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Review Article

Current Status of Animal Models to Promote Preclinical Research on Covid 19

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Abstract

At present, coronavirus disease 2019 (COVID-19), infected by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an emerging respiratory infection caused by the introduction of a novel coronavirus into humans late in 2019 (first detected in Hubei province, China). It is feared that these viruses adapt to replication in humans and become transmissible from human to human. In addition, mutation in SARS-CoV-2 continue to cause outbreaks and cause an ever-increasing number of human cases with high fatality rates. The ongoing spread of this virus could potentially bring major challenges to worldwide health systems and consequences on the global economy and financial market if not controlled effectively. The development of effective drugs and vaccines against potentially pandemic viruses is therefore considered a priority. In this review, we discussed animal models that are used for the preclinical evaluation of drugs and novel candidate SARS-CoV-2 vaccines for the treatment and prevention of COVID 19 respectively. In most cases, a tier of multiple animal models is used before the evaluation of drugs and vaccine candidates in clinical trials is considered. Commonly, drugs and vaccines are tested for safety and efficacy in mice, ferrets, hamster and/or macaques. The use of each of these species has its advantages and limitations, which are addressed here.

INTRODUCTION

In December 2019, pneumonia of an unknown aetiology was confirmed in China [1]. The Chinese Centre for Disease Control and Prevention (CCDC), identified a novel coronavirus infection as the cause of this pneumonia [2].On February 11, 2020, the World Health Organization (WHO), named the virus 2019-nCoV (SARS-CoV-2), and the syndrome was named coronavirus disease 2019 (COVID-19), and on March 11, 2020, t WHO declared this disease pandemic as a global health emergency [3,4]. The ongoing spread of this virus could potentially bring major challenges to worldwide health systems and consequences on the global economy and financial market if not controlled effectively [5]. Multiple clinical trials are currently underway for prevention or intervention in the disease progression [6]. In parallel, it is also equally essential to carry out basic research on SARS-CoV-2 to support the efficient development of therapeutic agents.

The lack of an effective therapeutic/prophylactic treatment against SARS-CoV-2 is a major reason for the frightening COVID-19 pandemic in such a short period of time. About 450 COVID-19 therapeutics are in pre-clinical trials [7] .These include viral, RNA, DNA, protein, and nanoparticle-based vaccines as well as re-purposed therapeutics including vasodilator, immune modulator, steroids, anti-inflammatory agents, anti-coagulatory molecules, anti-parasitic and antiviral drugs [8].

Animal models are the essential tools for infectious diseases, and they can help us not only to understand the pathogenesis and mechanisms of SARS-CoV-2 disease biology but also to elucidate aspects of pharmacology, toxicology, and immunology of the therapeutic and vaccine strategies. Several animals used for SARS-CoV studies, including mice, hamsters, ferrets, and non-human primates (NHPs), have therefore been evaluated as models for SARS-CoV-2 infection [9-21].However, availability of an ideal animal model would help us in the assessment of the efficacy of investigational drugs before they entered into the clinical trials. Animal models are also essential to assess the efficacy of drugs in vivo since in vitro evaluation of drugs may not suffice for clinical use. For instance, hydroxychloroquine showed promising results against COVID-19 in vitro but failed to show any remedial effect in Cynomolgus macaques exposed to SARS-CoV-2 [22]. No single animal model recapitulates the totality of pathogenesis or predicts interventional responses faithfully as in human. Defining animal models and their use is a prerequisite to performing studies to compare vaccines and therapeutics so that the most promising ones advance to the next phase.

In this review article, a literature search was performed using PubMed and Google Scholar to identify relevant English-language articles published on animal models for Covid 19 research. We have outlined the animal models available for Covid 19 and their key role in elucidating the pathogenesis, disease transmission, host response to SARS-CoV-2 infection. The information included in this report provides a strong intellectual groundwork for evaluating the investigational drugs and vaccine candidates for covid 19.

COVID 19 IN HUMANS

Understanding the course of covid 19 in humans will help us to develop the model disease in animals. Therefore, we present a summary of human disease first. SARS-CoV-2 is believed to have a zoonotic origin [23] . The transmission of COVID-19 occurs through respiratory droplets and incubation period of COVID-19 lasts for 14 days. However, the median duration for the onset of symptoms after exposure is around 4-5 days [24]. Covid 19 in humans is characterized by mild symptoms, with most people remaining asymptomatic and infection thought to be confined to the URT, although they are capable of transmitting infection. The severity of clinical signs and symptoms of COVID-19 illness varies among patients. Symptoms, when they do occur, are typically acute viral respiratory illness with fever, cough, dyspnoea, fatigue, anosmia, myalgia, and confusion. In ~80% of people, the course remains mild and disease does not extend to the lower respiratory tract (LRT). However, ~20% develop more severe symptoms, with diffuse widespread pneumonia, with 5% having severe gas exchange problems, acute lung injury, and progress onto acute respiratory distress syndrome (ARDS) [25,26]. The clearest predictor of mortality is age, with the case fatality rate rising dramatically over 60 years of age. Other predisposing factors for heightened mortality are male sex, social deprivation, and chronic disease particularly chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), obesity, and diabetes [27].

The ability of the inflammatory and immunological responses to contain the infection to the URT is one aspect that explains why some people get more severe lower respiratory disease while others do not. The LRT expresses ACE2 to a lesser extent than the nasopharynx [27]. Furthermore, while ciliated airway epithelial cells are easily infected and spread to neighbouring cells, a decrease in ACE2 may act as a barrier to LRT infection. Due to the development of systemic inflammatory response or “cytokine storm” in these people, the condition will advance to a severe state. Pneumonia associated with severe infection bears all the pathological features of ARDS, with diffuse alveolar damage, interstitial pneumonitis and lymphocytic infiltrates [28,29]. Unique features of the critical disease are extravascular fibrin deposition, neutrophil trapping, microvascular thrombosis, and large vessel pulmonary emboli [29]. Critical COVID-19 patients have a higher rate of widespread thrombosis and microangiopathy than ARDS patients [30].

ANIMAL MODELS FOR COVID 19 RESEARCH

The development of an animal model to study the infection and to test vaccines or new forms of therapies, as well as to understand the molecular mechanisms of COVID-19 is the current need and has attracted the interest of researchers worldwide [31,32]. Animal models for the closely related SARS-CoV and MERS-CoV viruses have paved the way for SARS-CoV-2 infection and pathogenesis studies. A plethora of animal models (species, strains, mutants) exists for preclinical research, ranging from tiny insects to large livestock species [33].These models may be experimentally induced so that the study of the disease becomes possible, or they can be spontaneous models, including naturally existing genetic variants, genetically modified models, negative models, resistance to some diseases, or even orphan models, which suffer from certain natural disorders[34,35].As the virus-host interaction is very complex, it may require the use of more than one animal model, since the chances of a single model reproducing all aspects related to the disease in humans are low [33].

Predicting the course of the disease in animals is challenging because the highest level of pathology mainly occurs in first week of infection, which can be caused by variable immune responses, and the way the infection occurs and the virus replicates. When looking for an ideal model, we need to ensure that at the end of the experimental trials, the differences in the pathophysiology are not in doubt when extrapolating to the human situation [36].To assess the course of COVID-19 in the proposed animal models, histological, radiological, and visual inspection tests should be performed. The animal model must be able to indicate the presence of lung tissue damage and the development of an inflammatory process. In addition, changes in the function of the alveolar-capillary barrier and physiological changes should be detectable so that the effectiveness of the therapy implemented can be evaluated and determined [37].

SARS-CoV-2 has been reported to infect human beings through the angiotensin-converting enzyme -2 (ACE-2) receptors [38]. Therefore, the animals having ACE-2 receptors that are closely similar to human beings can act as a model for various studies on SARS-CoV-2. ACE2 represents the main receptor for SARS-CoV-2 entry into cells whereas lungs and bronchi are the main targets for viral infection (Figure 1). [39]. However, the heart, kidneys, liver, brain, gastrointestinal tract, and upper respiratory tract can also be affected [40]. Zeiss et al., signaled that some amino acid sequences present in ACE2, considered critical residues, are more important than the total number of similar amino acids when comparing the animal model to the human and they have a direct influence on the susceptibility to infection [41].

Another factor that directly impacts ACE2 affinity and differs from one species to another is the tissue distribution of ACE2 together with TMPRSS2 and furin. The TMPRSS2 cellular serine protease and the furin proprotein convertase act as cofactors for this binding, which can be considered as a target for the action of inhibitory drugs and a difference among animal models facing infection [41].The fact that the main targets (lungs and bronchi) express low levels of ACE2 pointed to the existence of other molecules involved in cell invasion. In addition, the presence of such molecules directly impacts viral tropism, including the AXL receptors (present in lung), CD147, and ASGL1, independent of ACE2, which facilitate viral entry into cells. Furthermore, heparan sulfate, sialic acid, lectin receptors, Neuropilin 1, and CD4 act as co-receptors while SRB1/cholesterol, Furin, PC-1, trypsin, matriptase, TMPRSS2, and cathepsin are cofactors, demonstrating that the engineering of the infection of SARS-CoV-2 in organs is a quite complex process, and the virus uses more than one pathway to cross the cell barrier [40].

(Multiple molecules at the cell surface are involved in the entry of SARS-CoV-2, including the major receptor ACE2 the membrane protease TMPRSS2, and other potential alternative/auxiliary receptors or cofactors .Membrane fusion can take place either at the cell surface (left) or in the endosome (right). Both

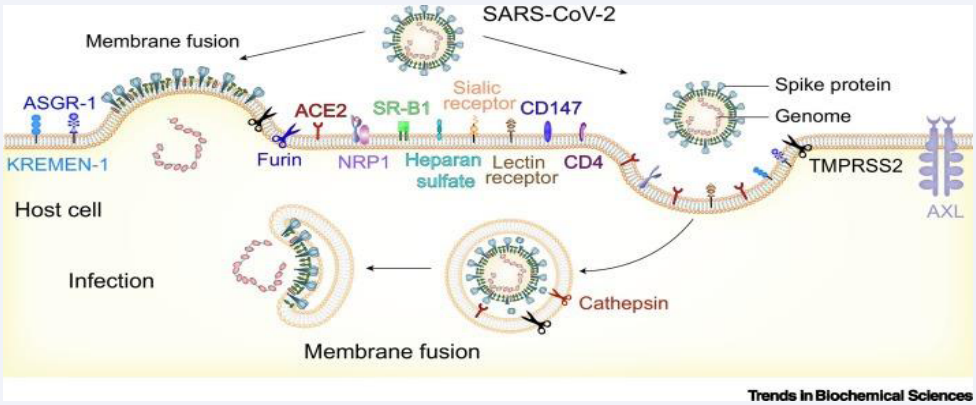

Figure 1 Schematic diagram of SARS-CoV-2 entry pathways.

entry pathways are utilized by SARS-CoV-2. Abbreviations: ACE2, angiotensin-converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome virus 2; TMPRSS2, transmembrane serine protease 2)

Currently, several animal models for SARS-CoV-2 are in development (Figure 2)[42]. Studies have shown that ferrets, monkeys, mice, hamsters, civet cats, camelids, and rabbits can be considered in animal models for coronavirus infection [43-50]. Animal models can be categorized as large or small. Larger animals models such as non-human primates (NHPs) - Rhesus Macaques, Cynomolgus macaque, African green monkeys are traditionally considered the most translational models to humans. NHPs bear close similarities to human genetic, neurological, cognitive, physiological, reproductive, anatomical, and immunological systems. Their susceptibility to most human pathogens is not surprising; they are therefore models for many of the most intractable acute and persistent pathogens. Their size and longevity make them excellent models for pathogenesis, allowing repeated sampling and imaging in vivo for longitudinal studies. However, their limited supply in the face of numerous drug and vaccine candidates makes them an even more precious resource. It is therefore imperative to prioritize agents to be tested by demonstrating tolerability and efficacy in smaller mammalian models like mice, hamsters, and ferrets are currently the small animal species of choice. Small animal models are presented first as the tractable models used in early discovery and development. Mice and hamsters are most commonly used animals for developing disease models [33].

SMALL ANIMAL MODELS

Mouse Models

Small animal models are widely used to study emerging viruses, but often they need to be genetically modified or the virus

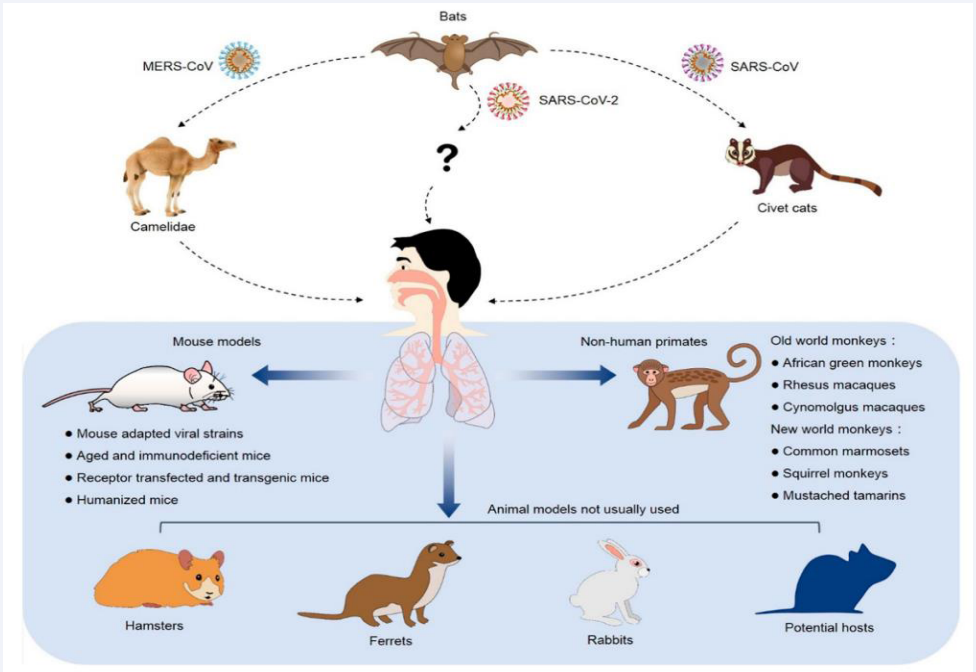

Figure 2 Animal models for SARS CO-V.

needs to be adapted for different species to be susceptible and this is the case for SARS-CoV-2 infection [38].Mice have several advantages over other experimental animal models in their small size, low cost, rapid breeding for reaching large group numbers, and availability of research tools. However, laboratory strains of the mouse are shown to be non-permissive to SARS-CoV-2 infection due to the lack of viral entry receptor hACE2 [12,51,52]. Viral spike protein doesn't bind to the murine ACE2 effectively due to amino acid difference in ACE2 receptor between mouse and human [53].

Several approaches have been employed to increase susceptibility to SARS-CoV-2 infection in laboratory mice. The first approach to solve the problem of lack of infectivity of laboratory animals is to use an adapted virus in which multiple passages/or and selected mutations in the virus make the mice more susceptible to infection. A recent publication demonstrated a mouse-adapted virus Q498T/ P499Y that was not only able to readily infect mice but was used to demonstrate the effectiveness of neutralizing antibodies to reduce viral replication *in vivo* [52]. Second approach is to express the human ACE2 gene (hACE2), either by viral transduction or genetic engineering. Two laboratories have used adenovirus transduction to express human ACE2 in the lungs of mice, resulting in mild disease noted by viral replication and weight loss. This model could be used to test neutralizing monoclonal antibodies or antivirals and convalescent sera [54,55].

Mouse-Adapted SARS-CoV-2 model

Standard inbred mouse strains such as WT BALB/c mice, C57BL/6 mice can be made susceptible for SARS-COV-2 infection by modifying the spike protein of SARS-CoV-2 and by effectively binding to mouse ACE2.In this approach, SARS-CoV -2 was serially passaged in the respiratory tracts of young BALB/c mice. This led to the generation of a mouse-adapted SARS-CoV strain (MA15), which was lethal to mice from 3 dpi [56].The infection resulted in high viral titers in the lungs from 1 dpi, followed by viremia and diffusion to extra-pulmonary sites including the brain, liver, and spleen, significant lymphopenia and neutrophilia, mild and focal pneumonitis, and necrotic cellular debris in the airways and alveoli [56]. Nevertheless, these mouse strains developed by adaptation can be used to study the development of neutralizing antibodies against the spike protein, pseudo viral vaccine candidate, and antiviral drugs [57]. Another mouse-adapted SARS-CoV strain (v2163), was produced by serial passage in 6-week-old BALB/c mice [58]. Infection resulted in more severe symptoms and a higher mortality rate than with MA15, with greater immune responses and lung pathology. mouse-adapted SARS-CoV strains lacking the critical viral envelope I protein induce varying degrees of protection against re-infection with virulent strains, highlighting the potential for live-attenuated vaccines [59,60].

The mouse adapted method by serial passaging of virus in mice is successful because populations of RNA viruses consist of a swarm of closely related viral quasispecies. Rare viruses in the swarm which contains mutations in the spike protein shows increase in their binding affinity to mouse ACE2 are expected to be selected, owing to their higher levels of replication in mouse lungs. Alternatively, SARS-CoV-2 can be adapted to infect mouse cells by using reverse genetics to modify the receptor-binding domain of the virus so that it can infect mouse cells via the mouse ACE2 protein. Their use is advantageous as they reduce biological risks to researchers and may more closely resemble natural host-pathogen interactions in mice. However, the use of mouse-adapted animals is limited because the mouse adaptation process can develop an infection in mice but that does not recapitulate all aspects of human disease.

Mouse-adapted SARS-CoV-2 has been reported in a preprint, with mutations in the receptor-binding domain (RBD) of the spike protein following serial passage, inducing productive infection of both young and aged WT BALB/c mice [61].Infected mice had high viral lung loads up to 7 dpi, and displayed mild pneumonia with inflammatory cell infiltration, alveolar damage, focal exudation, and hemorrhage, and endothelial cell denaturation. The efficacy of an RBD-Fc-based vaccine was examined in this model, which induced the production of neutralizing antibodies that potently inhibited the infection. A similar preprint describes the modification of the RBD of the SARS-CoV-2 S protein, which facilitated the efficient binding of the S protein to mACE2 for host cell entry [62].Infection with this virus resulted in viral replication in the upper and lower airways of young and aged BALB/c mice. Aged mice had greater weight loss and pulmonary function decline compared to young mice, reproducing important aspects of human disease.

Expression of human ACE2 in genetically altered mouse model

Transgenic mouse model expressing hACE2: Genetic alteration in the mouse will help in viral binding to mouse Ace2, expression of human ACE2 under a variety of tissue-specific promoters, and transfection of mice with human ACE2 cDNA using viral vectors [63]. All of these transgenic mice are susceptible to infection by SARS-CoV-2, but differences are there in expression of human ACE2 which results in a pathogenic range of mild to lethal disease. Given the resemblance of SARS-CoV-2 to SARS-Co V in its use of ACE2 as an entry receptor, several research teams have evaluated transgenic mice expressing hACE2 under the control of HFH1/FOXJ1, HFH4, K18, and mouse ACE2 promoters [15,20,64-67].These ACE2 transgenic mouse models will be useful to study SARSCoV-2 replication in the lungs and its pathogenesis. However, SARS-CoV-2 infection in some transgenic mouse models led to neuroinvasion with high viral replication in the brain, which may be related to high lethality [64-66,68].

K18 Promoter-hACE2 Transgenic Mouse Model

One of the best mouse models used for COVID-19 research is the K18-hACE2 transgenic mouse. A transgene of human ACE2 (hACE2), expression is driven into the mouse epithelial cells under the control of the human cytokeratin 18 (K18) promoter [69]. K18-hACE2 mice when treated with doses of SARS-CoV (2.3 9 104 PFU), that induced severe lung damage and neuronal damage of CNS. The transgenic mice showed replication of the virus in the lungs, weight loss, also developed pathological lung inflammation, and died at 4 dpi. However, SARS-CoV-2 at 105 TCID50 caused weight loss, evoked antibody responses, and developed histological evidence of lung inflammation in K18-hACE2 transgenic mice in a dependent manner with interstitial

congestion, inflammatory exudate, epithelial damage, etc [69,70]. These mouse models provide a stringent test for vaccine and therapeutic efficacy and may be useful for studies of pathogenesis.

HFH4/FOXJ1 Promoter-hACE2 Transgenic Mouse Model

Transgenic mice overexpressing hACE2 under the control of HFH4/FOXJ1 lung ciliated epithelial cell-specific promoters are also susceptible to SARS-CoV-2 infection [68,71]. Most infected HFH4-hACE2 mice had minimal weight loss over 7 dpi. However, mice that later became moribund showed significant weight loss from day 4 and significant lymphopenia and neutrophilia in peripheral blood at day 6, which recapitulates severe human disease [72,73]. Lung histology showed initial macrophage and lymphocyte infiltration and fibrin exudation from 1 dpi, which steadily progressed to severe pneumonia, blockage of terminal bronchioles, extensive fibroplasia, and alveolar necrosis by day 7[68].In contrast to previous findings of lung tissue specificity, HFH4-hACE2 infected mice had detectable viral titers in the lung, eyes, brain, and heart suggesting that the virus may have additional tissue tropisms following initial lung infection [70,74]. Re-infection following recovery from initial SARS-CoV-2 infection resulted in reduced weight loss and viral titres and improved survival indicating the development of protective immunity following initial challenge.

Murine mAce2 Promoter-hACE2 Transgenic Mouse Model

The first live SARS-CoV-2 infection model used is transgenic mice expressing hACE2 under the control of the mACE2 promoter [70,75]. Mice had significant weight loss over a 14-day infection period, and high viral lung titers 1–5 dpi. Histological lung examination revealed moderate interstitial pneumonia, infiltration of lymphocytes, mucus accumulation, and desquamation of bronchial epithelial cells from day 3 [70].There were no detectable viral titers or pathology in other tissues or organs, except on day 1 in the intestine, suggesting that infection is localized almost exclusively to the lungs.

Viral Transduction of hACE2 Using Adenoviral systems

An adeno-associated virus (AAV) delivery based mouse model that expresses the SARS-CoV-2 receptor in the mouse lungs is developed to study SARSCoV-2 pathogenesis [55,76,77]. The model is a more efficient and rapid, and reproducible murine model for SARS-CoV-2.

Transduction of BALB/c mice with adenovirus containing hACE2 (AdV-hACE2), led to stable hACE2 expression in the lungs from 10 h post-transfection [78]. SARS-CoV-2 infected AdVhACE2 mice had ~10% weight loss over 8 days, high viral lung titers and modest titers in the heart, brain, liver and spleen, extensive neutrophil accumulation in perivascular and alveolar locations and vascular congestion upon histological examination. Administration of anti-IFNAR1 monoclonal antibodies to transiently inhibit type-I IFN signaling resulted in up to 20% weight loss and more severe lung inflammation, compared to infection alone. In this system, neutralizing antibodies against SARS-CoV-2 S protein (1B07), were protective against severe disease. Mice lost less body weight and had lower viral titers in the lung, heart and spleen at 4 dpi, and reduced expression of pro-inflammatory cytokines and chemokines (Ifnb, Il6, Cxcl10, Cxcl1, Ccl2, Ccl5), and immune cell infiltrates in the lungs at 6 dpi. The limitations are that this model artificially expresses ACE2 in non-relevant cell types in the mouse respiratory system making pathology and immune response data hard to interpret in the situation of human SARS-CoV-2 infection. However, this model can be suitable for drug therapy and antibody testing [63].

Murine mAce2 Exon 2-hACE2 Knock in Mouse using g CRISPR-Cas9 technology

Using gene-editing CRISPR-Cas 9 technology, Sun et al inserted hACE2 cDNA into Exon 2 of the mAce2 gene to disrupt mAce2 gene expression and drive expression of hACE2 under the control of mAce2 promoter [73]. hACE2 expression occurs in the lung, small intestine, spleen, and kidney. In the lung, hACE2 has expressed in the CC10+ Clara cells in the airways as well as a subpopulation of surfactant protein C positive alveolar type II cells. High viral loads of SARS-CoV2 are evident in the lung, trachea, and brain (despite low hACE2 expression), on intranasal infection. Young inoculated animals do not display obvious clinical symptoms but develop interstitial pneumonia and vascular system injury. More severe disease is seen in aged hACE2. These exhibits more marked weight loss, more prolonged viral shedding (including from feces), and more severe pneumonia accompanied by stronger cytokine responses. Intragastric inoculation of SARS-CoV-2 induces productive infection and pulmonary disease.

Besides the above transgenic mouse, a few knock-out mouse model has been developed to understand the pathology of SARS-CoV-2. Among them, ACE-/- knockout mice can be used to study the effects of Angiotensin conversion enzyme during acute lung injury study [79].Using TMPRSS2-/-knockout mice the role of TMPRSS2 during SARS-CoV entry into cells can be studied for new drugs against SARS-CoV-2 infections [80]. Humanized DPP4 mice and STAT-1-/-knockout mice having susceptibility to coronavirus infection and used as a model for MERS can help in SARS-CoV-2 also [81,82].

The use of small animals in preclinical research on SARS-CoV-2 infection involves a broad spectrum of models, from infecting wild-type animals with adapted viruses to multiple methods of introducing human ACE2 receptors. A variety of murine models for mild and severe COVID 19 have been under development. The disease is typically mild in mice, although transgenic mice present with more severe diseases. Each model offers selected advantages that will be useful not only for the testing of therapies and diseases but also in understanding the disease enhancement and related comorbidities. No murine model at present recapitulates all aspects of human COVID 19, especially unusual features such as pulmonary vascular disease and hyperinflammatory syndrome observed in adults and children, respectively [30,83]. However, continued refinement may eventually result in models for these aspects of the human disease.

Syrian hamster model

Golden Syrian hamsters (Mesocricetus auratus), have been shown to have distinct advantages as small animal models for diseases involving respiratory viral infections including influenza virus, adenovirus, and SARS-CoV [56,84]. Following infection

by the intranasal route, golden Syrian hamsters demonstrate clinical features, viral kinetics, histopathological changes, and immune responses that closely mimic the mild to the moderate disease described in human COVID-19 patients [13,14,85].In this form of non-lethal disease, the clinical signs include rapid breathing, decreased activity, and weight loss that is most severe by day 6 post-infection. Airway involvement is evident, with histopathology showing a progression from the initial exudative phase of diffuse alveolar damage with extensive apoptosis to the later proliferative phase of tissue repair. Micro-CT analysis of infected hamsters revealed severe lung injury with the degree of lung abnormalities related to the infectious dose. Commonly reported imaging features of COVID-19 patients with pneumonia were present in all infected animals [79]. High-dose SARS-CoV-2 infection led to severe weight loss and partial mortality while older hamsters appear to exhibit more pronounced and consistent weight loss [86,87].Other findings include intestinal mucosal inflammation, degenerative changes, and lymphoid necrosis. There is a marked activation of the innate immune response, with high levels of chemokines/cytokines induced by the infection [85].

Transmission of COVID-19 from infected hamsters to naive cage mates suggests the utility of the model for studying transmission [14,85,88]. In addition, passive transfer studies with either convalescent sera or neutralizing monoclonal antibodies showed great promise for studies related to immunity and vaccine development [85,89]. The golden Syrian hamster model of SARS-CoV-2 infection appears to be a suitable model for the evaluation of antiviral agents and candidate vaccines [90,91,86].Hamsters carrying the hACE2 receptor under the control of the epithelial K18 promoter are also being evaluated as a model. In an initial study of SARS-CoV-2 infection of hACE2-hamsters, clinical signs were observed including elevated body temperatures, slow or reduced mobility, weight loss, and mortality (1 out of 4 animals). Virus titers were detected in lungs, heart, and brain tissues, with the highest titers observed in lungs on days 1–3. Hamsters with immune systems compromised by either cyclophosphamide treatment or RAG2-deficiency demonstrated more severe disease, longer in duration (cyclophosphamide induction) or resulting in mortality (RAG2).This could be protected by human antibody given prophylactically [92].

Ferret models

Ferrets are considered good models for respiratory diseases, as the physiology of their lungs and airways are close to humans, and they have been used extensively to model diseases caused by many respiratory viruses including influenza, RSV and SARS-CoV [93,94,95]. Unlike rodents, ferrets cough and possess a sneeze reflex, making them a particularly useful model in the study of disease transmission. Ferrets exhibit lethargy and appetite loss following infection with SARS-CoV-2 via the intranasal route, but the disease does not progress to acute respiratory disease, and the animals recover from the infection [96,97].Virus shedding from the upper respiratory tract (nasal washes, saliva) can persist for up to 21 dpi; the length of shedding appears to be dependent on the initial viral challenge dose and can be intermittent after 14 days. Mild multifocal bronchopneumonia is observed in early post-infection (day 3 in animals receiving 4 to 6 logs of virus).Fever has been reported in some studies, but neither coughing nor dyspnoea have been observed [96,97]. Ferrets re-challenged after 28 dpi appear to be completely protected [98]. SARS-CoV-2 was easily transferred to naive direct contact ferrets, but less so to naive indirect contact ferrets, resulting in productive infection and the identification of infectious virus in indirect receivers [96,99].To date, studies performed in ferrets strongly indicate that experimental SARS-CoV-2 infection results in a predominantly upper-respiratory tract infection in these animals. These findings make the ferret model well-suited to test the efficacy of mucosal vaccines and therapeutic agents that aim to prevent upper airway infection and/or transmission.

Non-human primates

Non-human primate models have been explored for COVID-19 in rhesus macaques, cynomolgus macaques, and African green monkeys. Nonhuman primates (NHP), in particular M. mulatta (Rhesus macaques) can be a good model to study COVID-19 pathogenesis. NHP models have been developed for SARS CoV-2 to resemble the condition seen in human pathogenesis. NPH models are considered the gold standard animal model for modelling human infectious diseases. The lack of suitable substitutes for NHP models for predicting response in humans serves as a bottleneck for the development of countermeasures against infectious diseases like SARS-CoV-2.

Rhesus Macaques

SARS-CoV-2 infects rhesus macaques (M. mulatta), which develop a moderate, non-lethal shedding disease phenotype with few to no clinical manifestations. If clinical observations are reported, they are typically transient and include reduced appetite, mild dehydration, tachypnoea, piloerection, and dyspnea [17].When reported, fever is mild and transient beginning shortly after 2 dpi and resolving within 2 or 3 days [17]. Bodyweight loss will be the findings in mild infection and transient drops in weight followed by recovery [17].Clinical chemistry and haematology are generally unremarkable. However, transient leucocytosis, neutrophilia, monocytosis, and lymphopenia are reported [17,100].Imaging (radiographs or PET/CT) confirms rhesus macaques are infected, with infiltrates and ground-glass appearances in radiographs, beginning early after exposure (2 or 3dpi), and resolution occurring by 10–14 dpi [17,100]. Anecdotal evidence suggests that older rhesus macaques develop a chronic infection, in which infiltrates persist throughout the study [101].When available, PET/CT images corroborate the radiograph findings [100].The virus is detected in nasal, throat, rectal, and ocular swabs and in bronchoalveolar lavage (BALs) via median tissue culture infectious dose (TCID50), beginning at approximately 2dpi, peaking around 4/5dpi, and decreasing after 6 dpi [16,17,100-103] . Finally, exposed rhesus macaques seroconvert, as demonstrated by a SARS-CoV-2 anti-spike ELISA and neutralization assays to various endpoint titers, depending on the laboratory and assay format utilized, and are protected from reinfection [17,104].

Cynomolgus Macaque

Cynomolgus macaques (M. fascicularis), have been used to study the pathogenesis of SARS-CoV in which aged animals were

more likely to develop disease. When exposed to SARS-CoV2, they become infected but show no overt clinical signs of disease [105].Weight loss is not observed, but in some studies, infected animals have a fever on days 2 to 3,[16,106].Virus shedding from the upper respiratory tract occurs, peaking early at 1dpi in young animals and 4 dpi in aged (15–20 years) animals, then decreasing rapidly but still detected intermittently up to 10dpi [105]. Overall, higher levels of virus shedding were measured in aged animals than young animals [105]. They develop mild to moderate lung abnormalities and macroscopic lesions in the lungs including alveolar and bronchiolar epithelial necrosis, alveolar edema, hyaline membrane formation, and accumulation of immune cells [106,107]. While infection is self-limiting, the disease in cynomolgus macaques does recapitulate many aspects of human COVID-19 and could be utilized to test preventative and therapeutic strategies [105].

African Green Monkeys

African green monkeys (AGMs), exposed to SARS-CoV-2 as young adults display a mild, non-lethal shedding disease phenotype that includes few to no clinical observations [108]. If clinical observations, such as fever, are reported, they are typically transient and mild with no serious manifestations [19,108]. Bodyweight findings are generally unremarkable. Clinical chemistry and haematology reveal mild and transient shifts in leukocyte populations, mild thrombocytopenia, and selected liver enzymes [19]. A measure of acute inflammation, CRP, is elevated early in infection. Imaging (radiographs or PET/CT) confirms that AGMs are infected with infiltrates and ground-glass appearances in radiographs beginning early after 2 or3 dpi and resolving by 10dpi. When available, PET/CT images corroborate the radiograph findings. Finally, plethysmography suggests respiratory disease, but there is no consistent trend [108].The presence of SARS-CoV-2 in bronchoalveolar lavage (BAL), may be detected using RT-PCR and a plaque assay by 3dpi and lasts at least until 7dpi [19,108].Finally, as proven by a SARS-CoV-2 anti-spike ELISA and neutralisation assays to various endpoint titers, exposed AGMs seroconvert to varied endpoint titers, depending on the laboratory and assay format used [19,108].

CONCLUSION

In this review article, we have explained the current status of animal models available for Covid 19 research. Currently, the dire need for the development of animal models are increasing in order to promote preclinical researches on Covid 19. Essentially, animal models are required to define the cause, effects, and elucidate pathophysiology and transmission processes that must be validated and translated into human studies. They need to replicate human disease features individually and collectively. Several models have been attempted thus far. However, there is no clear model that is preferred for studying SARS CoV-2 infection as the clinical signs, recovery, and transmission vary between and within species. Each animal model seems to have its own merits and demerits, and careful consideration is required before the selection of animal models. Mice that are modified genetically or with adenoviruses or CRISPR, or WT mice infected with mouse-adapted viruses will undoubtedly be the most widely used due to ease and costs but also because they replicate human features of pulmonary inflammation, histopathology and pneumonia. The researchers using these mouse strains should be careful in interpreting the data obtained from these mouse models. Possibly the study on the transgenic mouse can provide a proof of concept for understanding pathogenesis. Hamsters are ideal for studying the replication of mild SARS-CoV-2 infections seen in humans, as well as the virus's host defence response. They may also be utilised to better understand SARS-CoV-2 pathogenesis and test vaccines and antiviral medicines. Ferrets on the other hand can be appropriate for disease transmission and lung infections. Because SARS-CoV-2 infection in non-human primates, particularly the rhesus macaque, is most similar to that seen in humans, it could be a useful model for testing vaccinations and medication efficacy.

Available animal models are the scientific resources for researchers to elucidate preclinical data on investigational products to prevent and control COVID 19. However, these resources on animal models need to be more clear and validated by many more studies to understand the disease pattern as seen in humans .Standardization of animal models is critical for future research on Covid 19 in order to compare different medications and vaccine candidates, as well as to develop appropriate animal models for testing drug and vaccine potential.

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