**CORONAVIRUS**

**Cross-reactive neutralization of SARS-CoV-2 by serum antibodies from recovered SARS patients and immunized animals**

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The current coronavirus disease 2019 (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus genetically close to SARS-CoV. To investigate the effects of previous SARS-CoV infection on the ability to recognize and neutralize SARS-CoV-2, we analyzed 20 convalescent serum samples collected from individuals infected with SARS-CoV during the 2003 SARS outbreak. All patient sera reacted strongly with the S1 subunit and receptor binding domain (RBD) of SARS-CoV; cross-reacted with the S ectodomain, S1, RBD, and S2 proteins of SARS-CoV-2; and neutralized both SARS-CoV and SARS-CoV-2 S protein–driven infections. Analysis of antisera from mice and rabbits immunized with a full-length S and RBD immunogens of SARS-CoV verified cross-reactive neutralization against SARS-CoV-2. A SARS-CoV–derived RBD from palm civets elicited more potent cross-neutralizing responses in immunized animals than the RBD from a human SARS-CoV strain, informing strategies for development of universal vaccines against emerging coronaviruses.

**INTRODUCTION**

The global outbreak of the coronavirus disease 2019 (COVID-19) was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is a new coronavirus (CoV) genetically close to SARS-CoV that emerged in 2002 (1–3). As of 25 May 2020, a total of 5,307,298 confirmed COVID-19 cases, including 342,070 deaths, have been reported from 216 countries or regions, and the numbers are still growing rapidly (https://who.int). Unfortunately, even though 17 years passed, we have not developed effective prophylactics and therapeutics in preparedness for the reemergence of SARS or SARS-like CoVs. A vaccine is urgently needed to prevent the human-to-human transmission of SARS-CoV-2.

Like SARS-CoV and many other CoVs, SARS-CoV-2 uses its surface spike (S) glycoprotein to gain entry into host cells (4–6). Typically, the S protein forms a homotrimer with each protomer consisting of S1 and S2 subunits. The N-terminal S1 subunit is responsible for virus binding to the cellular receptor angiotensin-converting enzyme 2 (ACE2) through an internal receptor binding domain (RBD) that is capable of functional folding independently, whereas the membrane-proximal S2 subunit mediates membrane fusion events. While SARS-CoV-2 and SARS-CoV share about 80% homology in full-length genome sequences, their S proteins have about 76% amino acid identity (2, 3). The RBD sequences of the two viruses are only about 74% identical, with most mutations occurring in the receptor-binding motifs (RBM) (~50% amino acid identity). It was found that the ACE2-binding affinity of the SARS-CoV-2 RBD is 10- to 20-fold higher than that of the SARS-CoV RBD, which may contribute to the higher transmissibility of SARS-CoV-2 (7). Very recently, the prefusion structure of the SARS-CoV-2 S protein was determined by cryo–electron microscopy, which revealed an overall similarity to that of SARS-CoV (5, 7); the crystal structure of the SARS-CoV-2 RBD in complex with ACE2 was also determined by several independent groups, and the residues or motifs critical for the higher-affinity RBD–ACE2 interaction were identified (8–10). As seen, the SARS-CoV-2 RBD binds ACE2 in the same orientation with the SARS-CoV RBD and relies on conserved, mostly aromatic, residues. The structures have also provided evidence to support a mechanism of infection triggering that is thought to be conserved among the Coronaviridae, wherein the S protein undergoes distinct conformational states with the RBD closed (receptor-inaccessible) or opened (receptor-accessible).

The S protein of CoVs is also a main target of neutralizing antibodies (nAbs), thus being considered an immunogen for vaccine development (5, 11). During the SARS-CoV outbreak in 2002, we took immediate actions to characterize the immune responses in infected SARS patients and in inactivated virus vaccine– or S protein–immunized animals (12–20). We demonstrated that the S protein RBD dominates the nAb response against SARS-CoV infection and thus proposed an RBD-based vaccine strategy (11, 15–22). Our follow-up studies verified a potent and persistent anti-RBD response in recovered SARS patients (23–25). Although SARS-CoV-2 and SARS-CoV share substantial genetic and functional similarities, their S proteins, especially in the RBD sequences, display relatively larger divergences. Toward developing vaccines and immunotherapeutics against emerging CoVs, it is fundamentally important to characterize the antigenic cross-reactivity between SARS-CoV-2 and SARS-CoV.

**RESULTS**

**Serum antibodies from recovered SARS patients react strongly with the S protein of SARS-CoV-2**

A panel of serum samples collected from 20 patients who recovered from SARS-CoV infection was analyzed for the antigenic cross-reactivity with SARS-CoV-2. First, we examined the convalescent sera by a commercial diagnostic enzyme-linked immunosorbent assay (ELISA) kit, which uses a recombinant nucleocapsid (N) protein of...
SARS-CoV-2 as detection antigen. As shown in Fig. 1A, all the serum samples at a 1:100 dilution displayed high reactivity, verifying that the N antigen is highly conserved between SARS-CoV and SARS-CoV-2. As tested by ELISA, each of the patient sera also reacted with the SARS-CoV S1 subunit and its RBD strongly (Fig. 1B). Then, we determined the cross-reactivity of the patient sera with four recombinant protein antigens derived from the S protein of SARS-CoV-2, including S ectodomain (designated S), S1 subunit, RBD, and S2 subunit. As shown in Fig. 1C, all the serum samples also reacted strongly with the S and S2 proteins, but they were less reactive with the S1 and RBD proteins.

**Serum antibodies from recovered SARS patients cross-neutralize SARS-CoV-2**

Limited by facility that can handle authentic viruses, we developed a pseudovirus-based single-cycle infection assay to determine the cross-neutralizing activity of the convalescent SARS sera on SARS-CoV and SARS-CoV-2. A control lentivirus was pseudotyped with vesicular stomatitis virus G protein (VSV-G). Initially, the serum samples were analyzed at a 1:20 dilution. As shown in Fig. 2A, all the sera efficiently neutralized both the SARS-CoV and SARS-CoV-2 pseudoviruses to infect 293T/ACE2 cells, and in comparison, each serum had lower efficiency in inhibiting SARS-CoV-2 as compared to SARS-CoV. None of the immune sera showed appreciable neutralizing activity on VSV-G pseudovirus. The neutralizing titer for each patient serum was then determined. As shown in Fig. 2B, the patient sera could neutralize SARS-CoV with titers ranging from 1:120 to 1:3240 and could cross-neutralized SARS-CoV-2 with titers ranging from 1:20 to 1:360. In a highlight, the patient P08 serum had the highest titer to neutralize SARS-CoV (1:3240) when it neutralized SARS-CoV-2 with a titer of 1:120; the patient P13 serum showed the highest titer on SARS-CoV-2 (1:360) when it had a 1:1080 titer to efficiently neutralize SARS-CoV.

![Fig. 1. Cross-reactivity of convalescent sera from SARS-CoV–infected patients with SARS-CoV-2 determined by ELISA.](http://advances.sciencemag.org/)
Mouse antisera raised against SARS-CoV S protein react and neutralize SARS-CoV-2

To comprehensively characterize the cross-reactivity between the S proteins of SARS-CoV and SARS-CoV-2, we generated mouse antisera against the S protein of SARS-CoV by immunization. Here, three mice (M-1, M-2, and M-3) were immunized with a recombinant full-length S protein in the presence of MPL-TDM adjuvant (monophosphoryl lipid A plus trehalose dicorynomycolate), while two mice (M-4 and M-5) were immunized with the S protein plus alum adjuvant (fig. S1). Binding of antisera to diverse S antigens were initially examined by ELISA. As shown in Fig. 3A, the mice immunized by the S protein with the MPL-TDM adjuvant developed relatively higher titers of antibody responses as compared to the two mice with the alum adjuvant. It was expected that the adjuvanticity of alum formulation was weaker than that of MPL-TDM. Apparently, each of the mouse antisera had high cross-reactivity with the SARS-CoV-2 S and S2 proteins, but the cross-reactive antibodies specific for the SARS-CoV-2 S1 and RBD were relatively lower except that in mouse M-3. Subsequently, the neutralizing capacity of mouse anti-S sera was measured with pseudoviruses. As shown in Fig. 3 (B to F), all the antisera, diluted at 1:40, 1:160, or 1:640, potently neutralized SARS-CoV, and consistently, they were able to cross-neutralize SARS-CoV-2 although with reduced capacity relative to SARS-CoV.

Mouse and rabbit antisera developed against SARS-CoV RBD cross-react and neutralize SARS-CoV-2

As the S protein RBD dominates the nAb response to SARS-CoV, we sought to characterize the RBD-mediated cross-reactivity and neutralization on SARS-CoV-2. To this end, we first generated mouse anti-RBD sera by immunization with two RBD-Fc fusion proteins: one encoding the RBD sequence of a palm civet SARS-CoV strain SZ16 (SZ16-RBD) and the second one with the RBD sequence of a human SARS-CoV strain GD03 (GD03-RBD). Both the fusion proteins were expressed in 293T cells and purified to apparent homogeneity (fig. S1). As shown in Fig. 4A, all eight mice
developed robust antibody responses against the SARS-CoV S1 and RBD, and in comparison, four mice (M-1 to M-4) immunized with SZ16-RBD exhibited higher titers of antibody responses than the mice (M-5 to M-8) immunized with GD03-RBD. Each of the anti-RBD sera cross-reacted well with the S protein of SARS-CoV-2, suggesting that SARS-CoV and SARS-CoV-2 do share antigenically conserved epitopes in the RBD sites. Noticeably, while the SZ16-RBD immune sera also reacted with the SARS-CoV-2 S1 and RBD antigens, the cross-reactivity of the GD03-RBD immune sera was low. However, while the mouse anti-RBD sera at 1:50 dilutions were measured with increased coating antigens in ELISA, they reacted with the SARS-CoV-2 S1 and RBD efficiently, which verified the cross-reactivity (Fig. 4B). Similarly, the neutralizing activity of mouse antisera was determined by pseudovirus-based single-cycle infection assay. As shown in Fig. 4 (C and D), both the SZ16-RBD- and GD03-RBD-specific antisera displayed very potent activities to neutralize SARS-CoV; they also cross-neutralized SARS-CoV-2 with relatively lower efficiencies. As judged by the neutralizing activity at the highest serum dilution, the SZ16-RBD antisera were more potent than the GD03-RBD immune sera was low. However, while the mouse anti-RBD sera at 1:50 dilutions were measured with increased coating antigens in ELISA, they reacted with the SARS-CoV-2 S1 and RBD efficiently, which verified the cross-reactivity (Fig. 4B). Similarly, the neutralizing activity of mouse antisera was determined by pseudovirus-based single-cycle infection assay. As shown in Fig. 4 (C and D), both the SZ16-RBD- and GD03-RBD-specific antisera displayed very potent activities to neutralize SARS-CoV; they also cross-neutralized SARS-CoV-2 with relatively lower efficiencies. As judged by the neutralizing activity at the highest serum dilution, the SZ16-RBD antisera were more potent than the GD03-RBD antisera in neutralizing SARS-CoV; however, the two antisera had no significant difference in neutralizing SARS-CoV-2 (Fig. 4, E and F).

We further developed rabbit antisera by immunizations, in which two rabbits were immunized with SZ16-RBD (R-1 and R-2) or with GD03-RBD (R-3 and R-4). Each RBD protein elicited antibodies highly reactive with both the SARS-CoV and SARS-CoV-2 antigens (Fig. 5A), which were different from their immunizations in mice. As expected, all of the rabbit antisera potently neutralized SARS-CoV and SARS-CoV-2 in a similar profile with that of the mouse anti-S and anti-RBD sera (Fig. 5, B and C). Obviously, the neutralizing activity of rabbit anti–SZ16-RBD sera against both the viruses was higher than that of the rabbit anti–GD03-RBD sera (Fig. 5, D and E). Together, the results verified that the SARS-CoV S protein and its RBD immunogens can induce cross-neutralizing antibodies toward SARS-CoV-2 by vaccination.

Rabbit antibodies induced by SZ16-RBD but not GD03-RBD can block RBD binding to 293T/ACE2 cells

To validate the observed cross-reactive neutralization and explore the underlying mechanism, we purified anti-RBD antibodies from the rabbit antisera above. As shown in Fig. 6 (A and B), both purified rabbit anti–SZ16-RBD and anti–GD03-RBD antibodies reacted strongly with the SARS-CoV RBD protein and cross-reacted with the SARS-CoV-2 S and RBD but not S2 proteins in a dose-dependent manner. Moreover, the purified antibodies dose-dependently neutralized SARS-CoV and SARS-CoV-2 but not VSV-G (Fig. 6, C and D). Consistent with their antisera, the rabbit anti–SZ16-RBD antibodies were more active than the rabbit anti–GD03-RBD antibodies against both SARS-CoV and SARS-CoV-2 (Fig. 5, E and F). Next, we investigated whether the rabbit anti-RBD antibodies block RBD binding to 293T/ACE2 cells by flow cytometry. As expected, both the SARS-CoV and SARS-CoV-2 RBD proteins could bind to 293T/ACE2 cells in a dose-dependent manner and, in line with previous findings, that the RBD of SARS-CoV-2 bound to ACE2 more efficiently (fig. S2). Unexpectedly, the antibodies purified from SZ16-RBD–immunized rabbits (R-1 and R-2) potently blocked the binding of both the RBD proteins, whereas the antibodies from GD03-RBD–immunized rabbits (R-3 and R-4) had no such blocking functionality.
except a high concentration of the rabbit R-3 antibody on the SARS-CoV RBD binding (Fig. 7).

**DISCUSSION**

To develop effective vaccines and immunotherapeutics against emerging CoVs, the antigenic cross-reactivity between SARS-CoV-2 and SARS-CoV is a key scientific question that needs to be addressed as soon as possible. However, after the SARS-CoV outbreak more than 17 years ago, there are very limited blood samples from SARS-CoV–infected patients available for such studies. At the moment, Hoffmann et al. (26) analyzed three convalescent patient with SARS sera and found that both SARS-CoV-2 and SARS-CoV S protein–driven infections were inhibited by diluted sera, but the inhibition of SARS-CoV-2 was less efficient; Ou et al. (27) detected one patient with SARS serum that was collected at 2 years after recovery, which showed a serum neutralizing titer of >1:80 dilution for SARS-CoV pseudovirus and of 1:40 dilution for SARS-CoV-2 pseudovirus. While these studies supported the cross-neutralizing activity of the convalescent SARS sera on SARS-CoV-2, a just published study with the plasma from seven SARS-CoV–infected patients suggested that cross-reactive antibody binding responses to the SARS-CoV-2 S protein did exist,

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**Fig. 4.** Cross-reactive and neutralizing activities of antisera from mice immunized with the RBD proteins of SARS-CoV. (A) Binding activity of mouse antisera at a 1:100 dilution to SARS-CoV (S1 and RBD) and SARS-CoV-2 (S, S1, and RBD) antigens was determined by ELISA. A healthy mouse serum was tested as control. (B) The cross-reactivity of mouse antisera with the SARS-CoV-2 S1 and RBD proteins. The antisera were diluted at 1:50, and the S1 and RBD antigens were coated at 100 ng per ELISA plate well. (C and D) Neutralizing activities of mouse antisera at indicated dilutions against SARS-CoV, SARS-CoV-2, and VSV-G pseudoviruses were determined by a single-cycle infection assay. The experiments were performed in triplicate and repeated three times, and data are shown as means with SDs. (E and F) Comparison of neutralizing activities of the mouse anti–SZ16-RBD and anti–GD03-RBD sera. Statistical significance was tested by two-way ANOVA with Dunnett posttest. ns, not significant. *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001.
but cross-neutralizing responses could not be detected (28). In this study, we first investigated the cross-reactivity and neutralization with a panel of precious immune sera collected from 20 recovered SARS patients. As shown, all the patient sera displayed high titers of antibodies against the S1 and RBD proteins of SARS-CoV and cross-reacted strongly with the S protein of SARS-CoV-2. In comparison, the patient sera had higher reactivity with the S2 subunit of SARS-CoV-2 relative to its S1 subunit and RBD protein, consistent with a higher sequence conservation between the S2 subunits of SARS-CoV-2 and SARS-CoV than that of their S1 subunits and RBDs (3, 5). Each of the patient sera could cross-neutralize SARS-CoV-2 with serum titers ranging from 1:20 to 1:360 dilutions, verifying the cross-reactive neutralizing activity of the patient sera on the S protein of SARS-CoV-2.

Now, two strategies are being explored for developing vaccines against emerging CoVs. The first one is based on a full-length S protein or its ectodomain, while the second uses a minimal but functional RBD protein as vaccine immunogen. Our previous studies revealed that the RBD site contains multiple groups of conformation-dependent neutralizing epitopes: Some epitopes are critically involved in RBD binding to the cell receptor ACE2, whereas other epitopes have a neutralizing function but do not interfere with the RBD-ACE2 interaction (15, 18). Most neutralizing monoclonal antibodies (mAbs) previously developed against SARS-CoV target the RBD epitopes, while a few are directed against the S2 subunit or the S1/S2 cleavage site (29, 30). The cross-reactivity of such mAbs with SARS-CoV-2 has been characterized, and it was found that many

Fig. 5. Cross-reactive and neutralizing activities of antisera from rabbits immunized with the RBD proteins of SARS-CoV. (A) Binding activity of rabbit antisera at a 1:100 dilution to SARS-CoV (S1 and RBD) and SARS-CoV-2 (S protein and RBD) antigens was determined by ELISA. A healthy rabbit serum was tested as control. (B and C) Neutralizing activities of rabbit antisera or control serum at indicated dilutions on SARS-CoV, SARS-CoV-2, and VSV-G pseudoviruses were determined by a single-cycle infection assay. The experiments were done in triplicate and repeated three times, and data are shown as means with SDs. (D and E) Comparison of neutralizing activities of the rabbit anti–SZ16-RBD and anti–GD03-RBD sera. Statistical significance was tested by two-way ANOVA with Dunnett posttest. *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001.
SARS-CoV–neutralizing mAbs exhibit no cross-neutralizing capacity (8, 31). For example, CR3022, a nAb isolated from a convalescent patient with SARS, cross-reacted with the RBD of SARS-CoV-2 but did not neutralize the virus (31, 32). Nonetheless, a new human anti-RBD mAb, 47D11, has just been isolated from transgenic mice immunized with a SARS-CoV S protein, which neutralizes both SARS-CoV-2 and SARS-CoV (33). The results of polyclonal antisera from immunized animals are quite inconsistent. For example, Walls et al. (5) reported that plasma from four mice immunized with a SARS-CoV S protein could completely inhibit SARS-CoV pseudovirus and reduced SARS-CoV-2 pseudovirus to ~10% of control, thus proposing that immunity against one virus of the sarbecovirus subgenus can potentially provide protection against related viruses; two rabbit antisera raised against the S1 subunit of SARS-CoV also reduced SARS-CoV-2 S–driven cell entry although with lower efficiency compared to SARS-CoV S (26). Moreover, four mouse antisera against the SARS-CoV RBD cross-reacted efficiently with the SARS-CoV-2 RBD and neutralized SARS-CoV-2, suggesting the potential to develop a SARS-CoV RBD–based vaccine preventing SARS-CoV-2 (34). Differently, it was reported that plasma from mice infected or immunized by SARS-CoV failed to neutralize SARS-CoV-2 infection in Vero E6 cells (28), and mouse antisera raised against the SARS-CoV
RBD were even unable to bind to the S protein of SARS-CoV-2 (8). In the present studies, several panels of antisera against the SARS-CoV S and RBD proteins were comprehensively characterized. Although the use of pseudovirus-based neutralization assay might not fully reflect the complexity of authentic SARS-CoV-2 infection, our results, altogether, did provide reliable data to validate the cross-reactivity and cross-neutralization between SARS-CoV and SARS-CoV-2. Meaningfully, this work found that the RBD proteins derived from different SARS-CoV strains can elicit antibodies with unique functionalities: While the RBD from a palm civet SARS-CoV (SZ16) induced potent antibodies capable of blocking the RBD-receptor binding, the antibodies elicited by the RBD derived from a human strain (GD03) had no such effect despite their neutralizing activities. SZ16-RBD shares an overall 74% amino acid sequence identity with the RBD of SARS-CoV-2, when their internal RBMs display more marked substitutions (~50% sequence identity); however, SZ16-RBD and GD03-RBD only differ from three amino acids, all located within the RBM (fig. S3). Further research is needed to determine how these mutations change the antigenicity and immunogenicity of the S protein and RBD immunogens.

Three more questions invite further investigation. First, it would be intriguing to know whether individuals who recovered from previous SARS-CoV infection can direct their acquired SARS-CoV immunity against SARS-CoV-2 infection. To address this question, an epidemiological investigation of populations exposed to SARS-CoV-2 would provide valuable insights. Second, it would be important to determine whether a universal vaccine can be rationally designed by engineering the S protein RBD sequences. Third, although antibody-dependent infection enhancement was not observed during our studies with the human and animal serum antibodies, the possibility of such effects should be carefully addressed in vaccine development.

**MATERIALS AND METHODS**

**Recombinant S proteins**

Two RBD-Fc fusion proteins, which contain the RBD sequence of Himalayan palm civet SARS-CoV strain SZ16 (accession number: AY304488.1) or the RBD sequence of human SARS-CoV strain GD03T0013 (AY525636.1, denoted GD03) linked to the Fc domain of human immunoglobulin G1 (IgG1), were expressed in transfected 293T cells and purified with protein A–Sepharose 4 Fast Flow in our laboratory as previously described (15). A full-length S protein of SARS-CoV Urbani (AY278741) was expressed in expressSF+ insect cells with recombinant baculovirus D3252 by the Protein Sciences Corporation (Bridgeport, CT, USA) (16). A panel of recombinant proteins with a C-terminal polyhistidine (His) tag, including S1 and RBD of SARS-CoV (AAX16192.1) and S ectodomain (S-ecto), S1, RBD, and S2 of SARS-CoV-2 (YP_009724390.1), were purchased from the Sino Biological Company (Beijing, China) and characterized for quality control by SDS–polyacrylamide gel electrophoresis (fig. S4).

**Serum samples from recovered SARS patients**

Twenty patients with SARS were enrolled in March 2003 for a follow-up study at the Peking Union Medical College Hospital, Beijing. Serum samples were collected from recovered patients at 3 to 6 months after discharge, with the patients’ written consent and the approval of the ethics review committee (23, 24). The samples were stored in aliquots at −80°C and were heat-inactivated at 56°C before performing experiments.

**Animal immunizations**

Multiple immunization protocols were conducted in compliance with the Institutional Animal Care and Use Committee guidelines and...
are summarized in fig. S1B. First, five Balb/c mice (6 weeks old) were subcutaneously immunized with 20 μg of full-length S protein resuspended in phosphate-buffered saline (PBS; pH 7.2) in the presence of MPL-TDM adjuvant or alum adjuvant (Sigma-Aldrich). Second, eight Balb/c mice (6 weeks old) were subcutaneously immunized with 20 μg of SZ16-RBD or GD03-RBD fusion proteins and MPL-TDM adjuvant. The mice were boosted two times with 10 μg of the same antigens and the MPL-TDM adjuvants at 3-week intervals. Third, four New Zealand White rabbits (12 weeks old) were immunized intradermally with 150 μg of SZ16-RBD or GD03-RBD resuspended in PBS (pH 7.2) in the presence of Freund’s complete adjuvant and boosted two times with 150 μg of the same antigens and incomplete Freund’s adjuvant at 3-week intervals. Mouse and rabbit antisera were collected and stored at −40°C.

Enzyme-linked immunosorbent assay

Binding activity of serum antibodies with diverse S protein antigens was detected by ELISA. In brief, 50 or 100 ng of a purified recombinant protein (SARS-CoV S1 or RBD and SARS-CoV-2 S-ecto, S1, RBD, or S2) was coated into a 96-well ELISA plate overnight at 4°C. Wells were blocked with 5% bovine serum albumin in PBS for 1 hour at 37°C, followed by incubation with diluted antisera or purified rabbit antibodies for 1 hour at 37°C. A diluted horseradish peroxidase–conjugated goat anti-human, mouse, or rabbit IgG antibody was added for 1 hour at room temperature. Wells were washed five times between each step with 0.1% Tween 20 in PBS. Wells were developed using 3,3,5,5-tetramethylbenzidine and read at 450 nm after termination with 2 M H2SO4.

Neutralization assay

Neutralizing activity of serum antibodies was measured by pseudovirus-based single-cycle infection assay as previously described (35). The pseudovirus particles were prepared by cotransfecting 293T cells with a backbone plasmid (pNL4-3.luc.RE) that encodes an Env-defective, luciferase reporter-expressing HIV-1 genome and a plasmid expressing the S protein of SARS-CoV-2 (IPBCAMS-WH-01; accession number: QHU36824.1) or SARS-CoV (GD03T0013) or the VSV-G. Cell culture supernatants containing virions were harvested 48 hours after transfection, filtrated, and stored at −80°C. To measure the neutralizing activity of serum antibodies, a pseudovirus was mixed with an equal volume of serially diluted sera or purified antibodies and incubated at 37°C for 30 min. The mixture was then added to 293T/ACE2 cells at a density of 10⁴ cells/100 μl per plate well. After culture at 37°C for 48 hours, the cells were harvested and lysed in reporter lysis buffer, and luciferase activity (relative luminescence unit) was measured using luciferase assay reagents and a luminescence counter (Promega, Madison, WI). Percent inhibition of serum antibodies compared to the level of the virus control subtracted from that of the cell control was calculated. The highest dilution of the serum sample that reduced infection by 50% or more was considered to be positive.

Flow cytometry assay

Blocking activity of purified rabbit anti-RBD antibodies on the binding of RBD proteins with a His tag to 293T/ACE2 cells was detected by flow cytometry assay. Briefly, SARS-CoV-2 RBD protein (2 μg/ml) or SARS-CoV RBD protein (10 μg/ml) was added to 4 × 10⁵ cells and incubated for 30 min at room temperature. After washing two times with PBS, cells were incubated with a 1:500 dilution of Alexa Fluor 488–labeled rabbit anti–His tag antibody (Cell Signaling Technology, Danvers, MA) for 30 min at room temperature. After two washes, cells were resuspended in PBS and analyzed by FACSCantoII instrument (Becton Dickinson, Mountain View, CA).

Statistical analysis

Statistical analyses were carried out using GraphPad Prism 7 Software. One-way or two-way analysis of variance (ANOVA) was used to test for statistical significance. Only P values of 0.05 or lower were considered statistically significant [P > 0.05 (ns, not significant), *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001].

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/sciadv.abc9999/DC1

View/request a protocol for this paper from Bio-protocol.

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