Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development

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*Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, 108-8639 Tokyo, Japan; †Influenza Research Institute, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706; ‡Department of Pathology, National Institute of Infectious Diseases, 162-8640 Tokyo, Japan; §The gene involved in the emergence of new pathogens in humans can impose huge public health and economic burdens on a global scale, in large part, because of lack of preexisting immunity. At the end of 2019, a novel coronavirus (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) was detected in Wuhan, China, that spread rapidly around the world, with severe consequences for human health and the global economy. Here, we assessed the replicative ability and pathogenesis of SARS-CoV-2 isolates in Syrian hamsters. SARS-CoV-2 isolates replicated efficiently in the lungs of hamsters, causing severe pathological lung lesions following intranasal infection. In addition, microcomputed tomographic imaging revealed severe lung injury that shared characteristics with SARS-CoV-2–infected human lungs, including severe, bilateral, peripherally distributed, multilobular ground glass opacity, and regions of lung consolidation. SARS-CoV-2–infected hamsters mounted neutralizing antibody responses and were protected against subsequent rechallenge with SARS-CoV-2. Moreover, passive transfer of convalescent serum to naïve hamsters efficiently suppressed the replication of the virus in the lungs even when the serum was administered 2 days postinfection of the serum-treated hamsters. Collectively, these findings demonstrate that this Syrian hamster model will be useful for understanding SARS-CoV-2 pathogenesis and testing vaccines and antiviral drugs.

Syrian hamsters | SARS-CoV-2 infection | countermeasure

The emergence of new pathogens in humans can impose huge public health and economic burdens on a global scale, in large part, because of lack of preexisting immunity. At the end of 2019, a novel coronavirus (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) that causes respiratory disease in and transmits among humans was detected in Wuhan, China (1, 2). On 11 March 2020, the World Health Organization declared that the infections caused by this new coronavirus had reached pandemic proportions.

A key strategy to protect humans from this coronavirus pandemic is the development of effective vaccines and therapeutics. Therefore, animal models that closely resemble the pathogenesis of SARS-CoV-2–induced disease, coronavirus disease 2019 (COVID-19), in humans are essential for research on disease mechanisms and for the evaluation of potential vaccines and antiviral drugs. While this manuscript was in preparation, Chan et al. (3) reported that SARS-CoV-2 caused a severe lung disease in hamsters and suggested that hamsters could serve as a useful mammalian model for COVID-19. These authors evaluated the pathogenicity and tissue tropism of SARS-CoV-2 isolates in hamsters after intranasal infection, and found that the virus replicated efficiently in the respiratory tract. They determined the infectious titer of the virus in only the nasal turbinates and lungs and did not examine the dissemination of SARS-CoV-2 isolates following intranasal inoculation, which therefore remains largely unknown.

Here, we analyzed the replicative ability of SARS-CoV-2 isolates by evaluating infectious titers in various organs in two different age groups of hamsters following inoculation by both the nasal and ocular routes. Chan et al. (3) showed that the virus replicated to higher titers in the upper respiratory tract (nasal turbinates) than in the lower respiratory tract (lungs). In contrast to their findings, we observed that the virus replicated efficiently in the respiratory tracts of both young and older infected animals, with no difference in its growth in the upper and lower respiratory tracts. By using in vivo X-ray microcomputed tomographic (micro-CT) imaging, we also examined the progression of lung inflammation caused by SARS-CoV-2 infection and the lesion and pulmonary consolidation. SARS-CoV-2–infected hamsters mounted neutralizing antibody responses and were protected against subsequent rechallenge with SARS-CoV-2. Moreover, passive transfer of convalescent serum to naïve hamsters efficiently suppressed the replication of the virus in the lungs even when the serum was administered 2 days postinfection of the serum-treated hamsters. Collectively, these findings demonstrate that this Syrian hamster model will be useful for understanding SARS-CoV-2 pathogenesis and testing vaccines and antiviral drugs.

Significance

Since SARS-CoV-2 emerged in China, it has spread rapidly around the world. Effective vaccines and therapeutics for SARS-CoV-2–induced disease (coronavirus disease 2019; COVID-19) are urgently needed. We found that SARS-CoV-2 isolates replicate efficiently in the lungs of Syrian hamsters and cause severe pathological lesions in the lungs of these animals similar to commonly reported imaging features of COVID-19 patients with pneumonia. SARS-CoV-2–infected hamsters mounted neutralizing antibody responses and were protected against rechallenge with SARS-CoV-2. Moreover, passive transfer of convalescent serum to naïve hamsters inhibited viral replication in their lungs. Syrian hamsters are a useful small animal model for the evaluation of vaccines, immunotherapies, and antiviral drugs.


The authors declare no competing interest.

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subsequent recovery processes in hamsters. Moreover, we assessed whether primary infection or passive transfer of convalescent serum could suppress the replication of SARS-CoV-2 in hamsters. In addition, by using scanning transmission electron microscopy (STEM) tomography, we observed the internal structure of SARS-CoV-2 virions.

Results

Growth Kinetics of SARS-CoV-2 Isolates in Mammalian Cells. To characterize the new viruses, we examined the biological properties of two SARS-CoV-2 isolates: SARS-CoV-2/UT-NCGM02/Human/2020/Tokyo (UT-NCGM02) and SARS-CoV-2/UW-001/Human/Wisconsin (UW-001), which were both isolated from mild cases. These isolates were propagated in VeroE6 or Vero 76 cells to prepare virus stocks. Titers of virus stocks were determined by use of plaque assays on the VeroE6 cell line VeroE6/TMPRSS2, which constitutively expresses transmembrane protease serine 2 (TMPRSS2), which activates SARS-CoV-2 infection (4).

To identify cell lines capable of supporting the efficient replication of SARS-CoV-2 isolates, we first examined the growth kinetics of the two isolates in vitro (Fig. 1). Because the primary targets for SARS-CoV-2 are the epithelial cells in the airways and lungs in humans, the efficiencies of replication of the two isolates were compared in four human lung alveolar cell lines: Calu-3, A549, NCI-H322, and NCI-H358 cells. For comparison, we included VeroE6 and VeroE6/TMPRSS2 cells, which have previously been shown to support the efficient replication of SARS-CoV-2 (4, 5). These cells were infected with virus at a multiplicity of infection (MOI) of 0.05, and virus titers in the supernatant were measured by means of a plaque assay with VeroE6/TMPRSS2 cells. UT-NCGM02 grew efficiently in VeroE6 and VeroE6/TMPRSS2 cells, but it grew much faster and to higher titers (0.96 log units higher at 24 h postinfection) in the latter than in the former. UW-001 replicated with similar efficiency in VeroE6 and VeroE6/TMPRSS2 cells. Although the two isolates also grew in Calu-3 cells, the titers at 24 h post-infection were 0.81 to 1.89 log units lower than those in VeroE6 and VeroE6/TMPRSS2 cells, respectively. By contrast, these isolates did not show appreciable growth in A549, NCI-H322, or NCI-H358 cells. These findings are consistent with previous studies showing that VeroE6/TMPRSS2 and VeroE6 cells, respectively.

Pathogenicity and Replication of SARS-CoV-2 in Hamsters. SARS-CoV-2 is closely related genetically to the SARS-CoV-1 that emerged in 2003. Given that Syrian hamsters are susceptible to SARS-CoV-1 (11–13), we evaluated the replication and pathogenicity of SARS-CoV-2 in these hamsters. Epidemiologic data indicate that the elderly are more prone to severe outcomes with COVID-19 than younger individuals (14, 15), suggesting that the age of the host may influence the pathogenesis of SARS-CoV-2. We used two age groups of Syrian hamsters: 1 mo old (juvenile) and 7 mo to 8 mo old (mature adults). Hamsters were infected with $10^{5.8}$ plaque-forming units (PFU) or with $10^2$ PFU of UT-NCGM02 through a combination of the intranasal and ocular routes; the ocular inoculation route was included because conjunctivitis has been reported among COVID-19 patients (16). In the younger age group, the body weight of mock-infected hamsters had gradually increased by 14 d post-infection (Fig. 3A and SI Appendix, Fig. S1). In contrast, of the animals infected with the low dose, three showed slight weight loss by day 6 postinfection (6.1 to 8.8%). Although the remaining one hamster did not exhibit weight loss, it gained weight more slowly than the mock-infected animals. All four animals infected with the high dose exhibited weight loss (range, 7.9 to 15.4%) by day 6 postinfection. By day 14 postinfection, all eight animals infected with either dose had gained less weight than the mock-infected animals. In the older age groups, mock-infected Syrian hamsters exhibited little to no weight loss (<7.8%). Although three of the four animals infected with the low dose showed only modest weight loss by day 7 postinfection (8.9 to 10.4%), the remaining hamster exhibited severe weight loss at this time point (18.5%) and continued to lose weight for up to 14 d postinfection (23.3%). All four animals infected with the high dose experienced substantial weight loss by day 7 postinfection (13.8 to 21.9%).
We next investigated the replicative ability of UT-NCGM02 in Syrian hamsters (Fig. 3B and SI Appendix, Tables S1 and S2). In both age groups, infection of hamsters with UT-NCGM02 resulted in high virus titers in the nasal turbinates, trachea, and lungs, with no appreciable difference between the inoculation doses at day 3 postinfection. Viruses were also recovered from the brains of all eight animals infected with the high or low dose in the younger age group (note that the brain samples collected for virus titration included the olfactory bulb). On day 6 postinfection, virus was detected in the respiratory organs of animals infected with the high or low dose in both the younger and older age groups, but was not detected in any other organs tested. Virus titers in the respiratory organs of the infected animals were lower on day 6 than on day 3 postinfection. No substantial difference in viral titers in the respiratory organs on days 3 and 6 postinfection was observed between the two age groups. At day 10 postinfection, no virus was recovered from the organs of almost all of the infected animals, with the exception of the trachea of one of the four animals infected with the low dose in the younger age group.

**Micro-CT Imaging of the Lungs of SARS-CoV-2—Infected Hamsters.** Because no substantial difference in viral titers in the respiratory organs was observed between the two age groups, we performed qualitative and semiquantitative analysis of the lung abnormalities only in the younger age group after SARS-CoV-2 infection, by using a CT severity score adapted from a human scoring system (17). The micro-CT analysis revealed severe lung abnormalities in all infected animals that were not present in the mock-infected control animals (Fig. 4 and Movies S3–S7). CT lung abnormalities were first detected at day 2 postinfection (range 2 d to 4 d, mean 2.5 d) and began as ill-defined, patchy ground glass opacity (GGO) with a central, peribronchial distribution. Lung abnormalities then progressed to more severe, peripherally distributed, rounded, multiflobular GGO with regions of lung consolidation. The right cranial lung lobe was most commonly affected first, and lung abnormalities...
were more severe in right lung lobes compared to left throughout the study period (Fig. 4 A–H). The most severe lung changes occurred 7 d to 8 d postinfection (range 6 d to 8 d, mean 7.17 d), and correlated with the highest CT severity scores (Fig. 4I). High dose-infected animals had more severe lung abnormalities (CT severity score ranging from 0 to 18 [mean 8.62, median 8.50]) than low dose-infected animals (CT severity score ranging from 0 to 15 [mean 6.23, median 5.79]). All infected animals developed a pneumomediastinum 4 d to 6 d postinfection (mean 5.42 d) that resolved by 8 d to 10 d postinfection (mean 8.5 d). This finding was unexpected and likely secondary to severe pulmonary damage, micropulmonary rupture, and gas tracking into the mediastinum. High dose-infected animals developed a pneumomediastinum slightly earlier (mean 5.17 d) than low dose-infected animals (mean 6 d).

Improvement of CT lung abnormalities began 8 d to 10 d postinfection (range 8 d to 10 d, mean 9.45 d), with gradual decrease in GGO and lung remodeling evident by linear soft tissue bands at sites of lung injury. Residual, minimal lung abnormalities/remodeling including ill-defined GGO and linear bands remained in 10 of 11 infected animals imaged on day 14 postinfection, and in 6 of 10 infected animals imaged on day 20 postinfection. A higher percentage of high dose-infected animals (75%, 3/4) than low dose-infected animals (50%, 3/6) had minimal residual lung abnormalities on the final CT scan on day 20.

**Histopathological Examination of the Lungs of SARS-CoV-2–Infected Hamsters.** We further examined the histopathological changes in the respiratory organs of the younger age group after SARS-CoV-2 infection (Fig. 5). Pathological examination of
hamsters infected with UT-NCGM02 revealed severe lung lesions on day 3 postinfection that extended across larger areas for hamsters infected with the high dose compared with those infected with the low dose (Fig. 5 A and B), consistent with our micro-CT observations that the animals infected with the high dose developed a pneumomediastinum earlier than those infected with the low dose (Fig. 4 A–H). At 6 d postinfection, there were no differences in the histological changes between the lungs of the animals infected with the low dose and the lungs of the animals infected with the high dose (Fig. 5 A and B). Focal inflammatory cell infiltration in the interstitium and the alveolar cavity were prominent, and pulmonary edema and alveolar hemorrhage were evident in some areas of the lungs of animals infected with the high dose on day 3 postinfection and in the lungs of animals infected with either dose on day 6 postinfection. Viral antigen-positive cells were detected in the bronchi and/or lungs of all eight animals infected with either dose on days 3 and 6 postinfection; however, more virus antigen-positive cells were detected in the lungs of virus-infected animals on day 3 than on day 6 postinfection (Fig. 5 A and C), which is consistent with the results.
of virus titration (Fig. 3B). Virus antigens were also observed in the nasal mucosa and/or olfactory epithelium of all eight animals infected with either dose on days 3 and 6 postinfection (SI Appendix, Fig. S24). Our virological data revealed that SARS-CoV-2 may enter the brain of infected animals (Fig. 3B); however, viral antigens were not detected in the brain sections of the animals tested. Therefore, it is unclear whether SARS-CoV-2 can infect the brain. Of the animals infected with the high dose, one was humanely killed on day 7 postinfection because it had lost more than 25% of its initial body weight at this time point. Pathological examination of the lung showed a few virus-positive cells and severe inflammation (SI Appendix, Fig. S2B). By contrast, at 10 d postinfection, pathological changes were slight, and no viral antigen-positive cells were detected in the lungs of animals infected with either dose. This result was consistent with that of our micro-CT analysis in which improvement of CT lung abnormalities began 8 d to 10 d postinfection (Fig. 4F).

Rechallenge with SARS-CoV-2. We next asked whether hamsters that developed antibodies against SARS-CoV-2 were resistant to subsequent reinfection. Six hamsters that had previously received the high or low dose of UT-NCGM02 were rechallenged with 10^3.6 PFU of homologous virus on day 20 after the primary infection. At day 4 after infection, in the mock-infected control group, high virus titers were detected in the respiratory tract (Table 1). In contrast, no virus was isolated from any of the respiratory organs of all six animals that were previously infected and then reinfed with the virus. Enzyme-linked immunosorbent and virus neutralization assays with sera collected on day 19 after the primary infection revealed that all of the infected animals had seroconverted (Table 1). These observations indicate that primary SARS-CoV-2 infection provides protective immunity against subsequent reinfection.

Inhibitory Effects of Serum Antibodies on the Replication of SARS-CoV-2. We next assessed the protective efficacy of convalescent serum from infected animals on the replication of SARS-CoV-2 in the respiratory tracts of hamsters. Postinfection sera were collected from hamsters that had been infected with the high or low dose of UT-NCGM02 and then pooled. The pooled serum was then transferred intraperitoneally to three hamsters on day 1 or 2 after infection with 10^4 PFU of the virus. Normal uninfected hamster serum was
injected intraperitoneally into three naïve hamsters as a control. Virus titers in the nasal turbinates and lungs of the animals that received postinfection serum on day 2 postinfection were statistically significantly lower than the virus titers in those organs of animals that received normal serum at the corresponding time point postinfection (P < 0.05 and P < 0.01 for the virus titers in the nasal turbinates and lungs, respectively) (Table 2 and SI Appendix, Table S3). Although no statistically significant differences in the virus titers in the respiratory organs were found between the animals that received postinfection serum and those that received normal serum on day 2 postinfection, the virus titers in the lungs of animals that received postinfection serum and those that received normal serum (7.8 ± 0.1 PFU ± SD/g) were appreciably lower (5.9 ± 1.8 log10 PFU ± SD/g) than those in the lungs of animals that received normal serum (7.8 ± 0.1 PFU ± SD/g). We found that, of the animals that received postinfection serum, only animal #9, which received postinfection serum on day 2 after infection, had a lung virus titer similar to that of animals that received normal serum. This animal was the only animal that had undetectable levels of antibody to SARS-CoV-2, indicating that the serum was not successfully administered to this animal (SI Appendix, Table S3). We also found that viral replication was more effectively prevented in the lungs than in the nasal turbinates. Our findings are consistent with previous reports that passive transfer of immune serum to mice or hamsters prevents the replication of SARS-CoV-1 or SARS-CoV-2, respectively, in the lungs (3, 18). Taken together, these results indicate that the passive transfer of convalescent serum from infected animals could restrict viral replication in the respiratory tract of infected animals even if the serum is administered after infection has occurred.

**Discussion**

In COVID-19 patients with acute respiratory illness, the main clinical manifestation is severe lung inflammation. Consistent with a previous study using Syrian hamsters (3), our data demonstrated that SARS-CoV-2 replicates efficiently in the lungs of Syrian hamsters and causes severe pathological lesions in the lungs of these animals following SARS-CoV-2 infection. In addition, our micro-CT analysis revealed that severe lung injury occurs in infected hamsters and that the severity of the lung abnormalities is related to the degree of infectious dose. Commonly reported imaging features of COVID-19 patients with pneumonia (19) were present in all infected animals but not in mock-infected control animals. These findings indicate that the pathological features of the lungs of SARS-CoV-2–infected hamsters resemble those observed in COVID-19 patients (17, 19–21). The observed trends of CT lung changes in infected hamsters over time may provide valuable clinical insight into SARS-CoV-2 infection and recovery. Computational modeling suggests that ACE2 from Chinese hamster could interact with the S glycoprotein of SARS-CoV-2 (22). Thus, this animal would be a valuable model to improve our understanding of the pathogenesis of lung injury caused by SARS-CoV-2 infection. In addition, Chan et al. (3) demonstrated that this hamster model is a useful tool for studies on SARS-CoV-2 transmission.

We observed that the virus titers in the nasal turbinates and lungs of infected Syrian hamsters did not correlate with the dose of virus administered (Fig. 3B). Similar findings were obtained with Syrian hamsters infected with SARS-CoV-1; the viral titers in the lungs following intranasal administration of 10^3 or 10^6 50% tissue culture infective doses (TCID50) of SARS-CoV-1 were similar on day 3 postinfection regardless of the dose administered (13). Thus, in the hamster model, SARS-CoV-1 and SARS-CoV-2 can replicate to high titers in the respiratory organs, even when the virus is administered at a low dose. However, histopathology and body weight changes differ depending on the dose of virus administered; therefore, these parameters can be used to evaluate virus pathogenicity and the effectiveness of countermeasures when a high dose of virus is used to infect hamsters.

Chan et al. (3) showed that SARS-CoV-2 replicates to higher titers in the nasal turbinates than in the lungs of infected Syrian hamsters. In contrast, we observed that the level of virus replication in the lungs was comparable to that in the nasal turbinates (Fig. 3B). Chan et al. (3) and we used almost identical experimental designs, with no substantial differences in hamster age, gender, inoculation dose, or volume. Therefore, the reason for this discrepancy is unclear. However, it might be due to differences in the virus preparations; Chen et al. (3) used plaque-purified viruses amplified in VeroE6 cells, whereas we used

<table>
<thead>
<tr>
<th>Primary infection dose</th>
<th>Animal ID</th>
<th>Antibody endpoint titer in serum*</th>
<th>Neutralizing antibody titer in serum†</th>
<th>Nasal turbinate</th>
<th>Trachea</th>
<th>Lung</th>
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<tr>
<td>High dose (10^5.6 PFU)</td>
<td>#1</td>
<td>40,960</td>
<td>1,280</td>
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<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td>#2</td>
<td>40,960</td>
<td>640</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td></td>
<td>#3</td>
<td>40,960</td>
<td>1,280</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Low dose (10^3 PFU)</td>
<td>#4</td>
<td>40,960</td>
<td>640</td>
<td>—</td>
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<td>—</td>
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<td></td>
<td>#5</td>
<td>40,960</td>
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<td>1,280</td>
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<td>&lt;20</td>
<td>5.5</td>
<td>3.3</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>#8</td>
<td>&lt;10</td>
<td>&lt;20</td>
<td>5.4</td>
<td>4.7</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>#9</td>
<td>&lt;10</td>
<td>&lt;20</td>
<td>5.3</td>
<td>3.5</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Syrian hamsters were inoculated with 10^5.6 PFU (in 110 μL) of UT-NCGM02 via a combination of the intranasal (100 μL) and ocular (10 μL) routes on day 20 after the primary infection. Three Syrian hamsters per group were killed on day 4 after rechallenge for virus titration.

*Viral antibody endpoint titers against the receptor-binding domain expressed as the reciprocal of the highest dilution with an optical density at 490 nm (OD490) cutoff value >0.15; sera were collected on day 19 after the primary infection.
†Viral neutralization titers against inoculated virus; sera were collected on day 19 after the primary infection.
‡A dash denotes that virus was not detected.
isolates amplified in the same cells without plaque purification. The presence of quasispecies in patient samples has been documented for SARS-CoV-1 and SARS-CoV-2 isolates (23, 24). Therefore, it is possible that plaque purification may have resulted in the selection of viruses with reduced replication fitness in the lungs of hamsters.

Our data show that SARS-CoV-2 can replicate in the brain or olfactory bulb of animals (Fig. 3B); however, we detected viral antigens in neither the brain nor the olfactory bulb of infected hamsters. Previous studies have reported that coronaviruses, such as SARS-CoV-1, and mouse hepatitis viruses could enter the central nervous system following intranasal inoculation of mice (25, 26). Interestingly, acute olfactory impairment (anosmia) has been recognized as an early symptom of COVID-19 patients (27), suggesting that the virus may infect cells within the olfactory epithelium of humans, as we detected in hamsters (SI Appendix, Fig. S2A). Further investigations are required to determine whether SARS-CoV-2 isolates could spread from the nasal cavity to the nervous system via the olfactory route.

Our studies demonstrated that primary SARS-CoV-2 infection elicited neutralizing antibodies that protected hamsters from subsequent infection. Similar findings have been reported in reinfection experiments of SARS-CoV-1 using mice (18). These data support the concept that people who recover from COVID-19 would be protected from reinfection at least for a period of time while their immunity to SARS-CoV-2 lasted. These results serve as a rationale for the development of live attenuated vaccines and other vaccines that induce protective antibodies.

We also showed the protective effects of convalescent serum in our hamster model of SARS-CoV-2 infection (Table 2 and SI Appendix, Table S3); the levels of virus titer reduction were substantial in animals treated with convalescent serum (i.e., nearly 1,000-fold virus titer reduction in the lungs of animals inoculated with convalescent serum 1 d postinfection and nearly 100-fold virus titer reduction in those inoculated on day 2 postinfection). Chan et al. (3) also reported a protective effect of convalescent serum in their hamster model, although the level of protection was not substantial for reasons that are currently unclear. Nonetheless, both datasets suggest that anti–SARS-CoV-2 polyclonal hyperimmune globulin from convalescent sera from COVID-19 patients and monoclonal antibodies to SARS-CoV-2 could reduce viral load in patients.

In conclusion, our data indicate that hamsters are highly susceptible to infection with SARS-CoV-2, without the need for prior adaptation, and develop severe pneumonia similar to COVID-19 patients. Importantly, the use of this animal model would facilitate the rapid evaluation of vaccine or antiviral therapy candidates at a relatively low cost compared to other animal models such as ferrets and nonhuman primates, which have also been shown to be susceptible to SARS-CoV-2 infection (28, 29). In addition, the vast majority of hamsters did not die upon SARS-CoV-2 infection, which is consistent with human infections. It would be interesting and important to develop COVID-19 hamster models with comorbidities such as diabetes mellitus, hypertension, or obesity (30). Taken together, our findings demonstrate that this Syrian hamster model will be useful for understanding SARS-CoV-2 pathogenesis and testing vaccines and antiviral drugs.

### Methods

**Viruses.** SARS-CoV-2 isolates were propagated in VeroE6 cells in Opti-MEM I (Invitrogen) containing 0.3% bovine serum albumin (BSA) and 1 μg of l-tosylamide-2-phénylchloromethyl ketone treated-trypsin per mL or in Vero 76 cells in Eagle’s minimal essential medium (MEM) supplemented with 2% fetal calf serum at 37 °C.

All experiments with SARS-CoV-2 were performed in enhanced biosafety level 3 (BSL3) containment laboratories at the University of Tokyo, which are approved for such use by the Ministry of Agriculture, Forestry, and Fisheries, Japan, or in enhanced BSL3 containment laboratories at the University of Wisconsin-Madison, which are approved for such use by the Centers for Disease Control and Prevention and by the US Department of Agriculture.

**Experimental Infection of Syrian Hamsters.** One-month-old female Syrian hamsters (Japan SLC Inc.) and 7- to 8-mo-old female Syrian hamsters (Envigo) were used in this study. Baseline body weights were measured before infection. Under ketamine–xylazine anesthesia, four hamsters per group were inoculated with 10^5 PFU (in 110 μL) or with 10^6 PFU (in 110 μL) of UT-NCGM02 via a combination of the intranasal (100 μL) and ocular (10 μL) routes. Body weight was monitored daily for 14 d.

For virological and pathological examinations, two, four, or five hamsters per group were infected with 10^5.5 PFU (in 110 μL) or with 10^6 PFU (in 110 μL) of the virus via a combination of the intranasal and ocular routes; 3, 6, and 10 d postinfection, the animals were killed, and their organs (nasal turbinates, trachea, lungs, eyelids, brain, heart, liver, spleen, kidneys, jejunum, colon, and blood) were collected.

### Table 2. Effect of convalescent serum on the replication of SARS-CoV-2 in hamsters

<table>
<thead>
<tr>
<th>Serum was administered to recipient hamsters on:</th>
<th>Passively transferred serum</th>
<th>Neutralizing antibody titer in serum</th>
<th>Virus titers (mean log_{10} PFU ±SD/g) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 postinfection</td>
<td>Infected hamster serum</td>
<td>640</td>
<td>6.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Uninfected hamster serum</td>
<td>&lt;10</td>
<td>7.4 ± 0.3</td>
</tr>
<tr>
<td>Day 2 postinfection</td>
<td>Infected hamster serum</td>
<td>640</td>
<td>6.3 ± 0.7</td>
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<tr>
<td></td>
<td>Uninfected hamster serum</td>
<td>&lt;10</td>
<td>6.7 ± 0.3</td>
</tr>
</tbody>
</table>

Syrian hamsters (n = 3 for each group) were inoculated intranasally with 10^6 PFU of UT-NCGM02. On day 1 or 2 postinfection, the hamsters were injected intraperitoneally with postinfection serum or normal uninfected serum. Animals were killed on day 4 postinfection for virus titration.

*Viral neutralization titers against inoculated virus; sera were collected from eight hamsters on day 38 or 39 postinfection and then pooled.

†Statistical significance was calculated by using two-tailed unpaired Student’s t tests; the P value was <0.05 compared with the virus titers in the nasal turbinate of hamsters that received serum from uninfected hamsters.

‡Statistical significance was calculated by using two-tailed unpaired Student’s t tests; the P value was <0.01 compared with the virus titers in the lungs of hamsters that received serum from uninfected hamsters.
For the reinfection experiments, three hamsters per group were infected with 10^5.6 PFU (in 110 μL) or with 10^6 PFU (in 110 μL) of UT-NCGM02 or PBS (mock) via a combination of the intranasal and ocular routes. On day 20 postinfection, these animals were reinfeected with 10^5.6 PFU of the virus via a combination of the intranasal and ocular routes. On day 4 after reinfection, the animals were killed, and the virus titers in the nasal turbinates, trachea, and lungs were determined by means of plaque assays in VeroE6/TMPRSS2 cells.

For the passive transfer experiments, eight hamsters were infected with 10^5.6 PFU (in 110 μL) or with 10^6 PFU (in 110 μL) of UT-NCGM02 via a combination of the intranasal and ocular routes. Serum samples were collected from these infected hamsters on day 38 or 39 postinfection, and were pooled. Control serum was obtained from uninfected age-matched hamsters. Three hamsters per group were inoculated intranasally with 10^6 PFU of UT-NCGM02. On day 1 or 2 postinfection, hamsters were injected intraperitoneally with the postinfection serum or control serum (2 mL per hamster). The animals were killed on day 4 postinfection, and the virus titers in the nasal turbinates and lungs were determined by means of plaque assays in VeroE6/TMPRSS2 cells. All experiments with hamsters were performed in accordance with the Science Council of Japan's Guidelines for Proper Conduct of Animal Experiments and the guidelines set by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison. The protocol was approved by the Animal Experiment Committee of the Institute of Medical Science, the University of Tokyo (approval no. PA19-75) and the Animal Care and Use Committee of the University of Wisconsin-Madison (protocol no. V00806).

Detailed materials and methods for this study are described in SI Appendix. Date Availability. All data supporting the findings of this study are included in the main text and SI Appendix; any materials will be made available upon request.

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6. M. Hoffmann et al., SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181, 271–280.e8 (2020).