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Electronic Supplementary Material

The Infection and Pathogenicity of SARS-CoV-2 Variant B.1.351 in hACE2 Mice

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Supplementary Methods

Mouse and Virus

12-month-old female hACE2 mice used in this study were generated by inserting the *hACE2* gene into exon2, the first coding exon, of mouse ACE2 locating in chromosome X GRC m38.p6. of zygotes of C57BL/6 mice as we reported previously (Sun *et al.* 2020). The B.1.351 variant (501Y.V2, CSTR:16698.06.NPRC2.062100001) was isolated from an imported patient from South Africa and stored in National Pathogen Resource Center (NPRC), China. Viruses were amplified and titrated by standard plaque forming assay on Vero cells.

Mouse Challenge Experiments

For intranasal infection, mice were anesthetized with sodium pentobarbital at a dose of 50 mg/kg though intraperitoneal route, and then intranasally infected with 1.2×10^4 pfu of B.1.351. Mice were then weighed and monitored daily, and sacrificed on indicated time points for serum collection and tissue processing.

Measurement of Viral sgRNA

Tissue homogenates were clarified by centrifugation at 6,000 rpm for 6 min, and the supernatants were transferred to a new EP tube. RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's protocol. sgRNA quantification in each sample was performed by quantitative reverse transcription PCR (RT-qPCR) targeting the *S* gene of SARS-CoV-2. RT-qPCR was performed using One Step PrimeScript RT-PCR Kit (Takara) with the following primers and probes: sgRNA-F (5'- CGATCTCTTGTAGATCTGTTCTC-3'); sgRNA-R (5'- ATATTGCAGCAGTACGCACACA-3'); and sgRNA-P3 (5'- ACACTAGCCATCCTTACTGCGCTTCG-3').

RNA ISH Assay

SARS-CoV-2 genome RNA ISH assay was performed with RNAscope2.5 HD Reagent Kit (Advanced Cell Diagnostics) according to the manufacturer's instruction. Lung tissues were fixed in 4% PFA for 48 h, and paraffin-embedded in accordance with the standard procedure. The formalin-fixed paraffin-embedded tissue sections of 4 μm were deparaffinized by incubation for 60 minutes at 60 °C. Endogenous peroxidases were quenched with hydrogen peroxide for 10 min at room temperature. Sections were then boiled for 15 minutes in RNAscope Target Retrieval Reagents and incubated for 30 minutes in RNAscope Protease Plus before probe hybridization. Tissues were counterstained with Gill's hematoxylin and visualized with standard bright-field microscopy.

Histopathological Analysis

Paraffin tissue sections of 4 μm thickness were deparaffinized with xylene, rehydrated through successive bathes of ethanol/water and washed with distilled water at room temperature. The deparaffinized sections were stained with hematoxylin and eosin, and examined by light microscopy. The largest lung tissue lesions from each lung tissue were mainly assessed according to the degeneration and necrosis of bronchiole epithelial cells and alveoli pneumocytes, changes of alveoli structure, infiltration of inflammatory cells and hemorrhage. The degree of lung damage under the light microscopy was assessed by the degeneration and necrosis of bronchiole epithelial cells and alveoli pneumocytes, changes of alveoli structure, infiltration of inflammatory cells and hemorrhage. The semiquantitative assessment was performed as the following. For the degree of degeneration of alveolar epithelial

cells and bronchiolar epithelial cells, we scored 0 when no cell degeneration was observed, scored 1 when the cell degeneration was less than 10%, and scored 2 when the degeneration was 10%–50%. For the degree of alveolar septal thickening, we scored 0 when no alveolar septal thickening was observed, scored 1 when the alveolar septal thickening area was less than 10%, scored 2 when the area was 10%–50%, scored 3 when the area ＞50%, and 1 score was added when alveolar wall fusion was observed. For the degree of inflammatory cells infiltration, we scored 0 when no inflammatory cell infiltration, scored 1 when occasional infiltration of single inflammatory cell was visible, and scored 2 when focal infiltration of inflammatory cells was visible. For the degree of hemorrhage, we scored 0 when no hemorrhage was observed, scored 1 when occasional hemorrhage was visible, and scored 2 when focal hemorrhage was visible. The lesion of lung was observed by an experienced experimental pathologist who focuses on the pathogenesis study of virus infection, and the average of the three animals was taken as the total score for that group.

Immunofluorescence Staining

For immunostaining, paraffin tissue sections were deparaffinized as described above, and then put in EDTA (pH9.0) for 1 hour at 96℃for antigen retrieval. The endogenous peroxidases were inactivated with 3% hydrogen peroxide for 25 min, and the sections were then blocked with 3% BSA for 30 minutes. SARS-CoV/SARS-CoV-2 Nucleocapsid Antibody, Mouse Mab (Sino Biological, 1:500) was used as the primary antibody for incubating with the sections for 2 hours in a humidified chamber at 37°C. After 3 washes, the sections were incubated with the FITC-labeled secondary antibody (Zhongshan Biotechnology) for 1 hour followed by 5 min of DAPI staining.

Immunohistochemistry

For detecting inflammatory cell infiltration, the paraffin tissue sections were treated as above, and then incubated with rabbit anti-LY6G polyclonal antibody (Servicebio, 1:800), rabbit anti-CD68 polyclonal antibody (Servicebio, 1:200) or rabbit anti-CD3 polyclonal antibody (Servicebio, 1:200) overnight at 4 °C. After three washes, the sections were incubated with HRP-labelled secondary antibody (Servicebio, 1:200) at 37 °C for 1 h, followed by DAB (Servicebio). The sections were counterstained with hematoxylin for observation by microscopy.

Cytokine Assay

A total of 25 μL serum from each mouse was adopt for cytokine analysis with Mouse Cytokine & Chemokine Panel 1A (36 plex) (Invitrogen) according to the manufacturer's instructions. The data were collected on Luminex 200 and analyzed by Luminex PONENT (Thermo Fisher).

Statistical Analysis

Statistical analyses were carried out using Prism software (GraphPad Prism 7.0). Data of semiquantitative analysis of the H&E-stained lung sections are presented as mean. Other data are presented as mean ± SEM. Statistical details of experiments and animal replication numbers (n) are stated in the relevant figure legends and method details.