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Letter to the editor Open Access

Infectivity of SARS-CoV-2 and protection against reinfection in rats

DEAR EDITOR,

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic remains an important global public health issue. In this study, we unexpectedly found that wild-type Sprague-Dawley (SD) rats can be infected with the SARS-CoV-2 prototype. Our results showed direct experimental evidence of the infectivity of SARS-CoV-2 infection, subsequent pathogenicity, and protection against reinfection in rats.

Proper animal models for coronavirus disease 19 (COVID-19) are crucial to uncover disease mechanisms and develop appropriate vaccines and drugs. To determine the best model for studying COVID-19, a wide range of animals, including captive and wild animals (deer, lions, and gorillas), companion animals (cats and dogs), farm animals (pigs, chickens, ducks, and mink), and experimental animals (nonhuman primates, mice, hamsters, ferrets, and Chinese tree shrews), have been used to study susceptibility to SARS-CoV-2 infection, with each model harboring its own advantages and disadvantages (Fan et al., 2022; Muñoz-Fontela et al., 2020). Among these COVID-19 models, rhesus macaques well mimic the pathological characteristics of human patients; hamsters are the most suitable for understanding transmission of SARS-CoV-2; and human angiotensin-converting enzyme 2 (*hACE2*) transgenic mice are the most widely used for vaccine and drug evaluation (Fan et al., 2022).

Over the past century, rats have been the preferred rodent model in biomedical research, especially for studies in cardiovascular disease, diabetes, and nutritional metabolic disorders (Modlinska & Pisula, 2020). In the past four decades, mice have become increasingly used in research due to the establishment of mouse embryonic stem cells and gene knockout technology. Nonetheless, rats are still widely used for toxicological and pharmacological research, including assessment of SARS-CoV-2 vaccines and antiviral drugs (Cao et al., 2022). However, even if good antibody responses to vaccines or potential efficacy of drugs is achieved in rats, other susceptibility models are needed to study the effects of antiviral treatment on SARS-CoV-2 infection (Cohen, 2020). During our SARS-CoV-2 research, we created a *hACE2* transgenic rat model and unexpectedly found that wild-type Sprague-Dawley (SD) rats could be infected with the SARS-CoV-2 prototype. Thus, we designed two experiments to evaluate the susceptibility and subsequent pathogenicity of SARS-CoV-2 infection in rats.

In experiment I, SD rats aged 8–9 weeks were intranasally infected with a 1×10^5 median tissue culture infective dose ($TCID_{50}$; 50 μL) of the SARS-CoV-2 prototype (viral sequence available in the China National Microbiology Data Center under Accession No. NMDCN0000HUI) (Figure 1A). Rats were monitored for weight and temperature changes over 16 days (Figure 1B). We observed a gradual increase in body weight in both the infected and mock groups, consistent with the growth characteristics of SD rats at this age, and the increasing trend in body weight continued until the end of the study. However, there was a substantial difference in body weight between the two groups (Figure 1B). Average body temperature was similar in both groups (Supplementary Figure S1), and none of the infected rats exhibited the obvious clinical symptoms of COVID-19 as observed in humans (Wiersinga et al., 2020).

We characterized SARS-CoV-2 replication dynamics in the tracheal and lung tissues of rats. Viral loads were assessed by quantitative real-time polymerase chain reaction (RT-qPCR) of SARS-CoV-2 genomic (nucleoprotein gene (N) and envelope gene (E)) and subgenomic RNAs (sgRNA; subgenomic E (sgE) gene, marker of infectious virus (Dagotto et al., 2021)). Viral genomic RNA (N and E genes) was detected in throat

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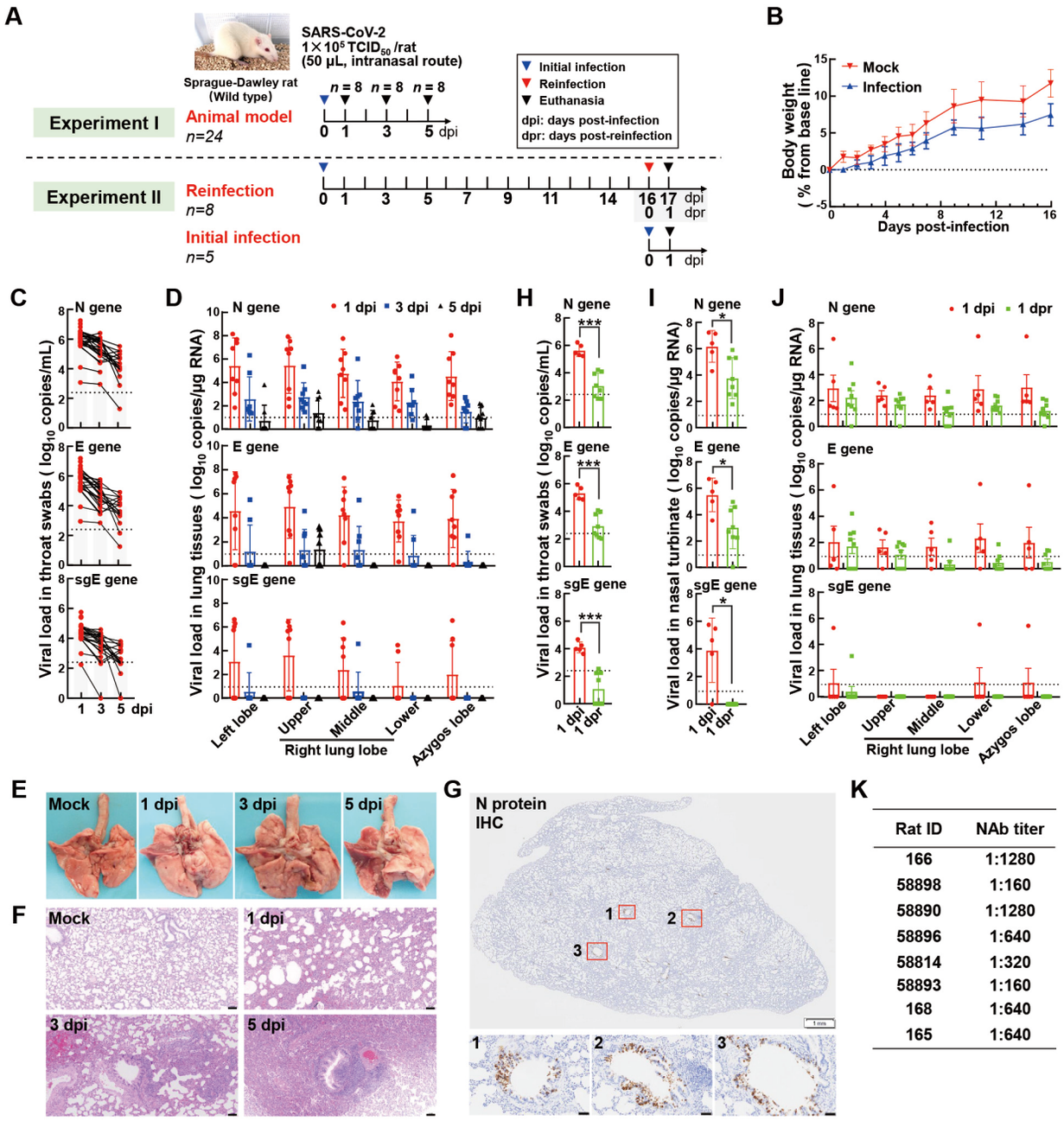

Rat ID	NAb titer
166	1:1280
58898	1:160
58890	1:1280
58896	1:640
58814	1:320
58893	1:160
168	1:640
165	1:640

Figure 1 Infection with SARS-CoV-2 prototype in Sprague-Dawley rats

A: Schematic of infection experiments. B: Alterations in body weight in rats with (infection group) or without (mock group) SARS-CoV-2 infection. Data are mean±standard error of the mean (*SEM*). C: Viral RNA levels in throat swabs of infected rats at 1 dpi (n=32), 3 dpi (n=24), and 5 dpi (n=16). Each dot indicates log copies of viral genomic N gene (top), E gene (middle), and subgenomic E (sgE) gene (bottom) per swab from an individual rat. D: Viral RNA levels in lungs of infected rats at 1, 3, and 5 dpi. Each red circle (1 dpi, n=8), blue square (3 dpi, n=8), and black triangle (5 dpi, n=8) refers to log viral RNA copies/μg total RNA of lung lobe of an individual rat. E: Gross pathological lung specimens at indicated time points after SARS-CoV-2 infection. F: Hematoxylin/eosin (H&E) staining of the lung sections in SARS-CoV-2-infected rats showing lesions with thickened alveolar septa and infiltration of lymphocytes. Scale bar: 100 μm. G: Immunohistochemical analysis of SARS-CoV-2 N protein in rat lung tissues on 1 dpi. Scale bar: 1 mm for entire lung lobe section (top), 50 μm for enlarged view of boxed areas labeled by numbers in entire section (bottom). H, I: Viral RNA loads in throat swabs (H) and nasal turbinate (I) of rats re-challenged with SARS-CoV-2 for 1 day (1 dpr, n=8) were significantly lower than in initial infection group rats (1 dpi, n=5). *: P<0.05; ***: P<0.001; two-tailed Student's t-test. J: Viral RNA levels in lungs of infected rats at 1 dpi (initial infection group) and 1 dpr (reinfection group). K: Neutralizing antibody (NAb) titers in SARS-CoV-2-infected rats for protection against re-challenge. Values in D, H, I, and J are presented as mean±standard deviation (*SD*). Dotted lines in C and H represent limit of quantification (250 copies/mL). Dotted lines in D, I, and J represent limit of quantification (8.7 copies/μg total RNA of lung).

swabs collected from each infected animal at 1 day and 3 days post-infection (dpi), followed by a sharp decline at 5 dpi, although most infected rats contained viral RNAs at this time (N gene, 15/16; E gene, 14/16; sgE gene, 10/16) (Figure 1C). We detected viral genomic RNA in the lung lobes of infected rats at 1 dpi, and viral sgE gene transcripts reached ~10^6 copies/μg RNA in the lung tissue. For animals euthanized at each time point (n=8), six out of eight infected rats at 1 dpi and two out of eight infected rats at 3 dpi had detectable levels of viral sgRNA, but sgRNA was not detected in lung tissue at 5 dpi (Supplementary Figure S2).

To exclude the possibility that the detected sgE transcripts were from residual input inoculum, we measured viral sgRNA levels in the input inoculum for comparison with viral sgRNA levels in the lung tissue of infected rats. Results showed that sgRNA abundance in 50 μL of input inoculum (1×10^5 $TCID_{50}$) was 5.5 $\log_{10}$ copies in total, much lower than the sgRNA level in infected lung tissue (mean viral load of 5.9 $\log_{10}$ copies/μg total RNA in lung tissue) at 1 dpi. Furthermore, infectious virus was present in the lung tissues of five ($TCID_{50}$/mL value ranges from $10^{3.8}$ to $10^{6.8}$) out of eight infected rats (Supplementary Figure S3). These results suggested that viral replication can occur at the early stage of SARS-CoV-2 infection in rats.

Gross necropsy revealed visible lung lesions, mainly mild edema and sporadic punctate hemorrhage, in the SARS-CoV-2-infected rats at the three time points (Figure 1E). Histopathological examination of the lung sections from infected animals from 1 dpi to 5 dpi showed pathological indicators of the severity of lung injury, including alveolar septal thickening, interstitial edema, hemorrhage, and mild diffused peribronchial infiltrates (Figure 1F). In addition, immunohistochemical staining of the lung sections detected high expression of the SARS-CoV-2 nucleocapsid (N) protein in the epithelial cells of the bronchioles at 1 dpi (Figure 1G; Supplementary Figure S4). However, N protein staining was not found in the lung sections at 3 dpi and 5 dpi. These results suggested that SARS-CoV-2 can replicate in the upper respiratory tract of rats in the early stages of infection.

In experiment II, we assessed the potential effects of SARS-CoV-2 infection on reinfection. Eight rats were infected with the SARS-CoV-2 prototype (1×10^5 $TCID_{50}$, 50 μL), then re-challenged with the same amount of SARS-CoV-2 prototype at 16 dpi (reinfection group). Five healthy rats were infected with the SARS-CoV-2 prototype (initial infection group) as a positive control (Figure 1A). Both groups were euthanized one day after infection with SARS-CoV-2. Consistent with the above observations, we detected viral genomic copies and sgRNA in the initial infection group (Figure 1H), with similar viral loads (sgE gene) in throat swabs to those infected animals at 1 dpi in experiment I (Figure 1C). We observed significantly lower levels of viral RNA in throat swabs and nasal turbinate samples from the reinfection group (Figure 1H, 1I). Notably, the sgE transcript was not detected in the nasal turbinate samples from the reinfection group (Figure 1I). Fewer viral genomic copies and sgRNA were found in the lung lobes of the reinfection group compared to the initial infection group (Figure 1J). Only one reinfected rat had detectable (low) levels of sgE transcript in the left lung lobe, whereas no detectable levels were found in the other seven reinfected rats (Figure 1J). We further measured neutralizing antibody titers in serum samples collected from the reinfected rats. Circulating neutralizing antibodies were detected in all reinfected rats (Figure 1K), indicating an enhanced immune response to SARS-CoV-2 infection. These results suggested that primary exposure to SARS-CoV-2 may have a protective effect against reinfection in rats. It may be worth detecting viral loads at later stages of infection (i.e., 3 days post-reinfection (dpr) and 5 dpr) to assess whether initial SARS-CoV-2 challenge delays viral replication kinetics of reinfection in SD rats.

Here, we provide multiple lines of evidence that the SARS-CoV-2 prototype can infect rats. First, we found high levels of viral genomic copies and sgRNA in throat swabs, nasal turbinate samples, and lung lobe tissues of infected rats at the early stage of infection. Second, we observed viral N protein expression and histopathological changes in the lung sections of infected rats. Third, SARS-CoV-2 infection induced circulating neutralizing antibodies in the rats, which also offered protection against reinfection. However, most infected rats experienced rapid viral clearance, suggesting that SD rats may exhibit a different immune response to SARS-CoV-2 than hamsters and rhesus macaques (Fan et al., 2022; Feng et al., 2022; Muñoz-Fontela et al., 2020).

The conventional view was that wild-type mice could not be infected with SARS-CoV-2 (Muñoz-Fontela et al., 2020). However, with the emergence of SARS-CoV-2 variants of concern, wild-type mice showed an infective and pathogenic phenotype to B.1.1.7 (alpha) and B.1.351 (beta) infection, likely due to mutations of N501Y in the receptor-binding domain (RBD) of the spike (S) protein (Huang et al., 2021). Similarly, wild-type rats could also be infected with the B.1.1.7 and B.1.351 variants (Shuai et al., 2021; Zhang et al., 2022), with those infected with the B.1.351 variant also able to transmit the risk to other rats (Zhang et al., 2022). However, screening samples of Norway rats inhabiting the sewers showed no SARS-CoV-2 infection (Colombo et al., 2022). During our efforts to create a *hACE2* transgenic rat model, we unexpectedly discovered that wild-type SD rats (serving as a control group for *hACE2* transgenic rats) can be infected with the SARS-CoV-2 prototype, thus providing direct evidence of SARS-CoV-2 infection in wild-type SD rats, although the exact reason remains to be explored. Whether other rat strains (particularly wild rats) are susceptible to SARS-CoV-2 infection remains unknown but raises potential ecological risks. Although the binding affinity of the SARS-CoV-2 S protein to rat ACE2 was not higher than that in mice (Cohen, 2020), the expression and distribution of ACE2 in rats may explain the infection. Warnings about hypertension as a moving target in COVID-19 stemmed from studies of pulmonary ACE2 expression in rat models of hypertension (Savoia et al., 2021). In addition, the expression of SARS-CoV-2 cell entry-associated molecules (e.g., furin, TMPRSS2, ADAM17) in rats with heart failure also indicates susceptibility to SARS-CoV-2 (Khoury et al., 2021). Our study not only supports the use of SD rats for assessing the toxicology,

pharmacology, and efficacy of SARS-CoV-2 vaccines and antiviral drugs (Cao et al., 2022), but also for modeling the infection process.

In conclusion, we found that SD rats can be infected with the SARS-CoV-2 prototype, with detectable viral loads in the upper respiratory tract and lung lesions. Initial SARS-CoV-2 infection may provide protection against reinfection.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

D.Y. and Y.G.Y. conceived and designed the study. D.Y., Y.L., L.X., J.B.H., X.L.F., W.Q., and M.H.L. performed the experiments and analyzed the data. J.Xi, J.Xu, L.X.Y., J.L., and Q.C.Z. prepared the animals. D.Y. performed data analyses, D.Y. and Y.G.Y. wrote the manuscript. All authors read and approved the final version of the manuscript.

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