MAJOR ARTICLE

Fully Human Monoclonal Antibody Directed to Proteolytic Cleavage Site in Severe Acute Respiratory Syndrome (SARS) Coronavirus S Protein Neutralizes the Virus in a Rhesus Macaque SARS Model

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Background. There is still no effective method to prevent or treat severe acute respiratory syndrome (SARS), which is caused by SARS coronavirus (CoV). In the present study, we evaluated the efficacy of a fully human monoclonal antibody capable of neutralizing SARS-CoV in vitro in a Rhesus macaque model of SARS.

Methods. The antibody 5H10 was obtained by vaccination of KM mice bearing human immunoglobulin genes with *Escherichia coli*–producing recombinant peptide containing the dominant epitope of the viral spike protein found in convalescent serum samples from patients with SARS.

Results. 5H10, which recognized the same epitope that is also a cleavage site critical for the entry of SARS-CoV into host cells, inhibited propagation of the virus and pathological changes found in Rhesus macaques infected with the virus through the nasal route. In addition, we analyzed the mode of action of 5H10, and the results suggested that 5H10 inhibited fusion between the virus envelope and host cell membrane. 5H10 has potential for use in prevention and treatment of SARS if it reemerges.

Conclusions. This study represents a platform to produce fully human antibodies against emerging infectious diseases in a timely and safe manner.

Severe acute respiratory syndrome (SARS) has been observed in ~30 countries, affecting >8000 persons and resulting in death in ~10% of cases [1]. The disease is caused by the newly identified SARS coronavirus (CoV) [1–3]. Although bats carry viruses similar to SARS-CoV [4], its natural host has not yet been identified, and no vaccine or drug treatment has yet been approved. Thus, there is still a risk of the reemergence of SARS in the human population, and it is necessary to develop effective measures to combat this disease.

SARS-CoV enters host cells by a mechanism involving the interaction between its spike (S) protein and human angiotensin-converting enzyme 2 (ACE2) expressed on host cells in the lung [5]. During entry, S is cleaved into the N-terminal S1 region and C-terminal S2 region. S1 mediates binding to ACE2, and S2 mediates fusion of viral and host cell membranes [6, 7]. A region named S791 in this study corresponding to amino acid

Received 23 June 2010; accepted 31 August 2010.
Potential conflicts of interest: none reported.
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The Journal of Infectious Diseases 2011;203:1574–81

0022-1899 (print)/1537-6613 (online)/2011/20311-0011$14.00
DOI: 10.1093/infdis/jir084

positions 620 to 900 of S contains cleavage sites for S1 and S2 domains [8]. S791 also contains an epitope recognized by serum samples from convalescing patients with SARS in Vietnam [9]. The S protein also plays an important role in inducing protective immunity [10–12]. Antibodies targeting the S1 domain, however, also induced antibody-dependent enhancement of SARS-CoV entry into the host cells when the amino acid sequences of S proteins were changed [13, 14].

Monoclonal antibodies against SARS-CoV have been generated in both human and murine backgrounds [15–17]. Some of the monoclonal antibodies (mAbs) were tested for passive immunization in mice, ferrets, and hamsters and showed the ability to prevent or limit infection [17–20]. Because all in vivo evaluations of the mAbs have been performed in models lacking detectable clinical symptoms and disease, it is difficult to assess whether the antibodies tested would actually provide reasonable protection in humans. The Rhesus macaque is one of the most valuable tools for evaluating the efficacy of antibodies against SARS-CoV, because this model showed virus propagation, immunological response, and pathological changes in the lungs of infected animals [21, 22].

In the present study, we produced a fully human monoclonal antibody, 5H10, by immunization of KM mice, which produce human antibodies [23], with fragments derived from the SARS-S protein prepared in *Escherichia coli* but not SARS-CoV to obtain human antibodies capable of neutralizing the virus. We examined the neutralizing activity of 5H10 against SARS-CoV in vivo in the Rhesus macaque SARS model.

MATERIALS AND METHODS

Expression and Purification of Recombinant S Proteins and Antibodies Against Them

All recombinant proteins (Supplemental Fig. 1) were expressed as His-tagged proteins using the TAGZyme pQE2 vector (Qiagen) and were affinity purified using the His tag. The His tags in the proteins were removed enzymatically, as described elsewhere [24], and used for vaccination of animals. All animal experiments were performed in accordance with the guidelines of the Ethics Review Committees of Animal Experiments of International Medical Center of Japan. Aliquots of 100 μg of purified proteins/animal were used for vaccination of rabbits or KM mice [23], transgenic animals that produce fully human antibodies, with Freund's adjuvant (Difco Laboratories). To produce human monoclonal antibodies, the spleen cells from KM mice vaccinated with the S791 fragment were fused with the mouse myeloma cell line SP2/O-Ag14, and hybridomas were screened for their ability to bind to recombinant S791 protein by conventional enzyme-linked immunosorbent assay as described elsewhere [23]. Mass production of 5H10 was performed in fed-batch culture of CHO cells, as described elsewhere [25]. Epitope mapping of mAbs was performed using a set of overlapping peptides (>70% purity) spanning the entire sequence of the S791 protein, each consisting of 15 amino acids overlapping the next peptide by 5 amino acids, as described elsewhere [24].

Neutralization of SARS-CoV In Vitro by 5H10

All experiments using SARS-CoV were performed in BSL3 level facilities. The neutralizing activities of mAbs against SARS-CoV Frankfurt01 were determined by plaque reduction assay. Monolayers of Vero E6 cells were grown in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 5% fetal calf serum in 6-well plates at 37°C with 5% carbon dioxide. Samples (100 mL) of 5H10 were incubated with an equal volume of SARS-CoV suspension (containing 200 plaque-forming units of virus) in a water bath at 37°C for 1 h. Samples of the incubated mixture were used to infect Vero E6 monolayers. After adsorption for 1 h, the cultures were overlaid with 1% methylcellulose-containing media and incubated for 6–8 days at 37°C. The cells were then fixed with 20% formalin, stained with 0.1% crystal violet in 0.1 M citric acid, and irradiated with UV light for 1 h. After drying, the numbers of virus plaques were counted. The neutralizing titer was defined as the reciprocal dilution of serum causing a 50% reduction in the plaque count, compared with the negative control. In the neutralization assay against SARS-CoV PUMC01, cell monolayers were stained with 100 μL of 0.15% neutral red (Sigma) in DMEM for 1 h at 37°C. After washing, 100 μL of acid alcohol (1% acetic acid in 50% ethanol) was added to each well. After incubation for 30 min at room temperature, the absorbance of neutral red-stained plates was read at 450 nm.

Cell–Cell Fusion Assay Mediated by Interaction Between SARS-S and Human ACE2

The full-length human *ace2* gene in pcDNA3.1 (−) (kindly provided by Dr H. Choe, Harvard Medical School), the human codon-optimized *spike* gene of SARS-CoV in the VRC8304 vector (kindly provided by Dr G. J. Nabel, National Institutes of Health), and pGL4.35 encoding luciferase, pACT-MyoD, and pBIND-Id plasmids (Promega) were used in the fusion assay. The human ace2 gene and the spike gene of SARS-CoV were inserted into pIRES2 DdRed-Express2 and pIRES2-AcGFP1 (Clontech), respectively, and used in the syncytium formation assay.

The spike gene and pBIND-Id plasmids were transfected into HEK293T cells with Lipofectamine LTX Reagent (Invitrogen) in one group. The human ace2 gene, pACT-MyoD, and pGL4.35 were transfected into the other HE293T cell group. Two days after transfection, HEK293T cells expressing S protein were detached with trypsin/EDTA solution (Sigma) or EDTA solution. HEK293T cells expressing ACE2 protein were detached with EDTA solution. The treatments with anti-SARS antibody were performed before and after detachment. These cells were mixed and cocultured for 1 day at 37°C. The cells were harvested with lysis buffer and centrifuged. The cell lysates were then mixed with the substrate PicaGene (Toyo Ink), and luciferase activity was measured using a plate reader (Infinite F200; Tecan).

In Western blot analysis, HEK293T cells expressing S protein were treated in the presence or absence of 5H10. Cells were detached using trypsin/EDTA or EDTA solution. After antibody treatment and detachment, these cells were lysed with radioimmunoprecipitation assay solution (0.1% sodium dodecyl sulfate, 0.5% sodium deoxycholate, 1% Nonidet P - 40, 150 mM sodium chloride, 50 mM Tris-HCl, pH 8.0). The cell lysates were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred onto Immun-Blot P polyvinylidene difluoride membranes (Bio-Rad Laboratories). The membranes were incubated with a mixture of in-house anti-S1-N, S1-C, S791, and S2 antibodies described above and anti-rabbit IgG horseradish peroxidase (GE Healthcare). After washing, the membranes were incubated with SuperSignal West Substrate (Thermo Scientific) according to the manufacturers' instructions.

Syncytium Formation Assay and Inhibition by Anti-SARS Antibodies

The *spike* gene in pIRES2-AcGFP1 and *ace2* gene in pIRES2 DdRed-Express2 were transfected into HEK293T cells separately. Two days after transfection, HEK293T cells expressing S protein were detached with trypsin/EDTA solution or EDTA solution. On the other hand, HEK293T cells expressing ACE2 protein were detached with EDTA solution. These cells were mixed and cocultured for 6 h at 37°C and observed with an Olympus IX-70 microscope.

Evaluation of 5H10 in Rhesus Macaques In Vivo

All the procedures were approved by the Institute of Animal Use and Care Committee of the Institute of Laboratory Animal Science, Peking Union Medical College. The sham administration, control human IgG administration, and 5H10 administration groups of Rhesus macaques (3–4 years of age) were used for experimental infection with SARS-CoV (PUMC01) by spraying of the virus (median culture tissue infective dose, 1×10^{-6}) into the nasal cavity of the animals separately, as described elsewhere [26]. The antibody was administered intravenously 1 h before virus infection and on days 1 and 3 after infection at a dose of 12.5 mg/kg (total dose, 37.5 mg/kg). Animals were sacrificed 6 days after infection, and lung tissue samples were collected. Organs were grossly examined and were photographed to record lung damage. Haematoxylin and eosin staining of the tissue sections, detection of viral antigens, and determination of SARS-CoV load by reverse-transcription polymerase chain reaction (RT-PCR) were performed as described elsewhere [22].

Preliminary safety tests of 5H10 in the Rhesus model: The 3 aforementioned groups of Rhesus macaques without SARS-CoV infection were used for the tests. The antibodies were injected intravenously 3 times at 24-h intervals at a dose of 12.5 mg/kg (total dose, 37.5 mg/kg). Full evaluation results are available upon request.

RESULTS

Recombinant Spike Fragments

To prepare antigen without using hazardous SARS-CoV, we used recombinant protein technologies using only genomic sequence information. The gene encoding SARS S protein was obtained from a commercial service and used for the expression of 4 S fragments separately (Supplemental Fig. 1a). Fragmentation of the S protein was based on the functional domain. No function has yet been assigned to S1-N, and S1-C is the domain responsible for ACE2 binding [5]. S791 and S2 contain cleavage sites for virus entry after binding of ACE2 [10] and heptad repeats for trimerization of S, supporting the interaction of S with ACE2 [7], respectively. These fragments were expressed in *E. coli* as His-tagged proteins and were purified to homogeneity using the tag. To avoid raising antibodies against the tag, the His tag was removed by enzymatic cleavage; N-terminal amino acid sequence analysis confirmed cleavage of the His tag at the expected amino acid position.

The S fragments obtained were evaluated for the ability to induce neutralizing antibodies in rabbits (Supplemental Fig. 1b). Although all fragments induced antibody responses when used as antigens for vaccination of rabbits, only the S791 fragment induced an antibody that neutralized SARS-CoV in vitro (Supplemental Fig. 1c).

Generation of Fully Human mAb Against SARS-CoV Using the S791 Fragment

On the basis of the ability to induce neutralizing antibodies, the S791 fragment was used to vaccinate KM mice to obtain fully human mAb. Three mAbs, 65B3, 63B10, and 5H10, reactive to the S791 fragment were chosen to characterize the epitopes recognized by them and the ability to neutralize SARS-CoV in vitro. 65B3 and 63B10 reacted with a peptide corresponding to amino acid positions 731 to 745 of the S protein (CANLLLQYGSFCTQL). In contrast, 5H10 reacted with a peptide corresponding to amino acid positions 791 to 805 of the S protein (PLKPTKRSFIEDLLF) (data not shown). The latter peptide was identical to that recognized by the serum samples from convalescing patients with SARS in Vietnam [9]. Furthermore, the amino acid R797 is the trypsin proteolytic site to enhance fusion of the virus [8]. Among the 3 mAbs, only 5H10 showed in vitro SARS-CoV neutralizing activity, and the 50% effective concentration was 5 μg/mL (Supplemental Fig. 1d). These results indicated that 5H10 recognizing the proteolytic site of the S protein is a potent fully human neutralizing mAb against SARS-CoV.

Inhibition of S-ACE2–Mediated Cell Fusion by 5H10

To examine the mechanism of SARS-CoV neutralization by 5H10, we analyzed cell–cell fusion between S-expressing HEK293 cells and ACE2-expressing HEK293 cells using

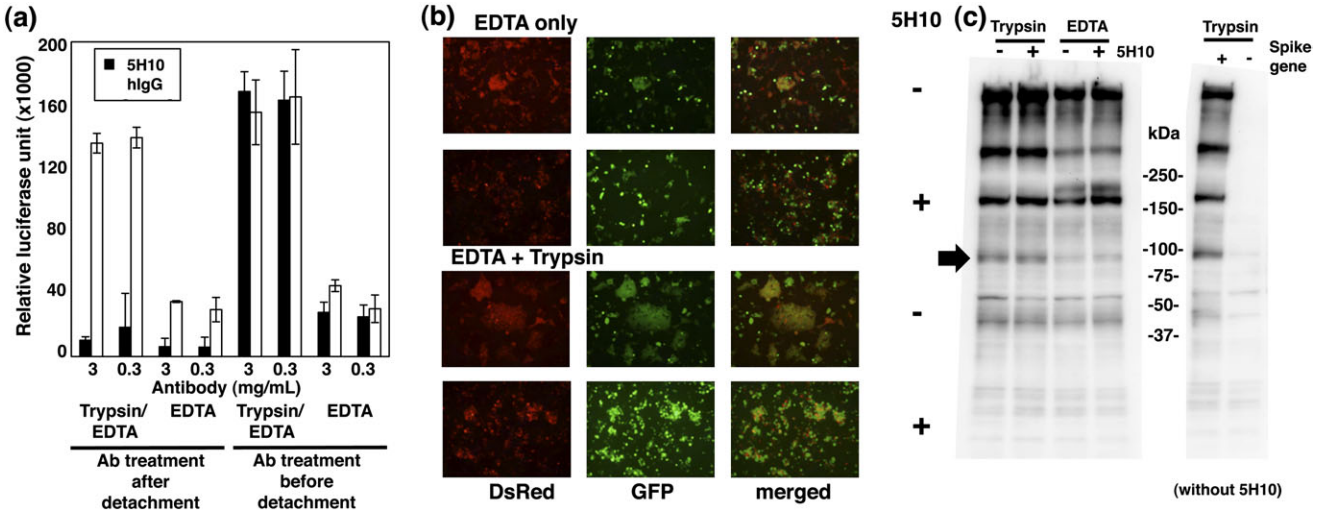

Figure 1. Inhibitory effects of 5H10 on cell–cell fusion mediated by S-ACE2 interaction. *A*, Effects of 5H10 in cell–cell fusion analysis using a dual-reporter system. Fusion of S-expressing 293 cells and human ACE2-expressing 293 cells was quantified as described in Materials and Methods. Cells were detached with or without trypsin in addition to EDTA. The antibody treatments were performed before or after detachment as indicated. *B*, Effects of 5H10 on syncytium formation by S-expressing cells and human ACE2-expressing cells. Syncytium formation of S- and ACE2-expressing cells labeled with GFP and DsRed, respectively, was visualized by confocal laser microscopy. Cells were detached with or without trypsin in addition to EDTA, then mixed, and 5H10 was added to the cultures as indicated. *C*, Western blotting analysis to determine the effects of 5H10 on S cleavage by trypsin treatment. S-expressing 293T cells were treated with trypsin or EDTA in the presence or absence of 5H10 (3 mg/mL) as indicated. After lysis of the cells, Western blotting using anti-S antibody mixture was performed. Control 293T cells, which did not harbor the *spike* gene, were used as a negative control.

a reporter system based on the mammalian 2-hybrid system. After cell–cell fusion mediated by S and ACE2, the dual-luciferase reporter system was activated and the amount of cell–cell fusion could be quantified. Because treatment with trypsin enhances cell–cell fusion via cleavage of a site located in the S791 region [10, 27], fusion efficiency of the cells treated with EDTA only was compared with that of cells treated with EDTA and trypsin in the presence or absence of 5H10 (Figure 1a). When S-expressing cells were detached from culture dishes by treatment with trypsin in addition to EDTA, cellular fusion reflected by relative fluorescence was significantly increased in comparison with detachment by EDTA only. Cell fusion was strongly inhibited by 5H10 but not human IgG when cells were treated with the antibodies after detachment from the dishes. In contrast, 5H10 did not show inhibition of cell fusion when added before detachment from the dishes.

We also analyzed cell–cell fusion mediated by S-ACE2 interaction using confocal laser microscopy (Figure 1b). S-expressing cells and ACE2-expressing cells were labeled with green fluorescent protein and DsRed, respectively. Although S-expressing cells and ACE2-expressing cells were partly fused even after detachment with EDTA only, syncytium formation was strongly enhanced by treatment with trypsin. Addition of 5H10 to the cell mixtures inhibited syncytium formation, at least in part, in those treated with or without trypsin. We also analyzed the cleavage of S by trypsin in the presence or absence of 5H10 (Figure 1c). By trypsin treatment, S was cleaved generating an additional fragment of ca. 90 kDa (indicated by an arrow). Addition of 5H10 during trypsin treatment did not significantly affect generation of the 90-kDa fragment. Furthermore, 5H10 reacted with the 90-kDa fragment as determined by Western blot (data not shown), suggesting that uncleaved S is not necessary for recognition by 5H10.

These results indicated that 5H10 inhibited the cell-cell fusion step but not cleavage of S or virus propagation in the virus replication cycle.

SARS-CoV Neutralizing Activity In Vivo Using a Rhesus Macaque Model

Before analyzing the efficacy of 5H10 in an animal model, we examined whether 5H10 has in vitro neutralizing activity against a SARS-CoV strain isolated in China (PUMC01). The results indicated that 5H10 neutralized the PUCM01 strain (data not shown).

To analyze the efficacy of 5H10 to protect against SARS-CoV infection in Rhesus macaques, 9 animals were separated into 3 groups (3 animals/group) consisting of sham administration, control IgG administration, and 5H10 administration. All of the animals were infected with the virus (PUMC01 strain) by spraying into the nasal cavity. The antibody was injected intravenously 1 h before virus infection and on days 1 and 3 after infection at a dose of 12.5 mg/kg (total dose, 37.5 mg/kg). The dose of 12.5 mg/kg was aimed to archive 10-fold 50% inhibition

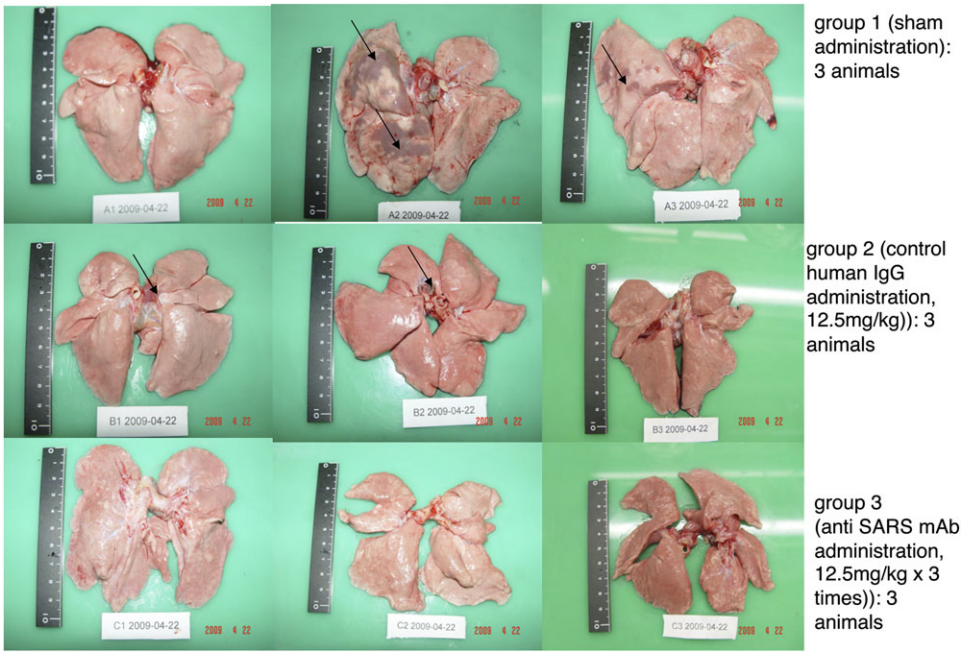

Figure 2. Gross pathological characterization of the lung damage in animals infected with SARS-CoV. Three animals were grouped as indicated and received sham treatment (top), control human IgG (middle), or 5H10 (bottom). Pathological changes found in the organs are indicated by arrows.

concentration in the blood according to the results of in vitro SARS-CoV neutralization assay. Gross pathological characterization of the lung on day 6 in animals administered 5H10 showed significant reduction of the severity of damage in all animals in comparison with those in the sham administration group (Figure 2).

Replication and Release of Virus

Viral load in the lung tissue samples from the animals were analyzed using both real-time RT-PCR and RT-PCR 6 days after infection (Figure 3). In real-time RT-PCR analyses, <10 copies of SARS-CoV were detected in the lung tissue samples from the anti-SARS-mAb administration group, and >600 copies of SARS-CoV were detected in the lung tissue samples from the sham administration and human IgG administration groups. RT-PCR amplification of a region located in the polyprotein 1ab of SARS-CoV showed amplified fragments from the samples from sham-administered animals and those given control human IgG, and no amplification of the fragment was observed in samples of 5H10-administered animals. These results indicated that 5H10, the human mAb, almost completely abolished the persistence of SARS-CoV in a Rhesus macaque model in vivo.

Immunohistochemical Analysis of the Infected Lung Tissue Samples With or Without 5H10 Administration

For immunohistochemical analysis, lung tissue sections were stained for SARS-CoV antigen, developed with d-amino benzidine, and counterstaining was performed with hematoxylin (Figure 4A). Lung tissue samples from all 3 animals in the sham administration or human IgG administration groups showed strong reactions for SARS-CoV antigen, whereas the positive reaction for the viral antigen was markedly diminished in all 3 animals administered 5H10.

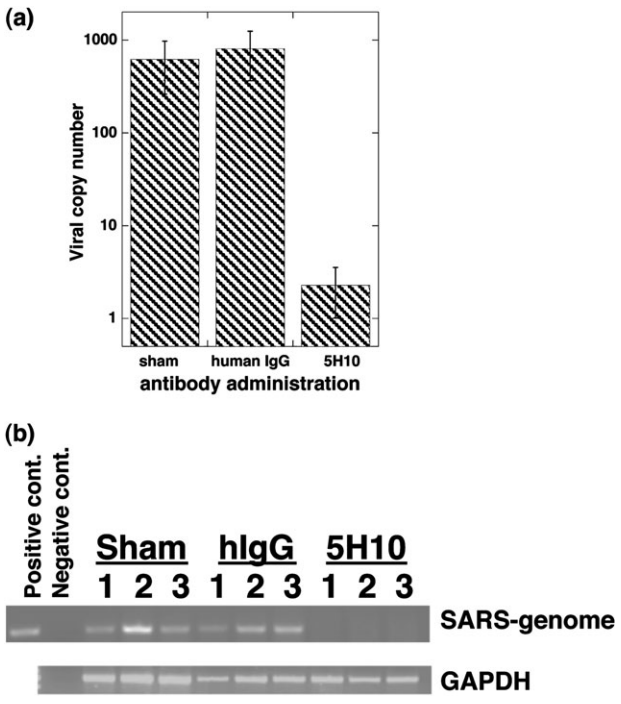

Figure 3. Viral load in lung tissues of animals administered 5H10. (a) SARS-CoV load was quantified by real-time RT-PCR. Data are presented as means ± SEM. (b) SARS-CoV genome fragments were amplified by RT-PCR. Data from each animal are presented and GAPDH was used to normalize the amounts of samples.

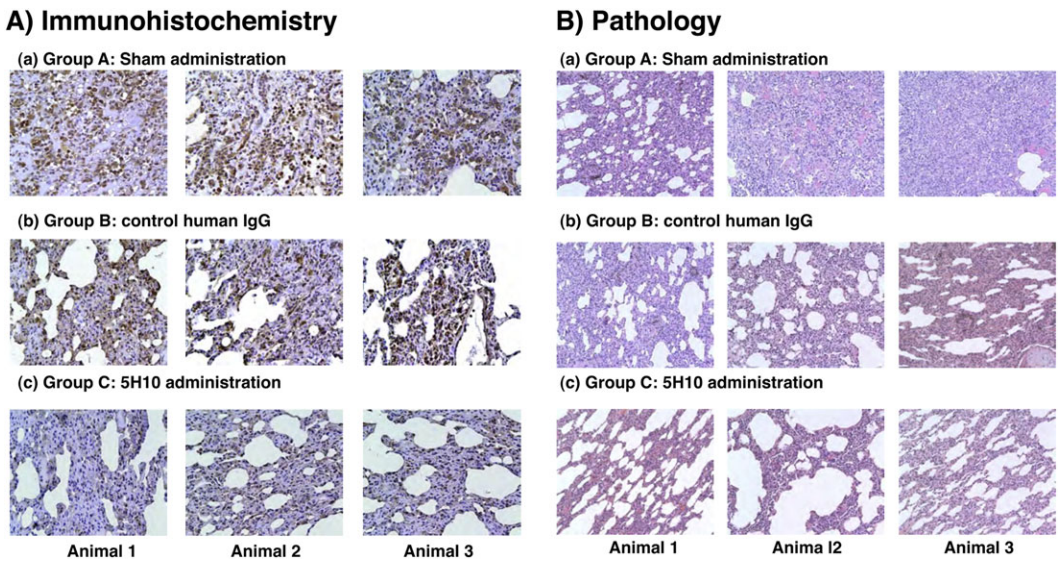

Figure 4. Immunohistochemical *(A)* and pathological *(B)* analyses of lung tissues from sham administration controls (a), those administered control human IgG (b), or those administered 5H10 (c). *(A)* Lung sections were treated with a mouse monoclonal antibody against SARS-CoV tissue sections were treated with the anti-SARS-CoV antibody, HRP-conjugated antibody, and DAB mix serially and stained with hematoxylin. (*B*) Histopathological analysis. (a) Sham administration group. Animal 1, +++, typical symptoms of acute DAD, extensive exudation and septal broadening, shrinkage of alveoli caused by pressure, restricted fusion of thick septa, ruptured elastic fibers of the alveoli, variably filled with protein-rich edema fluid, fibrin, erythrocytes, cellular debris, and a moderate number of inflammatory cells in alveolar cavities. Animal 2, ++++ severe acute DAD, massive cell infiltration and alveolar shrinkage, sheets of septal fusion, necrotic lesions at the hemorrhagic septa, and massive cell infiltration in alveolar cavities. Animal 3, ++++ severe acute DAD, massive cell infiltration and alveolar shrinkage, sheets of septal fusion, necrotic lesions at the hemorrhagic septa, and massive cell infiltration in alveolar cavities. (b) hIgG administration group. Animal 1, +++, typical symptoms of acute DAD, extensive exudation and septal broadening, shrinkage of alveoli caused by pressure, restricted fusion of thick septa, ruptured elastic fibers of alveoli, variably filled with protein-rich edema fluid, fibrin, erythrocytes, cellular debris, and a moderate number of inflammatory cells in alveolar cavities. Animal 2, +++, typical symptoms of acute DAD, extensive exudation and septal broadening, shrinkage of alveoli caused by pressure, restricted fusion of thickened septa, ruptured elastic fibers of alveoli, variably filled with protein-rich edema fluid, fibrin, erythrocytes, cellular debris, and a moderate number of inflammatory cells in alveolar cavities. Animal 3, ++ early symptoms of acute DAD, alveolar septal broadening with increasing inflammatory cell infiltration. (c) 5H10 administration group. Animal 1, + apparent inflammation, hemorrhage in septa, elastic fibers of alveolar wall distorted as shown by silver staining. Animal 2, ++ early symptoms of acute DAD, alveolar septal broadening with increasing inflammatory cell infiltration. Animal 3, ++ early symptoms of acute DAD, alveolar septal broadening with increasing inflammatory cell infiltration.

Pathological Examination of the Infected Lungs With or Without Administration of 5H10

As shown in Figure 4B, lung tissue samples from all infected animals in the sham administration group showed typical symptoms of acute diffuse alveolar damage (DAD), extensive exudation and septal broadening, shrinkage of the alveoli caused by pressure, restricted fusion of thick septa, ruptured elastic fibers of the alveoli, variably filled with protein-rich edema fluid, fibrin, erythrocytes, cellular debris, and a moderate number of inflammatory cells in the alveolar cavities. In the human IgG administration group, the lungs mainly showed typical symptoms of acute DAD, extensive exudation and septal broadening, shrinkage of alveoli caused by pressure, restricted fusion of thick septa, ruptured elastic fibers of the alveoli, variably filled with protein-rich edema fluid, fibrin, erythrocytes, cellular debris, and a moderate number of inflammatory cells in the alveolar cavities. In animal B3, the extent of DAD was slightly milder than in the former 2 animals. In contrast, lung tissue samples from the animals administered 5H10 showed apparent inflammation, hemorrhage in the septa, distortion of the elastic fibers of the alveolar wall, or signs of early symptoms of acute DAD, although the signs were markedly diminished in comparison with those in the tissue samples from the former 2 groups. The pathological findings are summarized in Table 1, based on the definitions of severity of lung damage.

These results indicated that the fully human mAb 5H10 inhibited viral replication and ameliorated the pathological changes in the lung of SARS-CoV–infected Rhesus macaques.

Preliminary Safety Evaluation of 5H10 in the Rhesus Model

Safety for administration of 5H10 was also analyzed in essentially the same Rhesus model as described above but without viral infection. Full results of the test are available on request. As expected from the nature of 5H10 as a fully human antibody, Rhesus monkeys administered 5H10 showed no abnormal behavior patterns, and examination of the organs of killed

Table 1. Summary of Pathological Examination

Group	Animal number	Grade
Sham administration	A1	+++
	A2	++++
	A3	++++
Control human IgG	B1	+++
	B2	+++
	B3	++
5H10 administration	C1	+
	C2	++
	C3	++

Pathological changes in the lungs were graded based on the following criteria: pathological characterization of the severity of lung damage in SARS Rhesus macaques

Grade	Pathological characterization of severity of lung damage in SARS Rhesus macaques
−: Normal lung section of macaque without SARS-CoV infection	
±: Minor inflammation, slight broadening of alveolar septa, and sparse monocyte infiltration	
+: Apparent inflammation, hemorrhage in septa, elastic fibers of alveolar wall distorted, as shown by silver staining	
++: Early symptoms of acute DAD, alveolar septal broadening with increasing inflammatory cell infiltration	
+++: Typical symptoms of acute DAD, extensive exudation and septal broadening, shrinkage of alveoli caused by pressure, restricted fusion of thick septa, ruptured elastic fibers of alveoli, variably filled with protein-rich edema fluid, fibrin, erythrocytes, cellular debris, and a moderate number of inflammatory cells in alveolar cavities	
++++: Severe acute DAD, massive cell infiltration and alveolar shrinkage, sheets of septal fusion, necrotic lesions at the hemorrhagic septa, and massive cell infiltration in alveolar cavities	

NOTE. DAD: diffuse alveolar damage.

animals did not show any safety concerns. Significant amounts of human anti-SARS antibody were detected even 2 weeks after administration (data not shown).

DISCUSSION

We obtained 5H10, a fully human mAb showing high SARS-CoV neutralizing activity in vitro and in vivo in a Rhesus macaque model. To our knowledge, this is the first report of the evaluation of a fully human antibody against SARS-CoV in a primate model.

The epitope of 5H10 was identified at amino acid position 791–805 in the S protein. This sequence is highly conserved among not only SARS-CoV strains from patients but also among CoV strains in general [28] and contains a critical cleavage site, which induces exposure of the fusion peptide on cleavage [10, 28]. 5H10 inhibited cell-cell fusion mediated by the interaction between S and ACE2 even in the presence of trypsin, although the inhibition was not effective if cells were treated with 5H10 before detachment by trypsin in addition to EDTA. Indeed, 5H10 did not efficiently inhibit the cleavage and reacted with cleaved S. Our observations suggested that 5H10 prevents viral fusion and entry into the host cells but not viral attachment to the host cells or cleavage of S. Antibodies recognizing epitopes located in the ACE2-binding domain induced antibody-dependent enhancement of SARS-CoV entry into the host cells when the amino acid sequences of the S proteins were changed [13, 14]. Our results indicated that targeting of the S protein region involved in mediating viral fusion would provide a feasible strategy to produce neutralizing antibodies against SARS-CoV, which is robust to mutation of the virus. Because synergistic effects of combinations of mAbs directed toward the ACE2 binding domain of S have been reported [15, 29], 5H10 directed toward the S cleavage site may serve as an alternative combination to provide stronger synergistic effects.

The Rhesus macaque model used here mirrors the sequence of pathogenesis in humans with SARS [22, 30, 31]. Clear clinical relevance of this model was observed in most of the parameters studied, such as elevated body temperature, lung pathology, and SARS-CoV antigen detection in the lung tissue samples, indicating that it is a valuable model for evaluation of candidate therapeutic agents. Administration of 5H10 significantly suppressed the SARS-CoV–induced pathogenesis in the SARS Rhesus model. Thus, 5H10 is sufficiently effective to prevent SARS-CoV infection in the primate model. Because significant amounts of human anti-SARS antibody were detected even 2 weeks after administration, 5H10 has a sufficient half-life for use in prophylaxis and for therapeutic purposes. Thus, 5H10 is a promising agent for use against the reemergence of SARS.

Taken together, our results suggest the potential usefulness of fully human antibody-based therapeutics against SARS-CoV based on evaluation in an in vivo non-human primate model. In this study, we produced 5H10 using a recombinant protein prepared in an *E. coli* expression system. This study represents a platform to produce fully human antibodies against emerging infectious diseases in a timely manner as (1) the recombinant proteins used in this study containing an antigenic epitope identified in the patients' serum samples and produced by *E. coli* to avoid the handling of large amount of hazardous SARS-CoV as the antigen, and (2) use of transgenic animals producing fully human antibodies makes it possible to rapidly produce fully human antibodies, which is essentially safe for clinical applications.

Supplementary Data

Supplementary data are available at http://jid.oxfordjournals.org online.

Funding

This work was supported by the National Institute of Biomedical Innovation (grant 04-02) and the Japan Science and Technology Agency.

Acknowledgments

We thank Mr Kurose for supporting the preparation of recombinant proteins.

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