Therapy with a Severe Acute Respiratory Syndrome–Associated Coronavirus–Neutralizing Human Monoclonal Antibody Reduces Disease Severity and Viral Burden in Golden Syrian Hamsters

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Background. Immunotherapy with monoclonal antibodies (MAbs) offers safe interventions for the prevention of infection in patients after organ transplantation and for the treatment of cancers and autoimmune diseases. MAb 201 is a severe acute respiratory syndrome–associated coronavirus (SARS-CoV)–specific MAb that prevents establishment of viral replication in vitro and prevents viral replication in vivo when administered prophylactically. The efficacy of MAb 201 in the treatment of SARS was evaluated in golden Syrian hamsters, an animal model that supports SARS-CoV replication to high levels and displays severe pathological changes associated with infection, including pneumonitis and pulmonary consolidation.

Methods. Golden Syrian hamsters that were intranasally inoculated with SARS-CoV were treated with various doses of MAb 201 or an irrelevant MAb 24 h after inoculation. Two to 7 days after infection, the hamsters were killed, and their lungs were collected for evaluation of viral titers and pathological findings.

Results. Postexposure treatment with MAb 201 can alleviate the viral burden and associated pathological findings in a golden Syrian hamster model of SARS-CoV infection. After a hamster is treated with MAb 201, its viral burden is reduced by 10^5–10^6 50% tissue-culture infectious doses per gram of tissue, and the severity of associated pathological findings, including interstitial pneumonitis and consolidation, is also remarkably reduced.

Conclusions. The demonstration of successful postexposure MAb 201 therapy in an animal model that demonstrates viral replication and associated pulmonary pathological findings suggests that MAb 201 may be useful in the arsenal of tools to combat SARS.
would necessitate the development of safe and efficacious vac-
cines, antiviral drugs, and immunotherapies. Proof-of-concept
studies of several SARS-CoV candidate vaccines have been re-
ported, including DNA-vectored vaccines; recombinant pro-
tein-subunit vaccines; whole, inactivated virus vaccines; and
live attenuated vectored vaccines [1–6]. Prototypes of each of
can also hold promise, but immunotherapy with a SARS-speci-
c monoclonal antibody (i.e., palivizumab) has been demon-
strated, for years, to safely and efficaciously prevent respira-
tory disease associated with respiratory syncytial virus infec-
tions [20]. Previously, MAb 201, a human monoclonal anti-
body generated from transgenic mice expressing human im-
munoglobulin genes (Medarex), was shown to specifically bind
to the angiotensin-converting enzyme 2 receptor–binding do-
main of the SARS-CoV spike protein, neutralize virus entry in
in vitro assays, and provide protection from SARS-CoV rep-
lication in the respiratory tissues of mice when administered
prophylactically [11]. The viral burden in the lungs of mice
that received MAb 201 treatment 24 h before intranasal infec-
tion with 10^5 TCID_{50} of SARS-CoV/mouse was reduced 1 mil-
ion-fold, to a level below the limit of detection [11].

The golden Syrian hamster provides a better model for spe-
cific evaluation of therapeutic effects than does the mouse. Like
the mouse, the hamster supports high levels of viral replication
in pulmonary tissues; however, unlike the mouse, which dem-
strates few to no remarkable pathological findings, hamsters
show moderate to severe interstitial inflammation and pul-
monary consolidation in association with replication of SARS-
CoV [21]. In the hamster model, immunotherapy may there-
fore be examined on 2 levels: the ability to alleviate viral burden
and the ability to reduce associated pathological findings. Ex-
amination of pulmonary tissues after SARS-CoV infection and
subsequent MAb therapy will establish whether a decrease in
the viral titer would be accompanied by a decrease in the se-
verity of associated pathological findings. Therefore, to explore
the immunotherapeutic potential of MAb 201, 10^5 TCID_{50} of
SARS-CoV was administered intranasally, and, 24 h later, ham-
sters were treated either with various doses of MAb 201 or with
an irrelevant human MAb administered intraperitoneally. We
found that treatment with SARS-CoV–specific human MAB
(i.e., MAb 201) can significantly reduce viral replication and
that this reduction in viral replication correlates with a reduc-
tion in the severity of observed pathological findings in the
pulmonary tissues of a SARS-CoV–susceptible host.

MATERIALS AND METHODS

All work with infectious virus and with infected animals was
performed in biosafety level 3 facilities by personnel wearing
positive-pressure air-purifying respirators (HEPA AirMate; 3M).
All animal protocols used in these studies have been approved
by the Animal Care and Use Committee of the National In-
institute of Allergy and Infectious Diseases.
**Animal studies.** Female golden Syrian hamsters (LVG [SYR]) were obtained from Charles River Laboratories and were housed in individually ventilated microisolator rodent cages. Hamsters were rested for ≥3 days before initiation of the following experiments.

In experiment 1, golden Syrian hamsters (age, 43 days) were lightly anesthetized by inhalation of isoflurane (USP-Baxter Healthcare) and were inoculated intranasally with $10^3$ TCID$_{50}$ of SARS-CoV in a total volume of 310 μL. The hamsters were treated, 24 h later, with intraperitoneal injections of 4 mg/kg or 40 mg/kg MAb 201 or with 40 mg/kg palivizumab, an irrelevant humanized MAB [22], which was used as a control (total volume, 1.0 mL; 12 hamsters/group). One day after MAB treatment, hamsters were bled, and sera were assayed for SARS-CoV–specific ELISA IgG antibodies (hereafter referred to as "IgG ELISA antibodies") and neutralizing antibodies. Hamsters were killed at 3 and 5 days after SARS-CoV infection (6 hamsters/group were killed on each of these days), and lungs were harvested for viral titer determination or histopathological evaluation. Lungs obtained from 3 hamsters from each group on each of these days were either (1) homogenized (10% wt/vol) and assayed in serial 10-fold dilutions on Vero cell monolayers for determination of viral titers (limit of detection, $10^{-3}$ TCID$_{50}$ of SARS-CoV/g of tissue), or (2) inflated with and stored in 10% formalin and then processed for histopathological examination.

In experiments 2 and 3, golden Syrian hamsters (age, 45–48 days) anesthetized by inhalation of isoflurane were inoculated intranasally with $10^3$ TCID$_{50}$ of SARS-CoV in a total volume of 100 μL. After 24 h, the hamsters were treated with various doses of MAB 201 or an irrelevant human MAB (total volume, 0.5 mL). One day after MAB treatment, hamsters were bled, and serum samples were assayed for SARS-CoV–specific IgG ELISA and neutralizing antibodies. Hamsters were killed 2 days after SARS-CoV infection, lungs were harvested, and 10% lung homogenates were assayed for determination of viral titers. Similarly, SARS-CoV–inoculated and MAB-treated hamsters were killed 5 or 7 days after infection, and lungs were inflated with and stored in 10% formalin for histopathological examination and then processed. Treatment strategies, the number of hamsters evaluated, IgG ELISA and neutralizing antibody titers, viral titers in lung homogenates, and pathological findings are summarized in tables 1 and 2.

**ELISAs.** The presence of SARS-specific IgG ELISA antibodies was determined by coating 96-well plates, at 4°C overnight, with 1 μg/mL S270-510 SARS-spike protein in PBS, as reported elsewhere [11]. A standard curve was generated from
serial dilutions of normal hamster serum samples spiked with SARS-spike protein 201 (100 μg/mL). Palivizumab (100 μg/mL) in PBS 0.1% Tween with 0.5% human serum albumin served as a negative control. All serum samples obtained from SARS-infected hamsters were heat-inactivated for 90 min at 56°C to inactivate SARS-CoV, and all dilutions (3-fold) were made in PBS 0.1% Tween 0.5% human serum albumin. Plates were washed with PBS 0.05% Tween. The limits of detection are reported as <0.4 μg/mL and are based on the highest concentration tested (dilution, 1:100 or 1:200) and the limit of quantitation of the assay (≈2 ng/mL). Each sample was run in duplicate, and assays were conducted in a blinded fashion. The plates were developed with a goat anti-human IgG FAb2 alkaline phosphatase conjugate.

Microneutralization assays for the determination of neutralizing antibody titers. Blood samples were collected by retro-orbital bleeding of hamsters that received isoflurane as general anesthesia and 0.5% tetracaine hydrochloride ophthalmic solution (Bausch & Lomb) as topical anesthesia. Serum samples were heat-inactivated at 56°C for 30 min and were assayed for the presence of SARS-CoV–neutralizing antibodies. Two-fold dilutions of serum samples in Leibovitz’s L-15 Medium (Invitrogen) media were tested in a microneutralization assay for the presence of antibodies that neutralized the infectivity of 100 TCID₅₀ of SARS-CoV in Vero cell monolayers, as described elsewhere [23].

Viral titer determination. Tissue samples were homogenized to a final 10% (wt/vol) suspension in L15 with piperacillin (Sigma Aldrich), gentamicin (Invitrogen), and amphotericin B (Quality Biological), which were added to the tissue culture medium at final concentrations of 0.4 mg/L, 0.1 mg/L, and 5 mg/L, respectively. Tissue homogenates were clarified by means of low-speed centrifugation, and viral titers were determined in Vero cell monolayers in 24- and 96-well plates, as described elsewhere [23]. Viral titers are expressed as the TCID₅₀ of SARS-CoV per gram of tissue, with a lower limit of detection of 10¹⁵ TCID₅₀ of SARS-CoV/g of tissue.

Histopathological evaluation. Lungs were fixed in 10% neutral buffered formalin for 3 days, routinely processed, and subsequently embedded in paraffin. The entire lung was studied histopathologically by use of hematoxylin-eosin–stained sections. In experiment 1, tissues were not coded before histopathological examinations. These data are not included in statistical evaluations of the efficacy of MAb 201 treatment in the reduction of the severity SARS-CoV–associated pathological findings. In experiments 2 and 3, tissues were coded before histopathological examination and were decoded before statistical analyses. Pathological lesions were classified as follows: (1) interstitial pneumonitis was defined by the presence of inflammation in the interalveolar walls and around the bronchioles, and (2) consolidation was defined by the presence of intra-alveolar inflammation and reactive proliferation of cuboidal epithelial cells.

Table 2. Findings of histopathological evaluations of lungs obtained from hamsters treated with monoclonal antibody 201 (MAb 201) after challenge with severe acute respiratory syndrome–associated coronavirus (SARS-CoV).

<table>
<thead>
<tr>
<th>Experiment, treatment group, dose</th>
<th>Hamsters, no.</th>
<th>Neutralizingb</th>
<th>IgG ELISAc</th>
<th>Interstitial pneumonia</th>
<th>Consolidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAb 201 Day 1, 40 mg/kg d</td>
<td>3</td>
<td>38 ± 8</td>
<td>131 ± 25</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>MAb 201 Day 2, 40 mg/kg e</td>
<td>3</td>
<td>26 ± 3</td>
<td>95 ± 6</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>MAb 201 80 mg/kg</td>
<td>4</td>
<td>45 ± 5</td>
<td>200 ± 14</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Subneutralizing, 40–80 mg/kg</td>
<td>2</td>
<td>&lt;8 ± 0</td>
<td>1 ± 1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Control, 40–80 mg/kg</td>
<td>8</td>
<td>&lt;8 ± 0</td>
<td>0 ± 0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>MAb 201 40 mg/kg</td>
<td>6</td>
<td>35 ± 8</td>
<td>207 ± 28</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Subneutralizing, 40 mg/kg</td>
<td>3</td>
<td>&lt;8 ± 0</td>
<td>8 ± 8</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Control, 40 mg/kg</td>
<td>8</td>
<td>&lt;8 ± 0</td>
<td>0 ± 0</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

a Severity of interstitial pneumonitis or consolidation observed on day 5 or 7 after infection: 0, no finding; 1, mild; 2, moderately severe; and 3, severe.

b Titters were measured using microneutralization assays performed on Vero cell monolayers and are expressed as reciprocal titers. Lowest dilution tested, 1:8.

c SARS-CoV–specific IgG antibodies detected by ELISA.

d Treatment administered 1 day after challenge with SARS-CoV.

e Treatment administered 2 days after challenge with SARS-CoV.

Palivizumab, an irrelevant humanized MAb.

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epithelial cells in the alveolar walls. Pathological lesions were not observed or ranged in severity from mild to severe, and a grade of 0–3 was assigned, as described below, for statistical evaluation. Interstitial pneumonitis and consolidation were graded independently, according to the extent of their severity, as is shown in figure 1. A grade of 0 indicated that no interstitial pneumonitis or consolidation was found. A grade of 1 (mild) denoted the presence of scattered, single foci of either interstitial pneumonitis or consolidation that did not involve >15–20 alveoli; a grade of 2 (moderately severe) denoted the presence of multiple areas where there was confluence of 2 foci of interstitial pneumonitis or consolidation; and a grade of 3 (severe) denoted the presence of large, confluent areas of interstitial pneumonitis or consolidation that involved more than half a lobe.

**Statistical analyses.** The nonparametric Mann-Whitney $U$ test, Kruskal-Wallis test, and Spearman’s rank correlation were the statistical methods used for ascertaining the significance of observed differences. Statistical significance was denoted by $P < .05$.

**RESULTS**

In an initial pilot experiment (experiment 1), we examined the potential of MAb 201 in the postexposure treatment of SARS-CoV infection. Within any single treatment group (4 mg/kg MAb 201, 40 mg/kg MAb 201, or the control treatment), no noticeable difference was observed in the levels of viral replication in the pulmonary tissues between 3 and 5 days after infection ($P > .05$). Therefore, data from days 3 and 5 after infection were combined and are presented, according to corresponding treatment groups, in tables 1 and 2. However, we did note significant differences in the virus levels in the lungs and in the degree of associated pathological findings in MAb 201–treated hamsters, compared with those in control hamsters (table 1, experiment 1). In the group treated with 4 mg/kg MAb 201, only 2 of 6 hamsters achieved measurable titers of neutralizing antibody and IgG ELISA antibodies after intraperitoneal administration of the treatment. The number of hamsters in this group was therefore too small for relevant statistical analysis to be done. Of the 6 hamsters in the group treated with 40 mg/kg MAb 201, 5 had measurable titers of neutralizing antibody and IgG ELISA antibodies. In a comparison of this group with the control group ($n = 6$), statistically significant reductions in the virus levels were achieved with MAb 201 treatment ($P = .04$, Mann-Whitney $U$ test). Furthermore, histopathological findings suggested that treatment with MAb 201 administered 24 h after SARS-CoV infection reduced the severity of interstitial pneumonitis observed 3 and 5 days after SARS-CoV infection, as well as the severity of consolidation observed 5 days after infection (data not shown).

The observations from this initial experiment led to 2 additional experiments in which lungs were collected for determination of viral titers 2 days after inoculation with SARS-CoV (i.e., at the time when peak viral titers are noted in unprotected hamsters [21]) and for histopathological evaluation 5 or 7 days after SARS-CoV infection (i.e., when both interstitial pneumonitis and consolidation are easily distinguished in infected hamsters) [21].

In experiment 2, the group of hamsters treated with 80 mg/kg MAb 201 had a nearly 10,000-fold reduction in the viral titer in the lungs, compared with the viral titer measured in the lungs of hamsters treated with an irrelevant MAb (table 1, experiment 2). The group treated with 40 mg/kg MAb 201 had an ~1000-fold reduction in the viral titer in the lungs, compared with that noted in hamsters treated with the irrelevant control MAb. Furthermore, and perhaps more importantly, the severity of the associated pathological findings was reduced in hamsters treated with MAb 201. Hamsters treated with MAb 201 had mild interstitial pneumonitis and moderately severe consolidation (regardless of the dose received, the median severity score for interstitial pneumonitis was 1, and that for consolidation was 2), compared with the severe interstitial pneumonitis and consolidation (median severity score of 3 for both) noted in hamsters treated with the irrelevant MAb. Figure 1 shows representative photographs of healthy lungs (showing no signs of interstitial pneumonitis or consolidation, panel G) or lungs demonstrating mild to severe interstitial pneumonitis (panels A–C, respectively) and consolidation (panels D–F, respectively).

In experiment 3, only 3 of 14 hamsters that received 10 mg/kg MAb 201 had a measurable titer of SARS-specific neutralizing antibody. We therefore withdrew this group from further evaluation (see Discussion). In the group treated with 40 mg/kg MAb 201, 4 of 6 hamsters had measurable titers of SARS-specific neutralizing antibody, and these animals had a 250-fold reduction in the viral titer noted in the lungs, compared with that noted for hamsters treated with the control MAb (table 1, experiment 3). As in previous experiments, the reduction in the severity of associated pathological findings was remarkable in the hamsters treated with MAb 201 (table 2, experiment 3). Mild interstitial pneumonitis and consolidation were seen in the lungs of hamsters treated with MAb 201 (median severity score of 1 for both), whereas hamsters treated with the irrelevant control MAb demonstrated severe interstitial pneumonitis and consolidation (median severity score of 3 for both).

In summary, in all 3 experiments, a very strong positive correlation ($r = 0.870; P < .0001$) was observed between neutralizing and IgG ELISA antibody titers, and a strong inverse correlation was observed between neutralizing antibody titers and viral burden ($r = −0.582; P < .0001$; mean values ± SEs are presented in tables 1 and 2). Furthermore, a very strong inverse correlation was noted between neutralizing antibody titers and the severity of interstitial pneumonitis ($r = −0.721$;
Figure 1. Hematoxylin-eosin staining of formalin-fixed hamster lungs after severe acute respiratory syndrome–associated coronavirus infection and antibody treatment. A–C, Differences in the severity of interstitial pneumonitis: mild (A), moderate (B), and severe (C). D–F, Differences in the severity of consolidation: mild (D), moderate (E), and severe (F). G, Photomicrograph of a normal hamster lung with no interstitial pneumonitis or consolidation. Original magnification, ×25.
DISCUSSION

Late in 2003, 4 cases of naturally acquired SARS were reported, most likely resulting from exposure to infected palm civets. The few cases of SARS that occurred during 2004 were limited to individuals who were exposed to SARS-CoV in laboratories and persons who came in contact with these individuals. Vaccination of laboratory workers could be a strategy to prevent infections, if an efficacious SARS vaccine were available. However, in the treatment and prevention of infectious diseases, a variety of interventions are desirable. If SARS were to recur, we may be best served by a combination of SARS-specific vaccines, drugs, and immunotherapies. Identification of SARS-CoV as the causative agent in an outbreak of respiratory disease might take 2 to several days, and it could take up to a few weeks after vaccination for protective immunity to develop. In these instances, individuals at greatest risk for infection (i.e., health care workers) and disease (i.e., elderly individuals) could be offered MAbs that could serve as prevention or treatment if exact exposure status were unknown. Also, depending on the nature of any future vaccine (e.g., live, attenuated viral vectors) and on the immune status of individuals considered to be at risk, treatment with MAbs or antiviral drugs may be preferred to vaccination.

Our findings indicate that SARS-CoV infections may be treated after exposure and that such treatment can reduce the viral burden and the severity of associated pathological findings. Systemic absorption of antibody from the peritoneum of hamsters was demonstrated by measuring serum neutralizing antibody titers 24 h after treatment with MAbs. In several of the hamsters treated with MAbs and, most notably, in hamsters that received lower doses of MAbs (4 or 10 mg/kg), SARS-specific neutralizing antibodies were undetectable in the serum 24 h after treatment. This may be explained by incomplete delivery of MAbs to the peritoneum, by delivery of MAbs to the gut, or, possibly, by loss of some of the antibody through leaking along the needle track. In several of these hamsters with subneutralizing anti–SARS-CoV antibody levels, reductions in the viral burden in the lungs and the severity of associated pathological findings were still observed (tables 1 and 2, “MAb 201 subneut.” groups, compared with controls). Perhaps more importantly, in hamsters with subneutralizing levels of anti–SARS-CoV antibodies, enhancement of disease severity was not observed (i.e., viral titers and associated pathological findings were not greater than those noted for control hamsters [tables 1 and 2, as well as unpublished data from experiment 3, for hamsters treated with 10 mg/kg MAb 201]).

Inconsistent delivery of antibody is not an issue for clinical trials of human MAbs administered intravenously. On the basis of the serum levels achieved in adequately treated hamster groups, we can predict the dose range (in milligrams per kilograms of body weight) for treatment of patients. Mean serum IgG concentrations of 125–175 μg/mL MAb 201 resulted in reduced severity of pathological findings and lowered viral concentrations in the hamsters (table 1), and we documented peak serum concentrations of 110–220 μg/mL in human subjects receiving doses of 5–10 mg/kg human IgG1 MAb (D.M.A., unpublished data). Thus, findings of diminished disease severity and a significant reduction in the viral burden in treated hamsters occurred at serum concentrations of MAb 201 that will be achieved using doses similar to those recommended for some licensed MAbs (5–10 mg/kg).

Another challenge in demonstrating effective immunotherapy for SARS-infected animals is that the course and kinetics of infection and disease are abbreviated in all animal models reported to date [21, 24–28], compared with the course of SARS in humans [7]. For instance, the hamster model supports early viral replication, which peaks at 2–3 days after infection. Viral replication and severe pathological findings persist in the lower respiratory tract for ~7 days, after which time they begin to clear. By 14 days after infection, little to no histopathological evidence of inflammation or disease persists, and virus is rarely detected in the upper respiratory tract [21]. Clinical symptoms are not observed beyond the first week after administration of virus in other animal models that demonstrate clinical signs of illness, including aged BALB/c mice, ferrets, and nonhuman primates, and infectious virus can no longer be recovered in respiratory tissues after this time. These observations are in contrast to the relatively prolonged course of infection associated with SARS cases in humans, for which the incubation period for SARS-CoV is 2–10 days. The peak viral load has been reported to occur ~7 [29] to 10 [30] days after the onset of illness, with peak severity of interstitial pneumonia occurring a few days later, at 6–13 days (median, 10.5 days) after the onset of illness [29]. Clinical findings may persist well into the second and third week after infection. In experiment 2, we treated an additional 3 hamsters with 40 mg/kg MAb 201 2 days after SARS-CoV infection, at the time that peak viral titers were observed in the lungs [21], and the lungs of these animals were harvested for histopathological analysis 5 days after infection. Even at this later point in time, the severity of associated pathological findings was reduced in hamsters treated with MAb 201. These hamsters, similar to those treated 1 day after infection, had mild interstitial pneumonia and moderately severe consolidation. Taking into account these limited observations in hamsters, as well as the longer period from initial exposure of SARS-CoV to the development of clinical symptoms of SARS in humans, suggests that the window for the initiation of MAb immunotherapy in human populations ex-
posed to SARS-CoV may be much longer than that indicated by the 24-h window for postexposure treatment used in these studies involving hamsters. MAb 201 has now been shown to have prophylactic and therapeutic potential for SARS-CoV infections in 2 animal models, and these data provide the basis for phase 1 clinical trials of MAb 201 in humans.

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References