Systematic profiling of antigen bias in humoral response against SARS-CoV-2

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ARTICLE INFO

ABSTRACT

We know little about the antigen bias in SARS-CoV-2 humoral response and the epitopes of spike recognized by the immune system in asymptomatic (AS) patients and symptomatic (S) patients. Here, we used a microarray to evaluate the humoral immune response in the sera collected from 33 COVID-19-recovered patients up to 1 year. We found that the levels of IgG and IgM induced by the 23 proteins differed significantly in the same patients, and were able to distinguish AS and S patients. The N- and S-specific antibodies were detected even at 12 months after onset. Five epitopes were identified to be associated with the clinical adverse events, and three peptides located in RBD. Overall, this study presents a systemic view of the SARS-CoV-2 specific IgG and IgM responses between AS and S recovered patients and provide insights to promote precise development of SARS-CoV-2 vaccines.

1. Introduction

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a pandemic and rapidly spread around the globe. Up to now, seven coronaviruses can infect humans, and four of them cause the common cold (Cui et al., 2018). Viruses can induce T-cell and B-cell immune responses. SARS-specific humoral immune response correlates with the T-cell immune response (Grifoni et al., 2020 Ni et al., 2020;). At the beginning of SARS-CoV-2 outbreak, most studies focused on cell entry and the binding receptor (Letko et al., 2020). Those studies showed that the interaction of spike of SARS-CoV-2 and human angiotensin-converting enzyme 2 (ACE2) plays the key role in SARS-CoV-2 infection (Ejemel et al., 2020 Yi et al., 2020;).

Recent studies have been focusing on the SARS-CoV-2-induced immune response. Cellular immunity to SARS-CoV-2 is also being evaluated. The number of SARS-CoV-2 specific–CD4+ T cells and CD8+ T cells increase during the first 7–9 days. Pre-existing SARS-CoV-2 cross-reactive T cells have been identified in healthy individuals (Braun et al., 2020 Le Bert et al., 2020;). T follicular helper (T_{fh}) cells are important in the formation of germinal centers and secretion of cytokines that regulate B-cell response (Kunzli et al., 2020). The neutralizing antibody titers against spike protein are positively associated with the frequencies of CXCR3+ T_{fh} cells (Crotty, 2011 Zhang et al., 2020;). Both antibodies and SARS-CoV-2–specific T cells are important for the control of primary infection (Dan et al., 2021).

The spike glycoprotein (S) and the nucleocapsid (N) protein of SARS-

CoV-2 are two main viral glycoproteins. The spike is cleaved into an N-terminal S1 and a C-terminal S2 subunit. In the N-terminal, there is a receptor-binding domain (RBD; residues 331–524). Monoclonal antibodies targeting spike have been purified and identified (Chi et al., 2020 Shi et al., 2020; Zost et al., 2020a;). Antibodies targeting spike have been used in clinical therapy, with the purpose of blocking the interaction of ACE2 and spike protein (Bojkova et al., 2020 Zost et al., 2020a;, 2020b). SARS-CoV-2 infection- and vaccine-elicited spike-specific antibodies are considered important for the control and clearance of SARS-CoV-2 (Poland et al., 2020b Yang et al., 2020;). Several antigens have been used for the evaluation of SARS-CoV-2 humoral immunity (Poland et al., 2020b).

According to the epidemiological characteristics and trends of SARS-CoV-2, a number of asymptomatic patients have been reported (Nikolai et al., 2020 Zhou et al., 2020;). However, little is known about the persistence and differences of antigen-specific antibodies between asymptomatic patients and symptomatic patients. A previously developed proteome microarray (Jiang et al., 2020) was modified and was used for the evaluation and screening of SARS-CoV-2–specific IgG and IgM from 33 convalescent patients, with 14 of them followed up to 12 months in this study. The high mutation rate of SARS-CoV-2 urged us to select conserved peptides in different mutants and develop peptide-based vaccines for the prevention of the disease. A peptide pool of spike, with 220 peptides included has also been designed into the proteome microarray for the identification of linear B-cell epitopes. To reduce the incidence of side effects and increase the level of neutralizing antibodies, we analyzed the antigen bias in humoral immune response and compared the humoral response between asymptomatic and symptomatic recovered patients.

2. Materials and methods

2.1. Human subjects

The cohort study was performed on patients with PCR-confirmed SARS-CoV-2 infection and the basic criteria to define the severity, i.e., mild, moderate, severe and critically severe, are according to the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7) (2020). Asymptomatic infection was determined as a positive result for SARS-CoV-2 RNA by PCR without showing any relevant clinical symptoms. COVID-19 patients were hospitalized and received treatment in Zhoushan Hospital, and were discharged when the standard criteria were met according to the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7). The vital points of the discharge criteria were: (1) normal body temperature; (2) obviously improved respiratory symptoms; (3) obvious absorption of inflammation in the pulmonary imaging; (4) twice consecutively negative for nuclei acid testing. The cohort included 19 asymptomatic and 14 symptomatic individuals with SARS-CoV-2 infection. In total, 30 samples (from 19 asymptomatic and 11 symptomatic) were collected at 1 month after infection, 6 samples (from 6 symptomatic) were collected at 4 months after infection, and 14 samples (from 14 symptomatic) were collected at 12 months after infection. Sera of the control group from healthy donors were collected from Zhoushan Hospital, Zhoushan, China. The serum was collected, processed, and stored by standard protocol (xiling et al., 2020). All the sera were stored at −80 °C for further analysis. The Institutional Ethics Review Committee of Zhoushan Hospital, Zhoushan, China approved the protocols used for blood collection from subjects with COVID-19.

2.2. Serum analysis with proteome array

Twenty-three proteins of SARS-CoV-2 and 220 peptides of spike were constructed into the proteome array. There were 14 chambers in each slide. Following the instruction previously reported (Jiang et al., 2020), the PBS buffer containing 0.1% Tween 20 (PBST) and 3% BSA was used

to block nonspecific interaction. The pretreated serum was diluted 1:200 in PBST. The diluted serum or buffer was incubated with each well at 4 °C overnight. The Cy3-conjugated goat anti-human IgG and Alexa Fluor 647-conjugated donkey anti-human IgM (Jackson ImmunoResearch, PA, USA, Cat#109–165–008 and Cat#709–605–073, respectively) were used to detect the binding antibodies after washing with PBST. The arrays were scanned by LuxScan 10K-A (CapitalBio Corporation, Beijing, China), and the data were analyzed using GenePix Pro 6.0 software (Molecular Devices, CA, USA).

2.3. Spike and ACE2 blocking assay

We used the SARS-CoV-2 Surrogate Virus Neutralization Test Kit (Cat. No. L00847) to detect spike-specific neutralizing antibodies (Tan et al., 2020). To evaluate the level of peptides induced neutralizing antibodies, the S1–61, S1–66, S1–82, and BSA (100 ng/well) were pre-coated in 96-well plate at 4 °C overnight. After washing, 200 μL serum (1:20 diluted with samples dilution buffer) was added, followed by incubation at 37 °C for 30 min. After three rounds of absorption, the supernatant was collected for the analysis of inhibitory ability. Briefly, we mixed 60 μL of negative control, positive control, or samples (pre-incubated or not) with the HRP-RBD solution in a volume ratio of 1:1 and incubated at 37 °C for 30 min. Next, 100 μL of each mixture was added to the corresponding wells and incubated at 37 °C for 15 min. The plate was washed with washing solution for four times. Then, 100 μL TMB Solution was added to each well and kept in dark at 25 °C for 15 min, followed by adding 50 μL stop solution to each well. The absorbance was read at 450 nm immediately using Synergy™ LX Multi-Mode Microplate Reader ((Bio Tek, VT, USA) with Gen5 software (Version 3.05.11). The inhibitory ability was calculated using the formula:

$$Inhibition = \left(1 - \frac{OD\ value\ of\ Sample}{OD\ value\ of\ Negative\ Control} \right) \times 100\%.$$

2.4. Statistics

The fluorescence intensities of IgG and IgM were defined as the medians of the foreground for each spot. Then, log2 of the data was taken, and we calculated the value for each protein per group. IgG and IgM data were analyzed separately. One-way ANOVA, Two-way ANOVA and *t*-test were used for the data analysis. Q-values or adjusted *p*-values were obtained using BH (Benjamini and Hochberg) method. The mean signal + three standard deviations (SD) of the negative sera were used to set a cutoff threshold. The values of the samples greater than the threshold were judged as positive (Jiang et al., 2020). All the Signal intensities were analyzed by the software GraphPad Prism version 8.0. The SARS-CoV-2 proteome microarray data are deposited on Protein Microarray Database under the accession number PMDE258 (http://www.proteinmicroarray.cn/index.php/experiments). Additional data related to this paper may be requested from the authors.

3. Results

3.1. Microarray-based igg and igm antibody analysis

Humoral immunity and cellular immunity can be detected in SARS-CoV-2–infected patients (Ni et al., 2020 Zhang et al., 2020;). Nevertheless, we know little about the differences and persistence of antibodies in asymptomatic and symptomatic SARS-CoV-2 infection. A total of 33 patients and 10 age-matched healthy controls were recruited to this study. To identify SARS-CoV-2–specific linear B-cell epitopes, the microarray was modified by adding 220 peptides (Fig. 1A). The collected samples were incubated at 56 °C for 30 min and stored at −80 °C for further analysis (Fig. 1B).

To explore and analyze IgG and IgM antibodies in asymptomatic and symptomatic patients, 33 COVID-19-recovered patients were assessed using microarray. The detailed information of patients and healthy
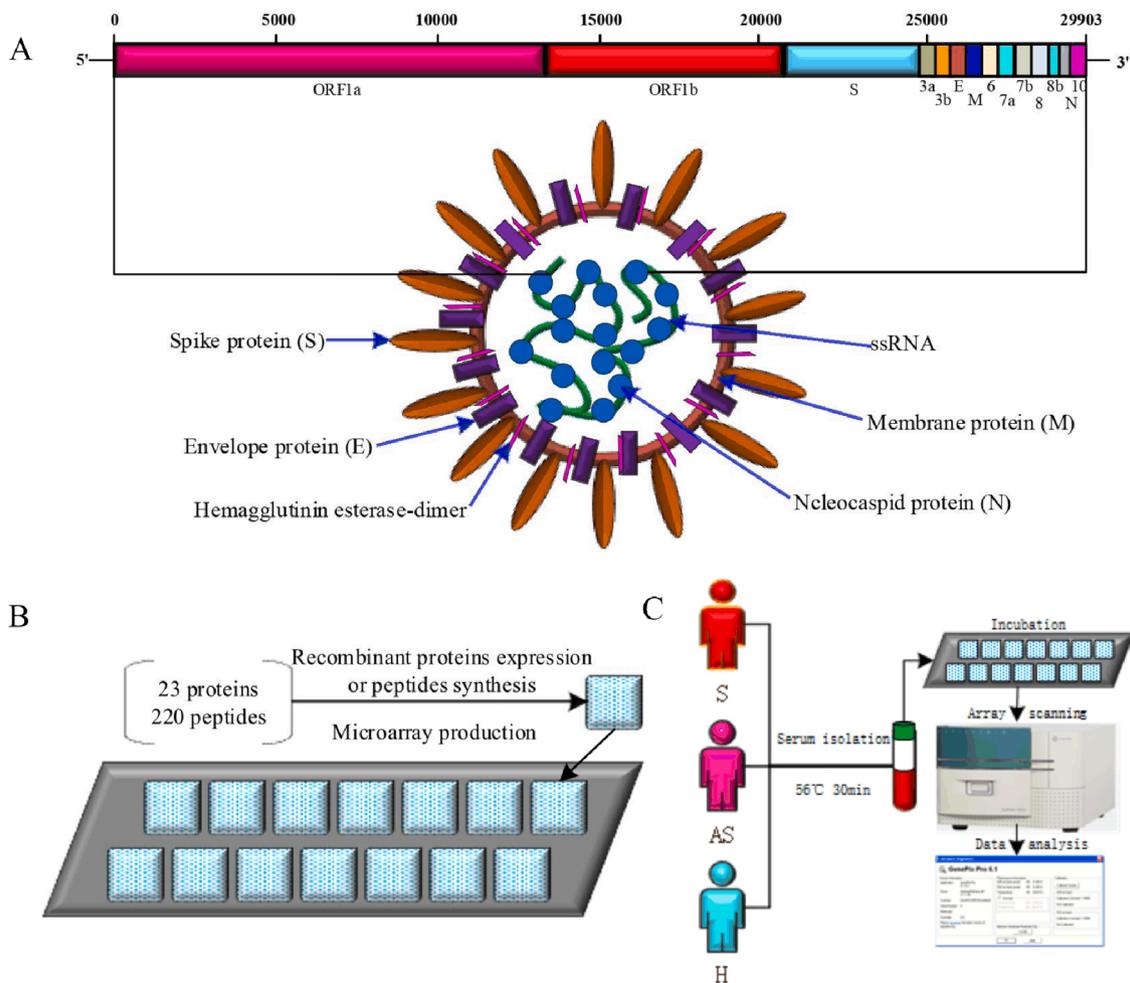
Fig. 1. The evaluation and comparison of humoral immunity of SARS-CoV-2–infected patients using a modified microarray. (A) The structure diagrams of SARS-CoV-2. (B) The construction and modification of microarray. (C) The schematic diagram of SARS-CoV-2–specific IgG and IgM detection.

controls is shown in Table 1. The recovered patients and healthy controls presented clearly separate clusters for both IgG and IgM data

Table 1
Detailed information of serum samples tested in this study.

Cohort	Gender	Average age (years)	Days after symptom onset for sampling	Disease severity	Sample name
Symptomatic Patients with COVID-19 (S)	Male (n = 5) Female (n = 6)	47.9	1 month	Mild	S1-S11
Symptomatic Patients with COVID-19 (S)	Male (n = 4) Female (n = 2)	40.5	4 months	Mild	S4M-1-S4M-6
Symptomatic Patients with COVID-19 (S)	Male (n = 7) Female (n = 7)	43.9	12 months	Mild	S12M-1-S12M-14
Asymptomatic Patients with COVID-19 (AS)	Male (n = 18) Female (n = 1)	44.8	1 month	NA	AS1-AS5, AS7-AS20
Healthy donors (H)	Male (n = 6) Female (n = 4)	45.7	_	_	H1-H10

NA: No symptom.

(Fig. 2A and Fig. 3A), and several proteins including N—Cter, N—Nter, RBD, S1 and S distinguished asymptomatic from symptomatic infection (Fig. 2C-2D and Fig. 3C-3F). SARS-CoV-2 proteins showed different immunogenicity to elicit humoral IgG and IgM responses (Fig. 2B and Fig. 3B). The levels of N—Nter- and N—Cter-specific IgG antibodies in symptomatic patients were higher than those in asymptomatic patients (Fig. 2C and 2D). The levels of S-specific IgM antibodies were higher than antibodies elicited by other proteins. Both N- and S-specific IgM antibodies were higher in symptomatic than in asymptomatic patients (Fig. 3C-3F).

According to the data, S and N proteins have the highest sensitivity for the diagnosis, so these two proteins have been applied for the diagnosis of COVID-19 infection (Grzelak et al., 2020 Ni et al., 2020;). The IgM and IgG may be associated with the clinical outcome. Persistent virus stimulation may involve stages of both silent and productive infection without rapidly clearing or even inducing excessive damage to the body (Govindan, 2015). In clinical studies, SARS-CoV-2 infection has been shown to lead to the impairment of several organs (Robba et al., 2020). Our present data suggest that the clinical symptoms may be associated with the levels of S-and N-specific antibodies.

3.2. The dynamics of SARS-CoV-2–specific antibodies over time

The receptor-binding domain (RBD)-specific antibodies have attracted great interest because RBD is the key domain binding human ACE2 (Yi et al., 2020). Previous studies have mainly focused on the durability of the S-specific antibodies (Zost et al., 2020a, 2020b).
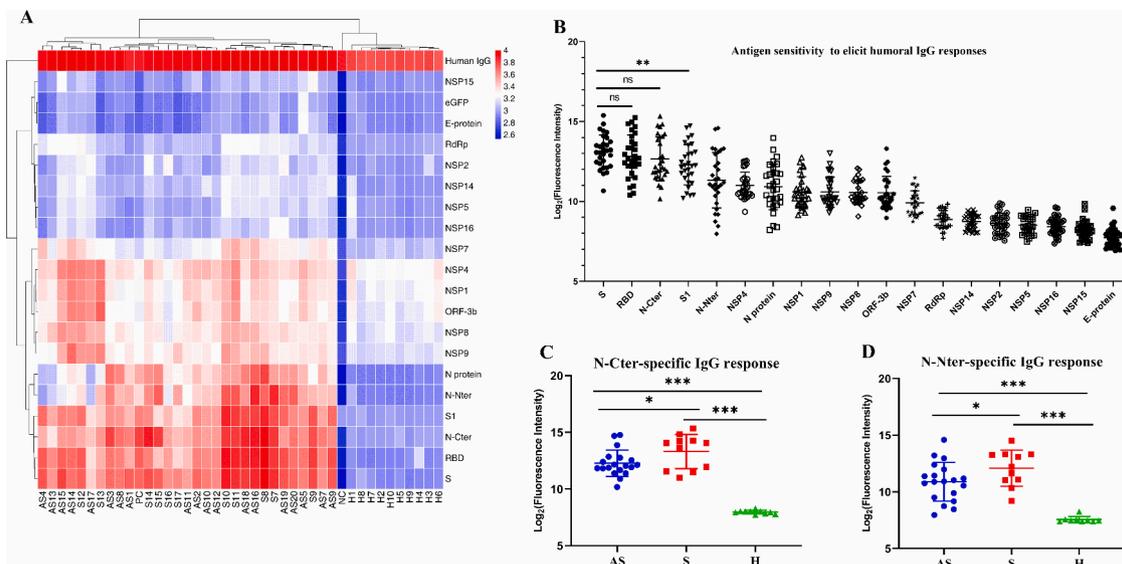
Fig. 2. The comparison of SARS-CoV-2–specific IgG fluorescence intensity profiles. (A) Each square presents the IgG antibody response against the protein (row) in the serum (column). (B) The intensity of antibodies induced by SARS-CoV-2 proteins. The intensity of antibodies against N—Cter (C) and N—Nter (D) was different between asymptomatic and symptomatic infection. AS: Asymptomatic infection ($n = 19$), S: Symptomatic infection ($n = 11$), H: Healthy controls ($n = 10$). One-way ANOVA were used for the data analysis. P values were calculated by the One-way ANOVA. Q values were adjusted p-values using BH method. Q-values < 0.05 were considered significant. ns: not significant, * $q<0.05$, ** $q<0.01$, **** $q<0.0001$.
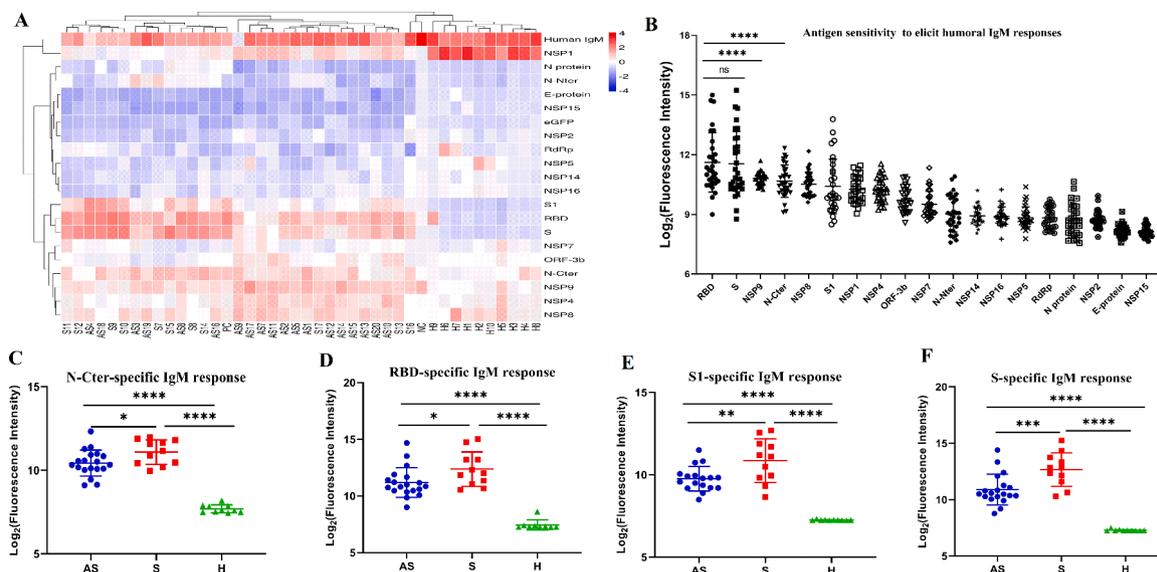

Fig. 3. The comparison of SARS-CoV-2–specific IgM fluorescence intensity profiles. (A) Each square presents the IgM antibody response against the protein (row) in the serum (column). (B) The intensity of IgM antibodies induced by SARS-CoV-2 proteins. The intensity of antibodies against N—Cter (C), RBD (D), S1 (E), and S (F) was different between asymptomatic and symptomatic infection. AS: Asymptomatic infection ($n = 19$), S: Symptomatic infection ($n = 11$), H: Healthy controls ($n = 10$). P values were calculated by the multiple t-test. Q values were adjusted p-values using BH method. Q-values < 0.05 were considered significant. ns: not significant, * $q<0.05$, ** $q<0.01$, *** $q<0.001$, **** $q<0.0001$.

However, nothing is known about dynamics of antibodies induced by other proteins. We utilized the microarray to examine the serum isolated from symptomatic patients at 1 month, 4 months, and 12 months after infection. We analyzed the IgG and IgM profiles for each recovered patient and created heat maps to display the fluorescence intensity (Fig. 4A and 4C). The N- and S-specific IgG and IgM antibodies significantly decreased over time (Fig. 4B and 4D). The NSP9- (50% percentage of all the patients), N protein- (100%) and S- (92.9%) specific IgG antibodies were still detected 12 months after infection (Fig. 4B). The antibodies against SARS-CoV-2 significantly decreased over time, and most of the antigens-induced IgM antibodies were not detectable 12 months post-infection (Fig. 4D). Except for RBD- and S- specific IgM, the

levels of all the other proteins- specific IgM decreased to the threshold at 12 months after the onset of infection (Fig. 4D). Although the serum samples were isolated from COVID-19-recovered patients, the level of RBD- and S-specific antibodies at 4 months post-infection were higher compared with those collected at 1 month. These results indicate that the RBD- and S-specific IgG antibodies can persist for at least one year after infection.

3.3. The identification of linear B-cell epitopes

The clinical symptoms appeared at the early phase of primary infection, and IgM was the main antibody subtype. In order to explore
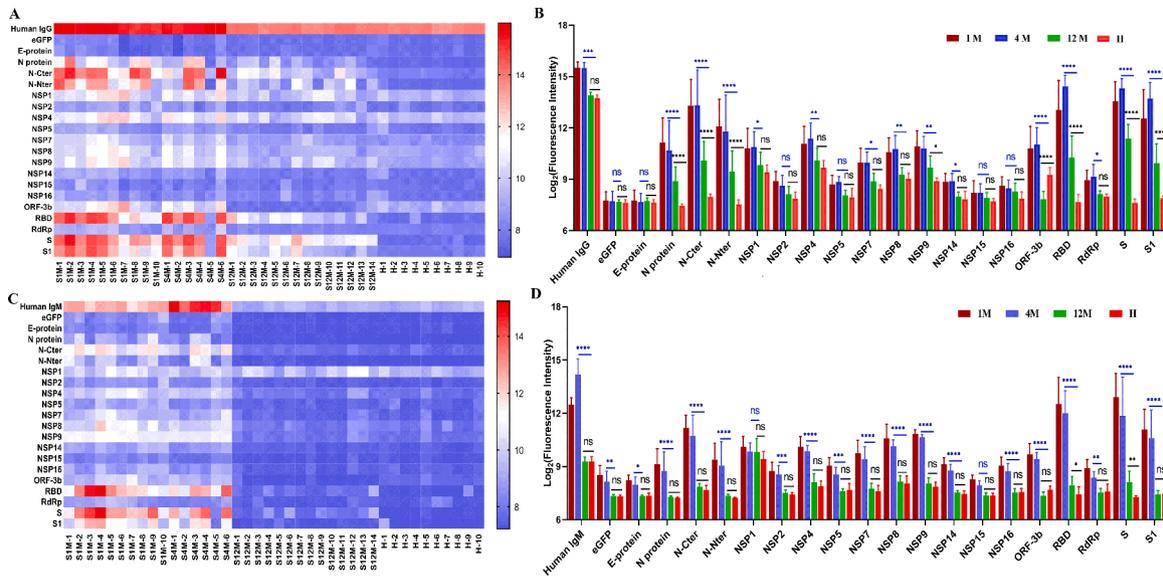
Fig. 4. The IgG and IgM response of the S recovered sera against the proteins at 1 month, 4 months and 12 months after infection onset. (A) The heat map of IgG response. Each square indicates the IgG antibody response against the protein (column) in the serum (row). (B) The IgG response was compared between different time points. (C) The heat map of IgM response. Each square indicates the IgM antibody response against the protein (column) in the serum (row). (D) The IgM response was compared between different time points. *P* values were calculated by the Two-way ANOVA. Q values were adjusted *p*-values using BH method. Q-values < 0.05 were considered significant. ns: not significant, * $q<0.05$, ** $q<0.01$, *** $q<0.001$, **** $q<0.0001$.

the correlation between IgM against spike and the clinical symptoms, we used the microarray containing 220 peptides of spike. The top 20 peptides were selected, and the relative intensities of IgM against the peptides were compared (Fig. 5A and 5B). The ratio of the total 20 peptides recognized by IgM antibodies isolated from AS and S patients did not show any significant difference (Fig. 5C). Four epitopes (S1–4, S1–82, S1–106, and S2–71) exhibited strong discriminatory ability between the AS and S patients using IgM response (Fig. 5D). These data indicate that the level of IgM is associated with clinical symptoms and the IgM against

SARS-CoV-2 with high heterogeneity must be considered in vaccine development.

The Spike–ACE2 interaction plays an important role in SARS-CoV-2 infection. Identification of antibodies targeting spike has been reported. Yet, we know little about the linear B-cell epitopes of spike recognized by the antibodies. The microarray containing 220 peptides distributed in different areas of spike was used. The top 20 peptides with strong IgG response were identified and analyzed (Table 2). The relative fluorescence intensity is shown in heat map (Fig. 6A), demonstrating that the
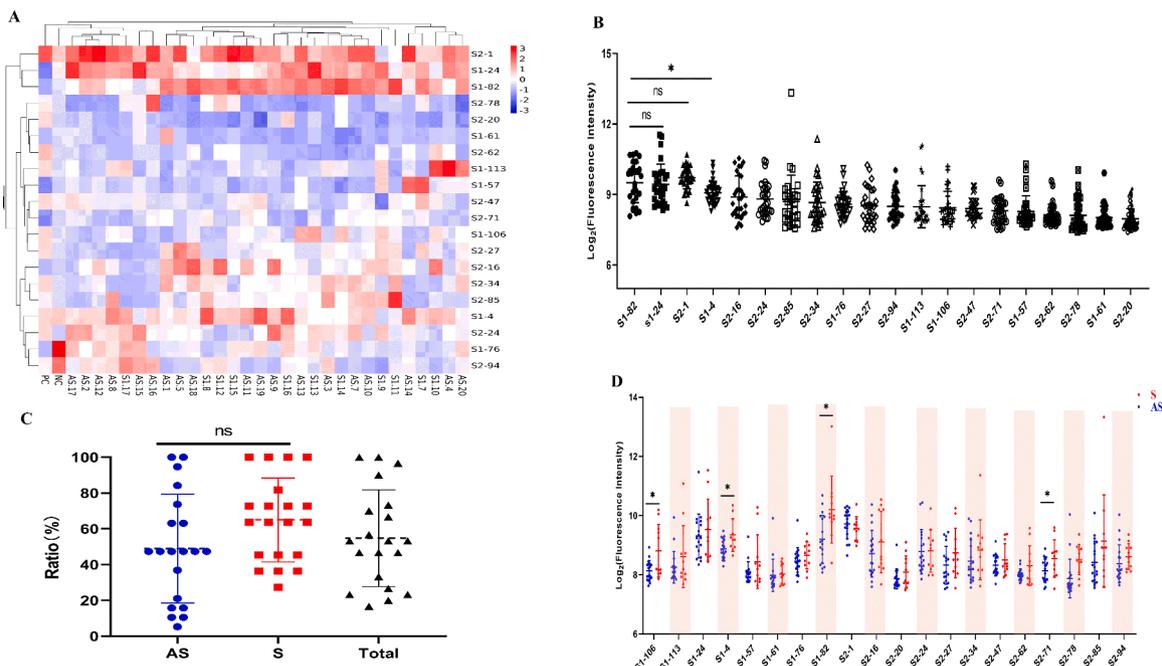

Fig. 5. The top 20 peptides recognized by SARS-CoV-2–specific IgM. (A) The heat map of IgM, where each square presents the IgM antibody response against the peptides (row) in the serum (column). (B) The intensity of IgM antibodies induced by the 20 peptides. (C) The percentage of positive patients recognized by the 20 peptides. (D) The level of IgM against the top 20 peptides. *P* values were calculated by the One-way ANOVA and multiple *t*-tests. Q values were adjusted *p*-values using BH method. Q-values < 0.05 were considered significant. ns: not significant, * $q<0.05$.

Table 2
Detailed information of the top 20 peptides.

Name	Sequence	Chemical formula	Length	PI	GRAVY	MW (g/mol)
S1–4	TTRTQLPPAYTN	$C_{59}H_{95}N_{17}O_{20}$	12	9.84	−1.1	1362.48
S1–5	PPAYTNSFTRGV	$C_{59}H_{88}N_{16}O_{18}$	12	9.84	−0.53	1309.42
S1–24	PFLGVYYHKNNK	$C_{71}H_{102}N_{18}O_{17}$	12	9.94	−0.98	1479.67
S1–61	CVADYSVLYNSA	$C_{57}H_{85}N_{13}O_{20}S$	12	3.12	0.59	1304.42
S1–66	CFTNVYADSFVI	$C_{64}H_{91}N_{13}O_{19}S$	12	3.12	−1.08	1378.54
S1–82	NCYFPLQSYGFQ	$C_{69}H_{91}N_{15}O_{19}S$	12	5.25	−0.33	1466.61
S1–96	DTTDAVRDPQTL	$C_{54}H_{90}N_{16}O_{23}$	12	3.6	−1.03	1331.38
S2–1	SVASQSIIAYTM	$C_{55}H_{91}N_{13}O_{19}S$	12	7	0.9	1270.44
S2–16	KNTQEVFAQVKQ	$C_{62}H_{102}N_{18}O_{20}$	12	9.88	−1.08	1419.57
S2–22	PSKRSFIEDLLF	$C_{68}H_{106}N_{16}O1_9$	12	7	−0.08	1451.66
S2–24	NKVTLADAGFIK	$C_{58}H_{97}N_{15}O_{17}$	12	9.88	0.25	1276.47
S2–47	LVKQLSSNFGAI	$C_{58}H_{97}N_{15}O_{17}$	12	10.09	0.67	1276.47
S2–62	FPQSAPHGVVFL	$C_{63}H_{91}N_{15}O_{15}$	12	7.88	0.71	1298.48
S2–71	QRNFYEPQIITT	$C_{68}H_{104}N_{18}O_{21}$	12	7	−0.92	1509.65
S2–78	FKEELDKYFKNH	$C_{75}H_{108}N_{18}O_{21}$	12	7.66	−1.73	1597.76
S2–94	LKGCCSCGSCCK	$C_{43}H_{78}N_{14}O_{15}S_5$	12	8.14	0.51	1191.48

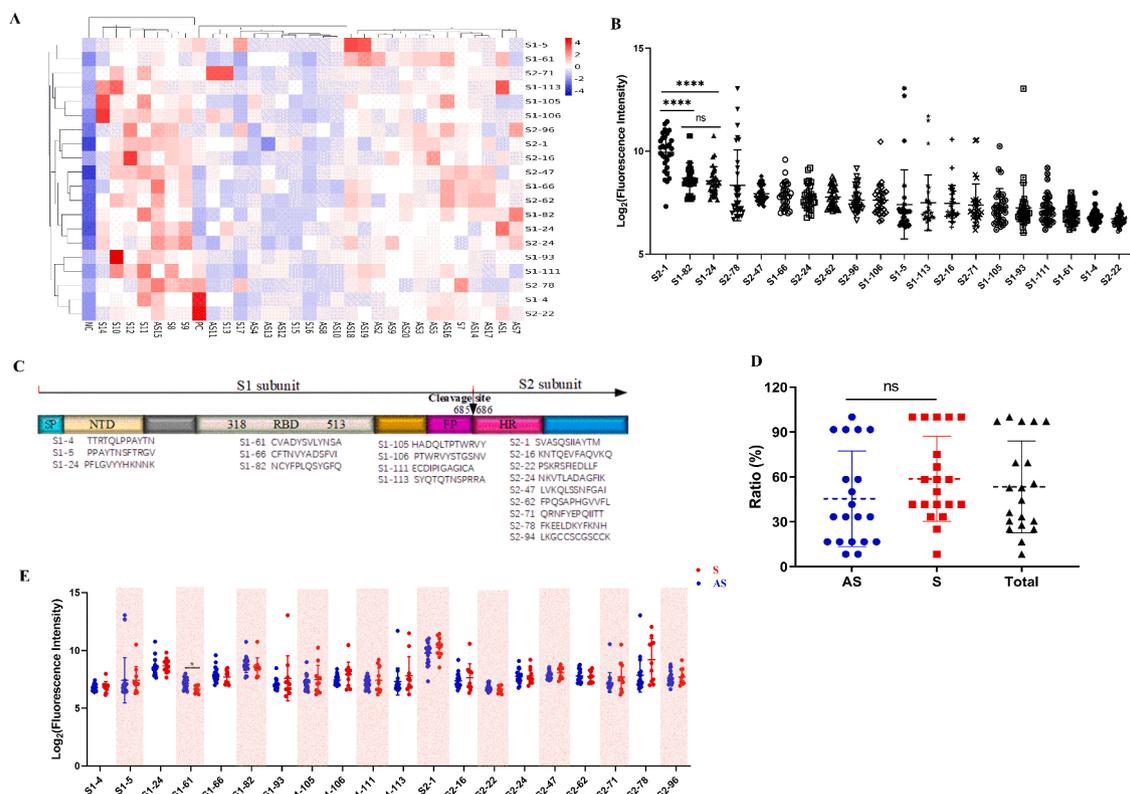PI: Isoelectric point; GRAVY: Grand average of hydropathicity; MW: Molecular weight.

Fig. 6. The lgG immune response against the top 20 peptides. (A) The heat map of top 20 peptides response to serum isolated from asymptomatic and symptomatic patients. (B) The sensitivity of the top 20 peptides. (C) The distribution of the peptides in spike. (D) The 20 peptides-positive ratio in asymptomatic and symptomatic patients. (E) The level of IgG response against the top 20 peptides. *P* values were calculated by the One-way ANOVA and multiple *t*-tests. Q values were adjusted *p*-values using BH method. Q-values < 0.05 were considered significant. ns: not significant, ** $q < 0.01$, *** $q < 0.0001$, **** $q < 0.0001$.

20 peptides have different immunogenicity (Fig. 6B). Although, 20 peptides were possible to be recognized by most of the sera isolated from both asymptomatic and symptomatic patients, there were only three peptides (S1–61, S1–66, and S1–82) located in the RBD (Fig. 6C). The ratio of the total 20 peptides recognized by IgG isolated from AS and S patients did not show any significant difference (Fig. 6D). Of all different epitopes recognized by IgG isolated from both AS and S patients, S1–61 exhibited strong discriminatory ability between the AS and S patients using IgG response (Fig. 6E). Amrun, Lee et al. designed a peptide pool to explore the association between the response of linear B-cell epitopes and disease severity (Amrun et al., 2020). Four peptides in spike (S6P2, S14P5, S20P2, S21P2) have been identified in the previous study, and

three (S14P5, S20P2, S21P2) of them were confirmed in our study. Moreover, we found that three peptides distributed in RBD were not conserved in different coronaviruses (Fig. 7A). The crystal structure of spike was downloaded from the Protein Data Bank with accession codes EMD: 30,701 and PDB: 7DK3 (closed SARS-CoV-2 S) (Xu et al., 2021). We utilized Discovery studio 4.1 Client to analyze the location of the three peptides in spike, and found that S1–82 was located on the surface of spike (Fig. 7B and Table 2). There are nine amino acid residues reported to be associated with the spike–ACE2 binding affinity (Shang et al., 2020 Yi et al., 2020;). The S1–82 peptide contains two key residues F490 and Q493. The ROC (Receiver Operating Characteristic) curve was constructed, and the peptides S1–82 and S1–61 identified
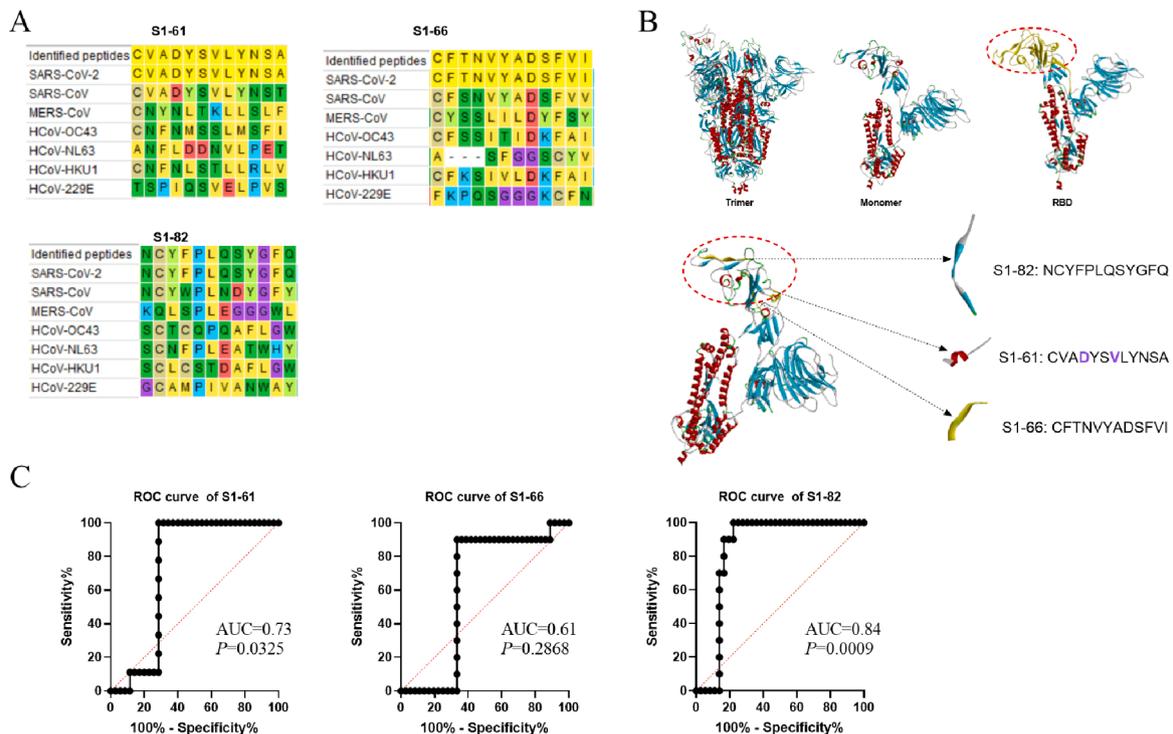
Fig. 7. Characteristics of the three peptides identified in RBD. (A) Sequence alignment of S1–61, S1–66, and S1–82 in different coronaviruses. (B) The location of S1–61, S1–66, and S1–82 in spike. Purple marked amino acids represent key residues of predominant binding forces. (C) The sensitivity and specificity of S1–61, S1–66, and S1–82.

were suitable for detection (Fig. 7C). These data indicate that linear B-cell epitopes should be considered in the development of SARS-CoV-2 vaccine.

3.4. The inhibitory ability of neutralizing antibodies

To evaluate the level of neutralizing antibodies in asymptomatic and symptomatic patients and the peptide-induced neutralizing antibodies, the Virus Neutralization SARS-CoV-2 Surrogate Test Kit purchased from GenScript was used in this study. We found that the level of neutralizing antibodies in symptomatic patients was significantly higher than that in asymptomatic patients (Fig. 8A). The level of neutralizing antibodies was associated with the antibodies induced by S1 ($r = 0.68$, $P = 0.0002$) and RBD ($r = 0.68$, $P = 0.0002$) (Fig. 8B). The peptides identified in RBD were able to induce antibodies, but the inhibition abilities of these antibodies have no significance (Fig. 8C). We also compared the inhibitory ability of serum isolated from symptomatic patients at 1 month, 4 months, and 12 months post-infection. The inhibitory ability of serum isolated at 1 month and 4 months was similar; however, the inhibitory ability of serum isolated at 12 months significantly decreased (Fig. 8D). The epitopes identified by antibodies of spike are potential candidates for the development of SARS-CoV-2 vaccine, especially the conserved sequences in spike.

4. Discussion

SARS-CoV-2 infection causes a spectrum of symptoms and signs and impairs several organs. Unfortunately, there has been scarce evidence of the long-term effect and the persistence of antibodies (Del Rio et al., 2020). We recruited 33 recovered patients to evaluate the dynamics of SARS-CoV-2–specific antibodies and identify spike-specific linear B-cell epitopes. Our data revealed that the level of antibodies against SARS-CoV-2 proteins was different. S and N proteins elicited the strongest humoral immune response, compared with other antigens. At the acute primary infection phase, antibodies against S and N continue to

rise (Norman et al., 2020). The antibodies against SARS-CoV-2 were stable at least 4 months, but they significantly decreased 12 months post-infection. A previous study showed that the N- and S-specific antibodies decreased after 6.2 months (Gaebler et al., 2021), while Dan et al. found that they were still detecTable 8 months post-infection (Dan et al., 2021). According to our results, SARS-CoV-2–specific antibodies persist at least 12 months. In the future study, we intend to pay more attention to the differences and duration of antibodies elicited by live virus and SARS-CoV-2–based vaccines. There are at least five vaccines that can be used for SARS-CoV-2 prevention in clinical setting, according to the WHO report.

We compared the IgG and IgM response between asymptomatic patients and symptomatic patients. The levels of IgG and IgM in symptomatic patients were higher than those in asymptomatic patients. The difference in IgG response is consistent with a previous report (Long et al., 2020). In contrast, IgM response is not, which may be due to different age ranges (8–75 years in the previous study vs 33–67 years in the present study) (O'Driscoll et al., 2021). The differences in humoral response between symptomatic and asymptomatic patients may be associated with the heterogeneity of clinical presentations, in particular the higher levels of IgM and IgG in symptomatic patients. Antibody-dependent enhancement (ADE) has been reported in many viruses. However, ADE has not been confirmed in SARS-CoV-2 (Lee et al., 2020). As previously reported, a specific range of antibody titers enhanced dengue virus replication and disease severity (Katzelnick et al., 2017). In vitro studies and animal models should be designed to verify whether there is a threshold or a range of SARS-CoV-2–specific antibody titers that cannot clear the virus but could rather cause ADE-enhanced viral infection and organ injury. Yet, the level and function pathogen-specific B cells were closely related to antibody titers (Dorner and Radbruch, 2007).

Circulating IgM antibodies play a key role in controlling infection (Racine and Winslow, 2009). In this study, we identified five epitopes and five proteins. The level of IgM against these antigens distinguishes asymptomatic from symptomatic patients. IgM is an important regulator
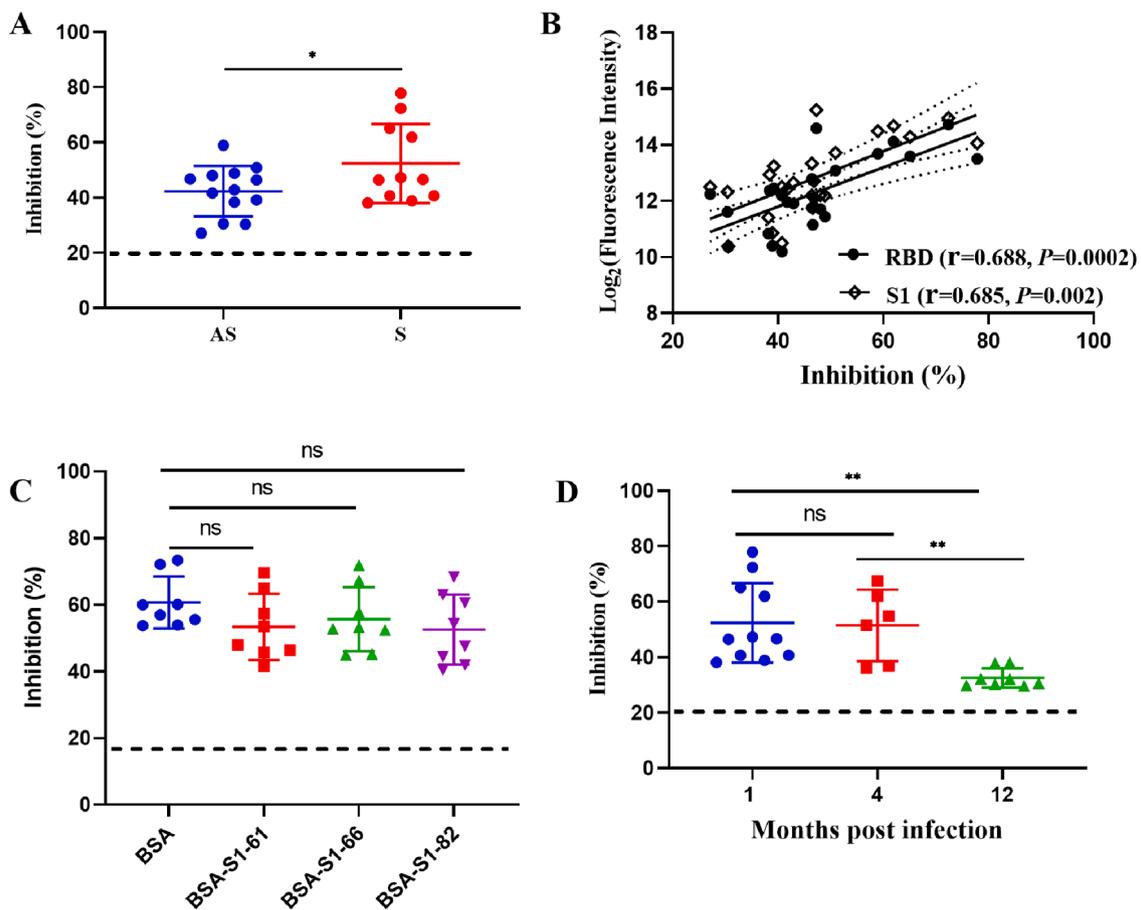
Fig. 8. The inhibitory ability of neutralizing antibodies. (A) The inhibitory ability of serum isolated from AS and S patients to inhibit the binding of spike and ACE2. (B) The association between the humoral immune response and inhibitory ability. (C) The inhibitory ability of neutralizing antibodies after absorption of S1–61, S1–66, and S1–82 specific antibodies. For A, p-value was calculated by unpaired t-test, * p <0.05. For C-D, p values were calculated by the One-way ANOVA. Q values were adjusted p-values using BH method. Q-values < 0.05 were considered significant. ns: not significant, * q < 0.05, ** q < 0.01.

of the complement system (Noris and Remuzzi, 2013 Sharp et al., 2019;). A previous study found that the dysfunction of immune complement was associated with the adverse outcomes (Ramlall et al., 2020). A hypothesis proposed that the antigen bias in eliciting IgM antibodies against SARS-CoV-2 and the abnormal dynamics of IgM may lead to adverse events. However, the depletion of IgM memory B cell impaired the immune response and increased mortality in COVID-19 (Lenti et al., 2020). The selected depletion of IgM or reduction of the level of IgM may relieve the adverse events at the early infection phase. IgM isolated from reverse genetics can be used for the development of modified vaccines, through deleting or replacing the adverse event-associated epitopes.

An increasing number of SARS-CoV-2 variants are being reported worldwide. Recently, a variant named B.1.1.7, dominant in UK, presented reduced neutralization by the antibodies of convalescent and vaccinated people (Supasa et al., 2021). To improve the precision and efficacy of SARS-CoV-2 vaccines, linear B-cell epitopes of spike should be considered in the development of SARS-CoV-2 vaccines. We identified three epitopes in RBD, especially the S1–82 peptide, with great sensitivity and specificity (Fig. 6C). S1–82 is located on the surface of spike (Fig. 7B). Scientists have reported plenty of B-cell epitope-based vaccines (Zhang, 2018). Peptide pool or bioinformatics has been utilized in the prediction and identification of SARS-CoV-2 B-cell epitopes (Amrun et al., 2020 Lon et al., 2020;). Several studies have also focused on prediction and identification of T-cell epitopes (Braun et al., 2020 Le Bert et al., 2020;).

The level and affinity of neutralizing antibodies are the two key points to evaluate the vaccines (Krammer, 2020 Poland et al., 2020a;).

In the present study, we detected the neutralizing antibodies in AS and S patients and found that the levels of neutralizing antibodies were associated with the intensity of humoral immune response. Because of the low immunogenicity of peptides, the ratio of peptides-induced neutralizing antibodies was low. The application of adjuvants and carrier proteins can improve the immune response and the level of peptide induced neutralizing antibodies (Leroux-Roels et al., 2016 Radtke et al., 2017;). Besides, peptide-based vaccines can reduce non-neutralizing antibodies and avoid ADE. Although we found that the level of IgM was related to the adverse events and several antigens were identified, we did not evaluate the contribution of the antigens to ADE. Further studies should pay more attention to this aspect.

There are two antigen bias phenomena in SARS-CoV-2–induced humoral immune response. First, the level of antibodies (IgG and IgM) induced by different antigens was different in the same patient. Besides antigen bias, the expression levels of antigens by SARS-CoV-2 may contribute to the difference. Second, the levels of antibodies induced by one antigen varied among different individuals. The genetic background and immune status likely also determine the antigen bias. The B-cell activation and maturation are also regulated by T cells. In a future study, antigen bias in T cells and B cells activation in clinical infection and vaccine development should be considered. In conclusion, accurate identification of the contribution of proteins or peptides in humoral response is important for the optimization of SARS-CoV-2 vaccines.

5. Conclusions

Our data indicate that SARS-CoV-2–induced antibodies may provide

12 months of protection. Increasing the humoral response against neutralizing antibody-inducing antigens and decreasing the humoral response against clinical symptom-associated antigens may promote the vaccine efficacy and reduce the incidence of side effects. Our findings in this study will shed light on the development of the precise and effective vaccine to combat the global COVID-19 crisis.

CRediT authorship contribution statement

Nana Wei: Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition. **Qiujing Wang:** Resources, Data curation, Formal analysis. **Zhibing Lin:** Conceptualization, Methodology, Formal analysis. **Liyun Xu:** Resources, Data curation. **Zheen Zhang:** Resources, Data curation. **Yan Wang:** Resources, Data curation. **Zhejuan Yang:** Resources, Data curation. **Lue Li:** Resources, Data curation. **Tingxiao Zhao:** Resources, Data curation. **Lu Wang:** Resources. **Haifei Lou:** Resources. **Mingfang Han:** Resources. **Mingliang Ma:** Methodology, Validation, Formal analysis. **Yaosheng Jiang:** Investigation. **Jinmiao Lu:** Investigation. **Shilan Zhu:** Investigation. **Li Cui:** Supervision, Funding acquisition. **Shibo Li:** Conceptualization, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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