Genes & Diseases (2023) 10, 664–667

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

RAPID COMMUNICATION

Two human monoclonal SARS-CoV-2 antibodies that maintain neutralizing potency against the SARS-CoV-2 Omicron BA.1 and BA.2 variants

The pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to sweep the globe with devastating consequences on human lives and world economy. As an RNA virus, SARS-CoV-2 has a relatively high mutation rate and is rapidly evolving. Thus, new SARS-CoV-2 variants continued to emerge, 5 of which were designated by the World Health Organization (WHO) as variants of concern (VOCs), Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and, recently, Omicron (B.1.1.529).[1] First identified in Botswana and South Africa in November 2021, the original Omicron, BA.1, then spread to every corner of the world and quickly replaced the previously dominant Delta strain to become the most prevalent SARS-CoV-2 circulating variant across the world. BA.1 is reported to escape most therapeutic monoclonal antibodies against SARS-CoV-2.[2] Consistently, sera from convalescent donors and vaccinated individuals contain very low to undetectable levels of neutralizing antibodies against BA.1.[3] Therefore, new therapeutic agents are urgently needed.

By phage display technique, antibody libraries generated from RNAs extracted from peripheral lymphocytes of fully or booster-vaccinated individuals in our research group were constructed. The recombinant wild-type (WT) receptor-binding domain (RBD) of the SARS-CoV-2 spike (S) glycoprotein was used as the target protein to screen the phage antibody library for potential hits. A panel of high-affinity binders to the RBD in single chain variable fragment (scFv) format were identified and two high-affinity candidate scFvs were converted into and expressed as two full-size IgG1 antibodies, XK01 and XK02. XK01 and XK02 bound strongly to the WT SARS-CoV-2 RBD with half-maximal effective concentration (EC_{50}) values of 0.012 and 0.018 μg/mL, respectively, as measured by enzyme-linked immunosorbent assay (ELISA), suggesting both monoclonal antibodies have a higher binding avidity than that of ACE2 (EC_{50} = 0.048 μg/mL) (Fig. 1A–C). To verify this result and further evaluate the binding affinity, we monitored real–time association and dissociation of XK01 and XK02 binding to the WT SARS-CoV-2 RBD (RBD_{WT}) by surface plasmon resonance (SPR). XK01 and XK02 exhibited tight binding to RBD_{WT} with equilibrium dissociation constants (K_D) of 93 nM for XK01 and of 109 nM for XK02, respectively, both of which are superior to that of ACE2 with RBD_{WT} (K_D = 348 nM) (Fig. 1D–F).

Based on the data obtained by ELISA and SPR, both XK01 and XK02 monoclonal antibodies probably have potential neutralization efficacy against SARS-CoV-2. As expected, both antibodies showed potent neutralizing activities against virus pseudotyped with the WT SARS-CoV-2 S with half-maximal inhibitory concentration (IC_{50}) values of 0.098 μg/mL for XK01 and 0.072 μg/mL for XK02, respectively (Fig. 1G, H).

In the case of BA.1, the binding kinetics of the Omicron SARS-CoV-2 RBD ($RBD_{BA.1}$) to the ACE2 receptor was measured by SPR, which showed that $RBD_{BA.1}$ bound potently to ACE2. The K_D values of $RBD_{BA.1}$ with ACE2 were 245 nM for XK01 and 438 nM for XK02, respectively (Fig. 1I, J), indicating the binding affinity of both antibodies for $RBD_{BA.1}$ was higher than that of the ACE2 receptor (K_D = 527 nM) (Fig. 1K). Notably, this observation is very similar to that is observed for RBD_{WT}. Unexpectedly, both monoclonal antibodies showed no loss but increased potency of neutralization against BA.1 S pseudotyped virus with IC_{50} values of 0.011 μg/mL for XK01 and 0.024 μg/mL for XK02, respectively (Fig. 1L, M).

Soon after the emergence and global spread of BA.1, BA.2 has initiated outcompeting BA.1 and has become the

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2022.05.027

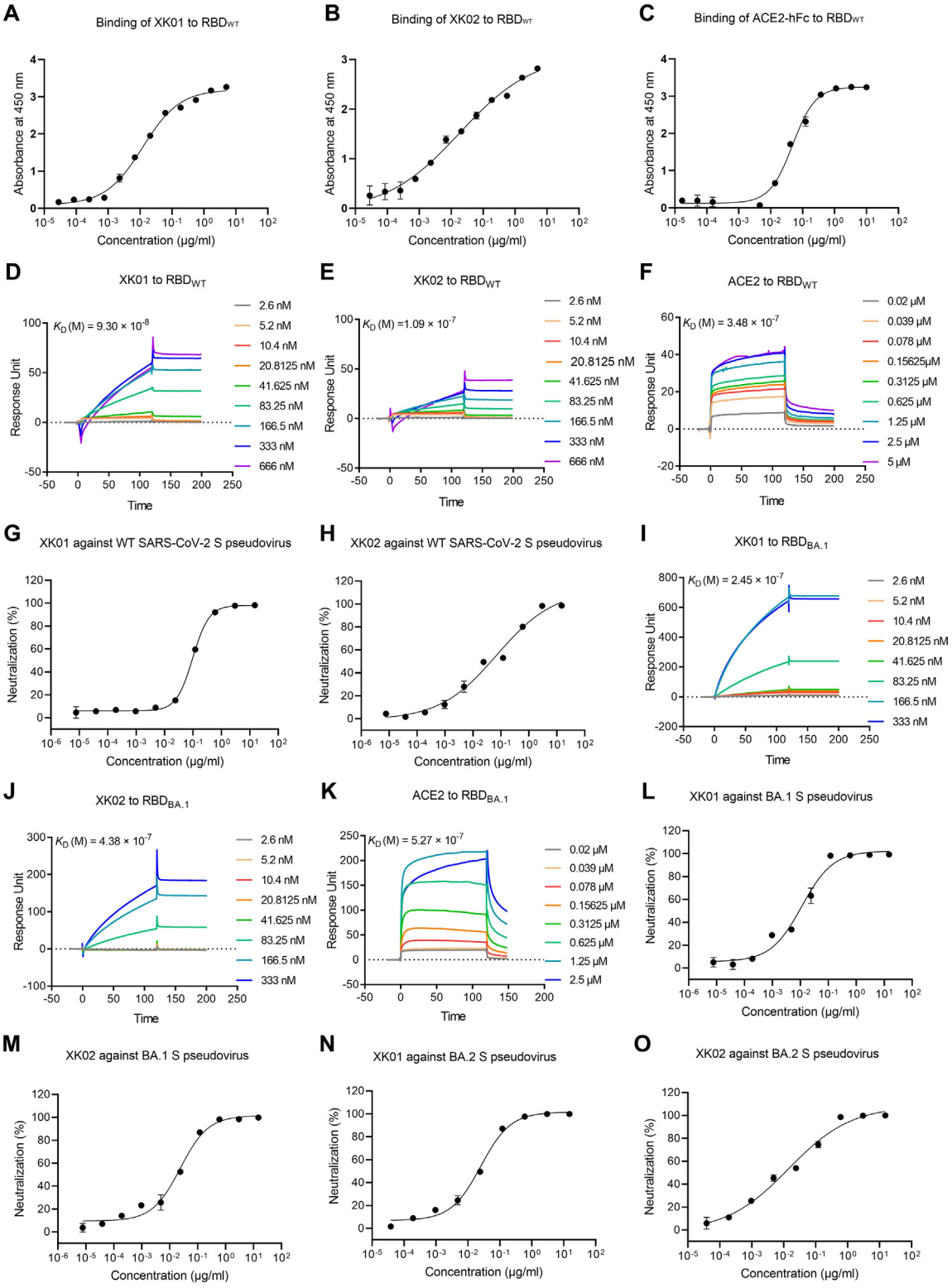

Figure 1 Binding and neutralization activities of XK01 and XK02 against SARS-CoV-2 wild-type (WT) and Omicron variants. **(A—C)** Binding curve of XK01 (A), XK02 (B) and ACE2-hFc (C) to the WT SARS-CoV-2 RBD (RBD_{WT}) measured by ELISA. **(D—F)** Binding kinetic of XK01 (D), XK02 (E) and ACE2-hFc (F) with immobilized RBD_{WT}, measured by SPR. The experiments were performed in duplicate with similar results and a representative experiment is shown. **(G, H)** WT SARS-CoV-2 S pseudovirus neutralizing activities of XK01 (G) and XK02 (H). **(I—K)** Binding kinetic of XK01 (I), XK02 (J) and ACE2-hFc (K) with immobilized $RBD_{BA.1}$, measured by SPR. The experiments were performed in duplicate with similar results and a representative experiment is shown. **(L, M)** Potent neutralization of Omiron BA.1 S pseudovirus by XK01 (L) and XK02 (M). **(N, O)** Potent neutralization of Omiron BA.2 S pseudovirus by XK01 (N) and XK02 (O).

dominant Omicron subvariant circulating worldwide, which prompts us to evaluate the ability of XK01 and XK02 monoclonal antibodies to neutralize the BA.2 subvariant. Still, both monoclonal antibodies maintained high neutralizing potency against virus pseudotyped with BA.2 S with IC_{50} values of 0.025 μg/mL for XK01 and 0.015 μg/mL for XK02, respectively (Fig. 1N, O), despite there are as many as 20 different mutations between BA.2 and BA.1 S proteins.

Unfortunately, XK01 and XK02 did not function synergistically with each other to enhance neutralization activities against WT and Omicron BA.1 SARS-CoV-2 (data not shown). Furthermore, both XK01 and XK02 exhibited little neutralizing potency against SARS-CoV S pseudovirus (data not shown) at the highest tested concentration (15.0 μg/mL). Structural studies clearly revealed that the RBD of SARS-CoV-2 is composed of a core subdomain and an external subdomain also known as receptor binding motif (RBM) which loops out of the core subdomain to directly engage ACE2.[4] The amino acid sequence identity of the RBD core subdomain and RBM between SARS-CoV and SARS-CoV-2 is more than 85% and less than 50%, respectively.[4] Almost all isolated neutralizing antibodies recognizing epitopes in the conserved core subdomain can cross-neutralize SARS-CoV-2 and SARS-CoV infections and are thought to maintain neutralizing potency against previous SARS-CoV-2 VOCs, while those targeting the RBM region with high sequence variations always exhibit no or limited cross—neutralization activity against both viruses. Based on these observations, it can be speculated that both XK01 and XK02 monoclonal antibodies neutralize WT and Omicron SARS-CoV-2 infections by competing with the ACE2 receptor for binding to the RBM and thus blocking attachment of the virus to the host cell surface, although the exact mechanisms of action of both neutralizing antibodies remain to be defined.

Universal vaccine and broadly neutralizing antibodies and/or variant-specific vaccines and neutralizating antibodies are urgently needed to counteract the emerging SARS-CoV-2 variants of immune escape.[5] S proteins of the Omicron BA.1 and BA.2 variants have approximately 37 and 31 mutations compared with the ancestral SARS-CoV-2 virus. The RBDs alone have 15 and 16 mutations, respectively, of which 5 and 8 lie in the conserved core domains, respectively, and the rests are in the RBM regions, enabling both Omicron subvariants to escape the majority of existing RBD-targeted neutralizing antibodies. Furthermore, use and development of broadly neutralizing antibodies may be challenged by the emergence of growing mutations in the conserved core of current and future emerging variants under the immune pressure exerted by vaccines or previous infections. For example, Sotrovimab (VIR-7831), the prototypic member of a canonical class of broadly neutralizing antibodies, has markedly reduced efficacy against BA.2 while maintaining much of it's neutralizating activity against BA.1 in pseudovirus neutralization assays, and thus its emergency use authorization has been withdrawn. Therefore, discovering variant-specific neutralizing antibodies is a more feasible strategy. Here, we isolated two such neutralizing antibodies, for which affinity maturation can be used to further improve their affinity and increase the virus neutralization potency.

In conclusion, two SARS-CoV-2 neutralizing antibodies selected from vaccine recipients by phage display are successfully isolated. Importantly, these two monoclonal antibodies maintain high neutralizing potency against both BA.1 and BA.2, the two most common subvariants of Omicron. The identification of these two neutralizing antibodies in this study provides promising starting points to be added to the limited list of antibodies with a high potential to effectively counteract the dominant circulating SARS-CoV-2 Omicron VOCs.

Author contributions

L.D. and H.W. conceived the study and designed the experiments; Q.Z., L.D., Z.J. and T.G. performed the experiments with the assistance of B.Z., J.L., Y.Z., and S.Z.; Q.Z., L.D., Q.Z., L.D., L.Y., and H.W. analyzed the data; L.D., and H.W. wrote the manuscript with input from all authors. All authors read and approved the final version of the manuscript.

Conflict of interests

L.D., Z.J. and H.W. are listed as inventors on pending patent applications for XK01 and XK02. Z.J. is an employee of KMD Bioscience (Tianjin) Co, Ltd. The other authors declare that they have no competing interests.

Funding

This work was supported by The National Natural Science Foundation of China (No. 81871312), the Key Scientific and Technological Project of Henan Province (No. 222102310025), The International Joint Research Laboratory for Recombinant Pharmaceutical Protein Expression System of Henan (KFKTYB202210), and the 111 Project (No. D20036).

Acknowledgements

We would like to thank all study participants who donated blood to our research. We would like to thank Zhenlin Fan, Duan Li, Yunwei Lou, Chunlei Guo, Mengchao Cui, Ting Zhuang and Xin Li and for valuable advice and helpful discussions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.05.027.

References

1. He X, Hong W, Pan X, et al. SARS-CoV-2 *Omicron* variant: characteristics and prevention. *MedComm*. 2021;2(4):838—845.
2. Cao Y, Wang J, Jian F, et al. *Omicron* escapes the majority of existing SARS-CoV-2 neutralizing antibodies. *Nature*. 2022;602(7898):657—663.
3. Planas D, Saunders N, Maes P, et al. Considerable escape of SARS-CoV-2 *Omicron* to antibody neutralization. *Nature*. 2022;602(7898):671—675.
4. Duan L, Zheng Q, Zhang H, et al. The SARS-CoV-2 spike glycoprotein biosynthesis, structure, function, and antigenicity: implications for the design of spike-based vaccine immunogens. *Front Immunol*. 2020;11:576622.
5. Cameroni E, Bowen JE, Rosen LE, et al. Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. *Nature*. 2022;602(7898):664—670.

Qianqian Zheng [a,b,1], Liangwei Duan [a,b,*,1], Zhihua Jiang [c,1], Tingxuan Gu [d,e,1], Bojie Zhang [a,b], Jiaoyang Li [a,b], Yang Zhang [a,b], Shiyu Zhang [a,b], Yinming Liang [a,b], Hui Wang [a,b,**]

[a] *Henan Key Laboratory of Immunology and Targeted Drugs, School of Laboratory Medicine, Xinxiang Medical University, Xinxiang, Henan 453003, China*

[b] *Henan Collaborative Innovation Center of Molecular Diagnosis and Laboratory Medicine, Xinxiang Medical University, Xinxiang, Henan 453003, China*

[c] *KMD Bioscience (Tianjin) Company Limited, Tianjin 301723, China*

[d] *Department of Pathophysiology, School of Basic Medical Sciences, Academy of Medical Science, College of Medicine, Zhengzhou University, Zhengzhou, Henan 450001, China*

[e] *China-US (Henan) Hormel Cancer Institute, Zhengzhou, Henan 450008, China*

*Corresponding author. School of Laboratory Medicine, Xinxiang Medical University, 601 Jinsui Road, Xinxiang, Henan 453003, China. Fax/Tel.: +86 373 3831203.

**Corresponding author. School of Laboratory Medicine, Xinxiang Medical University, 601 Jinsui Road, Xinxiang, Henan 453003, China. Fax/Tel.: +86 373 3831203.

E-mail addresses: duanliangwei@xxmu.edu.cn (L. Duan), wanghui@xxmu.edu.cn (H. Wang)

8 March 2022

Available online 7 June 2022

[1] These authors contributed equally to this work.