Original Article

Development of a rapid neutralizing antibody test for SARS-CoV-2 and its application for neutralizing antibody screening and vaccinated serum testing

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ABSTRACT

Background: Since the outbreak of coronavirus disease (COVID-19), the high infection rate and mutation frequency of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent, have contributed to the ongoing global pandemic. Vaccination has become the most effective means of controlling COVID-19. Traditional neutralizing tests of sera are complex and labor-intensive, therefore, a rapid test for detecting neutralizing antibodies and antibody status post-immunization is needed.
Methods: Based on the fact that antibodies exhibit neutralizing activity by blocking the binding of the S protein receptor-binding domain (S-RBD) to ACE2, we developed a rapid neutralizing antibody test, ACE2-Block-ELISA. To evaluate the sensitivity and specificity, we used 54 positive and 84 negative serum samples. We also tested the neutralizing activities of monoclonal antibodies (mAbs) and 214 sera samples from healthy individuals immunized with the inactivated SARS-CoV-2 vaccine.
Results: The sensitivity and specificity of the ACE2-Block ELISA were 96.3% and 100%, respectively. For neutralizing mAb screening, ch-2C5 was selected for its ability to block the ACE2–S-RBD interaction. A plaque assay confirmed that ch-2C5 neutralized SARS-CoV-2, with NT_{50} values of 4.19, 10.63, and 1.074 μg/mL against the SARS-CoV-2 original strain, and the Beta and Delta variants, respectively. For the immunized sera samples, the neutralizing positive rate dropped from 82.14% to 32.16% within 4 months post-vaccination.
Conclusions: This study developed and validated an ACE2-Block-ELISA to test the neutralizing activities of antibodies. As a rapid, inexpensive and easy-to-perform method, this ACE2-Block-ELISA has potential applications in rapid neutralizing mAb screening and SARS-CoV-2 vaccine evaluation.

1. Introduction

Coronavirus disease (COVID-19) first appeared at the end of 2019, then spread rapidly worldwide. Common symptoms include fever, fatigue, a dry cough and breathing difficulties. Some patients develop life-threatening complications such as severe pneumonia, respiratory failure, acute respiratory distress syndrome or multiple organ failure [1].

The causative agent of COVID-19 is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel strain of human *β*-coronavirus that belongs to the *Coronaviridae* family. The genome comprises a plus-sense, single-stranded RNA of 29 kb that encodes four major structural proteins [spike (S), nucleocapsid (N), mem-

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brane (M) and envelope (E)] and several non-structural proteins [2]. The S protein, which mediates entry of SARS-CoV-2 into host cells, comprises two subunits (S1 and S2) [3]. The S1 subunit contains the N-terminal domain and the receptor-binding domain (RBD); the latter binds to human angiotensin converting enzyme 2 (ACE2) through the receptor-binding motif [4]. S2 promotes fusion between the virus and the host cell membrane. The S protein therefore plays an essential role in virus attachment, receptor binding, membrane fusion, tissue tropism and host range, and it induces the production of neutralizing antibodies, as well as T cell responses [5,6].

In some cases of mild and severe COVID-19 infection, treatment with convalescent plasma led to a marked clinical improvement [7]. Some studies also indicated that antibodies targeting the RBD of SARS-CoV-2 or Middle East respiratory syndrome coronavirus (MERS-CoV) exert effective neutralizing activity by blocking binding of the S protein to its corresponding receptor [8,9], suggesting that passive administration of neutralizing monoclonal antibodies (mAbs) could play a major role in controlling SARS-CoV-2. Many therapeutic mAbs are under development; therefore, the ability to screen and analyze the neutralizing activity of a large number of samples is crucial.

As an increasing number of people are vaccinated worldwide, the prevalence of antibodies specific for SARS-CoV-2 is increasing; however, data are lacking on the systemic surveillance of neutralizing antibody titers in the sera of inoculated individuals. The virus-based plaque reduction neutralization test (PRNT) is the conventional and most reliable test for neutralizing activity. However, it entails culturing infectious virus in a biosafety level 3 laboratory, and the assay takes 72–96 h to complete; therefore, it is unsuitable for most research and clinical laboratories. A pseudovirus-based neutralization test (pVNT) has been developed as a more convenient method of detecting neutralizing activity. Nie et al. [10] constructed a SARS-CoV-2 pseudovirus expressing the S protein on its surface, which simplified detection of SARS-CoV-2 neutralizing activity. The pVNT assay can be completed within 24 h and reduces the researcher's risk of infection. However, as for the PRNT, the pVNT is expensive and requires specialized equipment and operators. Therefore, neither is suitable for rapid testing of large numbers of samples.

Thus, there is a demand for a simple and rapid assay for detecting serum neutralizing antibody activity and/or screening of neutralizing mAbs. Therapeutic mAbs and drugs currently under development inhibit virus infection by blocking binding of the S1-RBD to ACE2 [11,19]. Based on this mechanism, we established a simple method of detecting neutralizing mAbs, ACE2-Block-ELISA, which measures the blocking activity of antibodies specific for S1-RBD protein. This assay was used to screen mAbs and serum samples for neutralizing activity against SARS-CoV-2.

2. Materials and methods

2.1 Cells and virus strains

The SARS-CoV-2 BetaCoV/Beijing/IME-BJ05/2020 strain, and variant strains Beta CSTR.16698.06.NPRC 2.062100001 and Delta CSTR.16698.06.NPRC 6.CCPM-B-V-049–2105–6, were preserved at the Institute of Microbiology and Epidemiology (Beijing, China). The pseudoviruses (SARS-CoV-2, Beta, and Delta strains) were purchased from Beijing Tiantan Biological Products Co., Ltd.

2.2 Clinical serum samples

Serum samples were obtained from healthy individuals ($n = 214$) who were vaccinated with the inactivated vaccine in Beijing, China. In addition, 82 serum samples collected from patients with common fever symptoms at the Air Force Medical Center (Beijing, China) before December 2019 were used for the specificity assay, and 54 serum samples collected from patients with COVID-19 from the CDC in Hubei province were used for the sensitivity assay. All samples were diluted in 10 times the volume of phosphate-buffered saline (PBS) and inactivated at 56°C for 30 min before testing.

Written informed consent was obtained before sample collection. This study was approved by the ethics committee of the Academy of Military Medical Science (Number: AF/SC-08/02.91).

2.3 ACE2-Block-ELISA

The S1-RBD proteins (Sino Biological Inc., Beijing, China) derived from the original SARS-CoV-2 strain and expressed in the insect eukaryotic system were coated onto ELISA plates overnight at 4°C. After blocking with PBS containing 0.05% Tween-20 (PBS-T) and 3% bovine serum albumin (BSA), 50 μL of serially diluted mAb or serum were mixed with 50 μL of biotin-labeled ACE2 and then applied to the wells. Equivalent concentrations of biotin-ACE2 protein without mAb or serum were applied to the wells as controls, and wells containing dilution buffer (PBS-T containing 1% BSA) only were used as blanks. Horse radish peroxidise (HRP)-conjugated streptavidin (1:5000; Bioss,Suzhou, China) was added to each well for 1 h. Then, for color development, 100 μL of 3,−3′,5,−5′-tetramethylbenzidine (TIANGEN, China) was added to each well in the dark at room temperature (RT) for 15 min, and the reaction was stopped by adding stop solution (50 μL of 2 M H_2SO_4). The OD_{450} was measured using a spectrophotometer (BioTek SLEXFA, USA) [12].

The cutoff was set as the average OD_{450} value for the control plus three times the standard deviation. Samples with an average OD_{450} lower than the cutoff value were

considered positive, and the rate of inhibition was calculated as follows: inhibition rate = (OD_{450} of control – OD_{450} of sample) / OD_{450} of control × 100%.

2.4 Biolayer interferometry

The affinity assay was conducted at RT using a Gator^{TM} Label-Free Bioanalysis system (Gator Bio). For all measurements, proteins were exchanged into Q Buffer (PBS at pH 7.4 with 0.02% Tween 20, 0.2% BSA and 0.05% NaN_3) in advance. The blank channel of the chip served as the negative control. To measure the affinity of ch-2C5 for the S1-RBD protein, the S1-RBD protein was diluted to 192 nM and pre-coated onto an anti-probe chip. Next, ch-2C5 antibody was serially diluted two-fold (from 125 nM to 1.95 nM) and loaded onto the sensor chip. After each cycle, the sensor was regenerated with Gly-HCl (pH 1.5). Affinity was calculated by the Gator evaluation software using a 1:1 (Rmax local fit) binding model [13].

2.5 PRNT

The PRNT was performed to measure the neutralizing activity of mAbs ch-2C5 and MW05, as described previously [14]. Briefly, the mAbs were serially diluted three-fold (100–0.14 µg/mL), mixed with an equal volume of virus culture (10^3 plaque-forming units (pfu)/mL), then incubated at 37°C for 1 h. Then, the mixture (200 µL per well) was added to Vero cells in a 24-well culture plate (duplicate wells per sample). A mixture comprising 100 µL of virus and 100 µL of Dulbecco's modified Eagle's medium (DMEM; Gibco) was applied to the virus-only control wells. After incubation at 37 °C for 1 h in an incubator containing 5% CO_2, the culture media in each well were discarded and 1 mL of agarose mixture (DMEM containing 2% fetal bovine serum (v/v) and 50% low melting agarose solution; Promega) was added to each well. Cytopathic effects were observed daily under a light microscope (Olympus). After 72 h, the cells were fixed by adding 4% (v/v) poly formaldehyde (1 mL per well) at RT for 30 min. Then, the cultured cells were washed with PBS and bathed in 1% crystal violet solution. After washing with water, plaques were observed and counted. The inhibition rate for each mAb concentration was calculated as follows: inhibition rate = (number of plaques in virus-only wells −number of plaques in the mAb and virus mixture wells) / number of plaques in virus-only wells × 100%. A Probit regression curve was drawn and the concentration resulting in 50% inhibition of binding (NT_{50}) was calculated using GraphPad Prism software [15].

2.6 Pseudovirus-based neutralization test

The SARS-CoV-2 pVNT was performed as previously described [10]. Briefly, mAb MW05 or ch2C5 was serially diluted three-fold (100–0.14 µg/mL) and incubated with an equal volume of SARS-CoV-2 pseudovirus (1.3×10^4 $TCID_{50}$/mL) for 1 h at 37°C in a humidified 5% CO_2 incubator. Next, 100 µL of Huh-7 cells (2×10^5 cells/mL) were added and cultured for 24 h, then luciferase substrate (PerkinElmer) was added for 2 min. The mixture was transferred to a white plate and the relative light units (RLU) were read using a luminometer (Promega). In the assay, wells containing only cells with SARS-CoV-2 pseudovirus were set as the positive control, wells containing only cells were set as the blank. The inhibition rate was calculated as follows: Inhibition (%) = [1 − (sample RLU − blank RLU) / (positive control RLU − blank RLU)] × 100.

2.7 Statistical analysis

Statistical analysis was performed using GraphPad Prism 7.0 software. Neutralization rates were plotted against concentration, and the points were fitted using a linear interpolation model. The NT_{50} was defined as the reciprocal of the concentration resulting in 50% neutralization activity.

To determine the sensitivity and specificity of the ACE2-Block-ELISA, one-way analysis of variance was performed to compare the results for SARS-CoV-2-uninfected cases and SARS-CoV-2-infected cases. The level of significance was set at $p < 0.05$.

3. Results

3.1 Development of the ACE2-Block-ELISA for the rapid detection of neutralizing activity

MW05 is a human monoclonal neutralizing antibody specific for the RBD of SARS-CoV-2. This antibody inhibits the interaction between the virus and target cells by binding specifically to a neutralizing epitope [16]. In this study, MW05 was used as a positive control and 20 serum samples negative for SARS-CoV-2, identified by PRNT, were used as negative controls to optimize and validate the ACE2-Block-ELISA.

The results from ACE2-Block-ELISA are affected by several factors, including the concentration of the coating antigen (i.e., S-RBD protein) and the biotin-labeled ACE2 protein. Therefore, to optimize the ACE2-Block-ELISA, we used the PRNT assay results as a standard (Fig. 1A), and compared the inhibition rates of mAb MW05 and negative serum samples in the presence of different concentrations of S1-RBD protein (two-fold dilutions from 1.6 to 0.2 µg/mL) and biotin-labeled ACE2 (0.5, 1 and 2 µg/mL). Tests revealed that the optimal concentration of S1-RBD protein was 0.8 µg/mL and that of ACE2 was 1 µg/mL (Fig. 1B, C). The results using these concentrations were similar to those obtained in the PRNT. In the ACE2-
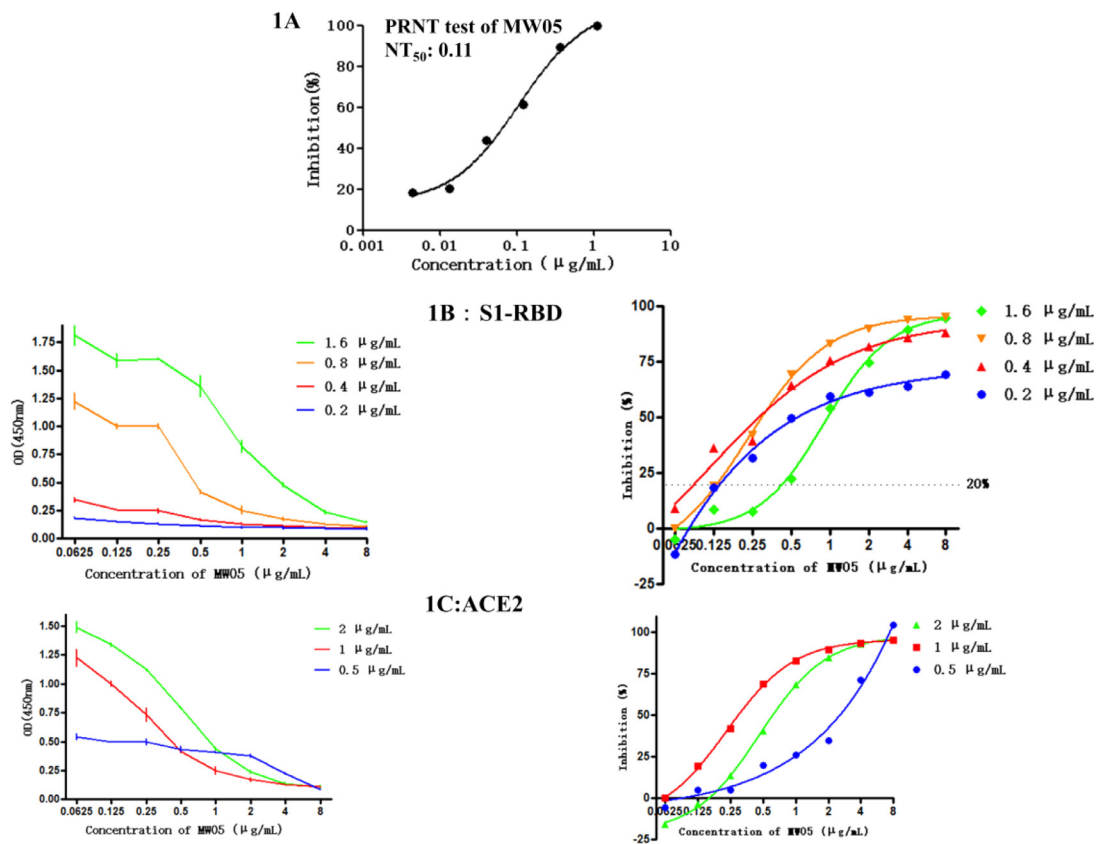
Fig. 1. Optimization of the ACE2-Block-ELISA. (**A**) Evaluation of the neutralizing activity of the MW05 antibody by PRNT. MW05 was serially diluted three-fold (from 10 to 0.004 μg/mL) and mixed with an equal volume of SARS-CoV-2 (10^3 pfu/mL). Plaques were counted under a microscope. The inhibition rate at different concentrations of mAb MW05 was calculated as follows: inhibition rate = (number of plaques in the virus-only wells – number of plaques in the mAb and virus wells) / number of plaques in the virus-only wells × 100%. A Probit regression curve was drawn and the 50% inhibitory concentration (NT_{50}) was calculated using GraphPad Prism software. The NT_{50} of MW05 for SARS-CoV-2 was 0.11 μg/mL. (**B**) Evaluation of the inhibition rate of MW05 antibody in the presence of different concentrations of coating antigen in the ACE2-Block-ELISA. The S1-RBD protein was coated onto ELISA plates at concentrations of 0.2–1.6 μg/mL. Biotin-labeled ACE2 (1 μg/mL) was added and the OD_{450} value was measured. The inhibition rate was calculated as follows: inhibition rate = (OD_{450} of control – OD_{450} of sample) / OD_{450} of control × 100%. (**C**) Inhibition rate of the MW05 antibody in the presence of different concentrations of biotin-labeled ACE2. Plates were coated with S1-RBD protein (0.8 μg/mL), followed by the addition of biotin-labeled ACE2 at concentrations of 0.5, 1 and 2 μg/mL. The OD_{450} was measured and the inhibition rate was calculated as described in B.

Block-ELISA, all of the negative samples showed an inhibition rate lower than 15%. MW05 inhibited binding of S1-RBD by 20% at 0.125 μg/mL, equivalent to an NT_{50} of 0.11 μg/mL in the PRNT. The rate of inhibition increased in a dose-dependent manner, reaching 90% at 1 μg/mL, a concentration equivalent to the NT_{90} in PRNT. After comparing the inhibition rate of MW05 in the ACE2-Block-ELISA with that in PRNT, we decided on a cutoff value for a positive antibody test in the ACE2-Block-ELISA of 20% inhibition, meaning that an antibody could be regarded as having neutralizing activity if it showed an inhibition rate >20% in the ACE2-Block-ELISA.

3.2 Sensitivity and specificity of the ACE2-Block-ELISA

To examine the specificity of the ACE2-Block-ELISA, we tested 84 SARS-CoV-2-negative sera (collected before December 2019 that were negative for SARS-CoV-2 IgM/IgG in an ELISA assay). The lack of a positive result suggested 100% specificity, the rate of inhibition varied from –9.31% to 18.32%, with no false positives (Fig. 2).
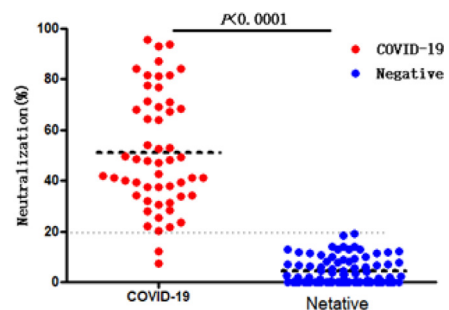

Fig. 2. Sensitivity and specificity of the ACE2-Block-ELISA. SARS-CoV-2 negative sera ($n = 84$; collected before December 2019) were applied to the ACE2-Block-ELISA to assess specificity. In addition, 54 serum samples from COVID-19 patients (positive by PRNT) were used to test sensitivity. The inhibition rate was calculated as follows: Inhibition rate = (OD_{450} of control – OD_{450} of sample) / OD_{450} of control × 100%. Samples were regarded as having neutralizing activity if they exhibited >20% inhibition in the ACE2-Block-ELISA.

Next, 54 serum samples from COVID-19 patients were tested using the ACE2-Block-ELISA, all of which were positive in the PRNT assay with titers >50. Of the 54 samples, 52 tested positive, with inhibition rates varying from

20.48% to 95.50% (Fig. 2), indicating that the sensitivity of the AC2-Block ELISA was 96.3%.

3.3 Use of the ACE2-Block-ELISA to select a neutralizing antibody, ch-2C5

We screened a panel of mAbs with SARS-CoV neutralizing activities. A humanized mAb, ch-2C5, diluted from 0.125 to 8 μg/mL exhibited an inhibition rate for the original S1-RBD of 20%–90% (Fig. 3A, B).

To determine the inhibitory effect of the ch-2C5 antibody on SARS-CoV-2 variant strains, we used the recombinant S1-RBD protein from the Beta and Delta variants as detection antigens in the ACE-2-block-ELISA (Fig. 3A, B). The results demonstrated that ch-2C5 blocked the S1-RBD protein of both variants from binding to the ACE2 receptor, suggesting a broad spectrum of antiviral neutralizing activity.

Next, we performed biolayer interferometry to measure the affinity of ch-2C5 for the S1-RBD protein of SARS-CoV-2. The results demonstrated that ch-2C5 has high affinity for the S1-RBD protein of all SARS-CoV-2 variants tested: $K_D = 9.90 \times 10^{-11}$ M for the SARS-CoV-2 original strain; 1.87×10^{-10} M for the Beta variant; and 8.39×10^{-11} M for the Delta variant (Fig. 3C, E).

To verify the neutralizing activity of ch-2C5 against SARS-CoV-2, we performed both pVNT and PRNT assays(Fig. 3F, G, respectively). The PRNT results showed that ch-2C5 exhibited marked neutralizing activity against all 3 SARS-CoV-2 variant strains tested. The antibody inhibited infection of Vero cells by the SARS-CoV-2 BetaCoV/Beijing/IME-BJ05/2020 strain from 0.1 μg/mL, and the NT_{50} values for ch-2C5 against the SARS-CoV-2 original strain, the Beta variant, and the Delta variant were 4.19, 10.63 and 1.072 μg/mL, respectively (Fig. 3G).

In the pVNT assay, ch-2C5 neutralized all three SARS-CoV-2 variants, with NT_{50} values of 30.39, 29.62, and 24.39 μg/mL, respectively (Fig. 3F).

3.4 Use of the ACE2-Block-ELISA to measure neutralizing antibody levels in serum from healthy individuals inoculated with a SARS-CoV-2 vaccine

For this experiment, we used 214 serum samples obtained from healthy individuals. Samples were taken within 1–4 months of vaccination. The results by ACE2-Block-ELISA demonstrated that among the 214 serum samples tested, 134 showed neutralizing activity, with an inhibition rate from 20% to 90%, and approximately 62.62% of the vaccinated individuals had neutralizing antibodies against SARS-CoV-2 (Fig. 4). Then, we correlated the time of sampling with the level of neutralizing antibodies, as shown in the Table 1, and the neutralizing positive rate dropped from 82.13% to 32.26% within 4 months post-vaccination, which confirmed the weakening of humoral immunity over time after vaccination.

Table 1
Analysis of serum samples by ACE2-Block-ELISA.

MPV	Total	Positive(%)
1	56	46 (82.14%)
2	51	38 (74.51%)
3	76	40 (52.6%)
4	31	10 (32.26%)
total	214	134 (62.62%)

MPV, months post-vaccination.

4. Discussion

The COVID-19 pandemic caused by SARS-CoV-2 has seriously affected human populations worldwide [17]. Prevention and control of SARS-CoV-2 mainly follows the principle of 'early identification, early detection, early reporting, early treatment'; timely detection and diagnosis are now the most essential steps. Detection of viral nucleic acid is the 'gold standard' for monitoring SARS-CoV-2 infections [18,27]. Detection of IgM antibodies is also a widely used and effective approach to diagnosing infection because such assays are quick and easy to perform; detection of IgG antibodies is used mainly for epidemiological investigations. As more of the population are being inoculated with SARS-CoV-2 vaccines, many individuals possess some level of SARS-CoV-2-specific antibodies. Among the polyclonal antibodies in serum, neutralizing antibodies exert the main protective function. However, the neutralizing activity of antibodies, expressed in terms of the neutralizing antibody titer, cannot be measured directly by detecting the binding activity of IgG/IgM antibodies. Traditional methods of detecting neutralizing activity are based on live virus and pseudovirus neutralization trials, which are complicated, expensive, and pose a potential risk to researchers [19]. Hence, it is necessary to find a safe and reliable neutralizing antibody detection method for the prevention and control of SARS-CoV-2 infection.

Tan et al. established a surrogate SARS-CoV-2 (sVNT) neutralization test based on antibody-mediated blockade of the ACE2–S protein interaction [24]. Benjamin et al. and Ranawaka et al. validated this sVNT using an extensive panel of sera. Ranawaka et al. proved that this sVNT kit had a sensitivity of 98.9% and a specificity of 98.8% using $PRNT_{90}$ as the reference; and Benjamin et al. showed that samples with a titer ≥160 were always positive in the sVNT [25,26]. Thus, the sVNT can be used as an additional neutralization assay to determine the immune status of COVID-19-infected vaccinated individuals.

Herein, we developed an ACE2-Block-ELISA for the rapid detection of neutralizing antibodies, which is similar to the sVNT mentioned above. The main difference is that in the sVNT, HRP-labeled S1-RBD is used in the competition step, and a substrate is added to generate color in the plates to obtain the result. In our ACE2-Block-ELISA,
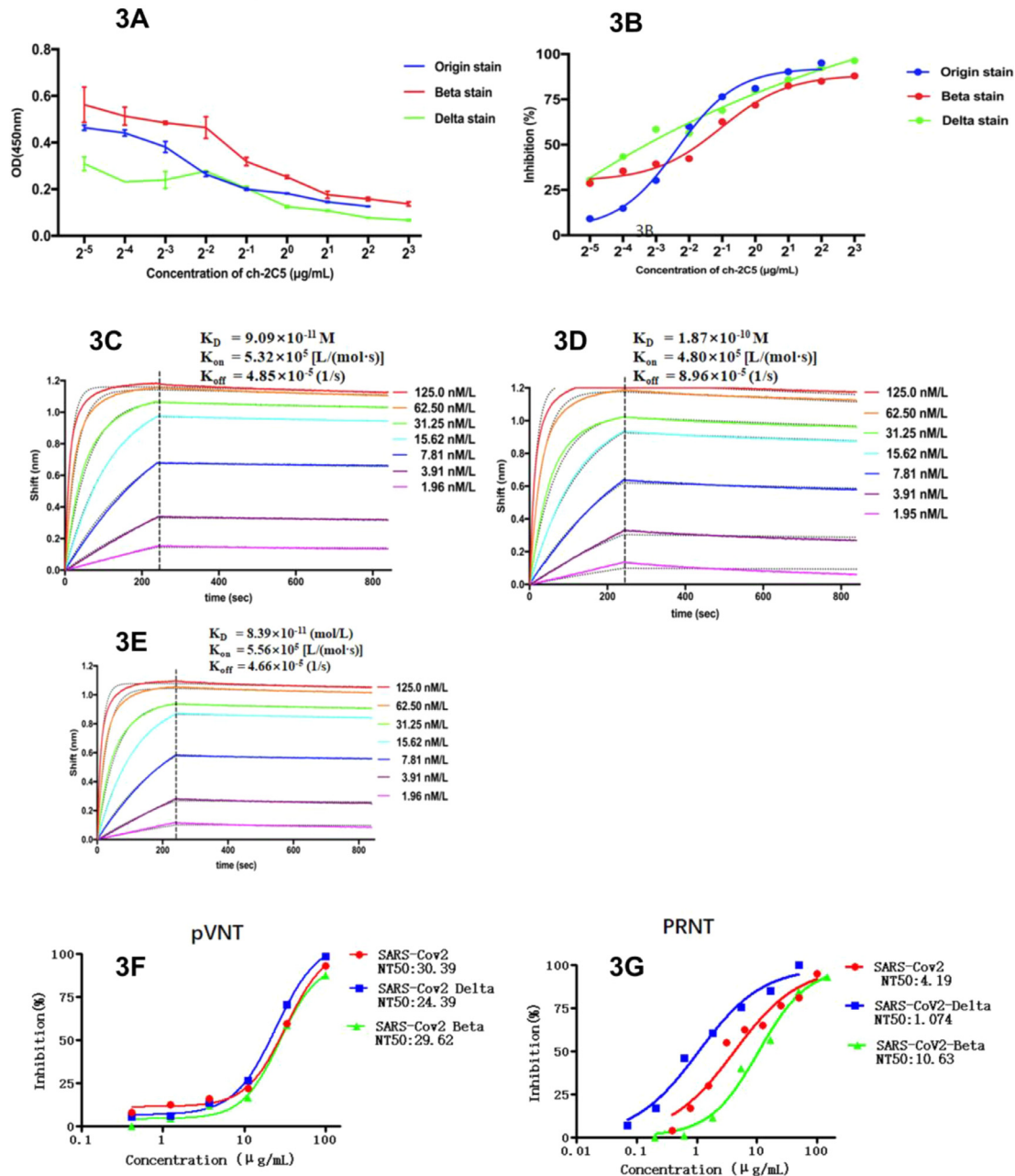
Fig. 3. Testing of the neutralizing monoclonal antibody ch-2C5. (**A, B**) Inhibitory effect of ch-2C5 on the binding of S1-RBD to the ACE2 receptor. The S1-RBD from the SARS-CoV-2 original and variant strains was used as the coating antigen for the ACE2-Block-ELISA. Different concentrations (8 to 0.3125 µg/mL, two-fold diluted) of ch-2C5 were tested and the OD_{450} value was measured. The inhibition rate was calculated as follows: inhibition rate = (OD_{450} of control − OD_{450} of sample) / OD_{450} of control × 100%. (**C–E**) Affinity of ch-2C5 for the S1-RBDs from the SARS-CoV-2 original, Beta variant and Delta variant strains. Affinity was measured on a Gator™ Label-Free Bioanalysis system. The S1-RBD was diluted and pre-coated onto an anti-probe chip, followed by the application of ch-2C5 at 125 nM – 1.95 nM (two-fold dilutions). Affinity was calculated by the Gator evaluation software using a 1:1 (Rmax local fit) binding model. The dissociation constant was calculated as follows: KD = K_{on} / K_{off}. The KD values for the SARS-CoV-2 original, Beta variant and Delta variant strain S1-RBDs were 9.90×10^{-11} M, 1.87×10^{-10} M and 8.39×10^{-11} M, respectively. F. Neutralizing activity of ch-2C5 in the pVNT. MAb ch-2C5 was serially diluted three-fold (100 to 0.14 µg/mL) and incubated with an equal volume of SARS-CoV-2 pseudovirus (1.3×10^4 $TCID_{50}$/mL). The number of relative light units (RLU) was read using a luminometer (Promega, USA). The neutralization rate was calculated as follows: inhibition (%) = [1 − (sample RLU − blank RLU) / (positive control RLU − blank RLU)] (%). A Probit regression curve was drawn and the 50% inhibitory concentration (NT_{50}) was calculated using GraphPad Prism software. The NT_{50} values of ch-2C5 for the SARS-CoV-2 original, Beta variant and Delta variant strains were 30.39, 29.62, and 24.39 µg/mL, respectively. G. Neutralizing activity of ch-2C5 in the PRNT. The ch2C5 mAb was serially diluted three-fold (100–0.14 µg/mL) and mixed with an equal volume of the SARS-CoV-2 original, Beta variant or Delta variant strain (10^3 pfu/mL). Plaques were counted. The inhibition rate at different concentrations of ch2C5 was calculated as follows: inhibition rate = (number of plaques in virus groups − number of plaques in the experimental groups) / number of plaques in the virus groups × 100%. A Probit regression curve was drawn and the 50% inhibitory concentration (NT_{50}) was calculated using GraphPad Prism software. The NT_{50} values of ch-2C5 for the SARS-CoV-2 original, Beta variant and Delta variant strains were 4.19, 10.63 and 1.072 µg/mL, respectively.
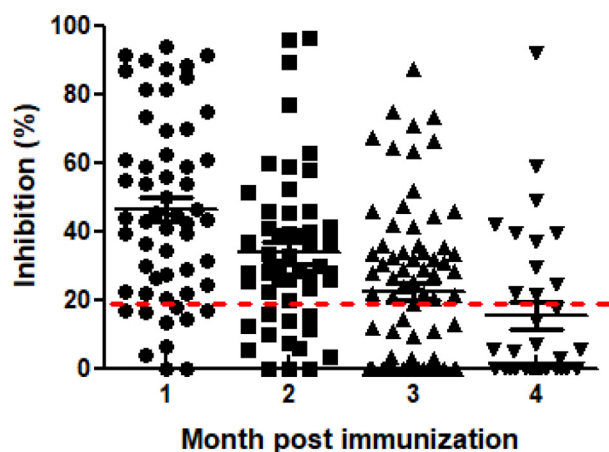
Fig. 4. Application of immune serum to the ACE2-Block-ELISA. Serum samples from 214 vaccinated healthy individuals were collected and applied to the ACE2-Block-ELISA to measure neutralizing activity. Of these, 134 showed marked neutralizing activity, with inhibition rates varying from 20% to 90%.

biotin-labeled ACE2 is used for the competition step, followed by the application of HRP-labeled streptavidin and a substrate; thus, our assay has one more step than the sVNT. This extra step may make the ACE2-Block-ELISA more sensitive than the sVNT since the HRP-labeled streptavidin will enhance the positive signal.

The ACE2-Block-ELISA was used to screen a panel of neutralizing mAbs for SARS-CoV developed in 2003, which identified ch-2C5 as a neutralizing humanized mAb. This antibody inhibited binding of all three SARS-CoV-2 strains to ACE2, not only the original strain of SARS-CoV-2, but also the variants including Beta and Delta strains. The PRNT and pVNT experiments confirmed that ch-2C5 has a broad spectrum of neutralizing activity. Thus, ch-2C5 is of great value for studying neutralization epitopes and for developing vaccines and therapeutic antibodies against SARS-CoV-2.

Immunization is regarded as the most effective method of obtaining herd immunity against SARS-CoV-2 [20,21]. However, antibody titers decline significantly after 6 months [22], and vaccinated individuals can be re-infected, which may be related to the decrease in antibody titers and the low level of neutralizing Abs generated after vaccination. In this case, there is a risk of rebound. Patients who recover from COVID-19 and individuals inoculated with two or three doses of vaccine show a strong immune response, particularly a humoral immune response, which is often associated with a 10–20-fold increase in the antibody titer; such individuals also show more broad spectrum neutralization of multiple variants, such as Delta [23]. These data indicate that timely monitoring of neutralizing antibody titers in the vaccinated population will help to guide immunization strategies.

Therefore, we also used the ACE-Block-ELISA to detect neutralizing antibodies in individuals vaccinated with SARS-CoV-2 vaccines. Of the 214 serum samples tested, the neutralizing positive rate dropped from 82% to 32%

within 4 months post-vaccination, with 62.62% showing inhibition of binding between the S1-RBD protein and the ACE2 receptor. Since the neutralizing antibodies declined greatly after inoculation, booster immunization and some prevention measures may still be necessary for effective control of SARS-CoV-2 infection. However, we cannot directly deduce that immunization has no protective effect as a result of the absence of neutralizing antibodies, because neutralizing antibodies are only part of the mechanism by which vaccination fights infection. When individuals are re-exposed, memory B and T cells can respond and mount an appropriate response even in the presence of low neutralizing antibodies, which can attenuate the course of the disease. In summary, the immune response after COVID-19 vaccination is complex and deserves further study.

In this study, we show that the ACE2-Block-ELISA is faster, cheaper and easier to perform than conventional assays for the rapid qualitative and quantitative detection of neutralizing activity. The ACE-Block-ELISA therefore has potential applications in evaluating the immunity induced by vaccines, identifying candidate therapeutic antibodies and serum testing.

Author contributions

Declaration of competing interest

The authors declare no competing interests.

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References

[1] L. Perico, A. Benigni, F. Casiraghi, et al., Immunity, endothelial injury and complement-induced coagulopathy in COVID-19, Nat. Rev. Nephrol. 17 (1) (2021) 46–64, doi:10.1038/s41581-020-00357-4.

[2] B.J. Bosch, R. van der Zee, C.A. de Haan, et al., The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex, J. Virol. 77 (16) (2003) 8801–8811, doi:10.1128/jvi.77.16.8801-8811.2003.

[3] N.N. Zhang, X.F. Li, Y.Q. Deng, et al., A thermostable mRNA vaccine against COVID-19, Cell 182 (5) (2020) 1271–1283, doi:10.1016/j.cell.2020.07.024.

[4] F.A. Lempp, L. Soriaga, M. Montiel-Ruiz, et al., Lectins enhance SARS-CoV-2 infection and influence neutralizing antibodies, Nature (2021), doi:10.1038/s41586-021-03925-1.

[5] L. Du, Y. He, Y. Zhou, et al., The spike protein of SARS-CoV–a target for vaccine and therapeutic development, Nat. Rev. Microbiol. 7 (3) (2009) 226–236, doi:10.1038/nrmicro2090.

[6] W. Song, M. Gui, X. Wang, et al., Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2, PLoS Pathog. 14 (8) (2018) e1007236, doi:10.1371/journal.ppat.1007236.

[7] L. Li, W. Zhang, Y. Hu, et al., Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: a randomized clinical trial, JAMA 324 (5) (2020) 460–470, doi:10.1001/jama.2020.10044.

[8] Y. Li, Y. Wan, P. Liu, et al., A humanized neutralizing antibody against MERS-CoV targeting the receptor-binding domain of the spike protein, Cell Res. 25 (11) (2015) 1237–1249, doi:10.1038/cr.2015.113.

[9] Q. Wang, G. Wong, G. Lu, et al., MERS-CoV spike protein: targets for vaccines and therapeutics, Antiviral Res. 133 (2016) 165–177, doi:10.1016/j.antiviral.2016.07.015.

[10] J. Nie, Q. Li, J. Wu, et al., Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2, Emerg. Microbes Infect. 9 (1) (2020) 680–686, doi:10.1080/22221751.2020.1743767.

[11] M. Prajapat, N. Shekhar, P. Sarma, et al., Virtual screening and molecular dynamics study of approved drugs as inhibitors of spike protein S1 domain and ACE2 interaction in SARS-CoV-2, J. Mol. Graph. Model 101 (2020) 107716, doi:10.1016/j.jmgm.2020.107716.

[12] X. Kang, Y. Li, L. Fan, et al., Development of an ELISA-array for simultaneous detection of five encephalitis viruses, Virol. J. 9 (2012) 56, doi:10.1186/1743-422X-9-56.

[13] J.F. Li, L. He, Y.Q. Deng, et al., Generation and characterization of a nanobody against SARS-CoV, Virol. Sin. 17 (2021) 1–8, doi:10.1007/s12250-021-00436-1.

[14] E.H.Y. Lau, O.T.Y. Tsang, D.S.C. Hui, et al., Neutralizing antibody titres in SARS-CoV-2 infections, Nat. Commun. 12 (1) (2021) 63, doi:10.1038/s41467-020-20247-4.

[15] E. Lau, O. Tsang, D. Hui, et al., Neutralizing antibody titres in SARS-CoV-2 infections, Nat. Commun. 12 (1) (2021) 63, doi:10.1038/s41467-020-20247-4.

[16] S. Wang, Y. Peng, R. Wang, et al., Characterization of neutralizing antibody with prophylactic and therapeutic efficacy against SARS-CoV-2 in rhesus monkeys, Nat. Commun. 11 (1) (2020) 5752, doi:10.1038/s41467-020-19568-1.

[17] M. Hasöksüz, S. Kiliç, F. Saraç, Coronaviruses and SARS-COV-2, Turk. J. Med. Sci. 50 (SI–1) (2020) 549–556, doi:10.3906/sag-2004-127.

[18] Y. Li, J. Li, Y. Zhang, et al., Development of an automatic integrated gene detection system for novel severe acute respiratory syndrome-related coronavirus (SARS-CoV2), Emerg. Microbes Infect. 9 (1) (2020) 1489–1496, doi:10.1080/22221751.2020.1782774.

[19] Q. Li, Q. Liu, W. Huang, et al., Current status on the development of pseudoviruses for enveloped viruses, Rev. Med. Virol. 28 (1) (2018) e1963, doi:10.1002/rmv.1963.

[20] M.Z. Tay, C.M. Poh, L. Rénia, et al., The trinity of COVID-19: immunity, inflammation and intervention, Nat. Rev. Immunol. 20 (6) (2020) 363–374, doi:10.1038/s41577-020-0311-8.

[21] A. Awadasseid, Y. Wu, Y. Tanaka, et al., Current advances in the development of SARS-CoV-2 vaccines, Int. J. Biol. Sci. 17 (1) (2021) 8–19, doi:10.7150/ijbs.52569.

[22] J. Mateus, J.M. Dan, Z. Zhang, et al., Low-dose mRNA-1273 COVID-19 vaccine generates durable memory enhanced by cross-reactive T cells, Science (2021) eabj9853, doi:10.1126/science.abj9853.

[23] H. Zhang, B. Li, Q. Gao, et al., A third dose of inactivated vaccine augments the potency, breadth, and duration of anamnestic responses against SARS-CoV-2, medRxiv (2021) 21261735, doi:10.1101/2021.09.02.21261735.

[24] C.W. Tan, W.N. Chia, X. Qin, et al., A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction, Nat. Biotechnol. 38 (9) (2020) 1073–1078 doi: 10.1038/s41587-020-0631-z. Epub 2020 Jul 23. PMID: 32704169.

[25] B. Meyer, J. Reimerink, G. Torriani, et al., Validation and clinical evaluation of a SARS-CoV-2 surrogate virus neutralisation test (sVNT), Emerg. Microbes Infect 9 (1) (2020) 2394–2403 doi: 10.1080/22221751.2020.1835448. PMID: 33043818; PMCID: PMC7605318.

[26] A.P.M.Perera Ranawaka, Ko Ronald, T.Y.Tsang Owen, et al., Evaluation of a SARS–CoV-2 surrogate virus neutralization test in human, canine, cat, and hamster sera, J. Clin. Microbiol. 59 (2021) e02504-20.

[27] R. A, H. Wang, W. Wang, et al., Summary of the detection kits for SARS–CoV-2 approved by the National Medical Products Administration of China and their application for diagnosis of COVID-19, Virol. Sin 35 (6) (2020) 699–712 doi: 10.1007/s12250-020-00331-1. Epub 2020 Dec 22. PMID: 33351166; PMCID: PMC7754703.