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Letter

Isolation and characterization of spike S2-specific monoclonal antibodies with reactivity to pan-coronaviruses

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Dear Editor,

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the seventh coronavirus known to jump from intermediate hosts to humans, has resulted in a worldwide pandemic and caused immense economic damage (Tan et al., 2020; Zhu et al., 2020). It is believed that coronavirus spillover may occur again in the future. Human exposure to a bat coronavirus has been serologically confirmed in a rural area of China, indicating that the coronavirus spillover occurred, although the origin and frequency of spillovers are unclear (Wang et al., 2018). Domestic animals and pets could be infected with coronaviruses and may serve as a reservoir and an intermediate host for further virus adaptation before spilling over to humans. Cats and ferrets are susceptible to SARS-CoV-2, and cat-to-cat transmission through respiratory droplets has been reported (Meekins et al., 2020; Shi et al., 2020). Coronaviruses enter the host cell by interacting with surface spike (S) proteins and host receptor proteins. Broad-spectrum monoclonal antibodies (mAbs) for multiple human and animal coronaviruses have been shown to recognize the spike proteins of coronaviruses (Klompus et al., 2021). We previously constructed 274 mAbs against the SARS-CoV-2 S from COVID-19 convalescents and inactivated vaccine recipients, and identified five broad neutralizing antibodies against SARS-CoV-2 Omicron subvariants (Chen et al., 2023). In this study, we conducted screening to evaluate the recognizing ability of these mAbs on S proteins derived from both human and animal coronaviruses, with the aim to identify mAbs that can recognize pan-coronaviruses.

First, we tested the binding activity of the 274 mAbs to S proteins of seven human coronaviruses and found that five mAbs (VSM7-28, VSM7-75, VSM6-5, VSM6-39, and SCM14-3) bound weakly to the SARS-CoV-2 spike S1 subunit, but had strong binding potency with the spike S2 subunits of SARS-CoV-2, other β-coronaviruses (SARS-CoV, MERS-CoV, OC43, and HKU1), and α-coronaviruses (229E and NL63) (Fig. 1A and B; Supplemental Fig. S1A–G). Of the five mAbs, VSM7-28 had the most potent binding potency to all seven human coronavirus spike extracellular domain (S-ECD) proteins (EC_{50} ranging between 0.0082 and 0.029 μg/mL), followed by SCM14-3 (EC_{50}, 0.0066–0.2005 μg/mL), VSM7-75 (EC_{50}, 0.037–3.849 μg/mL), VSM6-5 (EC_{50}, 0.019–5.725 μg/mL), and VSM6-39 (EC_{50}, 0.0543–8.969 μg/mL). VSM6-5 and VSM6-39 had weak binding potency with HKU1 and NL63 ($EC_{50} > 10$ μg/mL), respectively (Fig. 1A). Next, we tested the neutralization activity of the mAbs and found that none of them could neutralize a SARS-CoV-2 pseudotyped virus (Fig. 1C).

Since these mAbs had no neutralizing activity, we examined whether they had a broad binding activity with other SARS-CoV-2 variants. The binding potency of these mAbs with S-ECDs from SARS-CoV-2 variants of concern (VOCs), harboring mutations to escape from antibody binding and neutralization, were tested by ELISA. The results showed that VSM7-28 and VSM7-75 had strong reactivity to the S-ECD of eight VOCs [Alpha, Beta, Gamma, Delta, and Omicron variants (BA.1, BA.2, BA.3, and BA.2.75)]. VSM6-5 bound to Alpha, Beta, Gamma, Delta, and Omicron variants (BA.2, BA.3, BA.4/5, and BA.2.75). VSM6-39 only bound to Alpha, Beta, Gamma, Delta, and Omicron variants (BA.1, BA.2, and BA.3), with no binding to Omicron variants BA.4/5 and BA.2.75 (Fig. 1A, Supplemental Fig. S1H–P). Omicron variants BA.2, BA.3, BA.4/5, and BA.2.75 shared the same S2 sequence; however, the mAbs exhibited significant differences in binding activity to these proteins, indicating that these distinctions may be attributed to the different spatial configurations.

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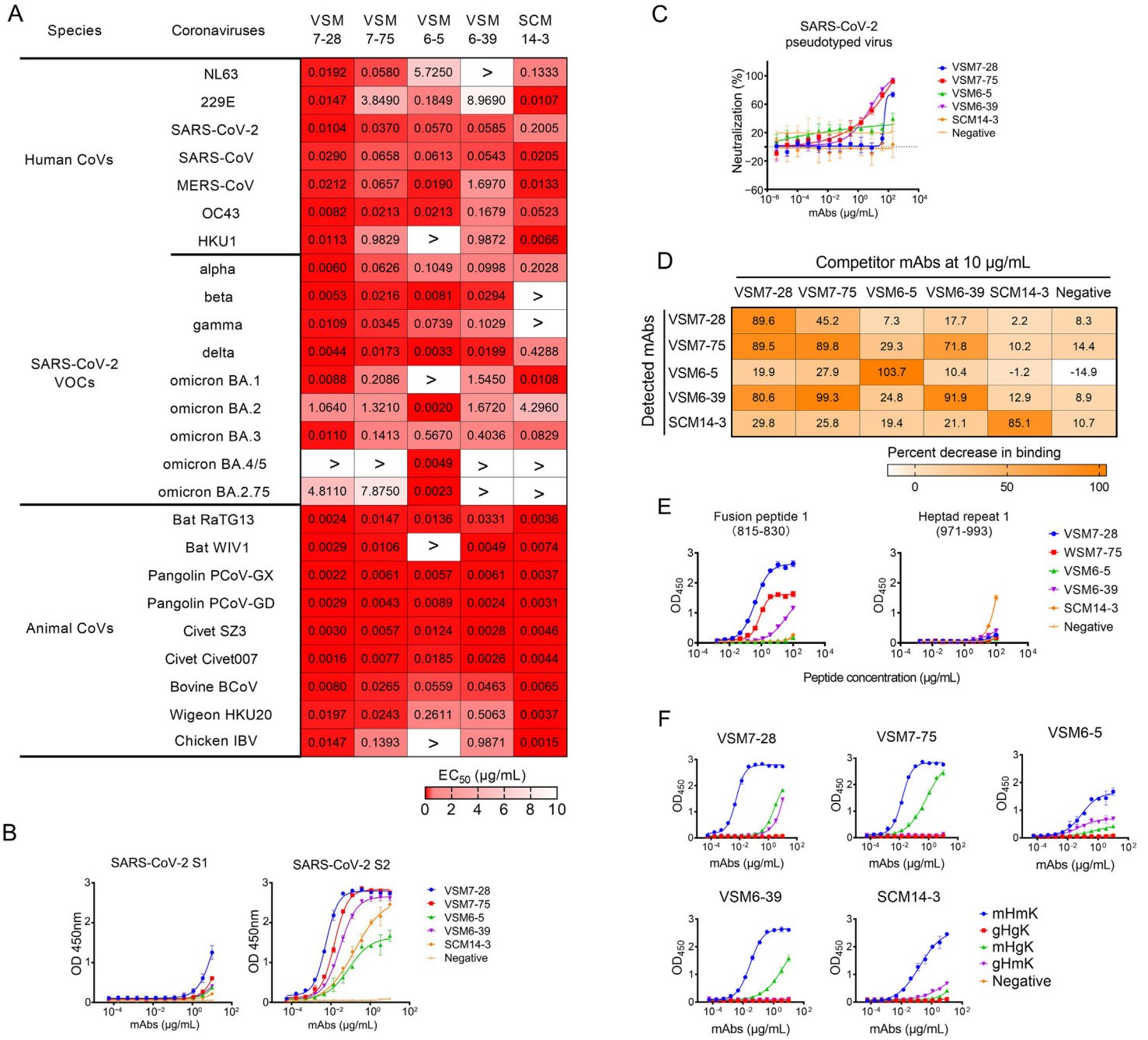
A
Species
Coronaviruses
VSM 7-28
VSM 7-75
VSM 6-5
VSM 6-39
SCM 14-3
Human CoVs
NL63 0.0192 0.0580 5.7250 > 0.1333
229E 0.0147 3.8490 0.1849 8.9690 0.0107
SARS-CoV-2 0.0104 0.0370 0.0570 0.0585 0.2005
SARS-CoV 0.0290 0.0658 0.0613 0.0543 0.0205
MERS-CoV 0.0212 0.0657 0.0190 1.6970 0.0133
OC43 0.0082 0.0213 0.0213 0.1679 0.0523
HKU1 0.0113 0.9829 > 0.9872 0.0066
SARS-CoV-2 VOCs
alpha 0.0060 0.0626 0.1049 0.0998 0.2028
beta 0.0053 0.0216 0.0081 0.0294 >
gamma 0.0109 0.0345 0.0739 0.1029 >
delta 0.0044 0.0173 0.0033 0.0199 0.4288
omicron BA.1 0.0088 0.2086 > 1.5450 0.0108
omicron BA.2 1.0640 1.3210 0.0020 1.6720 4.2960
omicron BA.3 0.0110 0.1413 0.5670 0.4036 0.0829
omicron BA.4/5 > > 0.0049 > >
omicron BA.2.75 4.8110 7.8750 0.0023 > >
Animal CoVs
Bat RaTG13 0.0024 0.0147 0.0136 0.0331 0.0036
Bat WIV1 0.0029 0.0106 > 0.0049 0.0074
Pangolin PCoV-GX 0.0022 0.0061 0.0057 0.0061 0.0037
Pangolin PCoV-GD 0.0029 0.0043 0.0089 0.0024 0.0031
Civet SZ3 0.0030 0.0057 0.0124 0.0028 0.0046
Civet Civet007 0.0016 0.0077 0.0185 0.0026 0.0044
Bovine BCoV 0.0080 0.0265 0.0559 0.0463 0.0065
Wigeon HKU20 0.0197 0.0243 0.2611 0.5063 0.0037
Chicken IBV 0.0147 0.1393 > 0.9871 0.0015
EC50 (μg/mL)
0 2 4 6 8 10
B
SARS-CoV-2 S1
SARS-CoV-2 S2
OD 450nm
mAbs (μg/mL)
VSM7-28
VSM7-75
VSM6-5
VSM6-39
SCM14-3
Negative
C
SARS-CoV-2 pseudotyped virus
Neutralization (%)
mAbs (μg/mL)
VSM7-28
VSM7-75
VSM6-5
VSM6-39
SCM14-3
Negative
D
Competitor mAbs at 10 μg/mL
VSM7-28 VSM7-75 VSM6-5 VSM6-39 SCM14-3 Negative
Detected mAbs
VSM7-28 89.6 45.2 7.3 17.7 2.2 8.3
VSM7-75 89.5 89.8 29.3 71.8 10.2 14.4
VSM6-5 19.9 27.9 103.7 10.4 -1.2 -14.9
VSM6-39 80.6 99.3 24.8 91.9 12.9 8.9
SCM14-3 29.8 25.8 19.4 21.1 85.1 10.7
Percent decrease in binding
0 50 100
E
Fusion peptide 1 (815-830)
Heptad repeat 1 (971-993)
OD450
Peptide concentration (μg/mL)
VSM7-28
WSM7-75
VSM6-5
VSM6-39
SCM14-3
Negative
F
VSM7-28
VSM7-75
VSM6-5
VSM6-39
SCM14-3
OD450
mAbs (μg/mL)
mHmK
gHgK
mHgK
gHmK
Negative

Fig. 1. Cross-binding reactivity of the five mAbs with human and animal coronaviruses. **A** Heatmap of the EC_{50} values of the five mAbs against spike protein of seven human coronaviruses, nine SARS-CoV-2 VOCs and nine animal coronaviruses. Binding ability of mAbs to spike protein of human and animal coronaviruses was measured by enzyme linked immunosorbent assay (ELISA), and EC_{50} values were calculated from concentration-response curves by sigmoidal fitting using GraphPad Prism 8.0 software. ">" means EC_{50} values > 10 μg/mL. **B** Five mAbs specifically bind to the prototype SARS-CoV-2 spike S2 subunit. The binding curves of five mAbs reactive to the SARS-CoV-2 S1 (left) and S2 (right) subunits. The prototype SARS-CoV-2 spike S1 or S2 proteins were coated on an ELISA plate at a concentration of 2 μg/mL; mAbs were serially diluted three-fold starting at 10 μg/mL and added to the plate, and then peroxidase-AffiniPure goat anti-human IgG (H + L) antibody was added to detect the binding. An anti-HCV mAb (HNC5) used as a negative control. **C** Neutralization activities of five mAbs against prototype SARS-CoV-2 pseudotyped virus. Pseudotype-based virus assays were used to test for neutralization. mAbs were serially diluted five-fold starting at 200 μg/mL and incubated with pseudotyped viruses, and then neutralization activities were scored by the reduction in luciferase intensity level after the infection of the HEK293T-ACE2 cells. An anti-HCV mAb (HNC5) used as a negative control. **D** Cross-competition patterns between five mAbs. Competition ELISA was used to determine whether the binding of one antibody affected the binding of another. The prototype SARS-CoV-2 spike S-ECD protein was coated on an ELISA plate at a concentration of 2 μg/mL; unlabeled competitor antibodies were added at 10 μg/mL, and then biotin-labeled detected antibodies were added at 0.1 μg/mL. The percentage of binding reduction compared to the binding potency without a competitor mAb is shown. **E** The binding curves of mAbs reactive to SARS-CoV-2 S2 fusion peptide 1 and heptad repeat 1 peptide. ELISA was employed to measure the binding activity between mAbs and peptides. The mAbs were coated on an ELISA plate at a concentration of 2 μg/mL; biotinylated peptides were serially diluted three-fold starting at 100 μg/mL and added to the plate, and then peroxidase-AffiniPure streptavidin was added to detect the binding. **F** The binding curves of mature mAbs (mHmL) and corresponding germline-reverted and heavy or light chain switched mAbs (gHgL, gHmL, mHgL) reactive to SARS-CoV-2 S2 subunit. Binding ability of mature mAbs and switched mAbs to prototype SARS-CoV-2 spike S2 protein was measured by ELISA. The prototype SARS-CoV-2 spike S2 protein was coated on an ELISA plate at a concentration of 2 μg/mL; mAbs were serially diluted three-fold starting at 10 μg/mL and added to the plate, and then peroxidase-AffiniPure goat anti-human IgG (H + L) antibody was added to detect the binding. An anti-HCV mAb (HNC5) was used as a negative control. Data are shown as the mean with standard deviation, representing technical replicates of three independent experiments. Data were analyzed and plotted by GraphPad Prism (version 8.0).

Given the zoonotic nature of coronaviruses, we examined the reactivity of these mAbs against the S2 of nine animal coronaviruses, including six SARS-like coronaviruses from bats (RaTG13 and WIV1), civets (SZ3 and Civet007) and pangolins (PCoV-GX and PCoV-GD) (Xiao et al., 2020), which could infect human epithelial cells using the ACE2 receptor (Ge et al., 2013; Xu et al., 2022), and three coronaviruses from bovine (BCoV), wigeon (HKU20), and chicken (IBV). VSM7-28, VSM7-75, VSM6-39 and SCM14-3 efficiently bound to the spike S2 of the nine coronaviruses. VSM6-5 showed binding ability with seven viruses but did not bind to S2 of WIV1 and IBV (Fig. 1A, Supplemental Fig. S1Q–Y). The data revealed that the five spike S2-binding mAbs also exhibited broad-spectrum reactivity with coronaviruses from animals.

To determine whether these mAbs share overlapping epitopes, we performed competition ELISA experiments. The mAbs VSM7-28, VSM7-75, and VSM6-39 had competitive binding with SARS-CoV-2 S-ECD (Fig. 1D), indicating that they may share overlapped epitopes. However, the Fc domain of antibodies occupies a significant space, leading to steric hindrance and potential antibody competition. To address this, we utilized papain to remove the Fc region, thus generating Fab fragments of three mAbs (VSM7-28, VSM7-75, and VSM6-39). Subsequently, we repeated competitive experiments using these Fab fragments. The obtained results exhibited high consistency with those of the full-length antibodies, suggesting that the observed competition remained unaffected by the steric hindrance imposed by the Fc domain of the antibody (Supplemental Fig. S2). In contrast, VSM6-5 and SCM14-3 had no competitive binding with other mAbs, suggesting that they may target distinct epitopes (Fig. 1D). In general, broad-spectrum mAbs target conserved coronavirus epitopes, such as the fusion peptide 1 and heptad repeat 1 (Klompus et al., 2021; Sun et al., 2022), which are essential for membrane fusion. Therefore, we synthesized the peptides that are conserved across Alpha, Beta, Gamma, and Delta coronaviruses (Supplemental Table S1), including the fusion peptide 1 and heptad repeat 1 peptide, and characterized the binding activity of the five mAbs to these peptides. VSM7-28, VSM7-75 and VSM6-39 bound with the fusion peptide 1 (^{815}RSFIEDLLFNKVTLAD^{830}) but not with the other peptides. Among them, VSM7-28 had the most potent binding to the fusion peptide 1 (EC_{50}, 0.4126 μg/mL), followed by VSM7-75 (EC_{50}, 0.7782 μg/mL) and VSM6-39 (EC_{50}, 35.7 μg/mL) (Fig. 1E, Supplemental Table S2). SCM14-3 had weak binding with the heptad repeat 1 peptide (^{971}GAISSVLNDILSRLDKVEAEVQI^{993}). VSM6-5 did not bind to the fusion peptide 1, fusion peptide 2, and heptad repeat 1 peptide, indicating that it recognized an unknown conserved epitope (Fig. 1D and E, Supplemental Fig. S3).

We further characterized the antibody germline by using IMGT/V-QUEST. The heavy chains of the five mAbs were from IGHV3, three of which (VSM7-28, VSM6-5, and VSM6-39) shared the same germline of VH3-30 (Supplemental Table S3), one of the most prevalent genes in SARS-CoV-2 mAbs (Kreer et al., 2020; Andreano et al., 2021). These mAbs had different light chains: four of them (IGKV1-12, IGKV3-15, IGKV3D-15, and IGKV4-1) had κ chains, and one (IGLV1-47) had a λ chain. The heavy-chain complementarity-determining region 3 (H-CDR3) was 8–19 amino acids in length (median, 17 aa), and L-CDR3 was 9–11 amino acids in length (median, 10 aa). The nucleotide sequences encoding the antibody *VH* were 84.1%–95.3% identical to the germline (median, 91.5%), and the *VL* genes were 92.3%–96.1% identical to their germline sequences (median, 93.0%) (Supplemental Table S3), indicating that these mAbs underwent a high level of somatic mutations. In a previous report, low level of somatic mutations were observed in the antibodies elicited in the early response to SARS-CoV-2 infections (Zost et al., 2020). Therefore, the mAbs tested in this study were probably produced after the priming by endemic coronavirus infection, before further boosted by SARS-CoV-2 infection or vaccination.

To further assess the role of the somatic mutations in the maturation of these mAbs, we generated germline reverted antibodies (gHgL) and compared their binding activity with the mature ones (mHmL). In addition, heavy or light chain switched antibodies (gHmL or mHgL) were also constructed for comparison with each other. All the gHgL antibodies had no binding with the prototype SARS-CoV-2 spike S2 protein, but chain-switched antibodies VSM7-28/gHmL (EC_{50}, 2.704 μg/mL), VSM7-75/mHgL (EC_{50}, 0.6925 μg/mL), and VSM6-39/mHgL (EC_{50}, 0.5656 μg/mL) had modest binding potency (Fig. 1F, Supplemental Table S4). These findings suggest that the somatic mutations of both heavy and light chains are required for the potent binding activity of these antibodies.

In summary, we isolated and characterized five spike S2-binding mAbs with broad reactivity with multiple coronaviruses from humans and animals. As these mAbs bind to at least three distinct epitopes, it is worthwhile to determine their accurate binding sites in future studies. It has been reported that an S2 booster after two doses of a full-length S DNA vaccine can induce a broader mAb response in mice, compared to a booster of the same S DNA vaccine (Ng et al., 2022). This study implies that the S2 region could be an immunogen for eliciting broad-spectrum antibodies against multiple coronaviruses. Mapping and identifying the epitopes on S2 subunit will facilitate the development of mAbs-based intervention strategies and antigen designs toward a broad immune response. Since these mAbs broadly react with the S2 of some or all of the 25 coronaviruses from humans and animals tested in this study, it is worth testing more viruses and the combination of mAbs recognizing different epitopes, which may further broaden the binding profiles of these mAbs. Coronavirus spillover may occur again in the future. The

mAbs targeting the S protein, especially receptor binding domain (RBD), have been proven essential in controlling and treating COVID-19. However, from the perspective of developing methods for diagnoses and surveillance purposes, the variable nature of RBD makes it unsuitable as a target. The nucleocapsid protein is the most common target of the commercial antigen test kits. This is because the nucleocapsid proteins rarely share the same motifs in different coronaviruses, which minimizes the likelihood of cross-reactivity and enhances the specificity of detection. However, this specificity also limits their detection spectrum. In contrast, the S2 region is highly conserved across most coronaviruses and contains several common motifs, including the fusion region 1 and heptad repeat 1 (Supplemental Fig. S4). Consequently, these S2-targeting mAbs, possess an advantage over current reagents in the diagnosis of pan-coronavirus infections. Collectively, we have identified five mAbs that broadly recognize human and animal coronavirus, laying a foundation for the development of reagents and broad-spectrum vaccines for the surveillance of unknown coronavirus infections.

Footnotes

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