LETTER

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

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Dear Editor,

Since the outbreak of COVID-19 at the end of 2019, its causative agent SARS-CoV-2 has been spreading around the world for one and half a year. During the long global circulation of SARS-CoV-2, mutations in the viral genome gradually emerged and accumulated, which have led to the emergence of Variants of Concerns (VOC). The mutation linage B.1.351 (also termed as 501Y.V2 or Beta variant by WHO) was announced in December 2020 and rapidly became the predominant lineage in South Africa. The B.1.351 is characterized by eight mutations in S protein, including K417N, E484K and N501Y in RBD (Tegally *et al*. 2021). The N501Y and E484K mutations have been demonstrated reducing neutralizing titer of antibodies, vaccine-induced and SARS-CoV-2-infected sera *in vitro*,

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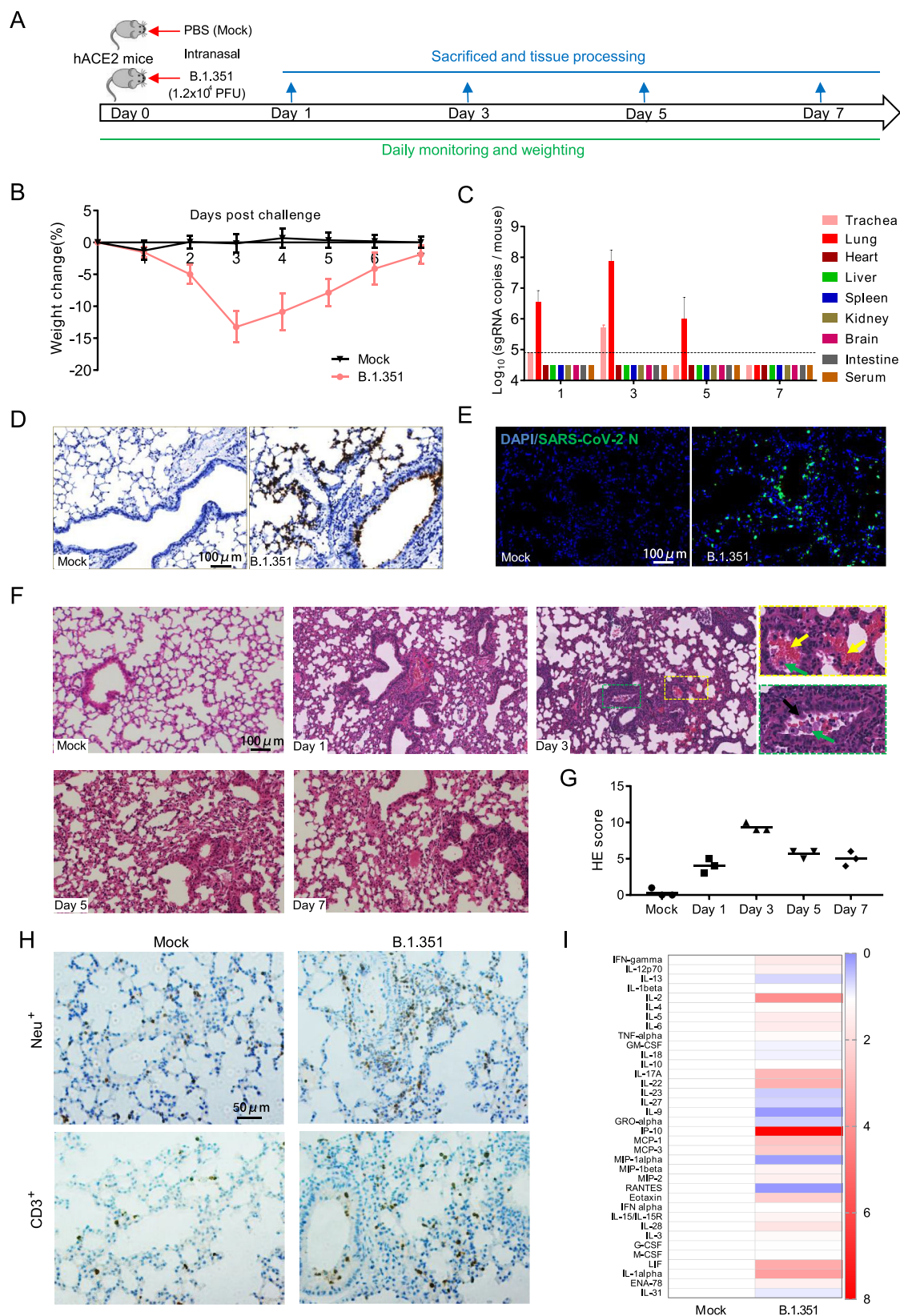
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which raised concerns of current vaccine efficacy against the mutation lineages (Li *et al*. 2021; Wang *et al*. 2021).

Previously, we have demonstrated the ACE2 humanized mice (*hACE2*), whose *ACE2* (*mACE2*) genes were replaced with the human *ACE2* by using CRISPR/Cas9 knock-in technology, are highly susceptive to SARS-CoV-2 isolated in early 2020 (Singh *et al*. 2020; Sun *et al*. 2020). Thereafter, the hACE2 mice model has been widely used to investigate the pathogenic mechanism and evaluate antivirals against SARS-CoV-2 (Lv *et al*. 2020; Ye *et al*. 2020; Zhu *et al*. 2020). However, the infectivity and pathogenicity of B.1.351 variant remains not determined.

Herein, we characterized the replication dynamic and clinical outcome of B.1.351 variant in hACE2 mice with the same protocol as previously described. Accordingly, two groups of 12-month-old female hACE2 mice were intranasally challenged with the B.1.351 variant at a dose of 1.2×10^4 plaque forming unit (pfu) per mouse for daily monitoring (n = 8) and tissue collection (n = 12), respectively (Fig. 1A). The 501Y.V2-infected mice began to show ruffled fur and reduced activity on 3 days post infection. Meanwhile, B.1.351 variant infection caused significant weight loss, and the average weight loss reached ∼ 13% on 3 days post infection (Fig. 1B). Then the mice began to recover and no death occurred during the seven-day observation period. These results indicate that the B.1.351 variant is pathogenic for the hACE2 mice, and leads more severe clinical symptoms than the prototype strain (IME-BJ05), which led only ∼ 10% weight loss and no obvious clinical symptoms (Sun *et al*. 2020).

SARS-CoV-2 subgenomic RNA (sgRNA) quantitation was further performed for detecting the viral loads in the main tissues and serum of mice on day 1, 3, 5 and 7 post infection. Results showed that high level of SARS-CoV-2 sgRNA was detected in the lungs and tracheas from B.1.351-infected mice (Fig. 1C). An obvious sgRNA increasing trend was observed in lung tissues and tracheas during 1–3 days post infection, and then the sgRNA loads

began to decrease (Fig. 1C). No viral sgRNA was detected
in serum and other tissues. *In situ* hybridization (ISH) assay
by RNAScope for the lung sections showed that the viral
RNA mainly located in bronchiolar epithelial cells and at
the alveolus walls (Fig. 1D). Immunostaining of lung
sections also showed that the SARS-CoV-2 N protein
expressed in both bronchiolar epithelial cells and alveolar
cells (Fig. 1E). Taken together, these results indicate that
the B.1.351 variant can effectively infect hACE2 mice,
with robust viral replication in the respiratory system.

More importantly, histopathological observation showed
that lung tissues from B.1.351-infected mice began to show
injury as early as on day 1 post infection and became more
severe on day 3 (Fig. 1F-G), characterized with denatured
and collapsed bronchiolar epithelial cells (black arrow),
thickened alveolar septa, alveolar damage, hemorrhage
(yellow arrow), and macrophages infiltration (green arrow).
Immunohistochemistry (IHC) staining indicated that the
infiltrated inflammatory cells also include Neu$^+$ neu-
trophils and CD3$^+$ T cells (Fig. 1H), as well as the mac-
rophages. The degree of alveolar septa thickening, alveolar
and bronchiolar epithelial damage became lower, and the
macrophages infiltration and hemorrhage were less on day
5 and 7 post infection compared with that on day 3 post
infection, indicating a recovery process. Furthermore,
Luminex cytokine analysis showed that SARS-CoV-2
infection led to elevated cytokine production in the sera,
mainly including IL-1alpha, IL-2, IL-5, IL-6, IL-17A, IL-
28, IL-22, IP-10, MCP-1, MCP-3, Eotaxin, and IFN-
gamma (Fig. 1I). These results indicate that the B.1.351
infection can lead hACE2 mice to produce similar pul-
monary pathology features as those observed in COVID-19
patients.

In conclusion, our present study confirmed that the
SARS-CoV-2 variant B.1.351 can also effectively infect

the hACE2 mice we generated previously, and lead to
obvious pulmonary pathology. Notably, compared with the
prototype strain IME-BJ05 we reported previously
(4×10^5 pfu per mouse), the variant B.1.351 infection led
more obvious clinical symptoms and weight loss at a much
lower dose (1.2×10^4 pfu per mouse), indicating the
increased virulence of the variant B.1.351 for hACE2
mice (Sun *et al.* 2020). Previous studies demonstrated that
the N501Y and K417N mutations of SARS-CoV-2 S pro-
tein enhance the binding affinity to human *ACE2* (Fratev
2020; Starr *et al.* 2020; Liu *et al.* 2021), which may con-
tribute, at least partially, to the higher virulence of the
variant B.1.351 for hACE2 mice. The variant B.1.351
effectively infects hACE2 mice and replicates in its res-
piratory system, and mainly distributes in bronchiolar
epithelial cells and alveolar cells, which is similar to the
prototype strain IME-BJ05 (Sun *et al.* 2020). Interestingly,
in this study, no SARS-CoV-2 RNA (data not shown) or
sgRNA was detected in the brain of mice infected with
B.1.351, while high level of SARS-CoV-2 RNA in the
brain of mice infected with IME-BJ05 was detected in our
previous study. Whether the amino acid mutations in the
mutation lineage affect this infection characteristic or
which one plays the important role in the process needs to
be further investigated.

In summary, our results showed that the hACE2 mice
are susceptible to intranasal infection of the SARS-CoV-2
variant B.1.351, and robust viral replication was detected in
respiratory system. Especially, the pathological changes
observed in the hACE2 mice closely resemble those
observed in COVID-19 patients. Thus, using the variant
B.1.351 to infect the hACE2 mice described in our
manuscript provides a small animal model for studying the
transmission, pathogenesis, as well as understanding the
unexpected clinical manifestations of the mutation lineage.
This model will also be valuable for testing vaccines and
therapeutics to combat the variant B.1.351.

Compliance with Ethical Standards

Conflict of interests The authors declare no competing interests.

Animal and Human Rights Statement All procedures involving
infectious virus were conducted in Biosafety Level 3 laboratory
(BSL-3) in Beijing Institute of Microbiology and Epidemiology and
approved by the Animal Experiment Committee of Laboratory

Animal Center, Beijing Institute of Microbiology and Epidemiology (approval number: IACUC-DWZX-2020–002). All institutional and national guidelines for the care and use of laboratory animals were followed.

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