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# A spike protein S2 antibody efficiently neutralizes the Omicron variant

Jia Hu[1,4]✉, Xiang Chen[2,4], Xingbing Lu[3,4], Lijuan Wu[3], Liyuan Yin[1], Lingling Zhu[1], Hao Liang[1], Feng Xu[1] and Qinghua Zhou[1]✉



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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant B.1.1.529, also named Omicron, is becoming the main circulating strain in many countries worldwide and brings new challenges to preventing COVID-19 [1–5]. The latest data show that the Omicron variant contains more than 50 mutations. Among them, there are 32 mutations in the spike protein [6], which is the key component that determines the infectivity and antigenicity of the virus. Furthermore, 15 mutations are located in the receptor-binding domain (RBD), which is key for viral-cell interactions mediated by angiotensin-converting enzyme 2 (ACE-2) [7, 8]. Due to the large number of mutations, especially in the RBD domain, there is concern that the Omicron variant can escape antibody neutralization induced by the current coronavirus disease 2019 (COVID-19) vaccines or neutralizing antibodies. This concern is supported by the fact that the Omicron variant exhibits significantly more neutralization resistance [2–4, 9]. In particular, RBD mutations are most prevalent. As reported previously, the RBD region of the S1 protein plays a critical role in novel coronavirus-infected cells, directly binding to the host receptor ACE2 [7, 8]. Therefore, almost all neutralizing antibodies have been designed to target the S1 protein [10]. However, in addition to the S1 subunit, the SARS-CoV-2 surface S protein comprises the S2 subunit, which mediates viral cell membrane fusion, though the probability of mutation in the S2 region is relatively low [11]. Based on these findings, we screened and validated S2 protein antibodies against the Omicron variant and explored the possibility of developing an S2 antibody to protect against SARS-CoV-2 variants.

Antibody phage/yeast display libraries are a powerful tool for generating human antibodies against tumors and infectious diseases [12–14]. Previously, we successfully used this technology to develop diagnostic and therapeutic reagents against a series of tumor targets [15, 16] and neutralizing antibodies against the RBD and NTD of SARS-CoV-2 (unpublished data). In this work, we screened a specific scFv against spike protein S2 of SARS-CoV-2. By validating specific binding with S2 and neutralization of Omicron pseudovirus, we identified an S2-specific antibody, HCLC-031, that efficiently neutralizes the Omicron variant in vitro. Our findings indicate that the S2 subunit is a potential target for the development of neutralizing antibodies and vaccines against SARS-CoV-2 Omicron.

To generate S2-specific antibodies, we screened an scFv phage display library derived from four recovered COVID-19 patients using recombinant SARS-CoV-2 S2 (Fig. 1A). After three rounds of panning, 192 top clones were picked for ELISA, and then the top 50 clones according to OD value were chosen for sequencing (Fig. 1B). Finally, 10 clones containing unique sequences were obtained. To validate whether the S2 antibodies identified through library screening bind to the S2 domain, we transiently expressed S2 on the surface of 293-T cells and detected the binding between antibodies and S2 by flow cytometry. We found that 90% (9/10) of the clones could specifically bind to S2, but not the RBD, on the surface of cells (Fig. 1C).

The Omicron variant is more transmissible and infectious. Although several anti-RBD antibodies we isolated showed strong neutralization of the original SARS-CoV-2 and Delta variants, their neutralizing potential was largely reduced with the Omicron variant (unpublished data). Because the S2 subunit is considered a potential target for neutralizing antibodies, we next assessed the neutralization activity of anti-S2 antibodies toward Omicron pseudovirus. The neutralizing ability of HXLC-031 toward Delta pseudovirus was significantly decreased, though it still exhibited comparable neutralizing ability against Omicron pseudovirus (IC50 = 9.61 nM) and original SARS-CoV-2 pseudovirus (IC50 = 5.18 nM) (Fig. 1D, E). In addition, sequence alignment showed that the CDR3 region of the heavy chain and light chain of HXLC-027 (Fig. 1F), which exhibit neutralizing ability against the Omicron strain (IC50 = 74.06 nM), differs from that of HXLC-031, indicating distinct anti-S2 antibody epitopes suitable for the development of neutralizing antibodies against the Omicron variant.

In summary, COVID-19, caused by SARS-CoV-2 and its variants, threatens human health worldwide. The spike protein on the surface of SARS-CoV-2 plays a critical role in viral infection of target cells and is divided into S1 and S2 subunits. The S1 subunit, with a high mutation rate, recognizes and binds to the receptor on the surface of target cells. In contrast, the S2 subunit, with a relatively low tolerance for sequence variation, mediates viral envelope and target cell membrane fusion. The Omicron variant, which was identified at the end of 2021, has been proven to exhibit significantly strong resistance to neutralization by most current neutralizing antibodies and vaccines [3, 9] due to the large number of mutations. However, most current therapeutic antibodies focus on the poorly conserved RBD domain of the S1 subunit, targeting neutralizing epitopes on the more conserved S2 subunit may provide a great option for the development of neutralizing antibodies against Omicron. Based on these findings,

[1]Lung Cancer Center, West China Hospital, Sichuan University, Chengdu, China. [2]Institute for Immunology, Tsinghua University, Beijing, China. [3]Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China. [4]These authors contributed equally: Jia Hu, Xiang Chen, Xingbing Lu. ✉email: hujia627@wchscu.cn; zhouqh135@163.com

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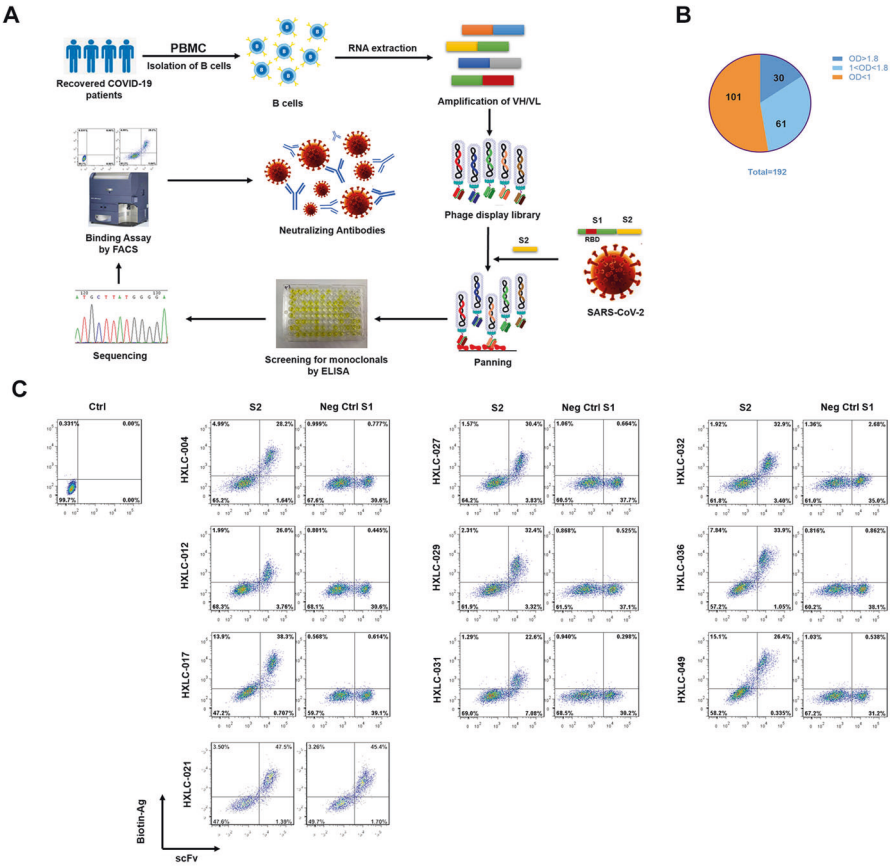


**E**

| | WT (nM) | Beta (B.1.351) (nM) | Delta (B.1.617.2) (nM) | Omicron (B.1.1.529) (nM) |
|---|---|---|---|---|
| HXLC-027 | 12.22 | 17.17 | 66.44 | 74.06 |
| HXLC-031 | 5.18 | 10.60 | 24.19 | 9.61 |
| HXLC-032 | 32.18 | 58.26 | 64.38 | $1.3x10^4$ |

**F**

| Ab name | $CDR3_H$ | $CDR3_L$ |
|---|---|---|
| HXLC-027 | AAWDDRLNGRV | VRERWLQLHY |
| HXLC-032 | SSYAGSASWV | GRSLNN |

**Fig. 1** Development of efficient neutralizing antibodies for Omicron variant. **A** Flow chart of the construction and panning of the scFv phage display library. **B** The pie chart shows that 192 clones were selected for ELISA; the top 50 clones according to OD value were then sequenced. **C** To verify whether the antibodies produced specifically bind to S2, 293 T cells were transfected with each scFv expression plasmid. After 48 h, the cells were incubated with biotinylated RBD or S2. Finally, binding between antibodies and S2 was detected by flow cytometry. **D** HXLC-031 efficiently neutralizes Omicron variant pseudovirus. Omicron variant pseudovirus was incubated with 10-fold serially diluted S2-specific antibodies. The mixtures were mixed with Huh-7 cells. After 48 h of incubation, the neutralization rate of each antibody was evaluated using a luciferase assay system. **E** The chart shows the IC50 of S2-specific antibodies (HXLC-027, HXLC-031 and HXLC-032) for SARS-CoV-2 and variant (beta, delta and submicron) pseudoviruses. **F** CDR3-H and CDR3-L show the amino acid sequences of CDR3 for both chains of the antibodies HXLC-027 and HXLC-032

we screened specific scFvs against spike protein S2 of SARS-CoV-2. By validating specific binding with S2 and neutralization of Omicron pseudovirus, we identified an S2-specific antibody, HCLC-031, that efficiently neutralizes the Omicron variant in vitro. Our findings indicate that the S2 subunit is a potential target for the development of neutralizing antibodies and vaccines against the SARS-CoV-2 Omicron variant.

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## AUTHOR CONTRIBUTIONS

HJ designed and performed the experiments and wrote the manuscript. CX designed the experiments, analyzed the data and wrote the manuscript; LXB performed parts of the experiments and analyzed the data. WLJ, YLY and ZLL participated in parts of the experiments. LH assisted with data analysis. XF and ZQH supervised this study and edited the manuscript.

## COMPETING INTERESTS

The authors declare no competing interests.

## ADDITIONAL INFORMATION

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**Correspondence** and requests for materials should be addressed to Jia Hu or Qinghua Zhou.