

REVIEW

Review of selected animal models for respiratory coronavirus infection and its application in drug research

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Abstract

Numerous viral pneumonia cases have been reported in Wuhan, Hubei in December 2019. The pathogen has been identified as a novel coronavirus, which was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The biological characteristics and pathogenesis mechanism of SARS-CoV-2 are unclear and under progress. At present, no specific preventive and therapeutic drugs are available. Animal models can reproduce the viral replication cycle and the significant functions of respiratory coronavirus infection and are urgently needed to evaluate the efficacy of drugs and vaccines, the transmission route of respiratory coronavirus, clinical features, and so on. We reviewed the current animal models of respiratory coronavirus (SARS-CoV, MERS-CoV, and SARS-CoV-2) infection and made a comparative analysis of the route of inoculation, virus replication, clinical signs, histopathology, application, advantages, and disadvantages. Animal models of respiratory coronavirus include susceptible animal models, genetically modified models, and various animal models of infected virus adaptation strains, such as nonhuman primates, mice, hamsters, ferrets, New Zealand rabbits, cats, and other animal models, all of which have distinct advantages and limitations. This review will provide relevant information and important insights for disease management and control.

KEYWORDS

animal model, coronavirus infection, mechanism, SARS-CoV-2

1 | INTRODUCTION

In late December 2019, the pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) became a serious threat to human and public health. Viral pneumonia caused by SARS-CoV-2 is mainly transmitted by respiratory droplets and contact routes. The incubation period following infection is usually between 1 and 14 days. Fever, cough, myalgia, and weakness are the most common

symptoms. A few patients had symptoms of nasal discharge, headache, and diarrhea. Most patients developed dyspnea within 8 days or so. The most severe patients developed acute respiratory distress syndrome (ARDS), chest computed tomography showed bilateral ground-glass opacity, and the severity of the disease is related to the cytokine storm.¹

SARS-CoV-2 is a new type of human infectious β coronavirus, and until now, there have been seven kinds of coronavirus that have

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caused human respiratory diseases. Coronaviruses are enveloped viruses containing single-stranded RNA and can be divided into α , β , γ , and δ genera, among which α and β coronaviruses are related to human diseases. HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU-1 coronavirus can cause mild respiratory disease and even pneumonia and lower respiratory tract diseases in immunocompromised individuals.³ SARS-CoV, MERS-CoV, and SARS-CoV-2 viruses have high variability and infectivity and can cause SARS. The undefined antigenic variability of coronaviruses makes the development of vaccines and drugs extremely difficult.

Suitable animal models are important experimental tools for studying pathogenic mechanisms, vaccines, and drug development. For example, Remdesivir is a nucleotide analog prodrug that targets the RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 and was the first to be approved by the United States Food and Drug Administration (FDA) for the treatment of hospitalized COVID-19 patients.⁴ The antiviral activity against SARS-CoV-2 was evaluated in mice,⁵ ferret,⁶ hamsters,⁷ and rhesus macaques model.⁸ Molnupiravir (EIDD-2801, MK-4482) is an orally bioavailable NHC-prodrug (β -D-N⁴-hydroxycytidine-5'-isopropyl ester), which is effective against SARS-CoV-2 infections in Syrian hamsters,⁹ mice,¹⁰ and ferrets.¹¹

Current animal models for respiratory coronavirus infection include nonhuman primates, mice, hamsters, ferrets, New Zealand rabbits, cats, and so on. We reviewed the animal models of respiratory coronavirus SARS-CoV (Table 1), MERS-CoV (Table 2), and SARS-CoV-2 (Table 3) infection and made a comparative analysis from the route of inoculation, virus replication, clinical signs, histopathology, application, advantages, and disadvantages.

1.1 | Nonhuman primate animal models

Nonhuman primates, such as macaca mulatta, cynomolgus macaques, the common marmosets, African green monkeys, are generally susceptible to human respiratory coronavirus regarded as ideal experimental models. Among them, Macaca mulattas are the most widely used.

Macaca mulattas were sensitive to SARS-CoV, SARS-CoV-2, and MERS-CoV, and the infection response was apparent.³⁴ In Macaca mulatta, fever develops 2–3 days after nasal inoculation with SARS-CoV. Within 5–60 days, pathological changes in the lungs, including hemorrhage, edema and diffuse lesions, septal macrophages, and lymphocyte infiltration, are continuously observed. SARS-CoV virus can be detected in the throat and nasal swab samples within 1–16 days after infection.¹²

Macaca mulatta infected with SARS-CoV-2 developed respiratory diseases lasting from 8 to 16 days, including cough, weight loss, loss of appetite, pallor, and dehydration, as well as changes in respiratory patterns. Pathological changes included mild to moderate interstitial pneumonia and X-rays of the lungs revealing infiltrates. SARS-CoV-2 replicates in the respiratory tract, and high viral load can be detected in bronchoalveolar lavage. Additionally, the virus can also be recovered from lymph and gastrointestinal tissues and shed for up to 27 days via the nose and feces.^{22,23}

TABLE 1 Animal models for SARS-CoV infection

Models	Route of inoculation	Virus replication	Clinical signs	Histopathology	Advantages	Disadvantages	Application	References
Macaca mulattas model	Nasal inoculation	Pharyngeal and nasal swab samples	Temporary rise in temperature	(Hemorrhagic) interstitial pneumonia, tracheaectasy, lung edema	To reflect the early clinical pathological changes of SARS	No fatal	Vaccines and therapeutics	¹²
Cynomolgus monkeys model	Mucosal inoculation	Nasal swabs, urine, blood	Decreased activity, decreased feeding, snuffling, and mildly labored breathing	Unifocal or multifocal pneumonia	Reflects the clinical and pathological manifestations	May not have observed the entire spectrum of disease severity	Examine the underlying mechanism of disease	¹³
Mouse model (MA15)	Nasal inoculation	2–5 dpi in pulmonary tissues, spleen, brain, and liver	Weight loss	Interstitial pneumonitis	Replicate acute (and chronic) cases of SARS infection in humans	Clinical signs of infection were not observed	Vaccines and antivirals	¹⁴
Ferret model	Nasal inoculation	Detectable infectious virus in the lungs	Temperature increase, sneezing	Perivascular and peribronchial inflammation, interstitial pneumonia, alveolar edema	Replicates the fever seen in human patients and infection by the respiratory route, clinical signs, viral replication in the respiratory tract and lung damage	Immune system is not well defined, dearth of reagents and larger numbers are needed to assess statistical significance	Testing vaccine efficacy	¹⁵

TABLE 2 Animal models for MERS-CoV infection

Models	Route of inoculation	Virus replication	Clinical signs	Histopathology	Advantages	Disadvantages	Application	References
Macaca mulatta model	Intratracheal inoculation	Virus was limited to the lung	Temperature increase, weight loss	Mild-to-moderate interstitial pneumonia and exudative pathological changes	Support viral growth, it also manifested respiratory and generalized illness along with tissue pathology	No fatal, no pathological changes in the kidneys	Vaccine and antiviral drugs	¹⁶
Common marmosets model	Inoculation combined intratracheal, intranasal, oral and ocular	Viral RNA is present in swabs and blood, viral titer highest in lung 3 dpi	Increased respiration rates, loss of appetite, temperature decrease, some degree of kidney involvement	Progressive severe pneumonia, bronchointerstitial pneumonia	Fatal MERS-CoV model	Disease process is rapid and transient	Diagnostic or prognostic tests	¹⁷
African green monkeys model	Aerosol inoculation	Higher viral titers in the serum were observed in the 10 ⁵ PFU group and 10 ⁴ PFU group	Elevated respiratory rates, lymphadenopathy, dehydration	Interstitial pneumonia, liver damage	Model of highly pathogenic coronavirus infection by aerosol	Viral replication is limited and difficult to detect, no recapitulate severe disease or lethality	Vaccines and other medical countermeasures	¹⁸
Ad5-hDPP4-transduced mice model	Nasal inoculation	Virus was limited to the lung	Mice developed pneumonia characterized by extensive inflammatory cell infiltration, virus clearance occurring 6–8 days after infection	Interstitial pneumonia	Easily reproducible	hDPP4 expression may not be targeted to the correct organ	Enable drug screening and vaccine validation	¹⁹
hDPP4 transgenic mice model	Nasal inoculation	In the lungs	Decreased survival, extreme weight loss, decreased pulmonary function	Decreased pulmonary function, pulmonary hemorrhage	Demonstrated widespread infection of pneumocytes and pathology consistent	The use of high viral loads to achieve severe	Vaccines and antiviral	²⁰
New Zealand rabbit model	Nasal inoculation	Largely limited to the respiratory tract until Day 5	No clinical sign	Inflammation and congestion in the lungs	Monitored the antibody and inflammatory response	Lack any discernible clinical signs of infection	Vaccines	²¹

TABLE 3 Animal models for SARS-CoV-2 infection

Models	Route of inoculation	Virus replication	Clinical signs	Histopathology	Advantages	Disadvantages	Application	References
Macaca mulattas model	Nasal inoculation	In respiratory, lymphoid, and gastrointestinal tissues. 3 dpi, high viral loads in the lungs	Cough, weight loss, loss of appetite, pale face, dehydration, breathing mode change	Interstitial pneumonia, lung edema	Reflects the clinical and pathological manifestations similar to COVID-19	Do not comprehensively reflect the influence of sex and age on SARS-CoV-2 infection	Testing of medical countermeasures	^{22,23}
African green monkeys model	Inoculation combined intratracheal and intranasal	Highest in the upper and lower respiratory tracts	Fever, decreased appetite	Pulmonary consolidation with hyperemia and hemorrhage in the lungs	Develop mild, moderate, or severe pulmonary lesions	Do not reflect the high viral replication and/or shedding kinetics observed in humans	Pathogenesis and testing medical countermeasures	²⁴
hACE2 transgenic mice model	Nasal inoculation	Highest at 3 dpi, in lung (106.77 copies per ml), viral RNA could also be found in the eye, heart, and brain in some mice	Typical interstitial pneumonia and pathology	Moderate interstitial pneumonia	Exhibits in the lungs similar to initial clinical of pneumonia	Not found clinical symptoms in any of the mice	Testing potential vaccines and therapeutics	²⁵
Ad5-hACE2 transduced mouse model	Nasal inoculation	Robust viral replication in lung	Weight loss	Perivascular to interstitial inflammatory cell infiltrates, necrotic cell debris, and alveolar edema	Very reproducible	Infection is nonlethal	Evaluate new therapies and vaccines	²⁶
VEEV-VRP-hACE2 transduced mouse model	Nasal inoculation	High level (10^6 – 10^7 copies/g) in lung	No clinical sign	Interstitial pneumonia	Can be rapidly established without any genetic manipulation	No obvious clinical symptoms	Testing vaccine efficacy	²⁷
MASCp6 model	Nasal inoculation	Higher in the lungs (109.16 – 10.42 RNA copies/mouse)	The manifestations of mild to moderate acute clinical cases	Presenting denatured trachea, changes of inflammation in pulmonary alveoli with detection of viral antigen in trachea, bronchiole and some type II pneumocytes	Conveniently, economically, and effectively	No fatal	Testing vaccine efficacy	²⁸
SARS-CoV-2 MA model	Nasal inoculation	Replicated in both the upper and lower airways of both young adult and aged BALB/c mice	Reproducing the age-related increase in pathogenesis observed in humans	Inflammation of small conducting airways on 2 dpi, associated with high levels of viral antigen staining	Accessibility, ease of use, availability of reagents, cost and utility are more favorable	Attenuate the function of select human monoclonal antibodies or vaccines in mice	Caccine and clinical candidate interferon (IFN) lambda-1a efficacy	²⁹

(Continues)

TABLE 3 (Continued)

Models	Route of inoculation	Virus replication	Clinical signs	Histopathology	Advantages	Disadvantages	Application	References
SARS-CoV-2 MA10 model	Nasal inoculation	Virus replication in the lung peaked 1–2 dpi, in the upper respiratory tract remained high on 1–3 dpi	Weight loss	Acute lung injury, diffuse alveolar damage	Elucidate the underlying host genetics and molecular mechanisms governing SARS-CoV-2 disease pathogenesis, host expression networks, and immunity after infection	May have more limited use for studies of alveolar disease pathogenesis	Evaluate vaccine and antiviral drug performance	³⁰
Golden hamsters model	Nasal inoculation	Highest in the lungs on 2 dpi, followed by rapid viral clearance by 7 dpi	Weight loss	Infiltration of mononuclear inflammatory cells in the lungs	Highly susceptible, inoculation support direct contact or via aerosols, the pathological features of the lungs resemble those observed in COVID-19 patients	No fatal	Understand the transmission dynamics for coronavirus	³¹
Roborovski Dwarf Hamster model	Nasal inoculation	Higher in the lungs	Weight loss, temperature decrease, snuffling, dyspnea, ruffled fur	Acute diffuse alveolar damage and hyaline microthrombi in the lungs, thrombosis	Highly susceptible, rapid and fatal course of experimental infection	Lack of generally available tools and reagents	Pathogenesis and testing vaccines and antiviral drugs	³²
Ferret model	Nasal inoculation	In nasal washes and lung tissue and peaked at 4 dpi	Temperature increase, cough	Acute bronchiolitis	Transmitted to naive ferrets by direct contact at high efficiency	Only mild clinical symptoms and relatively lower virus titers in lungs of infected animals	Therapeutics and vaccines	³³

Intratracheal inoculation of *Macaca mulatta* with MERS-CoV resulted in an increase in body temperature, decreased water intake, mild to moderate interstitial pneumonia, and exudative pathological changes. Renal failure is the main symptom of MERS, but there was no pathological change in the kidneys of *Macaca mulatta*. The virus could be detected in lung homogenate.¹⁶

In cynomolgus monkeys, diseases caused by SARS-CoV infection of the mucosa are more prominent than those caused by intravenous inoculation, and mild to moderate symptoms, including decreased activity, decreased food intake, nasal congestion, and mild dyspnea, begin 2–4 days after mucosal inoculation. Imaging can detect lung cavity-like lesions. Moreover, viruses have been found in the blood, as well as the nasopharynx, urine, and feces.¹³

It is worth noting that DPP4 from common marmosets are highly similar to that from humans and bind well to the MERS-CoV spike protein, implying that common marmosets are susceptible to the MERS-CoV. Inoculating common marmosets with the MERS-CoV through a combination of the trachea, nose, mouth, and eyes resulted in a loss of appetite and reduced activity levels, as well as moderate to severe respiratory diseases such as increased respiratory rate and dyspnea. An autopsy showed significant interstitial lung disease, including edema, bronchiolar congestion, interstitial infiltration, and a very high viral load in the lungs.¹⁷

In African green monkeys infected with MERS-CoV by aerosol, pulmonary disease symptoms increased in a dose-dependent manner. After infection, they presented clinical manifestations of dehydration, wheezing, and lung rales. The pathological changes were multifocal interstitial pneumonia, and the virus can be detected in the nose swab serum.¹⁸ Importantly, African green monkeys also support the replication of SARS-CoV-2 in vivo and exhibit severe respiratory disease. African green monkeys infected with SARS-CoV-2 showed decreased appetite, increased body temperature, and higher partial pressure of oxygen dioxide. Significant inflammation and clotting were found in the tissue and blood, and autopsies also showed pulmonary congestion and histological differing degrees of multifocal lung disease. Simultaneously, high viral load can be detected in nasal and rectal swabs.²⁴

1.2 | Mouse model

The receptor-binding domain of the spike protein is important for the entry of the virus into the cell. The spike protein is split into S1 and S2 by protease after entering the host cell, and S1 binds to the receptor of the host cell, allowing the virus to enter the cell. As structural differences exist between mouse and human respiratory coronavirus-related receptors, the receptor recognition mechanism of coronavirus is complex so that mice are not susceptible to respiratory coronavirus. Therefore, some researchers alter the mouse gene through gene modification or the virus transduction technology, allowing the mouse to express the virus's corresponding receptor, or modify the virus's adaptability, thus making the mouse susceptible to the virus.

1.3 | Transgenic mouse model

1.3.1 | hAPN transgenic mice

Double transgenic mice (HAPN+/+STAT1-/- mice) were generated by crossing homozygous males of hAPN with immunocompromised female mice of STAT1 α/β . The mice were susceptible to the HCoV-229E virus that expressed the hAPN receptor. The HCoV-229E virus can be detected in the lungs, intestines, livers, and spleens of infected mice.³⁵

1.3.2 | hACE2 transgenic mice

There are many kinds of transgenic mouse models generated by using molecular biology techniques, such as injecting the hACE2 promoter into the prokaryon of ICR mouse fertilized eggs³⁶; hACE2 receptor transgenic mice driven by K18-hACE2 promoter of cytokeratin 18(K18) gene³⁶; mouse model expressing human ACE2 (hACE2) using CRISPR/Cas9 knock-in technique.²⁶ After infection with SARS-CoV-2, hACE2 transgenic mice developed typical interstitial pneumonia with infiltration of inflammatory cells. The virus replicated at high levels in the lungs and then spread to other organs.^{25,36–38}

1.3.3 | hDPP4 transgenic mice

(1) By using a plasmid-mediated gene targeting strategy and replacing mouse DPP4 exon 10–12 with the corresponding human DPP4 codon (HDPP4-KI), homogeneous C57BL/6 mice were developed and retained the natural promoter to regulate the gene. The HDPP4-KI mice supported moderate replication of the MERS-CoV in the lungs but lacked underlying signs of infection, and their lung virus titers were 100 times higher after 30 successive generations of the adapted strain, which can lead to fatal infection in mice and diffuse lung injury.³⁹ (2) Using the VELOCIGENE technique, the mouse DPP4 gene was replaced by 82KB of its human homolog, and the full human DPP4 gene was expressed under the control of regulatory elements. Besides, pathological changes in the lungs were observed.⁴⁰ (3) Using CRISPR-Cas9 gene-editing technique, A288L and T330R were introduced into MDPP4 to predispose mice to infection with MERS-CoV, and then the MERS-CoV was passed in 288/330+/+ mice to develop a mouse-adapted virus, Mers-15, which can cause ARDS symptoms.²⁰

1.4 | Mouse model of viral transduction

The long research cycle of transgenic mice can be shortened by using virus transduction technology to express virus-related vectors in mice. In comparison to transgenic mice, virus transduction technology enables the effective and rapid construction of animal models. The Ad5-hACE2 transduced mouse phase, for example, can result in high levels of virus replication in the lungs.²⁶ BALB/C and C57BL/6 mice infected with SARS-CoV-2 by VEEV-VRP-hACE2 developed interstitial pneumonia,

according to histopathology, and the virus maintained a stable and high level of replication in the lungs for 1–5 days after infection.²⁷ Transfection of BALB/C mice with a recombinant nonreplicating adenovirus expressing the hDPP4 receptor followed by infection with MERS-CoV resulted in weight loss and interstitial pneumonia in mice, with virus clearance occurring between 6 and 8 days post infection.¹⁹

1.5 | Animal model of virus-adapted strain

By molecular modeling and reverse genetics, the recombinant virus SARA-CoV-2 MA was able to replicate in upper and lower airways of BALB/c mice, and caused more severe disease in aged mice, which can reproduce the age-related increase in disease severity observed in humans.²⁹ Mice vaccinated in the nose with SARS-CoV-2 in the respiratory tract of older BALB/c mice for six consecutive generations showed an inflammatory response associated with interstitial pneumonia.²⁸ Infection with SARS-CoV-2 MA10 in C57BL/6J mice resulted in milder symptoms, while infection with immunodeficiency type I and II interferon-like double-knocking out of C57BL/6J mice led to severe weight loss and morbidity. Besides this, infected BALB/C mice can also cause acute lung damage in mice, with the highest titration in the lungs occurring between 1 and 3 days after viral infection.³⁰ After 15 transmissions, the virus (MA15) produced by SARS-CoV is lethal to mice, and its lethality could be due to mutations in six nucleotides in MA15. Following nasal inoculation of BALB/c mice, a clinical sign of weight loss and intensive pneumonia were developed. In the lungs, the virus replicated rapidly and was highly virus titer, and it spread to other tissues including the brain and spleen.¹⁴

1.6 | Hamster model

Golden hamsters are highly susceptible to SARS-CoV-2 infection and can be infected directly by contact or aerosols.⁴¹ Severe lung changes occur 7–8 days after infection and virus replication peaks in the lungs at 3 days. Although there was no obvious linear relationship between the virus titration in the lungs and the dose of infection, the degree of lung damage was positively related to the dose size of the infection.³¹ However, MERS-CoV cannot replicate in Golden hamsters, possibly due to virus replication being limited at cellular levels.⁴² In addition, Roborovski Dwarf Hamster is also highly prone to SARS-CoV-2. On Day 1 post infection, clinical symptoms, such as hypothermia, reduced activity, and breathing difficulties, were observed, which was consistent with pathological findings of severe acute diffuse vesicle damage, and hyaluronic acid microthrombosis.³²

1.7 | Ferret model

The ferrets infected with SARS-CoV showed elevated body temperature, runny nose, sneezing, and other clinical manifestations, and pathological changes in interstitial pneumonia, pus pneumonia,

and pulmonary edema were observed in the upper respiratory tract.¹⁵ Ferrets can be infected with SARS-CoV-2 through direct and indirect contact.⁴³ After infection with SARS-CoV-2, the temperature of the ferret rises. Viruses replicate in the host body and can be detected in the blood, nasal armor, trachea, lungs, and intestines. The highest level of virus drips in lung tissue can be eliminated within 8 days post-infection by nasal lotion, saliva, urine, and feces.³³

1.8 | Other models

In addition to the above animal models, New Zealand rabbits and cats are also susceptible to certain respiratory coronavirus. A transient dose-dependent lung infection occurs in New Zealand rabbits following infection with MERS-CoV, and the absence of meso-antibodies leads to increased inflammation caused by reinfection of its susceptible virus.²¹ Cat vaccination against SARS-CoV did not result in clear signs of infection, but interestingly, a titration test of the virus in the cat's lung plasma revealed a lower replication of the virus in the lungs.⁴⁴ Besides this, SARS-CoV-2 can be transmitted between cats, but there is no evidence that cats can infect humans.⁴⁵

1.9 | Application in drug research

The rhesus macaque was used to evaluate the efficacy of remdesivir intravenously against MERS-CoV, SARS-CoV, and SARS-CoV-2 infection. All animals were inoculated with a total dose of 7×10^6 TCID₅₀ of MERS-CoV via intranasal, oral, ocular (1×10^6 TCID₅₀), and intratracheal (4×10^6 TCID₅₀) routes. The results showed that prophylactic remdesivir treatment initiated 24 h before infection can completely prevent virus-induced clinical symptoms, inhibited virus replication in respiratory tissues, and prevented pulmonary lesions. Therapeutic administration remdesivir 12 h postinfection can reduce clinical signs and virus replication in the lungs, decrease lung lesions.²²

The total dose of 2.6×10^6 TCID₅₀ SARS-CoV-2 was administered intranasally, orally, intraophthalmally, and intravenously. Remdesivir was given intravenously at 10 mg/kg at 12 h after infection and intravenously at 5 mg/kg every 24 h thereafter. The results confirmed that early treatment with remdesivir reduced lung viral load and lung injury in rhesus monkeys.⁸

Ferrets were used to evaluate the efficacy of oral antiviral drugs. Ferrets were inoculated intranasally with 1×10^5 pfu SARS-CoV-2. After infection, remdesivir was given orally at 10 mg/kg twice daily for 4 days, indicating that replication of the virus could be blocked.⁶ MK-4482/EIDD-2801, a kind of orally ribonucleoside analog inhibitor, has also been shown to reduce SARS-CoV-2 load in ferrets' nasal secretions and stop the spread of the virus.¹¹

Rhesus macaques were aged 4–6 years with a total of 7×10^6 TCID₅₀ of MERS-CoV. The drug treatment was initiated at 8 h after infection. The initial ribavirin loading dose (30 mg/kg) was delivered intravenously and the subsequent doses (10 mg/kg) were delivered

intramuscularly every 8 h. Human interferon- α 2b (5 MIU/kg) was delivered subcutaneously every 16 h. The results demonstrated that treated animals did not develop breathing abnormalities and showed lower levels of systemic and local pro-inflammatory markers, less severe histopathological changes in the lungs. These data suggest that treatment of MERS-CoV infected rhesus macaques with IFN- α 2b and ribavirin reduces virus replication, moderates the host response, and improves clinical outcome.⁴⁶

The clinical and radiographic changes of rhesus monkeys following infection with 5×10^6 PFU MERS-CoV. Two groups of NHPs were treated with either a human anti-MERS monoclonal antibody 3B11-N or E410-N, an anti-HIV antibody. MERS-CoV infection resulted in quantifiable changes by computed tomography, but limited other clinical signs of disease. 3B11-N-treated subjects developed significantly reduced lung pathology when compared to infected, untreated subjects, indicating that this antibody may be a suitable MERS-CoV treatment.⁴⁷

Cynomolgus macaques were infected intratracheally with 1×10^6 TCID₅₀ SARS-CoV, prophylactic treatment with the antiviral agent pegylated interferon- α (IFN- α) can significantly reduce viral replication and excretion, viral antigen expression by type 1 pneumocytes and pulmonary damage, compared with untreated macaques.⁴⁸

Twelve healthy male common marmosets (*Callithrix jacchus*; 3 years old; 230–395 g) were inoculated with 5×10^6 TCID₅₀ of MERS-CoV. Each treatment group included three animals, the clinical symptoms were minimal pulmonary infiltrates and mild bronchointerstitial pneumonia, and lower mean viral loads in necropsied lung tissues in the lopinavir/ritonavir-treated and interferon- β 1b-treated animals. In contrast, all MMF-treated animals developed severe diseases with higher viral loads. The mortality rate at 36 h postinfection was 67% (untreated and MMF-treated) versus 0–33% (lopinavir/ritonavir-treated and interferon- β 1b-treated).⁴⁹

The antiviral activity of 80R immunoglobulin G1 (IgG1), a human monoclonal antibody against severe acute respiratory syndrome coronavirus (SARS-CoV) spike (S) protein was investigated in a mouse model. 80R IgG1 was given intraperitoneally to BALB/c mice 1 day before SARS-CoV (10^4 TCID₅₀) intranasal challenge and the virus titer of lung tissue was determined 2 days later. At the highest 80R dose tested (undiluted, 250 μ g/mouse), 100% of mice had a more-than-4-log reduction in viral load. At a dose of 50 μ g/mouse, 25% of mice showed a viral load reduction to below the limit of detection and 75% mice showed a nearly 4-log reduction in viral titer. At the lowest dose (10 μ g/mouse), 100% of mice became infected and the virus load was reduced about 10-fold. This excellent level of protection is comparable to that seen when the animals were injected with convalescent-phase sera from previously infected mice.⁵⁰

The hmAb IgG1m336 was administered to rabbits intravenously or intranasally at 1 or 10 mg/kg. Female New Zealand white rabbits aged 5–7 months were infected intranasally with 1 ml of 10^5 TCID₅₀ of MERS-CoV one day later. Prophylaxis with m336 resulted in a reduction of pulmonary viral RNA titers by 40–9000-fold, compared with an irrelevant control antibody with minimal inflammation and no

evidence of virus antigen. The neutralizing MERS-specific antibody titers in the sera of the rabbits that received 1 mg/kg of m336 ranged from 10 to 32 immediately before infection and remained in the range of 10–25 on Day 3 after infection. The group that received 10 mg/kg of m336 had serum titers ranging from 113 to 320 before virus inoculation and from 101 to 320 on Day 3 after infection.⁵¹

2 | DISCUSSION

The emergence of mutant SARS-CoV2 strains increases the difficulty of prevention and control. The biological characteristics, epidemiological characteristics, and pathogenesis of the virus warrant further investigation. An ideal animal model is an important tool for studying viral diseases, which cannot be confirmed in vitro culture experiments, and the top concern for animal models is to imitate well the key features observed under clinical conditions.

At present, animal models of respiratory coronavirus include susceptible animal models, genetically modified models, and various animal models of infected virus adaptation strains, such as Nonhuman primates, mice, hamsters, ferrets, New Zealand rabbits, cats, and other animal models, all of which have distinct advantages and limitations. Among them, mice, hamsters, ferrets, New Zealand rabbits, cats belong to small animal models.

The very commonly used animal models for viral diseases are mice, which have a highly characterized immune system, low cost, and rapid breeding cycle. As mice do not have the hACE2 receptor, they cannot be used directly and need to be infected with SRAS-CoV-2 by transgenic or mouse lung adapted strains, which are the limitations of the mice model. hACE2 transgenic mice model research cycle is long and difficult. The long research cycle of transgenic mice can be shortened by the Ad5-hACE2 transduced mouse model. Through a variety of ways, hDPP4 transgenic mice model can reproduce the severe pulmonary pathology caused by MERS-CoV. Ad5-hDPP4-transduced mice model can develop pneumonia characterized by extensive inflammatory cell infiltration, but the limitation is that hDPP4 expression may not be targeted to the correct organ. Although the mouse model could not reproduce the clinical manifestations of COVID-19, it was able to better evaluate the replication process of the virus in the lung tissue.

The Syrian hamster has been used to the viral infections' diseases for many years and it has similarities to humans on disease symptoms, pathogenesis, and immune reaction.⁵² Golden Syrian hamster's model and Roborovski Dwarf Hamster model are highly susceptible to SARS-CoV-2. Amino acid and binding energy comparison between human and other mammalian ACE2 results showed that the acid residues of Syrian hamster at the ACE2 interface may interact with the SARS-CoV-2 spike glycoprotein RBD. The clinical signs of rapid breathing, weight loss, histopathological changes of diffuse alveolar damage, airway and intestinal involvement with virus nucleocapsid protein expression, high lung viral load were observed within the first week of virus challenge. Hamsters have proven to be a useful animal model for SARS-CoV-2 infection due to the clinical signs of illness,

including lung damage similar to that seen in COVID-19 patients.⁵³ But the limitation is that the hamster model is not fatal and lacks generally available reagents.

Ferrets are a very important and useful model for the study of respiratory viruses in general, such as influenza viruses, SARS-CoV.⁵⁴ Ferret model also can be infected through direct and indirect ways and is a better model for evaluation of SARS-CoV-2 transmission, but it is only mild clinical symptoms and relatively lower virus titers in the lungs of an infected animal, and also, ferret-specific immunological reagents are scarcer.

These small animal models are smaller in size and small space required, they are easier to get and have better operability and repeatability, especially the mice which are easier to control, cheaper, and large numbers for data analysis.

Nonhuman primates belong to large animal models. These pathological changes of SARS-CoV infection in the *Macaca mulatta* model, such as pneumonia, diffuse alveolar damage, and fibrosis of pulmonary tissues reflect the changes that are seen in the early stage of clinical SARS cases, but these pathological changes are mild. The same as the *Cynomolgus* monkey model, it reflects the similar clinical and pathological results of mankind but fails to observe the severity of the disease.

These nonhuman primate models, such as *Macaca mulatta* model, Common marmosets model, and African green monkeys, were used to study MERS-CoV. Fever is one of the markers of MERS-CoV infection in humans. The body temperature of *Macaca mulatta* model increased after infection, but no repeated lethality was observed. The common marmosets model is a fatal model of MERS-CoV, which shows progressive severe pneumonia and bronchointerstitial pneumonia. African green monkey is a model of highly pathogenic coronavirus infection by aerosol, and observable disease signs include elevated respiratory rates and other respiratory disease signs. *Macaca mulatta* model and African green monkeys model can reflect the clinical and pathological manifestations similar to COVID-19.

Nonhuman primates animal models are more reliable models to replicate human disease pathogenesis and there are many clinical similarities to human infection, which are widely used for evaluating the protective efficacy of vaccines and drugs. But the primary limiting factor of nonhuman primates animal models is the expensive price, special experimental site, genetic backgrounds variability.

3 | SUMMARY

SARS-CoV2 and its variants are spreading fast and worldwide; however, there is still no animal model that can accurately recapitulate all the clinical features of coronavirus infection in humans.

Nonhuman primates, ferrets and hamsters, are susceptible to coronavirus and are widely used for researching the transmission and pathogenesis. Nonhuman primate models are physiologically, immunologically, and genetically similar to humans, share many clinical similarities with human infections, and are widely used to evaluate the efficacy of

drugs to improve clinical symptoms and alleviate pulmonary pathological changes, and ferrets are highly effective at transmitting the virus via droplets, so they are the preferred candidate for evaluating the effectiveness of drugs in blocking virus replication and transmission in the respiratory tract. The Syrian hamsters have similarities to humans in disease symptoms, pathogenesis, and immune reaction and have proven to be a useful animal model for SARS-CoV-2 infection due to the clinical signs of illness, seen in COVID-19 patients. Limited by gene, mouse models need Genetic modification or virus adapted. Thus, when selecting models based on the purpose of the study and evaluation indicators, we should choose those that can reliably, specifically, and repeatedly reproduce the primary symptoms of diseases and that can respond to coronavirus infection as closely as possible to humans, especially these reproduce characteristics of the disease. We need to integrate consequences from multiple animal model systems with data from human patients, which will provide a reliable foundation for developing effective strategies for the prevention and treatment of coronavirus diseases.

AUTHOR CONTRIBUTIONS

Shengle Qin completed the document collection and manuscript writing with the help of Zhaoguang Zheng and Xuxin Zeng. The revision of the manuscript was collaboratively finished by Yutao Wang and Runfeng Li and finally approved by Yutao Wang and Xinhua Wang.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Data are available from the corresponding author upon reasonable request.

PEER REVIEW

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