As of February 2024, the pandemic has led to over 774 million confirmed cases and more than 7 million deaths worldwide (https://covid19.who.int/). Despite the continuous evolution of the virus, the current prevalent variants are not associated with increased disease severity (Statement from the Fifteenth Meeting of the International Health Regulations (IHR) (2005) Emergency Committee on the COVID-19 Pandemic). Consequently, on 4 May 2023, the World Health Organization (WHO) declared that COVID-19 no longer constituted a “public health emergency of international concern” (PHEIC). Nonetheless, considerable research challenges remain, particularly in our understanding of the role of the host immune system in disease progression, as well as the development of broad-spectrum vaccines capable of targeting rapidly mutating SARS-CoV-2 strains and the implementation of different immunotherapies. Therefore, animal model research remains a priority for advancing our knowledge of COVID-19.

Studying the immunobiology of COVID-19 using only *in vitro* tools is difficult due to the complexity of the human immune system. Animal models offer significant advantages as they enable the simulation and control of various factors, such as host characteristics, viral dosage, and antiviral interventions. These models facilitate procedures such as transgene expression, cell selection, and tissue sampling, which are challenging in humans, thereby providing valuable experimental data. Hence, animal models are essential for the

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*Corresponding author, E-mail: zhengyt@mail.kiz.ac.cn

advancement of immunological research and immunotherapy for COVID-19, particularly those involving higher mammals. During the early stages of the pandemic, various animal models were established to support vaccine and drug development, and to investigate SARS-CoV-2 pathogenesis (Muñoz-Fontela et al., 2020). To date, naturally susceptible animals such as non-human primates (NHPs), Syrian hamsters (*Mesocricetus auratus*), ferrets (*Mustela furo*), and mice (*Mus musculus*) — either sensitized through transgenic techniques or infected with SARS-CoV-2-adapted strains — have been widely utilized. Various studies have documented the creation, pathology, and application of animal models for COVID-19 (Bi et al., 2021; Chu et al., 2022; Kane et al., 2023; Muñoz-Fontela et al., 2020; Shou et al., 2021; Zhang et al., 2023; Zhao et al., 2023), which will not be repeated here. Other reviews have also summarized the immune responses to SARS-CoV-2 infection in both patients and animals, highlighting the unique immune characteristics of diverse animal models (Chen et al., 2024; Saravanan et al., 2022). This review focuses on current advancements in our understanding of the immunological characteristics and mechanisms of animal models for COVID-19, as well as their applications in studying immune prevention and treatment strategies, emphasizing the effective use of each model to address specific problems (Figure 1).

ANIMAL MODELS OF COVID-19

In response to the rapid global spread of SARS-CoV-2, various animal species, including NHPs, mice, ferrets, tree shrews, and Syrian hamsters, have been established as models to facilitate COVID-19 research. The suitability of these animals as models predominantly depends on the molecular structure of the primary binding receptor (ACE2) for the virus. Catarrhine primates, including apes and African/Asian monkeys, have ACE2 amino acid residues identical to those in human ACE2 (hACE2), while New World monkeys exhibit over 90% similarity, making both groups highly susceptible to SARS-CoV-2 (Gao & Zhang, 2020; Karki et al., 2021; Melin et al., 2020). However, the high costs associated with using large animal models often limit their utility in research, prompting a shift towards smaller animals. Tree shrews, which possess certain primate-like characteristics, show a divergence of 10 amino acids in their ACE2 key domain compared to hACE2, resulting in lower susceptibility to SARS-CoV-2 infection, characterized by mild viral shedding and lung lesions (Xu et al., 2020a; Zhao et al., 2020). Furthermore, mice and ferrets have ACE2 sequences that differ from hACE2 by eight and seven amino acids, respectively. Although mice are naturally resistant to SARS-CoV-2, humanized mouse models are widely utilized in COVID-19 research due to advancements in gene-editing techniques, including transgenic expression, clustered

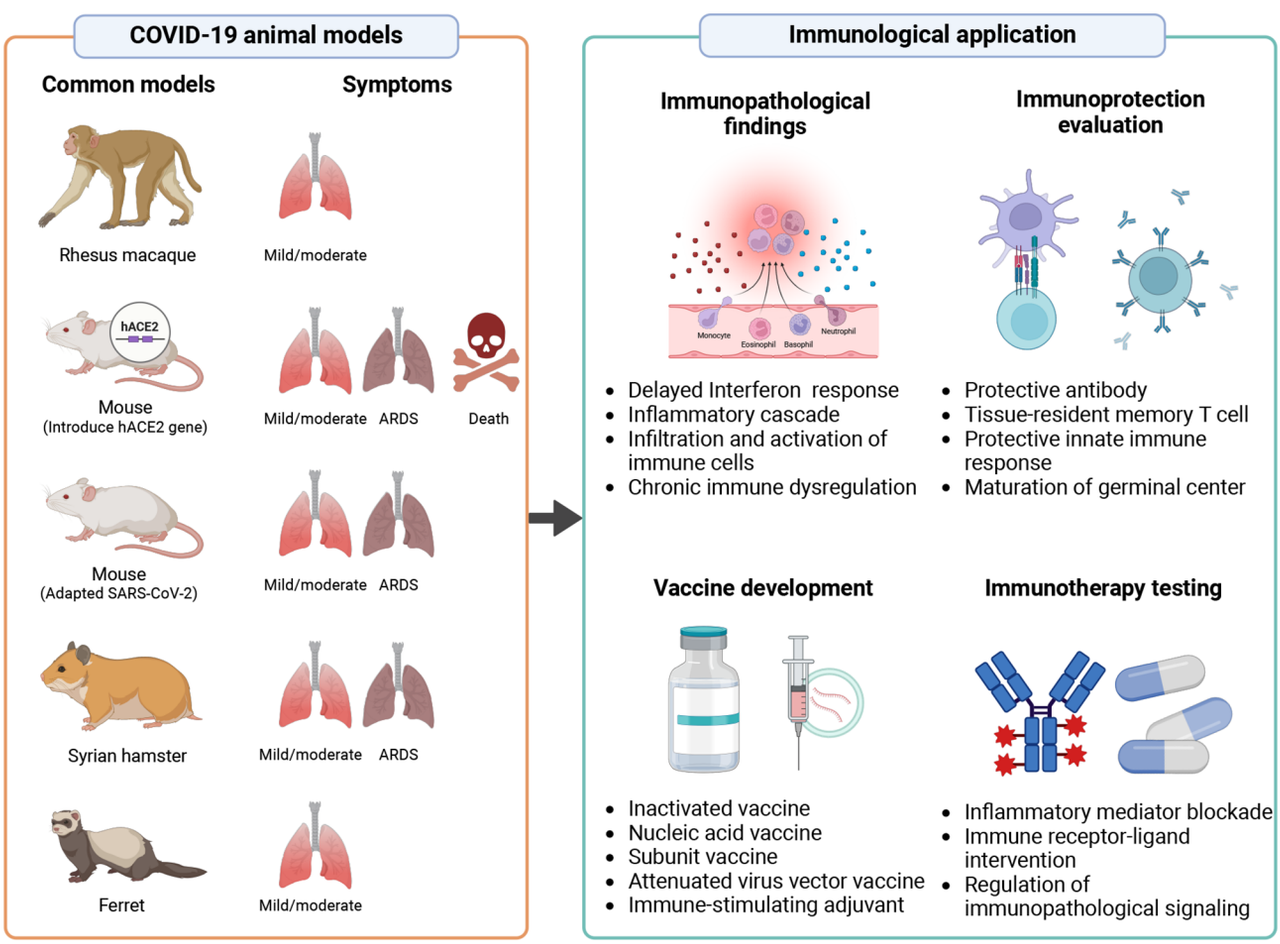

Figure 1 Immunological applications of common COVID-19 animal models

Rhesus macaques, mice transduced with human ACE2 gene, mice infected with adaptive SARS-CoV-2, Syrian hamsters, and ferrets are widely used to study immune pathogenesis and immune protection mechanisms of COVID-19, and also play important roles in COVID-19 vaccine and immunotherapeutic drug development. Figure created using BioRender (https://biorender.com/).

regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) editing, and transient overexpression of hACE2 (Sefik et al., 2022; Zhang et al., 2023; Zhou et al., 2023). Conversely, ferrets exhibit a natural susceptibility to SARS-CoV-2, manifesting with mild clinical symptoms and relatively low lung viral titers (Zhao et al., 2023). Syrian hamsters, with only two amino acid differences in ACE2 compared to hACE2, are a preferred model for *in vivo* preclinical assessment of SARS-CoV-2 virulence and for testing vaccines, antivirals, and therapeutics (Handley et al., 2023). Despite these advancements, however, no single animal model can fully replicate all aspects of COVID-19, highlighting the need for tailored research approaches that utilize the unique features of each animal model (Table 1).

NHPs

Compared to other laboratory animals, NHPs possess immune and respiratory systems, as well as tissue structures, that closely resemble those of humans (Estes et al., 2018). Old World monkeys, such as rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), African green monkeys (*Chlorocebus aethiops*), and baboons (*Papio hamadryas*), and New World monkeys, such as common marmosets (*Callithrix jacchus*), are frequently employed as models for SARS-CoV-2 infection, displaying mild to moderate respiratory symptoms. In rhesus macaques, SARS-CoV-2 infection causes acute localized to widespread pneumonia, but typically without pronounced clinical respiratory symptoms (Shan et al., 2020). Notably, age significantly impacts the severity of SARS-CoV-2 in rhesus macaques, with older animals demonstrating more severe interstitial pneumonia and cytokine storm, despite a delayed immune response, compared to their younger counterparts (Song et al., 2020; Yu et al., 2020b). Cynomolgus macaques, which are smaller and less expensive to maintain, are predominant in China's experimental primate population, with crab-eating monkeys (211 171) far outnumbering rhesus macaques (28 806). Post-infection, cynomolgus macaques show symptoms such as abnormal chest radiographs, elevated body temperature, and weight loss, although their cytokine response and lung disease are less severe than in rhesus macaques (Lu et al., 2020). Despite the abundance of cynomolgus macaques, rhesus macaques are preferred in COVID-19 research due to their higher suitability. Northern pig-tailed macaques (*Macaca leonina*), lacking the retroviral restriction factor tripartite motif-containing protein 5α (TRIM5α), exhibit more severe inflammation and physical impairment from SARS-CoV-2 than rhesus macaques (Kuang et al., 2009; Song et al., 2021). However, limited research on these macaques due to their small number hinders a full understanding of their potential in studies. Common marmosets exhibit lower susceptibility to SARS-CoV-2, with less pronounced and replicable manifestations of COVID-19 (Ireland et al., 2022; Lu et al., 2020). African green monkeys and baboons, though utilized less frequently, can also serve as SARS-CoV-2 models. Compared to macaques, baboons experience prolonged viral RNA shedding and more severe lung inflammation (Singh et al., 2020), while African green monkeys display higher SARS-CoV-2 replication and marked

Table 1 Common animal models for COVID-19 immunobiology research

Animal	Immunopathology	Immune response	Advantage	Disadvantages
NHPs	Diffuse alveolar damage Lung consolidation Infiltration of inflammatory cells Thoracic adhesion Glassy opacity Hepatosplenopathy Age-related pathological changes	Neutralizing antibody response Virus-specific T cell response Up-regulation of IL-1, IL-6, IL-10, and other inflammatory factors Transient neutropenia and lymphocytopenia	Closer to human immune response Essential models for preclinical trials	Lack of severe clinical symptoms High technical requirements for biosecurity High experimental costs
hACE2 transgenic mice	Severe interstitial pneumonia Infiltration of inflammatory cells Thickening of alveolar septum Unique vascular system damage	Lymphopenia Pulmonary immune cell recruitment Up-regulation of IFN-γ, IL-6, MCP-1 and other inflammatory factors Diffuse microglia activation	Excellent simulation of severe COVID-19 Dose-dependent respiratory symptoms and mortality Central nervous system infection	Vastly different ACE2 expression pattern from humans Complex and expensive transgenic operations Lack of genetic diversity and disease models
AdV/AAV-hACE2 mice	Infiltration of inflammatory cells from perivascular to mesenchymal Diffuse alveolar intraepithelial infection Alveolar edema Increased vascular congestion and bleeding	Virus-specific T cell response Up-regulation of TNF-α, IFN-γ, IL-10 and other inflammatory factors Reduced activation of $CD4^+$, $CD8^+$, or NK cells	Fast and simple model construction Diverse genetic backgrounds or disease models Suitable for large-scale drug and vaccine research	Interference from anti-Adv/AAV immune response Time and tissue limitations of viral infection
Mouse adapted SARS-COV-2	Interstitial pneumonia Edema Diffuse alveolar damage Mild to moderate lung inflammation	Proinflammatory and monocyte chemokine responses Up-regulation of IL-6, IL-1α, IL-1β and other inflammatory factors	Easy to simulate multiple COVID-19 symptoms Diverse genetic backgrounds or disease models	Clinical SARS-CoV-2 strains cannot be used
Syrian hamsters	Alveolar destruction Monocyte infiltration Alveolar collapse Lung consolidation Pulmonary hemorrhage	Neutralizing antibody response Infiltration of macrophages and T lymphocytes Up-regulation of IFN-γ, IL-4, IL-6 and other inflammatory factors	Naturally susceptible to SARS-CoV-2 Similar to human COVID-19 symptoms Suitable for large-scale drug and vaccine research	Lack of reagents and tools for immunology research Lack of genetic diversity and disease models

respiratory issues, including pronounced inflammation and clotting disorders (Woolsey et al., 2021). The correlation between age and COVID-19 severity is consistent across these NHP species, highlighting their utility in exploring the effects of aging and metabolic diseases on SARS-CoV-2 infection (Singh et al., 2020; Song et al., 2020; Yu et al., 2020b). Despite requiring biosafety level 3 (BSL-3) facilities and specialized personnel to handle SARS-CoV-2-infected NHPs, these models are essential for evaluating COVID-19 treatments and vaccines (Guebre-Xabier et al., 2020; Rabdano et al., 2023; Sui et al., 2021b; Vogel et al., 2021).

Mice

Due to their small size, large populations, and low feeding costs, mice are extensively used as model organisms for a variety of human diseases. However, significant structural differences between hACE2 and mouse ACE2 (mACE2) inhibit the natural replication of SARS-CoV-2 in wild-type mice (Mahdy et al., 2020). Consequently, current strategies for developing COVID-19-susceptible mouse models involve either introducing the hACE2 gene or adapting mACE2 through viral passage (Knight et al., 2021). The diverse array of genetic and immunological tools available for mice allows for a more effective exploration of COVID-19 pathogenesis, offering advantages not achievable with other animal models and contributing to a more profound understanding of this complex disease.

hACE2 transgenic mice: Animal susceptibility to SARS-CoV-2 is influenced by the structural characteristics of ACE2, while COVID-19 severity is closely associated with its expression (Ni et al., 2020). As a result, the transgenic introduction of hACE2 into mice under the control of efficient promoters has become a highly effective strategy for constructing COVID-19 models. In 2007, hACE2 transgenic mice were generated to evaluate SARS-CoV *in vivo* using cytomegalovirus (CMV) immediate enhancer/β-actin (CAG) and keratin-18 (K18) promoters, with the former enabling robust hACE2 expression across various mouse cell types and the latter restricting high hACE2 expression to epithelial cells (McCray et al., 2007; Tseng et al., 2007). The K18-hACE2 mouse model has also been extensively used in COVID-19 research, replicating dose-dependent characteristics of SARS-CoV-2 infection, including loss of olfaction, severe lung pathology, thrombosis, vasculitis, nerve damage, and mortality, similar to those observed in human COVID-19 patients (Zheng et al., 2021b). However, K18-initiated hACE2 is overexpressed in the brain, exceeding ACE2 expression levels in humans (Chen et al., 2021b). Consequently, K18-hACE2 transgenic mice exhibit a disparity in brain infection levels compared to COVID-19 patients (Winkler et al., 2020). To address this issue, hACE2 transgenic mice were developed using the mACE2 promoter to ensure equivalent hACE2 expression in $mACE2^+$ cells (Yang et al., 2007), resulting in a self-limiting and non-lethal course of infection, which is invaluable for assessing the efficacy of antiviral therapeutics and vaccines (Bao et al., 2020). Additionally, CRISPR/Cas9 knock-in technology has been employed to generate transgenic mouse models expressing hACE2, which display detectable SARS-CoV-2 in the lung, trachea, and brain, accompanied by interstitial pneumonia and elevated cytokine levels (Sun et al., 2020b).

Viral vectors for hACE2 transduction in mice: Employing adenoviral vectors (AdV) or adeno-associated virus vectors (AAV) to transiently express hACE2 in mouse lungs is considered highly effective for modeling SARS-CoV-2 infection in strains with distinct genetic characteristics or diseases. Notably, intranasal administration of AdV-encoded hACE2 in wild-type BALB/c or C57BL/6 mice, with subsequent SARS-CoV-2 infection at the peak of hACE2 expression at 5 days post-transduction, leads to weight loss, severe lung lesions, and high viral replication in the lungs (Sun et al., 2020a). In general, AAVs are preferred over AdVs due to their lower immunogenicity, prolonged expression of target genes, and enhanced suitability for *in vivo* gene delivery. Intranasal administration of hACE2 encoded by AAV9 and AAV-DJ results in robust hACE2 gene expression in the lungs of mice, while AAV6 yields relatively low expression (Gary et al., 2021; Glazkova et al., 2022; Israelow et al., 2020). Mice humanized with AAV-hACE2, specifically the MSTRG6 strain, exhibit chronic COVID-19 features, including weight loss, persistent viral RNA presence, fibrotic lung pathology, human-like macrophage responses, sustained interferon (IFN)-stimulated gene (ISG) expression, and T cell lymphopenia (Glazkova et al., 2022; Sefik et al., 2022). These AdV/AAV-hACE2 mouse models provide a readily available and genetically varied framework for studying SARS-CoV-2 infection, crucial for rapid preclinical assessments.

Mouse-adapted viruses: For research involving COVID-19 animal models where SARS-CoV-2 mutation is not a factor, using a mouse-adapted strain of SARS-CoV-2 may be a preferable option. Dinnon et al. (2020) engineered a mouse-adapted recombinant SARS-CoV-2 variant by modifying the Q498Y/P499T amino acid residue in the receptor-binding domain (RBD) of the spike (S) protein, enabling viral replication in both the upper and lower respiratory tracts of wild-type BALB/c mice, although young mice rapidly cleared the infection within 4 days. Leist et al. (2020) generated the MA10 variant by passaging mouse-adapted SARS-CoV-2 through mice 10 times, inducing pulmonary disease and potential acute respiratory distress syndrome (ARDS) in BALB/c mice, but only mild lung inflammation and weight loss in C57BL/6 mice. Another variant carrying the N501Y substitution, generated by serial passage of SARS-CoV-2 through the respiratory tract of elderly BALB/c mice, induces interstitial pneumonia without visible clinical symptoms or weight loss (Gu et al., 2020). The SARS2-N501Y MA30 strain, produced through successive passages of SARS2-N501Y in mouse lungs, shares spike protein mutations with the Omicron variant and can cause fatal disease in young BALB/c mice (Kibler et al., 2022; Wong et al., 2022). The generation of highly virulent mouse-adapted SARS-CoV-2 through *in vivo* passage alone mirrors unrestricted SARS-CoV-2 transmission in the population without preventative measures, providing a vital model for studying viral transmission dynamics.

Hamsters

Hamsters, particularly Syrian hamsters (*Mesocricetus auratus*), are highly susceptible to SARS-CoV-2 infection, making them a preferred model for viral study. SARS-CoV-2 effectively replicates in the pulmonary system of Syrian hamsters, targeting the lower respiratory tract within 12 hours post-inoculation and causing severe pathological alterations and olfactory dysfunction similar to symptoms observed in COVID-19 patients (Clancy et al., 2023; Imai et al., 2020; Merle-Nguyen et al., 2024). SARS-CoV-2 infection in Syrian hamsters is characterized by rapid viral clearance by day 7, resembling infection dynamics in mildly infected humans,

possibly attributed to varying viral infection titers (Sia et al., 2020). Few animal models of COVID-19 exhibit clinical features of ARDS, limiting our understanding of the molecular mechanisms underlying the host immune response to SARS-CoV-2 infection. However, SARS-CoV-2-infected Syrian hamsters display key ARDS characteristics, such as lung injury, increased permeability, acute inflammation, and hypoxemia, particularly when inflammatory mediators are up-regulated and persist after viral clearance (Bednash et al., 2022). Thus, Syrian hamster models of SARS-CoV-2 infection reflect several aspects observed in moderate, self-limiting cases of COVID-19, including respiratory and vascular inflammation and age-related patterns (Bednash et al., 2022; Gruber et al., 2022; Imai et al., 2020). In addition, Roborovski pygmy hamsters (*Phodopus roborovskii*) are highly susceptible to SARS-CoV-2, developing severe acute diffuse alveolar injury and pulmonary hyaline microthrombi similar to severe COVID-19 patients (Trimpert et al., 2020). Chinese hamsters (*Cricetulus griseus*) can also be infected with SARS-CoV-2, presenting with clinical symptoms akin to the Syrian hamster models but with prolonged symptom duration (Bertzbach et al., 2021). As valuable models for SARS-CoV-2 infection, hamsters, mice, and NHPs have been used in combination for the development of viral vaccines and drugs (Baum et al., 2020; Lu et al., 2023; Yu et al., 2023).

Other animal models

Various other rodents have been used in studies of SARS-CoV-2 infection. Guinea pigs (*Cavia porcellus*) do not appear to be susceptible to SARS-CoV-2 infection, as the virus cannot be detected despite early histological changes in the lungs by day 3 (Hewitt et al., 2020). In contrast, New Zealand white rabbits (*Oryctolagus cuniculus*) are sensitive to SARS-CoV-2 but remain asymptomatic (Mykytyn et al., 2021), making them unsuitable as a disease model. Interestingly, Yu et al. (2022a) established a SARS-CoV-2-susceptible hACE2-transgenic rat (*Rattus norvegicus*) model but found that wild-type Sprague-Dawley (SD) rats infected with the prototype strain of SARS-CoV-2 showed detectable viral loads in the upper respiratory tract and lung lesions. The increase in demand for COVID-19 research has expanded the range of animals used for testing and model development, beyond conventional primate and rodent models. Studies have indicated that SARS-CoV-2 exhibits limited replication in dogs, pigs, chickens, and ducks, but shows efficient replication in the upper respiratory tract of ferrets and in the respiratory and digestive systems of cats (Shi et al., 2020). The respiratory tract of ferrets, which is physiologically and anatomically similar to that of humans, is naturally sensitive to a variety of human respiratory viruses, including influenza virus, SARS-CoV, and SARS-CoV-2 (Belser et al., 2011; Kim et al., 2020; Shi & Hu, 2008). SARS-CoV-2 infection in ferrets results in fever, mild respiratory symptoms, acute bronchiolitis, and diffuse interstitial histiocytic pneumonia, but not death (Kim et al., 2020; Kreft et al., 2022). Minks (*Neovison vison*), also belonging to the weasel family, experience severe respiratory disease and death following SARS-CoV-2 infection, presenting with pulmonary edema, moderate vasculitis, and fibrinous interstitial pneumonia (Eckstrand et al., 2021). Domestic cats (*Felis catus*) also exhibit high susceptibility to SARS-CoV-2 infection, although their widespread use is impeded by the challenges of non-standard animal models (Shi et al., 2020). Additionally, in-depth study of infection immunology in these models is hindered by the scarcity of specialized immunological research tools.

INFECTION IMMUNOLOGY IN ANIMAL MODELS OF COVID-19

Immune pathogenesis of SARS-CoV-2 infection

The pathophysiology of COVID-19 primarily arises from host dysregulation in controlling SARS-CoV-2 replication and modulating the immune response, resulting in delayed viral clearance, inflammation, and tissue damage that extends beyond pulmonary involvement to systemic manifestations (Arish et al., 2023). The host innate immune system detects RNA viruses, like coronaviruses, through various pattern recognition receptors (PRRs). Toll-like receptor 3 (TLR3), TLR7, and TLR8 recognize viral genomic RNA, double-stranded RNA (dsRNA), and single-stranded RNA (ssRNA). Cytoplasmic RNA sensors, such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5), detect dsRNA intermediates formed during viral replication. This detection triggers the activation of IFN regulatory factor 3 (IRF3)/IRF7-dependent type I and III IFN transcription, as well as nuclear factor kappa B (NF-κB)-dependent proinflammatory cytokines and chemokines, initiating the antiviral process (Merad et al., 2022).

SARS-CoV-2 disrupts the innate immune system by targeting viral sensors and blocking downstream antiviral signaling molecules, facilitating successful transmission and adaptation to human hosts (Kasuga et al., 2021). Patients with moderate to severe COVID-19 exhibit impaired responses to both IFN-I and IFN-III, associated with persistent viral load in the bloodstream, while patients with mild disease show early induction and higher levels of IFN (Galani et al., 2021; Hadjadj et al., 2020). As the virus evades immune surveillance and efficiently infects neighboring target cells, leading to acute lung injury, it continues to activate the innate immune response via PRRs and damage-associated molecular patterns (Chu et al., 2020). Uncontrolled immune responses trigger secondary systemic inflammation and a cytokine storm, marked by immune cell overactivation, abnormal blood counts, and elevated levels of circulating cytokines, such as tumor necrosis factor (TNF)-α, IFN-I and IFN-II, interleukin (IL)-1, IL-6, IL-12, IFN-γ-inducible protein 10 (IP-10), and monocyte chemoattractant protein 1 (MCP-1), culminating in septic shock, ARDS, and multiple organ failure (Fajgenbaum & June, 2020; Kasuga et al., 2021).

Initial insights into the pathological mechanisms of COVID-19 were derived from patient blood samples, biopsies, and autopsy specimens. With the advancement of animal models replicating SARS-CoV-2 infection, a more comprehensive understanding of COVID-19 pathogenesis has been achieved (Figure 2). An ideal animal model should capture the key aspects of SARS-CoV-2 pathogenesis and associated immune responses, allow detailed study of multiple anatomical sites of viral infection through invasive sampling, and support experimental approaches to manipulate the immune system. Currently, NHPs, mice, and golden hamsters are commonly used as models of SARS-CoV-2 infection due to their similarity to human patients regarding virus sensitivity, anatomical sites of viral infection, immune system components, and clinical symptoms. However, no single animal model can perfectly replicate all characteristics of COVID-19. By effectively and thoughtfully utilizing each animal

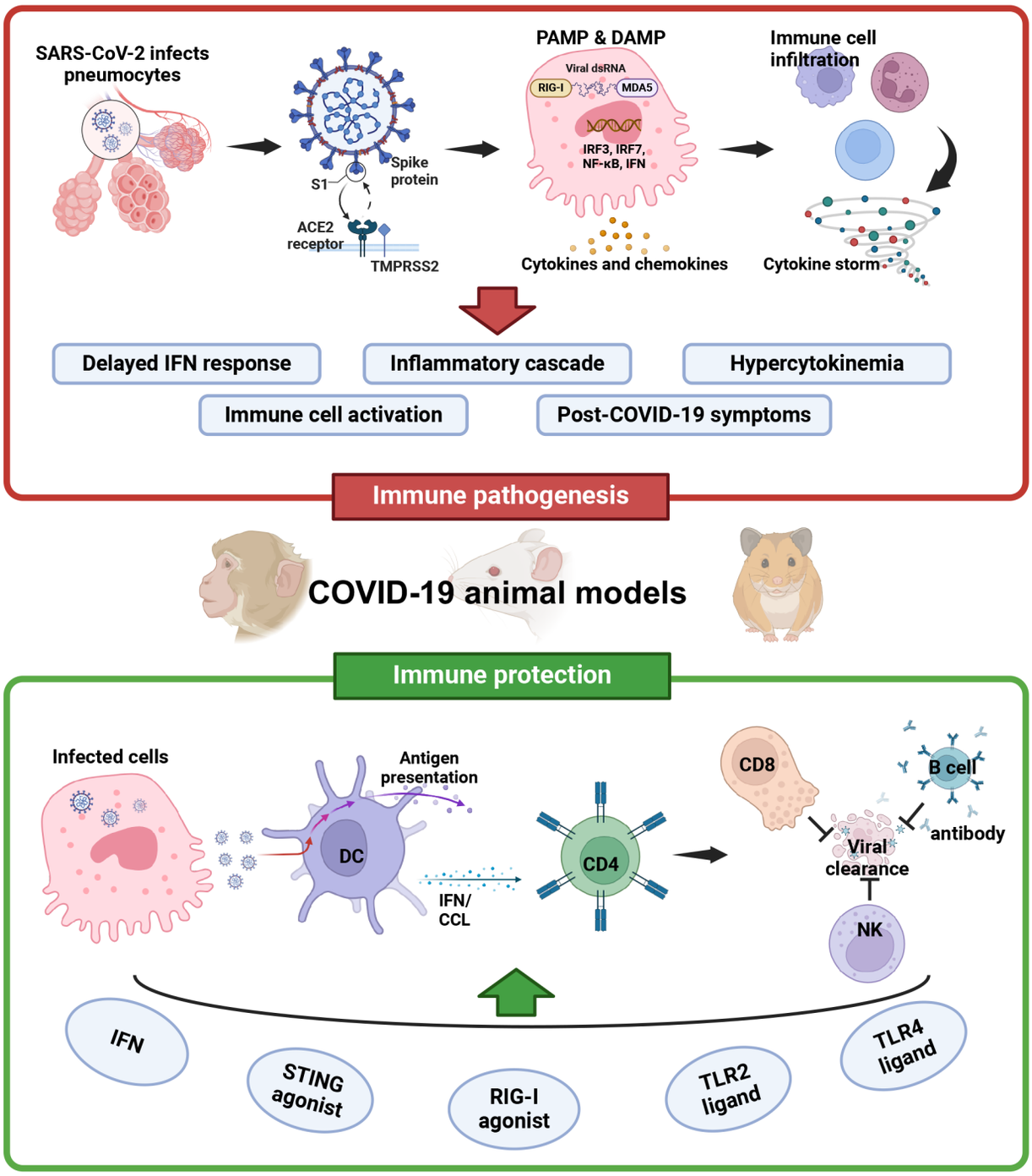

Figure 2 Infection immunology based on COVID-19 animal models

Application of animal models of SARS-CoV-2 infection has promoted systematic research on COVID-19 immunopathology. Animal experiments on vaccines and adjuvants have advanced our understanding of the immunoprotective mechanisms against SARS-CoV-2 infection. Figure created using BioRender (https://biorender.com/).

model, we can gain a deeper understanding of the immunopathological mechanism underpinning SARS-CoV-2 infection.

IFN-I response: The role of IFN-I in SARS-CoV-2 infection is complex and extensively studied. IFN-I is crucial for antiviral immunity, exerting various antiviral effects and promoting immune cell recruitment, thereby playing a protective role against SARS-CoV-2 infection (Zhang et al., 2020a). However, evidence also links a strong IFN-I response and increased ISG expression with the development of severe COVID-19 (Lee & Shin, 2020; Zhu et al., 2020). This abnormal IFN-I response in COVID-19 is further supported by animal models. Notably, C57BL/6J mice infected with the SARS-CoV-2 MA10 strain do not develop severe disease, while those lacking IFN-I and IFN-II receptors develop more pronounced and longer-lasting pulmonary dysfunction after infection, confirming the protective role of IFN-I signaling (Leist et al., 2020). Studies have shown that SARS-CoV-2 suppresses IFN-I expression, promoting infection in models involving rhesus macaques and Syrian hamsters (Francis et al., 2021; Sui et al., 2021a). However, most animal models have reported an association between IFN-I signal intensity and

disease severity. For instance, AAV-hACE2-transfected IFN-α/β receptor (IFNAR)$^{-/-}$ and IRF3/7$^{-/-}$ mice have revealed that the IFN-I response post-SARS-CoV-2 infection is essential for the recruitment of proinflammatory monocytes and macrophages to lung lesions, which induces inflammation (Israelow et al., 2020). Prolonged IFN signaling in mice with respiratory tract infection disrupts lung epithelial cell repair, thereby increasing disease severity and susceptibility to bacterial infection (Broggi et al., 2020; Major et al., 2020). In baboons, SARS-CoV-2 infection leads to a higher production of inflammatory factors, including IFN-α, compared to rhesus macaques, resulting in more severe lung pathology (Singh et al., 2020). K18-hACE2 transgenic mice infected with SARS-CoV-2 exhibit high and persistent IFN-I, IFN-II, and IFN-III expression, highlighting the correlation between IFN expression and COVID-19 severity (Oladunni et al., 2020; Zheng et al., 2021b).

Inflammatory response: Activation of inflammasomes by SARS-CoV-2 infection initiates an inflammatory cascade, which can, in severe cases, lead to a lethal cytokine storm and systemic organ failure (Mehta et al., 2020). Animal models have been instrumental in elucidating the inflammation caused by SARS-CoV-2 infection and its role in disease pathogenesis. NHPs infected with SARS-CoV-2 exhibit typical clinical symptoms, such as fever, cough, dyspnea, and interstitial pneumonia. However, severe lung injury and diffuse alveolitis are primarily observed in rhesus macaques and African green monkeys (Azkur et al., 2020; Yuan et al., 2021). This severe condition is marked by thickening of the alveolar wall and infiltration of numerous monocytes and lymphocytes, as well as a small number of eosinophils (Shan et al., 2020). Beyond the respiratory tract, NHP models also show pathological changes in the heart, liver, kidneys, spleen, lymph nodes, and brain, although direct evidence of SARS-CoV-2 infection in these tissues is still lacking (Lu et al., 2020; Philippens et al., 2022). Research involving K18-hACE2 transgenic mice has shown that lung function impairment is associated with the infiltration of monocytes, neutrophils, and activated T cells, leading to a heightened innate immune response (Winkler et al., 2020). Similarly, Syrian hamsters infected with SARS-CoV-2 exhibit symptoms such as interstitial pneumonia, inflammatory cell infiltration, alveolar septum thickening, and vascular system injury, as well as a pulmonary immune response driven by macrophages (Bednash et al., 2022; Mulka et al., 2022). These findings suggest that inflammatory injury induced by SARS-CoV-2 is more critical than the multiorgan damage resulting from direct viral infection.

The cytokine storm associated with COVID-19 is a key pathogenic factor and potential therapeutic target in COVID-19 patients (Zanza et al., 2022). Clinical studies have highlighted the significant role of cytokines such as IL-6, IL-1, IL-17, and TNF-α in lung injury (Montazersaheb et al., 2022). In cases of severe COVID-19, SARS-CoV-2 induces a chronic immune response mediated by transforming growth factor-β (TGF-β), which promotes fibrosis (Ferreira-Gomes et al., 2021). SARS-CoV-2-infected NHPs also show elevated expression of inflammatory cytokines, including IL-10, IL-1A, IL-8, IL-15, MCP-1, IFN-β, and IP-10, but unlike in severe COVID-19 patients, IL-6 is not highly expressed (Lu et al., 2020; Song et al., 2020), underscoring its critical role in cytokine storms. Transcriptomic analysis of lung tissue from SARS-CoV-2-infected mice with severe disease has shown that antiviral responses in younger mice are dominated by IFN and IL-6 pathway activation, while fatal outcomes in older animals are associated with TNF and TGF-β signaling, highlighting the significant impact of age on cytokine response in COVID-19 (Bader et al., 2023). In the Syrian hamster model of SARS-CoV-2 infection, various cytokines, such as IFN-α, IL-6, IL-1β, and TNF, are associated with severe lung disease, consistent with clinical observations (Fomin et al., 2023; Francis et al., 2021).

Immune cell activation: Clinical studies have demonstrated that cytokine storms in COVID-19 are driven by the robust activation of monocytes and macrophages, leading to the development of SARS-CoV-2-related complications, including ARDS, disseminated intravascular coagulation syndrome (DICS), edema, and pneumonia (Kosyreva et al., 2021; Liao et al., 2020). Similarly, SARS-CoV-2-infected K18-hACE2 transgenic mice exhibit pronounced recruitment of pulmonary immune cells, including dendritic cells (DCs), innate myeloid cells (IMMs), and CD4$^+$ and CD8$^+$ T lymphocytes (Zheng et al., 2021b). In rhesus macaques, SARS-CoV-2 infection induces rapid recruitment of macrophage subsets, particularly CD163$^+$ mannose receptor C-type 1 (MRC1)- and triggering receptor expressed on myeloid cells 2 (TREM2)$^+$, which are primary sources of inflammatory cytokines (Upadhyay et al., 2023). In ferrets, SARS-CoV-2 infection also results in notable alterations in the composition of macrophage subsets in bronchoalveolar lavage fluid (BALF), with monocyte-derived M1 and M2 macrophages playing pivotal roles in both early viral clearance and late-phase excessive inflammation (Lee et al., 2021). Furthermore, in Syrian hamsters, SARS-CoV-2 infection induces rapid and extensive infiltration of monocyte-derived macrophages (MDM) in the lungs, contributing to tissue remodeling and fibrosis via the up-regulation of prothrombotic factors, tissue repair, and alveolar cell proliferation (Bagato et al., 2024). Neutrophils, the most abundant white blood cells, are the first to infiltrate infected lungs following viral infection. Chemokine (C-X-C motif) ligand 5 (CXCL5) plays an important role in the process of neutrophil recruitment, with its knockout in SARS-CoV-2-infected mice found to significantly reduce pulmonary inflammation (Liang et al., 2020).

Hospitalized COVID-19 patients, especially those with severe disease, frequently exhibit a notable decrease in blood lymphocyte counts (Liu et al., 2020a; Sekine et al., 2020). Autopsies have revealed T cell infiltration and migration in inflamed tissues, suggesting that the decrease in blood lymphocytes may be due to their relocation to infection sites (Adamo et al., 2021; Jafarzadeh et al., 2020). However, the percentage of CD8 T cells in the BALF is lower in severe patients compared to those with moderate disease, possibly due to apoptotic signals (André et al., 2022; Liao et al., 2020). Despite variations in T cell numbers, their activation in SARS-CoV-2 infection-induced pulmonary inflammation is significantly increased. This heightened activation, characterized by overactivation, depletion, or apoptosis sensitivity, is identified through various cell surface markers, including CD38, human leukocyte antigen-DR isotype (HLA-DR), programmed cell death 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), T cell immunoglobulin and mucin domain-containing protein 3 (Tim-3), and natural killer (NK) cell receptor A (NKG2A). These activated T cells are closely linked to the development of severe COVID-19 (Du et al., 2021; Georg et al., 2022; Zheng et al., 2020), as supported by various animal models. For example, Zheng

et al. (2021a) observed a significant increase in T cell CXCR3 and CD38 expression in the lung tissues of elderly macaques infected with SARS-CoV-2, indicative of both activation and inflammation and potentially valuable for predicting severe COVID-19. Similarly, SARS-CoV-2-infected rhesus macaques exhibit a marked increase in T cell activation markers in their lung tissues and cerebrospinal fluid (Verma et al., 2021), while SARS-CoV-2-infected mice show a significant up-regulation in $CD44^+CD3^+CD8^+$T cell activation in their lungs, accompanied by alveolar macrophage loss (Winkler et al., 2020). In K18-hACE2 mice, T cell activation is correlated with diffuse microglial activation and plays a critical role in SARS-CoV-2 brain infection (Seehusen et al., 2022). Wu et al. (2021) found that the SARS-CoV-2 membrane (M) protein activates STAT1 phosphorylation and T-bet transcription via TREM-2, contributing to T cell activation. Experiments using CD4-specific conditional TREM-2 knockout mice have demonstrated that TREM-2 enhances the TH1 response and aggravates lung injury.

Immune protection against SARS-CoV-2

Following the SARS-CoV-2 outbreak, the development of vaccines and drugs necessitated relevant animal studies to evaluate their safety and efficacy *in vivo*. Various NHP models, particularly rhesus macaques, have been employed to test the efficiency of different SARS-CoV-2 vaccines developed using different technologies, including inactivation (A'La et al., 2023; Chen et al., 2021a), spike protein or RBD subunit expression (Guebre-Xabier et al., 2020; Prenafeta et al., 2023), DNA (Yu et al., 2020a), mRNA (Oh et al., 2023; Vogel et al., 2021), and attenuated viral vectors (Feng et al., 2020; Jacob-Dolan et al., 2021). Mouse and hamster models have primarily been applied for testing new vaccines and adjuvants and studying immunoprotective mechanisms, such as immune-stimulating adjuvant chimeric vaccines (Ashhurst et al., 2022), multivalent vaccines (Afkhami et al., 2022), lipid nanoparticle vaccines (Elia et al., 2021), adenovirus vaccines (Port et al., 2023), DC vaccines (Tada et al., 2023), novel adjuvants (Machado et al., 2023; Vijayanand et al., 2023), and cross-protection mechanisms (Liu et al., 2023a). The effects of different vaccine delivery modes on immune efficacy have also been verified using animal models. In addition to traditional intramuscular injection, oral (Beddingfield et al., 2021; Langel et al., 2022; Yu et al., 2022b) and intranasal vaccines (Langel et al., 2022; Sui et al., 2021b; Tokunoh et al., 2023) have also been shown to provide protection. These animal models have significantly enhanced our understanding of the immunoprotective mechanisms against SARS-CoV-2 (Figure 2).

Protective adaptive immune response: Animal models have been instrumental in elucidating the importance of adaptive immune protection in SARS-CoV-2 infection. Speranza et al. (2022) discovered that older rhesus macaques exhibit a sustained inflammatory innate response to SARS-CoV-2 infection, while younger animals initiate an earlier local effector T-cell response, leading to more rapid recovery of immune homeostasis. A key immunological feature of SARS-CoV-2-infected NHPs and hamsters is their ability to develop protective immunity, which not only protects against secondary infection but may also cross-react with other coronaviruses (Chandrashekar et al., 2020; Horiuchi et al., 2021; Jacob-Dolan et al., 2021). McMahan et al. (2021) demonstrated that the transfer of purified IgG from convalescent rhesus macaques protects recipient macaques from SARS-CoV-2, highlighting that $CD8^+$ T cell responses can confer protection even with low or weak antibody levels. The $CD4^+$ T cell response in rhesus macaques is strongly correlated with the efficacy of SARS-CoV-2 spike protein mRNA vaccines and neutralizing antibody levels (Corbett et al., 2021). In mice, the IFN-I pathway-mediated virus-specific T cell immune response is critical for protecting against severe SARS-CoV-2 infection (Zhuang et al., 2021). $CD8^+$ T cells are essential for early control and elimination of SARS-CoV-2, while $CD4^+$ T cells play a crucial role in eliciting antibody responses (Israelow et al., 2021), underscoring the importance of adaptive immunity in mitigating disease progression and preventing secondary infections. Despite the significant role of T cells in recovery from acute SARS-CoV-2 infection in rhesus macaques, their depletion does not result in severe disease, suggesting that T cells alone do not fully account for the innate resistance of rhesus macaques to severe COVID-19 (Hasenkrug et al., 2021).

The respiratory mucosal surface is a crucial site for interactions between SARS-CoV-2 and the immune system, where mucosal antibodies and tissue-resident memory T (TRM) and B cells provide early antiviral immune responses (Lee & Oh, 2022; Zheng & Wakim, 2022). However, challenges in sampling from human mucosal sites, especially the lower respiratory tract, have hindered studies on local immunity to SARS-CoV-2. Animal model vaccination experiments have offered vital insights into mucosal immune memory following SARS-CoV-2 infection. Tang et al. (2022) demonstrated that mice immunized with a combination of systemic mRNA vaccine and mucosal AdV-S protein vaccine exhibit a strong neutralizing antibody response, providing better respiratory mucosal protection than the systemic mRNA vaccine alone. Intratracheal inoculation of rhesus macaques with a bivalent SARS-CoV-2 vaccine based on Ad26 significantly induces humoral and cellular immunity in mucosa, with almost complete protection against SARS-CoV-2 BQ.1.1 challenge (McMahan et al., 2024). Furthermore, intranasal delivery of the ChAdOx1 vaccine induces potent respiratory RBD-specific IgA antibody titers, antibody-dependent neutrophil and monocyte phagocytosis, complement activation, NK cell activation, and reduced viral shedding following SARS-CoV-2 infection (Oh et al., 2021). Intranasal immunization with trivalent COVID-19 AdV vaccine elicits a robust multifunctional respiratory tract mucosal TRM response in K18-hACE2 mice, enhancing mucosal protection and conferring complete protection against SARS-CoV-2 infection-induced disease or mortality (Afkhami et al., 2022). Research on SARS-CoV-2-infected K18-hACE2 mice has also shown that respiratory memory T cells, both $CD4^+$ and $CD8^+$, induced by a subunit-based adjuvant system, can protect against SARS-CoV-2 infection, even in the absence of virus-neutralizing antibodies (Van Doremalen et al., 2021).

Protective innate immune response: The efficacy of COVID-19 vaccines may be reduced in certain populations, such as the elderly, highlighting the need for more personalized vaccination regimens. Targeted activation of innate immune signals has gained attention as a strategy to elicit strong immune responses in vaccine development. Studies have shown that mRNA vaccines can enhance the innate immune response in obese mice with impaired adaptive immunity by enhancing IFN-I signaling and providing protection against SARS-CoV-2 infection (Chen et al., 2023).

In rhesus macaques, mRNA-based vaccines can effectively stimulate all immune system components and improve the connection between innate and adaptive immunity after a second dose via key mediators, such as TNFα, IFNγ, IL-21, and chemokine (C-C motif) ligand 3 (CCL3) (Schramm et al., 2023). Marx et al. (2022) found that therapeutic RNA oligonucleotides, acting as RIG-I agonists, can trigger a robust NK cell response in K18-hACE2 mice, providing transient yet potent antiviral protection against SARS-CoV-2 infection. TLR2, an important innate immune receptor on respiratory epithelial and lung immune cells, is crucial for effective defense against infection (Beckett et al., 2012). The TLR2 ligand Pam 2 Cys, when used as a mucosal adjuvant in COVID-19 vaccines, enhances the recruitment of antigen-presenting cells (APCs), including DCs, alveolar macrophages, and monocytes, to the respiratory mucosa, leading to robust activation of the IL-17^{+}CD4^{+} T cell response and significantly boosting the protective efficacy of COVID-19 vaccines in murine models (Ashhurst et al., 2022). Lymphoblastoid cells play a crucial role in shaping the microenvironment of lymph nodes and the structure and function of the germinal center (GC). Age-related decline in GC response impedes the establishment of durable humoral immunity post-vaccination (Masters et al., 2018). Denton et al. (2022) discovered that TLR4 agonists can promote the maturation and expansion of follicular DCs (FDCs) and the activation and proliferation of stromal cells, thus enhancing GC immune response initiation and COVID-19 vaccine efficacy in aged mice. Additionally, the RBD dimer fusion protein carrying IFN exhibits potential as a candidate for COVID-19 vaccination by targeting DCs in lymph nodes and stimulating T follicular helper (Tfh) cell differentiation and GC formation, providing complete protection against high-dose SARS-CoV-2 challenge in rhesus macaques (Sun et al., 2021).

Protective immune response against novel SARS-CoV-2 variants: In late 2020, after nearly a year of transmission in humans, SARS-CoV-2 underwent significant adaptive changes. These highly mutated variants, referred to as “variants of concern” (VOC), exhibit higher transmission rates than earlier strains. To date, the WHO has identified five SARS-CoV-2 variants as VOCs, including Alpha, Beta, Gamma, Delta, and Omicron, all of which exhibit significant changes in transmissibility or immune escape, warranting close surveillance (Carabelli et al., 2023). As of 9 February 2024, the primary circulating variants of interest include XBB.1.5, XBB.1.16, EG.5, BA.2.86, and JN.1 (https://www.who.int/activities/tracking-SARS-CoV-2-variants). These variants have numerous nonsynonymous mutations in the spike protein and unique phenotypic characteristics, leading to changes in transmissibility and antigenicity, which facilitate evasion of host immune responses. This evolution may reduce the effectiveness of current SARS-CoV-2 vaccines (Kim et al., 2022). Therefore, it is crucial to use appropriate animal models to further study the etiology, transmission, and pathogenesis of the virus and to evaluate the efficacy of vaccines against the latest SARS-CoV-2 variants.

Compared to the Delta variant, the Omicron BA.1 and BA.2 variants have been shown to exhibit weakened lower respiratory tract replication and lower pathogenicity in a variety of animal models, including rodents and NHPs (Boon et al., 2022; Halfmann et al., 2022; Van Doremalen et al., 2022). Halfmann et al. (2023) reported that the Omicron subvariant XBB.1.5 exhibited greater airborne transmissibility than its predecessor BA.2 and partial immune escape from previous infection with BA.1 in Syrian hamsters. Tamura et al. (2024) found that nonsense mutations in ORF8 of XBB.1.5 impaired MHC suppression and were associated with reduced virulence in Syrian hamsters infected with this subvariant. XBB.1.16, an independent variant from XBB.1.5, has two amino acid substitutions in its spike protein compared to XBB.1.5: E180V in the N-terminal domain and T478R in the receptor-binding domain (Yamasoba et al., 2023). Pseudovirus experiments have shown that XBB.1.16 has similar infectivity to XBB.1.5 and similar sensitivity to XBB.1 in convalescent serum as XBB.1 and XBB.1.5 (Tamura et al., 2023). Meehan et al. (2023) developed a digital pathology algorithm to quantitatively assess respiratory lesions caused by SARS-CoV-2, confirming that Omicron subvariants BA.2.75 and EG.5.1 have regained some virulence, although not to the levels previously observed in Omicron. Similar to XBB.1.5, EG.5.1 is more transmissible among hamsters, but exhibits significantly enhanced immune evasion characteristics, and shows no significant differences in replication ability or pathogenicity compared to BA.2 (Uraki et al., 2023). Herder et al. (2023) evaluated the virulence of BA.2.86 and BA.2.75, among the most virulent Omicron subvariants, and found that BA.2.86 exhibited an attenuated phenotype in hamsters, suggesting no greater risk to public health than its parental Omicron subvariants. JN.1, derived from BA.2.86, harbors a key mutation (S:L455S) in the spike protein, which enhances its immune evasion ability and resistance to monovalent XBB.1.5 vaccine serum (Altamimi et al., 2024). However, the lack of animal models associated with JN.1 has hindered pathology and vaccine development.

Several animal-based vaccine studies have been conducted against novel SARS-CoV-2 variants. An Omicron (BA.1)-specific mRNA vaccine has shown effective protection in hamsters against BA.1 at 253 days after immunization, and moderate neutralizing activity against Omicron subvariants in rhesus macaques at 9 months after immunization (Wu et al., 2023b). Immunization of mice and rhesus macaques with the XBB.1.5-based monovalent recombinant spike protein COVID-19 vaccine induces neutralizing antibodies against the XBB.1.5, XBB.1.16, XBB.2.3, EG.5.1, and XBB.1.16.6 subvariants and elicits a CD4^{+} Th1 cell response specific to the XBB subvariant (Patel et al., 2023). The Omicron XBB.1.5 RBD dimer-based vaccine completely protects mice from Omicron XBB.1.16 and significantly reduces respiratory viral infection in Syrian hamsters, demonstrating excellent protective efficacy against SARS-CoV-2 and its variants (Wu et al., 2023a). Wang et al. (2024) identified a highly conserved neutralizing epitope targeted by the broad-spectrum neutralizing antibody BA7535, which was highly neutralizing not only to previous variants (e.g. Alpha, Beta, Gamma, Delta and Omicron BA.1-BA.5) but also to recently emerging Omicron subvariants BF.7, CH.1.1, XBB.1, XBB.1.5, XBB.1.9.1, and EG.5. BA7535 effectively protects female mice from Omicron BA.5 and XBB.1 variants. The novel ChAd-SARS-CoV-2 vaccine, consisting of a bivalent Ad36 vector vaccine encoding SARS-CoV-2 WA1 and BA.5 spike proteins, induces persistent antibody and T cell responses against XBB.1.16 infection in rhesus macaques immunized by intranasal spray or inhalation aerosol (Gagne et al., 2023). The new generation of RBD nano-antibodies neutralizes

Alpha, Delta and Omicron BA.2.75, BA.1, BA.2, BA.4/5 and XBB.1 variants, significantly reducing viral load, weight loss, and pathogenicity in infected hamsters (Aksu et al., 2024).

IMMUNOLOGICAL INSIGHTS OF POST-COVID-9 SYMPTOMS (LONG COVID-19) FROM ANIMAL MODELS

Development of long COVID-19

Although most individuals fully recover from SARS-CoV-2 infection within days or weeks, some experience persistent or new symptoms months or even years later, a condition known as long COVID-19 (Schmidt, 2021). This complex multisystem disorder is characterized by over 200 identified symptoms, including fatigue, dyspnea, arthralgia, myalgia, cardiac arrhythmias, and cognitive impairments, affecting up to 75% of those who have recovered from COVID-19 (Jansen et al., 2022). Huang et al. (2022) found that patients infected with the original strain of SARS-CoV-2 continued to experience long-term symptoms more than 2 years after hospital discharge, although most symptoms had improved. Significant risk factors for long COVID-19 include female sex, obesity, and severe COVID-19 disease; however, even outpatients with mild symptoms may experience prolonged COVID-19 manifestations (Frontera et al., 2022; Subramanian et al., 2022). The original SARS-CoV-2 strain is associated with a higher incidence of long COVID-19 compared to the Alpha or Delta variants (Fernández-de-las-Peñas et al., 2022). In contrast, the Omicron variant is associated with a lower risk of long-term pneumonia compared to the Delta variant (Antonelli et al., 2022; Wise, 2022). Older adults infected with SARS-CoV-2 are more likely to suffer from long COVID-19, presenting with fatigue, difficulty breathing, coughing, and joint pain, as well as abnormalities in chest imaging and lung function tests. The situation is more complex for children and adolescents (Daitch et al., 2022), with immunocompromised children showing a higher prevalence of gastrointestinal symptoms associated with long COVID-19, while immunocompetent children report increased levels of fatigue (Roessler et al., 2022).

The mechanisms behind long COVID-19 and reasons for differences in symptoms across viruses and populations are unclear. Key hypotheses include changes in the immune system, persistence of residual viral components driving chronic inflammation, endothelial dysfunction or activation, changes in the microbiome, mitochondrial dysfunction, abnormal metabolites, reactivation of pre-existing chronic viral infections, microbiota dysbiosis, and unrepaired tissue damage (Davis et al., 2023; Liu et al., 2023b; Santopaolo et al., 2023). From an immunological perspective, uncontrolled immune dysregulation following SARS-CoV-2 clearance is an important contributor to the development of long COVID-19. In-depth immunophenotyping analysis has revealed significant gene expression interference in innate immune cells (such as NK cells, low-density neutrophils, and $CXCR3^+$ monocytes) and adaptive immune cells (such as T helper (Th) cells, Tfh cells, and regulatory T cells) in long-term COVID-19 patients (Ryan et al., 2022). Klein et al. (2023) demonstrated that long COVID-19 patients exhibit an excessive humoral response to SARS-CoV-2 and higher levels of antibody response to non-SARS-CoV-2 viral pathogens, especially Epstein-Barr virus. Single-cell omics and serologic studies have also revealed systemic inflammation and immune dysregulation in patients with long COVID-19, characterized by an increased frequency of inflammatory $CD4^+$ T cells and an exhausted phenotype of virus-specific $CD8^+$ T cells, indicating disharmony between humoral and cell-mediated responses (Yin et al., 2024). Despite these findings, clinical challenges remain in identifying potential therapeutic targets for COVID-19, necessitating urgent *in vivo* intervention studies using animal models.

Animal models of long COVID-19

Animal models for long COVID-19 are still in the early stages. Paidas et al. (2022) inoculated mice with mouse hepatitis virus 1 (MHV-1), a member of the same β-coronavirus family as SARS-CoV-2, for 12 months to simulate long COVID-19, finding severe pathological changes and inflammation in their brain, lungs, and heart. Although not a direct SARS-CoV-2 infection model, these results imply that coronaviruses can have long-term effects, even after viral clearance. Sefik et al. (2022) examined innate and adaptive immune responses to SARS-CoV-2 infection in an AAV-hACE2 mouse model over 28 days, revealing characteristics of chronic COVID-19, such as progressive weight loss, persistent viral RNA presence, lung pathology accompanied by fibrosis, inflammatory macrophage response, sustained expression of ISGs, and depletion of T lymphocytes. Mice infected with SARS-CoV-2 for up to 120 days exhibit persistent inflammation and fibrosis, important features of chronic COVID-19 lung injury, as well as an increase in interstitial macrophages (Dinnon et al., 2022). The neurological symptoms associated with SARS-CoV-2 infection may potentially arise from cytokine storm, neuroimmune stimulation, and systemic infection, rather than direct viral mechanisms of injury (Efstathiou et al., 2022). Evidence suggests that SARS-CoV-2 infection-induced CCL11 evokes a selective microglial response in white matter, leading to dysfunction and structural dysregulation of multiple neuronal lineages in the central nervous system, contributing to cognitive impairment during the acute phase following COVID-19 (Li et al., 2022). In Syrian hamsters, 31 days of SARS-CoV-2 infection results in permanent lung and kidney damage, along with unique effects on the olfactory bulb and olfactory epithelium, including myeloid and T cell activation, proinflammatory cytokine production, and IFN responses, leading to neurobehavioral disorders such as anosmia and depression (Frere et al., 2022). Due to the lack of animal models of long COVID-19 and the high cost of BSL-3 laboratories, it is difficult to summarize the immunological mechanisms underlying COVID-19 development in a small number of cases, hindering the advancement of appropriate treatments. Solving this issue requires the development of pseudovirus and transgenic mouse models suitable for establishing animal models for long COVID-19.

APPLICATION OF ANIMAL MODELS IN COVID-19 IMMUNOTHERAPY

Following the COVID-19 outbreak, various new and repurposed compounds exhibiting *in vitro* anti-coronavirus activity have rapidly advanced towards clinical application. Prior to the development of an effective SARS-CoV-2 vaccine, the US Food and Drug Administration (FDA) granted emergency use authorization (EUA) for a nucleoside analog and three monoclonal antibodies (MAbs) (Tao et al., 2021). Despite widespread vaccination, developing drug therapy for SARS-CoV-2 remains crucial, particularly for immunocompromised individuals and those who may not respond to the rapidly mutating virus (Boby et al., 2023).

Current COVID-19 antiviral drugs are primarily divided into the following categories: ① Polymerase inhibitors, which act on RNA-dependent RNA polymerase (RdRps), mainly nucleoside analogs, including remdesivir, molnupiravir, and nelfinavir, and have been validated in rhesus monkeys (Johnson et al., 2023; Tao et al., 2021; Williamson et al., 2020) and Syrian hamsters (Abdelnabi et al., 2021); ② Protease inhibitors, which act on 3C-like proteinase protein (3CLpro), mainly nirmatrelvir, PF-07321332, S-217622, ensitrelvir, and have been validated in rhesus monkeys (Rosenke et al., 2023), Syrian hamsters (Sasaki et al., 2023; Tao et al., 2021), and mice (Jeong et al., 2022; Tao et al., 2021); ③ Entry inhibitors, including antibodies, fusion inhibitors, soluble recombinant hACE2, which have achieved good results in SARS-CoV-2 infected rhesus monkeys (Streblow et al., 2023), mice (Morgan et al., 2022; Tao et al., 2021), Syrian hamsters (Linsky et al., 2020; Uraki et al., 2022), and ferrets (De Vries et al., 2021). The rapid development of COVID-19 immunotherapy research, relying on *in vivo* testing, can be attributed to the establishment of diverse animal models for SARS-CoV-2 infection, leading to numerous potential therapeutic drugs and protocols. These findings will serve as a valuable resource for addressing future infections, even if COVID-19 ceases to be a major public health threat. This discussion will not delve into the efficacy and mechanisms of these antivirals but will instead focus on trial outcomes of immunomodulatory drugs in COVID-19 animal models (Figure 3).

Targeting immune receptors

Since the early stages of the global pandemic, research has focused on targeting immune receptors for COVID-19. Early studies revealed that severe COVID-19 patients often show reduced T-cell counts, linked to poor clinical outcomes and indicating potential T-cell exhaustion (Liu et al., 2020b; Zheng et al., 2020). Lymphopenia in severe COVID-19 is linked to the overexpression of immune checkpoint receptors PD-1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), TIGIT, and Tim-3 (Jin et al., 2023; Yin et al., 2024). With numerous inhibitory receptor antagonists already in clinical trials and available for use, their rapid implementation in COVID-19 trials is likely, minimizing the necessity for preclinical animal testing (Loretelli et al., 2021; Tan & Li, 2022). Given our advancing comprehension of the interactions between SARS-CoV-2 and immune receptors, the validation of novel immune receptor-targeted drugs will largely depend on animal models to confirm their efficacy.

SARS-CoV-2 enters host cells and is recognized by TLRs, NOD-like receptors (NLRs), and NLR protein 3 (NLRP3), initiating IFN-I production and innate immune reactions. Targeting these immune receptors therapeutically may help ameliorate infection-induced inflammation. TLRs, crucial for recognizing SARS-CoV-2 and initiating innate immunity, are expressed in various cell types, including immune cells, fibroblasts, epithelial cells, and ACE2-expressing type II lung cells (Jiang et al., 2022; Qi et al., 2020). TLRs, including those in human (TLR1–TLR10) and mouse cells (TLR1–TLR9 and TLR11–TLR13), are broadly categorized into cell surface TLRs, including TLR1, TLR2, TLR4–6, and TLR10, and intracellular somatic membrane TLRs, including TLR3 and TLR7–9 (O'Neill et al., 2013). Upon activation, TLRs recruit specific bridging molecules like myeloid differentiation primary response protein 88 (MyD88) and Toll/IL-1 receptor (TIR) domain-containing linker-induced interferon β (TRIF), which trigger the IRF and NF-κB signaling pathways to initiate IFN-I and inflammatory cytokine production (Jiang et al., 2022). TLR-mediated immune dysregulation plays a critical role in hyperinflammation and cytokine storm observed in COVID-19, making it a potential target for intervention (Liu et al., 2022). Zheng et al. (2021c) demonstrated that the SARS-CoV-2 E protein induces TLR2-related inflammatory responses in mice,

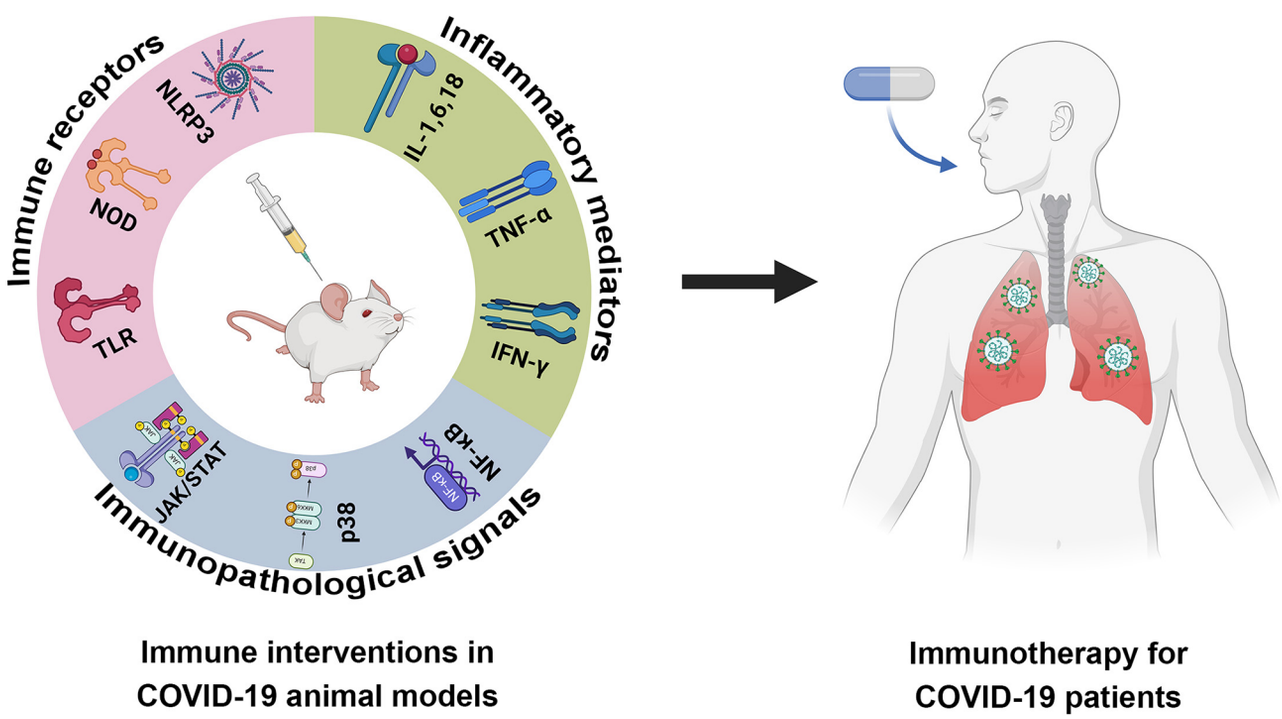

Figure 3 COVID-19 immunotherapy based on animal models

Validation of immune interventions targeting immune receptors, inflammatory mediators, and immunopathological signals in SARS-CoV-2-infected animal models is crucial for the development of COVID-19 immunotherapy. Figure created using BioRender (https://biorender.com/).

while inhibition of TLR2 with oxPAPC significantly attenuates the release of proinflammatory cytokines and chemokines. Fontes-Dantas et al. (2023) reported that intracerebral administration of the spike protein in mice induces neuroinflammation and synaptic loss through TLR4-mediated microglial activation, with early intervention with TLR4 inhibitors shown to prevent late neuronal damage in mice, identifying TLR4 as a potential target for long-term cognitive dysfunction induced by COVID-19. The role of TLRs in COVID-19 is complex, extending beyond immunopathology. Pre-COVID-19 studies indicated that MyD88- and TRIF-deficient mice infected with SARS-CoV experience higher mortality rates, weight loss, and viral loads, suggesting that MyD88 and TRIF are essential for preventing fatal SARS-CoV infection (Sheahan et al., 2008; Totura et al., 2015). Furthermore, combining a TLR9 agonist with mRNA enhances both T cell and neutralizing antibody responses against RBD in mice, compared to mRNA alone (Haabeth et al., 2021). Treating K18-hACE2 transgenic mice with a lethal SARS-CoV-2 dose and the synthetic agonist Poly(I:C) for TLR3/MDA5 temporarily boosts the innate immune response in the lungs, subsequently reducing viral load and cytokine storm levels, and significantly improving survival (Tamir et al., 2022).

Inflammatory processes at the cellular level are typically mediated by inflammasomes, particularly NLRP3 inflammasomes, which are highly responsive to RNA viruses. NLRP3 inflammasomes are composed of the NLRP3 receptor, apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (ASC), and caspase-1. Upon assembly, caspase-1 is activated by autoproteolytic cleavage, facilitating the maturation of proinflammatory cytokines such as IL-1β, IL-18, IL-6, TNF-α, and gasdermin-D (GSDMD), which all contribute to the development of COVID-19-induced cytokine storm (Broz & Dixit, 2016; Dutta et al., 2022). Increasing evidence suggests that hyperactivation of NLRP3 inflammasomes plays a role in the pathogenesis of SARS-CoV-2-induced multiorgan failure (Ratajczak & Kucia, 2020). Studies have indicated that $Nlrp3^{-/-}$ mice, which lack NLRP3 inflammasomes, exhibit reduced severity of SARS-CoV-2-induced lung pathology compared to wild-type C57BL/6 mice, while specific inhibition of MCC950 reduces excessive lung inflammation in SARS-CoV-2-infected hACE2 transgenic mice, confirming NLRP3 as a potential immunotherapeutic target for COVID-19 (Zeng et al., 2022). Albornoz et al. (2023) discovered that SARS-CoV-2 infection activates NLRP3 inflammasomes in microglia through the NF-κB and ACE2 signaling pathways, leading to neurodegeneration in hACE2 transgenic mice, with these effects significantly mitigated and survival rates improved with oral administration of an NLRP3 inhibitor. These findings suggest that targeting NLRP3 may be beneficial in alleviating neurocognitive disorders associated with long-term SARS-CoV-2 infection.

Targeting inflammatory mediators

In the context of acute lung injury in COVID-19, macrophage activation syndrome and ARDS arise from the release of proinflammatory cytokines, such as IL-1, IL-6, IL-18, and TNF-α. Consequently, targeting these cytokines presents significant therapeutic potential for reducing cytokine storm in COVID-19 patients (McGonagle et al., 2020). Rubsamen et al. (2020) noted that early administration of α-IL-6 mAbs in mice infected with Ebola more effectively combated cytokine release syndrome (CRS) than IL-6 receptor blockade alone, offering insights for potential SARS-CoV-2 treatments. Subsequently, Xu et al. (2020b) found that the recombinant human IL-6 mAb tocilizumab binds specifically to soluble and membrane-bound IL-6 receptors (sIL-6R and mIL-6R), inhibiting IL-6-mediated signal transduction and improving clinical outcomes in severe and critically ill COVID-19 patients. IL-18 is also implicated in SARS-CoV-2-induced inflammatory lung injury. Notably, IL-18 is associated with the excessive recruitment, infiltration, and activation of neutrophils observed in the lung tissue of COVID-19 patients, as well as progression of cardiovascular dysfunction (Liang et al., 2023). In hACE2 transgenic mice, exposure to the spike protein increases NF-κB activity and NLRP3-inflammasome-dependent IL-18 levels in heart and lung tissues, with subsequent improvement in cardiac function and reduced NF-κB activity following inhibition of IL-18 (Liang et al., 2023). In K18-hACE2 mice, SARS-CoV-2 infection activates the NLRP3 pyroptosis signaling pathway in the lungs, resulting in IL-1β release, pulmonary vascular injury, and associated protein-rich pulmonary edema, while the selective IL-1 receptor antagonist anastrozole blocks IL-1 receptor signaling and prevents VE-cadherin down-regulation and excessive pulmonary vascular permeability, significantly improving survival (Xiong et al., 2021). Clinical findings indicate that the bispecific IL-1β/IL-18 MAb MAS825 effectively inhibits biomarkers of clinical and inflammatory pathways associated with COVID-19 and promotes the rapid clearance of SARS-CoV-2 (Hakim et al., 2023). Dysregulation of TNF-α, an important proinflammatory cytokine in the innate immune response, can induce CRS (Ragab et al., 2020). Karki et al. (2021) demonstrated that inhibition of TNF-α and IFN-γ can effectively mitigate sepsis, hemophagocytic lymphohistiocytosis (HLH), and cytokine shock in K18-ACE-2 transgenic mice following SARS-CoV-2 infection. Several anti-TNF therapeutics, including Humira (adalimumab), Remicade (infliximab), Simponi (golimumab), Cimzia (certolizumab pegol), and Entyvio (vedolizumab), are currently undergoing clinical trials for COVID-19, with most studies confirming favorable impact on reducing or ameliorating disease progression (Hakim et al., 2023).

Targeting immunopathological signaling pathways

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway is pivotal in the regulation of inflammatory factors and formation of cytokine storms in COVID-19. Consequently, several JAK/STAT signaling inhibitors, including baricitinib, ruxolitinib, and tofacitinib, have been investigated as potential treatments for COVID-19 (Hall et al., 2023; Rein et al., 2022). Despite this, clinical trials have indicated that JAK/STAT signaling inhibitors are less effective than expected in alleviating disease severity in severe COVID-19 cases. Baricitinib effectively inhibits JAK1/2 kinase by competitively blocking adenosine triphosphate (ATP), essential for JAK1/2 activation by inflammatory cytokines such as IL-6, and disrupts various proinflammatory signaling pathways, including IL-6, IL-1β, IL-12, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-2 (Ravid et al., 2022; Zhang et al., 2020b). Additionally, baricitinib effectively attenuates pulmonary inflammation in SARS-CoV-2-infected rhesus macaques by suppressing cytokines and chemokines, which, in turn, reduce neutrophil recruitment and pulmonary macrophage inflammation. However, its effect on viral replication and the IFN-I response

is minimal, resulting in incomplete resolution of immunopathology (Hoang et al., 2021).

Bruton's tyrosine kinase (BTK) is essential for B cell development in the bone marrow and plays a key role in the proliferation and survival of leukemia B cells (Hendriks et al., 2014). Elevated BTK activation levels in blood monocytes from severe COVID-19 patients are associated with systemic inflammation (Roschewski et al., 2020). Ibrutinib, a BTK inhibitor, is widely used in the treatment of indolent B cell malignancies and chronic graft-versus-host disease (cGVHD) and shows potential in eliminating inflammatory cytokines in the lungs, minimizing lung injury, and lowering mortality risk in a lethal influenza mouse model (Florence et al., 2018). Ibrutinib effectively inhibits poly I:C and lipopolysaccharide (LPS)-induced activation of BTK, fms-like tyrosine kinase 3 (FLT3), and epidermal growth factor receptor (EGFR)-related pathways, significantly reducing acute lung injury in mice (Rao et al., 2022). These findings suggest that BTK inhibitors may serve as potential therapeutic agents for regulating SARS-CoV-2-induced acute lung injury. BTK inhibitors, including acalabrutinib, ibrutinib, and zanubrutinib, have been utilized in COVID-19 clinical trials, showing protection against lung injury and reduced inflammatory activity (Kifle, 2021; Treon et al., 2020).

Excessive up-regulation of p38 activity may drive the intense inflammatory response observed in COVID-19 infection through two potential mechanisms: first, the loss of ACE2 after viral entry may hinder the conversion of angiotensin II to angiotensin 1–7, thereby releasing the inhibition of p38 activity; second, SARS-CoV-2 may exploit p38 activity to facilitate its replication (Grimes & Grimes, 2020; Higgins et al., 2023). Therefore, p38 inhibition may be a promising therapeutic strategy for alleviating COVID-19. Jimenez-Guardeño et al. (2014) found that during SARS-CoV infection, syntenin binds to the E protein, leading to syntenin redistribution from the nucleus to the cytoplasm, activating the p38 mitogen-activated protein kinase (MAPK) pathway and promoting excessive inflammatory cytokine expression. Furthermore, the application of p38 MAPK inhibitors significantly improves survival in SARS-CoV-2-infected mice by reducing IL-1α and IL-6 levels in the BALF, decreasing neutrophil infiltration, and alleviating interstitial edema (Gu et al., 2021). Selective p38 signaling inhibitors, such as PH-797804 and VX-702, markedly reduce the expression of proinflammatory cytokines IL6, CXCL8, CXCL10, and TNF-α during SARS-CoV-2 infection, while concurrently reducing viral replication and modulating the IFN-mediated antiviral response (Faist et al., 2023).

The NF-κB pathway plays a central role as an immune regulator in COVID-19, contributing to excessive inflammation and cytokine storms. Inhibiting the NF-κB pathway can suppress the release of various proinflammatory cytokines, chemokines, and adhesion molecules, making it an important potential therapeutic target for COVID-19 (Jiang et al., 2022). During the early phase of infection, the SARS-CoV-2 nucleocapsid protein, present in body fluids, activates NF-κB p65 phosphorylation, promotes M1 macrophage polarization and proinflammatory cytokine expression, and induces acute lung injury. The NF-κB inhibitor pyrrolidine dithiocarbamate alleviates the effects of the N protein on acute lung injury in mouse models (Xia et al., 2021). Auranofin, another NF-κB inhibitor, also exerts inhibitory effects on SARS-CoV-2 replication and inflammatory cytokine expression, including IL-6, TNF-α, and IL-1β, *in vitro* (Cirri et al., 2021; Rothan et al., 2020). Oral administration of auranofin significantly attenuates lung tissue damage, cell infiltration, inflammatory response, and IL-6 production in Syrian hamsters infected with SARS-CoV-2 (Biji et al., 2021). Neufeldt et al. (2022) reported that in human epithelial cells, SARS-CoV-2 triggers an inflammatory immune response via cyclic GMP-AMP synthase (cGAS)-stimulator of IFN genes (STING)-mediated NF-κB activation, which can be attenuated by various STING-targeting drugs, suggesting that cGAS-STING may be a potential target for COVID-19 therapy. However, STING-deficient K18-hACE2 mice do not exhibit altered disease progression after SARS-CoV-2 infection, suggesting that STING deficiency does not impact viral replication or IFN and inflammatory cytokine production (Marino et al., 2023). In contrast, the small-molecule STING agonist diABZI limits SARS-CoV-2 replication in mice and effectively inhibits SARS-CoV-2 infection in multiple viral strains (Li et al., 2021). These findings emphasize the need to verify the effects of immunomodulatory drugs via *in vitro* and *in vivo* experiments and conduct comprehensive validations in animal models before advancing with COVID-19 treatment strategies.

CONCLUSIONS

The COVID-19 pandemic continues to pose unprecedented challenges to global health, particularly in terms of prevention and treatment efforts. While activation of the immune system is critical for defending against invading pathogens, it also plays a key role in COVID-19 pathogenesis. Understanding the interactions between SARS-CoV-2 infection and the host immune system is essential for developing effective prevention and treatment strategies. Animal models have been instrumental in studying COVID-19, uncovering the pathological and immunological mechanisms of SARS-CoV-2 infection and accelerating vaccine and drug development. Future research in COVID-19 immunobiology should focus on optimizing the existing animal model library, incorporating specialized animal strains to better represent diverse human populations, and developing relevant tools and methods to address the challenges posed by the high variability and continuous evolution of novel SARS-CoV-2 variants.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Y.T.Z. conceived the project; H.Y.Z., T.Z.S., and Y.T.Z. wrote the manuscript; Y.T.Z. edited the manuscript. All authors read and approved the final version of the manuscript.

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