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Brief Report

Inhibition of Severe Acute Respiratory Syndrome-Associated Coronavirus Infection by Equine Neutralizing Antibody in Golden Syrian Hamsters

GUANGYU ZHAO,[1,*] BING NI,[2,*] HAIYAN JIANG,[1] DEYAN LUO,[1] MAREK PACAL,[3] LILI ZHOU,[1] LIANGYAN ZHANG,[1] LI XING,[1] LIANGYAN ZHANG,[1] ZHENGCAI JIA,[2] ZHIHUA LIN,[4] LI WANG,[2] JINTAO LI,[2] YUNFEI LIANG,[2] XINFU SHI,[1] TINGTING ZHAO,[2] LIYUN ZHOU,[2] YUZHANG WU,[2] and XILIANG WANG[1]

ABSTRACT

Equine anti-severe acute respiratory syndrome-associated coronavirus $F(ab')_2$ has been verified to protect mice from infection with severe acute respiratory syndrome-associated coronavirus (SARS-CoV). However, before potential clinical application, the antibody needs to be tested in as many animal models as possible to ensure its safety and efficiency. In this study, after verification by various methods that the golden Syrian hamster constitutes a model susceptible to SARS-CoV infection, we confirmed that the antibody could protect animals completely from SARS-CoV infection in the preventive setting. More importantly, the antibody could reduce viral titers or copies by approximately 10^3- to 10^4-fold in animal lung after virus exposure, compared with negative control. These data provide further evidence to warrant clinical studies of this antibody in the treatment and prevention of SARS.

INTRODUCTION

SEVERE ACUTE RESPIRATORY SYNDROME (SARS) is now controlled, and widespread infections between humans and/or animals have not been observed since the initial outbreak in 2002 and 2003 (3,4). However, its mysterious animal origins (11) and strong infectivity necessitate further studies on how to control the replication of the SARS-associated coronavirus (CoV) in infected individuals. Furthermore, it is possible for an SARS outbreak to reoccur, for example, as a result of the virus evolving into variants that can be transmitted from human to human or as a result of laboratory accidents and biological warfare. These issues mandate further study of SARS prevention and treatment.

Various interventions to prevent and treat SARS

[1]Department of Immunology, Institute of Microbiology Epidemiology, Academy of Military Medical Sciences, Beijing, People's Republic of China.
[2]Institute of Immunology, Third Military Medical University, Chongqing, People's Republic of China.
[3]Molecular and Cellular Division, Toronto Western Research Institute, University Health Network, Toronto, ON, Canada.
[4]College of Bioengineering, Chongqing Institute of Technology, Chongqing, People's Republic of China.
*G.Z. and B.N. contributed equally to this work.

should be explored, including vaccines, passive immune therapy, RNA interference, and antiviral drugs. A prospective study of the humoral immune response of SARS patients indicated that patients with a longer illness showed a lower neutralizing antibody response than did patients with a shorter illness duration (5), indicating that neutralizing antibodies in patients play a pivotal role in SARS-CoV clearance *in vivo*. However, the neutralizing antibody titers decreased markedly after month 16 of infection (6), suggesting that persons who were exposed to SARS-CoV may become reinfected later. All this evidence indicates that humoral immunity is critical in preventing and treating SARS-CoV infection, which also suggests the direction of vaccine research. On the basis of this evidence, we developed an equine anti-SARS-CoV $F(ab')_2$ that was able to provide excellent protection from this virus in a BALB/c mouse model (15). However, before any possible future clinical applications, this kind of antibody must be tested rigorously in as many animal models as possible to ensure its efficacy and safety.

To date, several animal models susceptible to SARS-CoV infection, including mouse, ferret, golden Syrian hamster, rabbit, and monkey, have been established (1,2,8–10,13,14,16,17). Among these animals, hamster seems to be a good platform for evaluation of various anti-SARS strategies because it can maintain infection longer, with higher titers of SARS-CoV and more evident pathological changes in experimental animal lungs, than do other animals (9,10). In this study, we evaluated the equine anti-SARS-CoV $F(ab')_2$ in a golden Syrian hamster model to provide more evidence for potential clinical testing of this antibody.

MATERIALS AND METHODS

Virus, antibody, and animals

SARS-CoV (strain BJ01, GenBank accession number AY278488, isolated February 10 to March 15, 2003) was maintained at the Institute of Microbiology Epidemiology (Academy of Military Medical Sciences [AMMS], Beijing, China) and propagated in Vero cells. The virus was released from infected cells by three freeze–thaw cycles and the titer was determined to be 1.13×10^7 $TCID_{50}$ (50% tissue culture infective doses)/mL. All operations with SARS-CoV were performed in the Bio-Safety Level 3 (BSL-3) laboratory.

We prepared serum IgGs and their pepsin-digested $F(ab')_2$ fragments from horses inoculated with purified SARS-CoV (BJ01 strain), as described previously (15). The neutralizing titer of the $F(ab')_2$ was 1:1600–1:3200, determined in cultured Vero E6 cells by cytopathic effect (CPE), 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), and plaque-forming assays, respectively (15).

To evaluate the susceptibility of golden Syrian hamsters to SARS-CoV infection, after light anesthetization with isoflurane, an SARS-CoV particle suspension (1×10^4 $TCID_{50}$ in 100 μL) was administered intranasally to the animals on day 0. Four hamsters from each group were killed on days 1–7 postinfection. The lungs of experimental animals were removed and homogenized in a 10% (w/v) suspension of Leibovitz 15 medium (Invitrogen, Carlsbad, CA). Viral titers and copies in the homogenates were then determined in CPE and TaqMan real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assays, as described below. The pathology and localization of SARS-CoV in the lungs of infected animals were determined by pathological observation and immunohistochemistry (IHC), as described below.

To evaluate the therapeutic role of anti-SARS-CoV $F(ab')_2$ against SARS-CoV infection in golden Syrian hamsters, the animals received, intraperitoneally, a 100-μL suspension containing 1×10^4 $TCID_{50}$ of SARS-CoV on day 0, followed by intraperitoneal injection of 0.5 mL of the $F(ab')_2$ solution containing 70 or 35 mg/kg body weight of this kind of antibody, or normal horse antibody (70 mg/kg) lacking neutralizing activity as a negative control on day 1 postinfection. The viral titer, copy number, and localization, as well as pathologic changes in the infected animal lungs, were determined at the indicated times.

To investigate the preventive role of the equine anti-SARS-CoV $F(ab')_2$ against the SARS-CoV infection, after anesthetization, the hamsters were injected intraperitoneally with the anti-SARS-CoV $F(ab')_2$ (10 mg/kg body weight) or nonimmune normal horse antibody (10 mg/kg body weight), as a negative control, on day -1, the day before viral infection. Twenty-four hours later (day 0), the golden Syrian hamsters were challenged intranasally with 1×10^4 $TCID_{50}$ of SARS-CoV, and were killed on the indicated time points. The viral titer, copy number, and localization, as well as pathologic changes in the infected animal lungs, were then determined on the basis of CPE, qRT-PCR, IHC, and observation of pathology.

Cytopathic effect and qRT-PCR assay

Real-time quantitative TaqMan PCR and CPE assays were used to determine SARS-CoV titer and copy number in the lungs of SARS-CoV-infected or mock-infected hamsters. As described previously (15), the number of SARS-CoV N gene copies was determined by qPCR and the CPE assay was conducted on cultured Vero E6 cells to determine the viral titer.

Histopathology and immunohistochemistry

Hamsters were anesthetized with isoflurane and killed by cervical dislocation on the indicated day after virus administration or anti-SARS-CoV F(ab′)$_2$ injection. Lung tissue was fixed with 10% neutral buffered formalin, embedded in a paraffin block, and processed for routine histology and IHC (avidin–biotin–peroxidase complex [ABC] technique) detection as described (12). For IHC, the purified equine anti-SARS-CoV IgG (diluted 1:10,000) was used as the detecting antibody and 3,3′-diaminobenzidine (DAB) was used as the chromogenic substrate.

Statistical analysis

Statistical analyses were performed by one-way analysis of variance (ANOVA) and/or multiple comparison (Scheffé) and Student *t* test. All graphs represent means ± SEM.

RESULTS

As the golden Syrian hamster has not been studied extensively, we validated this animal model not only with the CPE assay used by Roberts and colleagues (9,10), but also with a quantitative TaqMan reverse transcription polymerase chain reaction (qRT-PCR) method that could provide accurate copy numbers of propagated virus, as described previously (7,15). Hamsters (weighing 80–100 g each) were inoculated intranasally with 1×10^4 $TCID_{50}$ of SARS-CoV (BJ01 strain) on day 0. We then determined viral titers and copies in the same lung tissue on days 1–7 postinfection, using the CPE assay and qRT-PCR assay, respectively. Viral titers increased from day 1 postinfection, and peaked (about $1 \times 10^{8.5}$ $TCID_{50}$/g lung tissue) on day 3 postinfection (Fig. 1A). Viral titers sustained a high level until day 5 postinfection (about 1×10^7 $TCID_{50}$/g), but decreased rapidly thereafter to

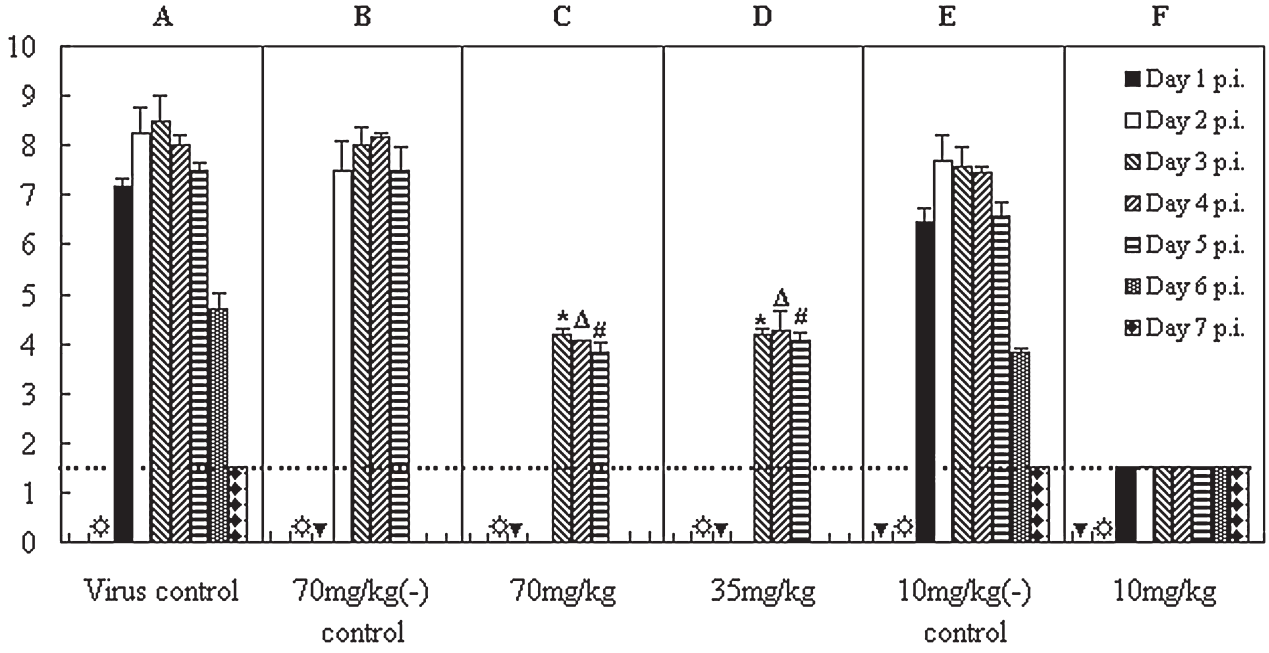

FIG. 1. Inhibition of SARS-CoV infection in the golden Syrian hamster, as determined by CPE assay. Animals were inoculated intranasally with 1×10^4 $TCID_{50}$ of SARS-CoV on day 0, and the titers of SARS-CoV were determined by CPE assay on days 1–7 postinfection (**A**). For therapeutic effect assays, 1×10^4 $TCID_{50}$ of SARS-CoV in 100 μL of phosphate-buffered saline (PBS) buffer was inoculated intranasally into each hamster on day 0. The indicated amount of nonimmune equine F(ab′)$_2$ control or equine anti-SARS-CoV F(ab′)$_2$ was injected intraperitoneally on day 1 postinfection (day 0), and viral titers were examined on the following 4 d (days 2–5; **B**), or on days 3–5 postinfection (**C** and **D**), respectively. For the preventive role investigation, 10 mg/kg body weight of equine anti-SARS-CoV F(ab′)$_2$ or nonimmune F(ab′)$_2$ in 0.5 mL of PBS buffer was administered intraperitoneally on day −1 and 1×10^4 $TCID_{50}$ of SARS-CoV was inoculated intranasally into each hamster on day 0. The viral titers were then investigated on days 1–7 postinfection (**F**), with 10 mg/kg body weight of nonimmune equine F(ab′)$_2$ as a negative control (**E**). Each experimental group contained three hamsters and the panels show mean viral titers from three independent experiments. Error bars indicate standard errors. Viral titers are expressed as $\log_{10}$ $TCID_{50}$ per gram lung tissue. The lower limit of detection of virus in a 10% (w/v) suspension of lung homogenate was 1.5 $\log_{10}$ $TCID_{50}$ per gram (dotted line). $^{*,\Delta,\#}p < 0.01$ (one-way ANOVA) compared with 70 mg/kg of nonimmune equine antibody control. Solid wedge, the day antibody was administered; sun symbol, the day virus was injected.

undetectable levels by day 7 postinfection (Fig. 1A), results that were similar to previously published results (9,10). The qRT-PCR assays verified that the course of the viral titer changes was concordant with the course of viral copy changes (Fig. 2A). On days 1–3 postinfection, the lung viral titers peaked at 1×10^{10} copies/g lung tissue and remained high until day 5 postinfection. However, on day 7 postinfection, when viral titers were undetectable by CPE assay, several thousand viral copies could still be detected by qRT-PCR (Figs. 1A and 2A), which reflects the higher sensitivity of real-time RT-PCR versus CPE. Thus, more information has been generated with respect to the golden Syrian hamster as a model of SARS-CoV infection.

Because of the close association between the degree of pathological change and viral titer (9,10), we believe that the efficiency of various interventions against SARS-CoV infection, such as antiviral drugs or passive antibody transfer, can be evaluated by detecting the viral titer or copy number in infected animal lungs. For assessment of therapeutic efficacy of the equine anti-SARS-CoV $F(ab')_2$, each hamster was inoculated intranasally with 1×10^4 $TCID_{50}$ of SARS-CoV on day 0, and received an intraperitoneal injection of the indicated dose of $F(ab')_2$ on day 1. The therapeutic role of the $F(ab')_2$ fragment was then investigated at the indicated times. We have determined that the half-life ($t_{1/2}$) of the $F(ab')_2$ fragment is about 60 h in rat and 40 h in macaque (18). As hamsters are more closely related to rats than to macaques, we assumed 60 h as the $t_{1/2}$ of our $F(ab')_2$ fragments. On the basis of the $t_{1/2}$, we measured the therapeutic role of the antibody on days 3–5 postinfection to provide exact data for its potential future clinical applications. The CPE assays indicated that equine anti-SARS-CoV $F(ab')_2$ at a concentration of 70 mg/kg body weight could exert a significant therapeutic effect on the inhibition of SARS-CoV propagation in animals. Animals protected with this dose of antibody could decrease about virus in their lungs by 4 $\log_{10}$ $TCID_{50}$ (Fig. 1C), compared with controls ($p < 0.01$) (Fig. 1B). As expected, the therapeutic efficiency of this antibody on day 4 postinfection was similar to that on day 3 postinfection (Fig. 1C). However, on day 5 postinfection, past the half-life of this antibody, the protective rate did not decrease,

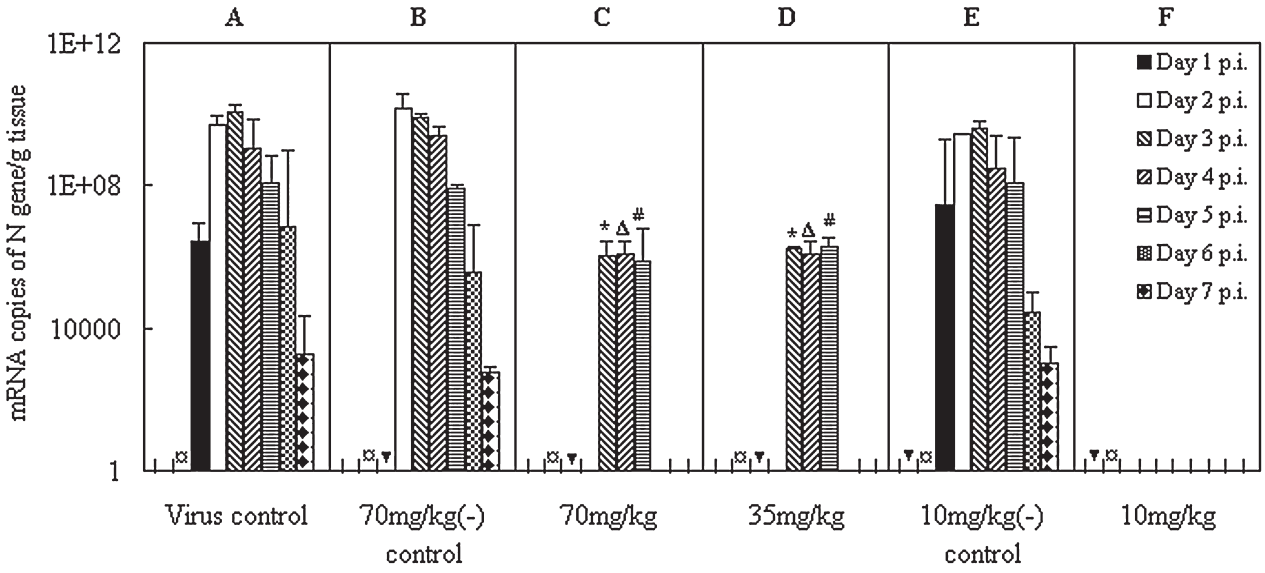

FIG. 2. Inhibition of SARS-CoV infection in the golden Syrian hamster, as determined by qPCR. Animals were inoculated intranasally with 1×10^4 $TCID_{50}$ of SARS-CoV in 100 μL of PBS on day 0, and SARS-CoV copies were determined by qPCR on days 1–7 postinfection (**A**). For therapeutic effect assays, 1×10^4 $TCID_{50}$ of SARS-CoV was inoculated intranasally into each hamster on day 0, the indicated nonimmune equine $F(ab')_2$ control or equine anti-SARS-CoV $F(ab')_2$ was injected intraperitoneally on day 1 postinfection, and viral copies were examined on the following 6 d (days 2–7, **B**), or on days 3–5 postinfection (**C** and **D**). For the preventive role investigation, 10 mg/kg body weight of equine anti-SARS-CoV $F(ab')_2$ or nonimmune $F(ab')_2$ was administered intraperitoneally on day -1 and 1×10^4 $TCID_{50}$ of SARS-CoV was inoculated intranasally into each hamster on day 0. Viral copies were then investigated on days 1–7 postinfection (**F**), with 10 mg/kg body weight of nonimmune equine $F(ab')_2$ as a negative control (**E**). Viral copies are expressed as N gene copies of SARS-CoV. Each experimental group contained three hamsters and the panels show mean viral copies from three independent experiments. Error bars indicate standard errors. $^{*,\Delta,\#}p < 0.01$ (one-way ANOVA) compared with 70 mg/kg of nonimmune equine antibody control. Solid wedge, the day the antibody was administered; sun symbol, the day the virus was inoculated.

suggesting that enough antibody was still present at this time in the blood to exert a protective effect in the animal. When we reduced the amount of injected antibody to 35 mg/kg, similar results were observed (Fig. 1D). Thus, the saturation concentration of this antibody for SARS therapy in the golden Syrian hamster model would be equal to or less than 35 mg/kg. In contrast, the 70-mg/kg body weight negative antibody control, nonimmune equine F(ab′)$_2$, did not exert any neutralizing effect on the virus (Fig. 1B). The qRT-PCR assays confirmed the CPE results (Fig. 2). The F(ab′)$_2$ dose of 70 mg/kg could significantly decrease viral copies in infected animal lungs (Fig. 2C), relative to the antibody-negative control and virus positive control (Fig. 2A and B). Viral copies in the inoculated hamster lungs were reduced at least 1000-fold (Fig. 2C). The F(ab′)$_2$ dose of 35 mg/kg had a similar protective effect on SARS-CoV infection as the 70-mg/kg antibody dose (Fig. 2D). Viral titers in the hamster lungs were reduced by $10^{2.4}$–$10^{3.9}$ $TCID_{50}$/g lung tissue when 40 mg/kg body weight of human anti-SARS-CoV (monoclonal antibody [mAb] 201) was used in the therapeutic setting (9). Thus, the equine anti-SARS-CoV F(ab′)$_2$ fragments seem to have similar or slightly better protective effect against SARS-CoV infection.

In the preventive setting, we injected intraperitoneally the indicated amounts of equine anti-SARS-CoV F(ab′)$_2$ into hamsters on day -1, and then inoculated hamsters intranasally with 1×10^4 $TCID_{50}$ of SARS-CoV on day 0 and investigated the role of this antibody in preventing SARS-CoV infection on days 1–7 postinfection, using the dose that previously protected BALB/c mice (15). Each hamster was protected with the antibody at 10 mg/kg body weight. CPE assays showed that this antibody dose could reduce the virus in inoculated animal lungs to an undetectable level (Fig. 1F). In contrast, the negative control of 10 mg/kg nonimmunized normal equine F(ab′)$_2$ did not protect the hamsters against SARS-CoV infection (Fig. 1E). Accordingly, the qRT-PCR assay also demonstrated that 10 mg/kg body weight of the SARS-CoV-specific antibody could completely clear the inoculated virus from the experimental lungs, because N gene copies of SARS-CoV were undetectable in lung homogenates at this dose (Fig. 2F). In contrast, about 10^{10} copies of SARS-CoV N genes could be detected in the negative antibody control group (Fig. 2E), similar to the viral levels observed in the positive control group (Fig. 2A).

We then examined the pathological changes in the hamster lungs to investigate the preventive and therapeutic roles of equine antibody against SARS-CoV in this animal model. Golden Syrian hamsters were inoculated intranasally with 1×10^4 $TCID_{50}$ of SARS-CoV on day 0 and pathological changes were analyzed by hematoxylin–eosin (H&E) staining and immunostaining on days 1, 3, and 5 postinfection. On day 1 postinfection, the pathological changes were mild and the lung tissue architecture remained roughly normal, except for some small vessel dilation and congestion, and edema in alveolar spaces (Fig. 3A), compared with normal mouse lung (see Fig. 3I). The virus invaded bronchial epithelial cells and, to a lesser extent, alveolar epithelial cells adjacent to the bronchi (Fig. 3B). On day 3 postinfection, there was a large degree of inflammatory cell infiltration, mainly involving mononuclear cells and lymphocytes (Fig. 3C). There also were focal hemorrhages around small vessels (Fig. 3C), and besides bronchial epithelial cells, alveolar epithelial cells were infected extensively (Fig. 3D). On day 5 postinfection, there was considerable inflammatory cell infiltration that surrounded bronchi (Fig. 3E and F). The interstitial pneumonia had become more severe than that observed on day 3 postinfection and there appeared to be apparent focal consolidation (Fig. 3E and F). A large viral load also could be observed in animal lungs on this day (Fig. 3F).

When the equine neutralizing antibody was administered after virus inoculation, antibody at 35 mg/kg apparently relieved the pathological lesions (Fig. 3G) on day 4 postinfection, compared with virus control (Fig. 3C and E). On the same day, animal lungs showed mild to moderate interstitial pneumonia and lung consolidation (Fig. 3G and H). The viral load in animal lungs on this day was much less than that in the viral control (Fig. 3D and F). For preventive effect assay, antibody at 10 mg/kg completely neutralized the inoculated virus. No pathological changes or virus could be detected in animal lungs (Fig. 3I and J).

DISCUSSION

Although the ideal immunoglobulin for adoptive transfer would be of autologous or homologous origin for the lowest anti-antibody response, the bottleneck of low mAb yield must be overcome before it can be used in practice. On the other hand, heterologous immunoglobulins can surmount the bottleneck of antibody yield. In general, 10,000–20,000 mL of whole blood can be obtained from one horse on a weekly basis, and about 100,000 mL of whole blood can be harvested if the horse is killed. One milliliter of blood contains about 7.23–16.85 mg of IgG and the recovery rate of F(ab′)$_2$ from IgG is about 50%. Thus a relatively high amount of antibody can be obtained from each horse. Furthermore, polyclonal IgGs would possess broader antigenic coverage and a lower likelihood of emergence of escape mutants, although this potential advantage of the equine anti-SARS-CoV F(ab′)$_2$ has not been studied here.

Heterologous immunoglobulins from several species including horse have been used for adoptive humoral therapy for several diseases such as toxicosis from spider and caterpillar, pure red cell aplasia, multiple sclerosis and systemic sclerosis, myelodysplasia, rabies, hepatitis virus, and human immunodeficiency virus (HIV), which have been discussed previously in detail (15). The immunogenicity of heterologous equine immunoglobulins must be given seriously consideration when used in humans. We can take some measures to reduce the immunogenicity of equine antibody, for example, by cutting off the Fc fragment. This way, the immune response of the host to the equine $F(ab')_2$ can be decreased to a tolerable level. Our experimental data (19) from 18 monkeys that were injected intravenously every day for 4 wk showed that equine $F(ab')_2$ had stimulated the host immune response against this kind of antibody itself and caused mild hypersensitivity. No experimental animals died and the motivated host secondary lymphoid organs, the spleen and lymph nodes, recovered 3 wk after the last injection. Because the host anti-$F(ab')_2$ antibody appears 2 wk after $F(ab')_2$ administration, there is enough time for the equine antibody to exert a protective effect at the early stage of SARS-CoV infection, thus producing a marked effect during a large-scale SARS outbreak.

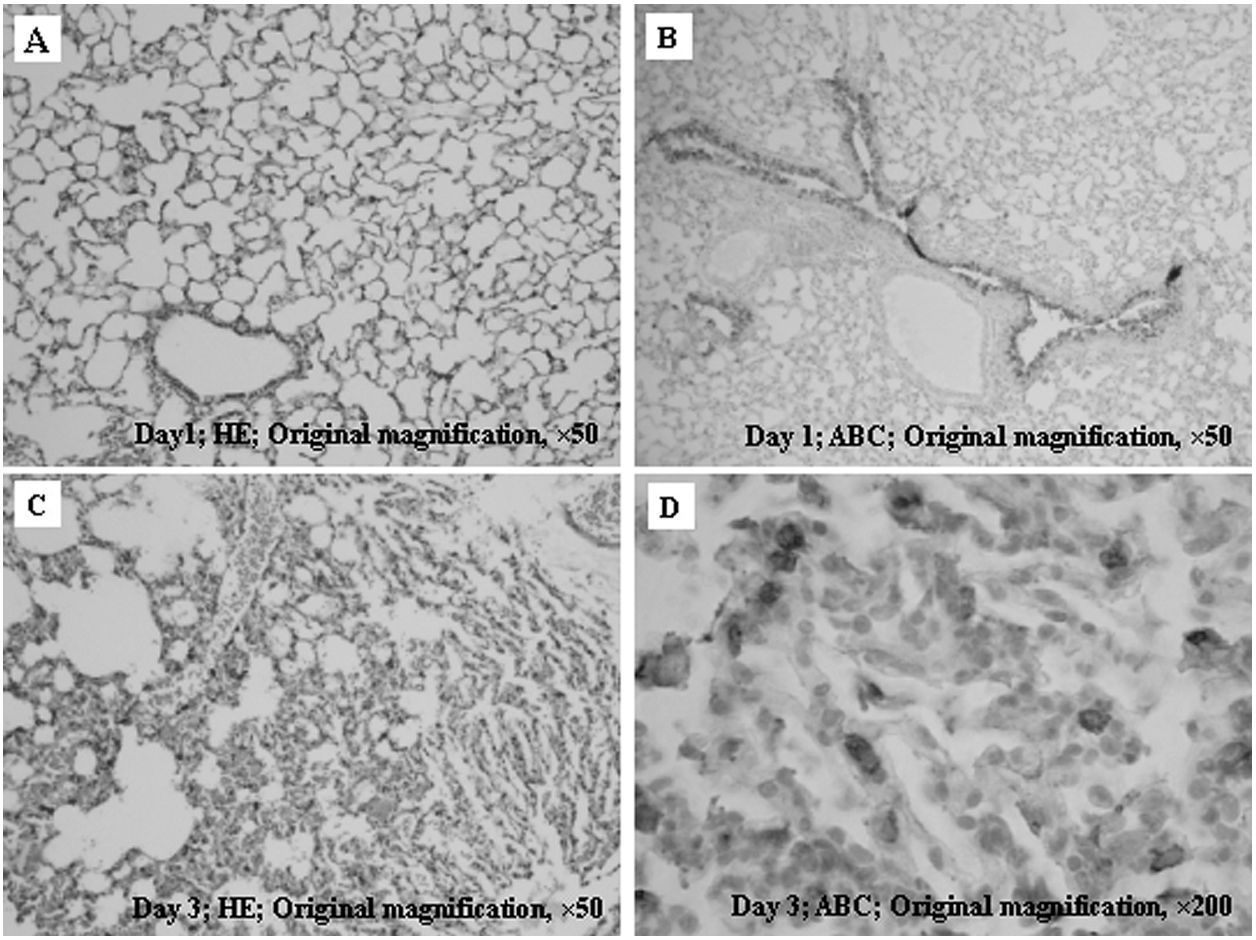

FIG. 3. Pathological effects on the golden Syrian hamster lung. Golden Syrian hamsters were inoculated intranasally with 1×10^4 $TCID_{50}$ of SARS-CoV on day 0, with or without antibody protection. At the indicated times, the experimental animal lungs were removed and fixed with formalin and then stained with hematoxylin and eosin (H&E) for pathological observation, or subjected to IHC with anti-SARS-CoV $F(ab')_2$ as the primary antibody (diluted 1:10,000) and DAB as the chromogenic substrate. (**A**–**F**) Establishment of the animal model. After viral inoculation, animal lungs were examined on days 1, 3, and 5, postinfection by H&E staining and by IHC assay. To assay the therapeutic effect, animals were treated with antibody at 35 mg/kg after viral exposure and the lungs were analyzed on day 4 postinfection (**G** and **H**). For preventive assays, animals were administered equine anti-SARS-CoV $F(ab')_2$ at 10 mg/kg before viral exposure and the lungs were analyzed (**I** and **J**); as no pathological changes were observed, this tissue was also used as a normal control. Data shown are representative of five animals per group.

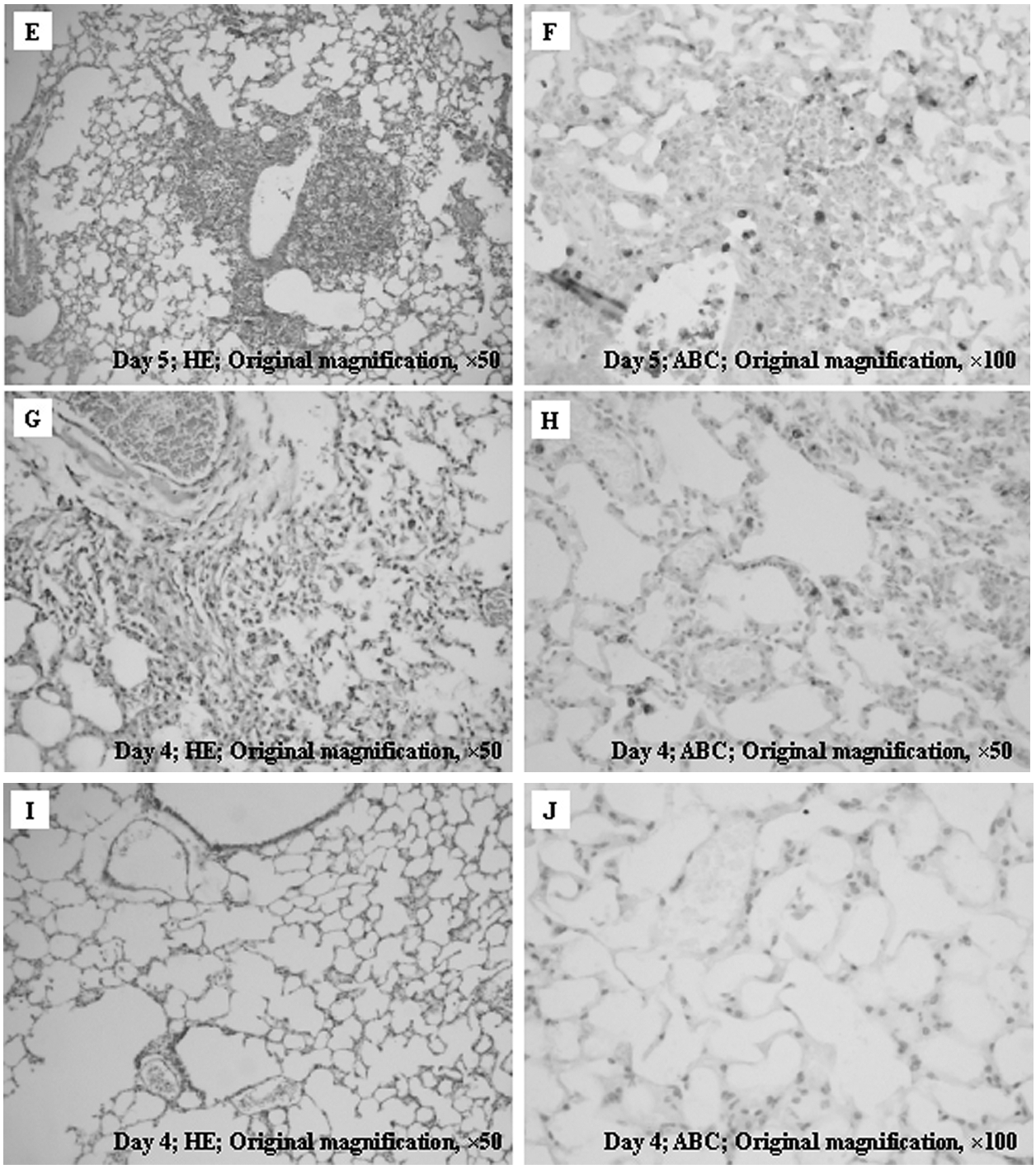

FIG. 3. (*Continued*).

In our animal model, equine F(ab′)$_2$ could only partially but significantly inhibit SARS-CoV propagation in the therapeutic setting. As SARS-CoV is an intracellular virus and equine anti-SARS-CoV F(ab′)$_2$ will neutralize mainly the extracellular virus, it is reasonable that a saturating concentration of this antibody, 35 mg/kg as determined in this study, could not completely neutralize all viral particles. However, this effective neutralization of extracellular virus will inhibit other normal tissue from being infected further and thus halt the transmission of the virus *in vivo*. This will enable the host to produce an immune response against the virus and clear it thor-

oughly. However, we currently cannot verify this scenario because there are no appropriate animal models that can be infected for so long a time to evaluate an active immune response to SARS-CoV infection. Finally, the rapid clearance of SARS-CoV by the $F(ab')_2$ *in vivo* will provide the chance for other interventions such as antiviral drugs, RNA interference (RNAi), and vaccines to eliminate the virus from the host.

Roberts *et al*. reported that some animals injected with neutralizing antibody did not display this antibody in the serum (9). However, we have not observed this phenomenon. It may be due to the following reasons. The antibody used in this study has been truncated by removing the Fc fragment from IgG, and therefore the specific neutralizing activity of the $F(ab')_2$ per dose of antibody solution used is higher than that used by Roberts *et al*., which consisted of intact immunoglobulin (9). This means the 35-mg/kg dose of $F(ab')_2$ used in this study will have higher neutralizing activity than the 40-mg/kg dose of IgG used by Roberts *et al*. (9), and thus $F(ab')_2$ at 35 mg/kg should exceed the dose needed to neutralize extracellular SARS-CoV in the golden Syrian hamster. In addition, the diffusible feature of $F(ab')_2$ may represent another advantage over intact IgG, thus presenting better protective efficiency than the latter.

In conclusion, in this study we have verified the golden Syrian hamster model by several methods. We have confirmed that equine anti-SARS-CoV $F(ab')_2$ can protect animals completely from SARS-CoV infection when adequate doses of antibody are administered before viral inoculation. More importantly, the antibody also exhibits an excellent therapeutic effect after viral exposure. Together with its proven protective role against SARS-CoV infection in mice (15), more evidence now exists to warrant clinical studies of this antibody in the treatment and prevention of SARS.

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REFERENCES

1. Buchholz UJ, Bukreyev A, Yang L, Lamirande EW, Murphy BR, Subbarao K, and Collins PL: Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. Proc Natl Acad Sci USA 2004;101:9804–9809.
2. Bukreyev A, Lamirande EW, Buchholz UJ, Vogel LN, Elkins WR, St Claire M, Murphy BR, Subbarao K, and Collins PL: Mucosal immunisation of African green monkeys (*Cercopithecus aethiops*) with an attenuated parainfluenza virus expressing the SARS coronavirus spike protein for the prevention of SARS. Lancet 2004;363:2122–2127.
3. Enserink M, and Normile D: Infectious diseases. Search for SARS origins stalls. Science 2003;302:766–767.
4. Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, Luo SW, Li PH, Zhang LJ, Guan YJ, Butt KM, Wong KL, Chan KW, Lim W, Shortridge KF, Yuen KY, Peiris JS, and Poon LL: Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science 2003;302:276–278.
5. Ho MS, Chen WJ, Chen HY, Lin SF, Wang MC, Di J, Lu YT, Liu CL, Chang SC, Chao CL, King CC, Chiou JM, Su IJ, and Yang JY: Neutralizing antibody response and SARS severity. Emerg Infect Dis 2005;11:1730–1737.
6. Liu W, Fontanet A, Zhang PH, Zhan L, Xin ZT, Baril L, Tang F, Lv H, and Cao WC: Two-year prospective study of the humoral immune response of patients with severe acute respiratory syndrome. J Infect Dis 2006;193:792–795.
7. Ni B, Shi X, Li Y, Gao W, Wang X, and Wu Y: Inhibition of replication and infection of severe acute respiratory syndrome-associated coronavirus with plasmid-mediated interference RNA. Antivir Ther 2005;10:527–533.
8. Roberts A, Paddock C, Vogel L, Butler E, Zaki S, and Subbarao K: Aged BALB/c mice as a model for increased severity of severe acute respiratory syndrome in elderly humans. J Virol 2005;79:5833–5838.
9. Roberts A, Thomas WD, Guarner J, Lamirande EW, Babcock GJ, Greenough TC, Vogel L, Hayes N, Sullivan JL, Zaki S, Subbarao K, and Ambrosino DM: Therapy with a severe acute respiratory syndrome-associated coronavirus-neutralizing human monoclonal antibody reduces disease severity and viral burden in golden Syrian hamsters. J Infect Dis 2006;193:685–692.
10. Roberts A, Vogel L, Guarner J, Hayes N, Murphy B, Zaki S, and Subbarao K: Severe acute respiratory syndrome coronavirus infection of golden Syrian hamsters. J Virol 2005;79:503–511.
11. Saif LJ: Animal coronaviruses: What can they teach us about the severe acute respiratory syndrome? Rev Sci Tech 2004;23:643–660.
12. Subbarao K, McAuliffe J, and Vogel L: Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice. J Virol 2004;78:3572–3577.
13. Takasuka N, Fujii H, Takahashi Y, Kasai M, Morikawa S, Itamura S, Ishii K, Sakaguchi M, Ohnishi K, Ohshima M,

Hashimoto S, Odagiri T, Tashiro M, Yoshikura H, Takemori T, and Tsunetsugu-Yokota Y: A subcutaneously injected UV-inactivated SARS coronavirus vaccine elicits systemic humoral immunity in mice. Int Immunol 2004;16: 1423–1430.

14. Tsunetsugu-Yokota Y, Ohnishi K, and Takemori T: Severe acute respiratory syndrome (SARS) coronavirus: Application of monoclonal antibodies and development of an effective vaccine. Rev Med Virol 2006;16:117–131.
15. Wang X, Ni B, Du X, Zhao G, Gao W, Shi X, Zhang S, Zhang L, Wang D, Luo D, Xing L, Jiang H, Li W, Jiang M, Mao L, He Y, Xiao Y, and Wu Y: Protection of mammalian cells from severe acute respiratory syndrome coronavirus infection by equine neutralizing antibody. Antivir Ther 2005;10:681–690.
16. Weingartl H, Czub M, Czub S, Neufeld J, Marszal P, Gren J, Smith G, Jones S, Proulx R, Deschambault Y, Grudeski E, Andonov A, He R, Li Y, Copps J, Grolla A, Dick D, Berry J, Ganske S, Manning L, and Cao J: Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets. J Virol 2004;78: 12672–12676.
17. Yang ZY, Kong WP, Huang Y, Roberts A, Murphy BR, Subbarao K, and Nabel GJ: A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. Nature 2004;428:561–564.
18. Luo D, Ni B, Zhao G, et al. Protection from infection of SARS-CoV in a Chinese hamster model by equine neutralizing F(ab′)2 and its safety, immunogenicty and pharmokinetic study in macaque (submiitted).
19. Zhou L, Ni B, Zhao G, et al. Inhibition of infection caused by severe respiratory syndrome-associated coronavirus by equine neutralizing antibody in aged mice. Int Immunopharmacol 2007;7:392–400.

Address reprint requests to:
Dr. Xiliang Wang
State Key Laboratory of Pathogen and Biosecurity
Institute of Microbiology and Epidemiology
Academy of Military Medical Science
Beijing 100071, People's Republic of China

E-mail: nibingxi@yahoo.com

or
Dr. Yuzhang Wu
Institute of Immunology
Third Military Medical University
Chongqing 400037, People's Republic of China

E-mail: wuyuzhang@yahoo.com

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