Structural Insight into SARS-CoV-2 Broadly Neutralizing Antibody, NT-193

The ongoing spread of the COVID-19 pandemic has caused many deaths and injuries worldwide. Monoclonal antibodies against the spike protein have been developed as therapeutic agents against SARS-CoV-2 and some are now in clinical use in many countries. We have identified a potent SARS-CoV-2 neutralizing antibody, NT-193, which can also neutralize SARS-CoV-1. The crystal structure of NT-193 complexed with SARS-CoV-2 RBD was determined using the X-ray diffraction dataset collected at BL-17A. The structure clearly explains that the NT-193 binding mode is reasonable for both potent neutralization and cross-reactivity. This structural insight into NT-193 recognition will substantially contribute to the rational design of antibodies in future.

Human monoclonal antibodies neutralizing the SARS-CoV-2 virus have been used in therapeutic agents including antibody cocktail therapy. Some of the SARS-CoV-2 variants that have emerged so far have acquired the ability to escape from neutralizing antibodies, thus the identification of cross-neutralizing antibodies that do not lose their activity to variants is required for the development of new therapeutic agents.

In this study, we identified a highly potent SARS-CoV-2 neutralizing antibody, NT-193, from TC-mAb mice. NT-193 has high neutralizing activity against SARS-CoV-2 variants (alpha and gamma variants) and can also neutralize SARS-CoV-1. Notably, NT-193 antibody was found to be unique in that its neutralizing activity against SARS-CoV-2 is enhanced by introducing an IgG3-type constant region. The IgG3-type NT-193 antibody also shows strong neutralizing activity against other SARS-related coronaviruses, SARS-CoV-1 and WIV-1, suggesting that this antibody is effective against a wide range of SARS-related viruses. *In vivo* infection experiments using hamsters revealed that the antibody exhibits superior prophylactic and therapeutic effects that are comparable to those of antibody drugs that have been clinically applied to date.

NT-193 antibody shows high binding activity to the receptor binding site (RBD) of the spike protein. Hence, we conducted crystallization of the NT-193 Fab fragment in complex with SARS-CoV-2 RBD. The crystal was successfully obtained in the condition containing PEG8000 as a precipitant [**Fig. 1(A)**]. The X-ray diffraction dataset up to 2.8 Å was collected at BL-17A. The structure of NT-193-RBD complex was determined by the molecular replacement method. NT-193 binds to the top areas of the RBD and showed similar binding mode with Angiotensin converting enzyme 2 (ACE2) [**Fig. 1(B)** and **(C)**]. NT-193 recognizes both the ACE2 binding area and highly conserved region of coronaviruses. NT-193 mainly uses light chains for recognizing the ACE2 binding area and heavy chains for the conserved area. It was thus clarified that the recognition mode of NT-193 by the light chains and the heavy chains contributes to the potent neutralization and the cross-reactivity, respectively [1].

The NT-193 antibody is expected to be developed as a therapeutic agent against SARS-related coronaviruses, including SARS-CoV-1, in addition to the therapeutic agents against new SARS-CoV-2 variants. It has the potential to become an antibody drug that can contribute to the treatment of emerging and re-emerging infectious diseases in the future.

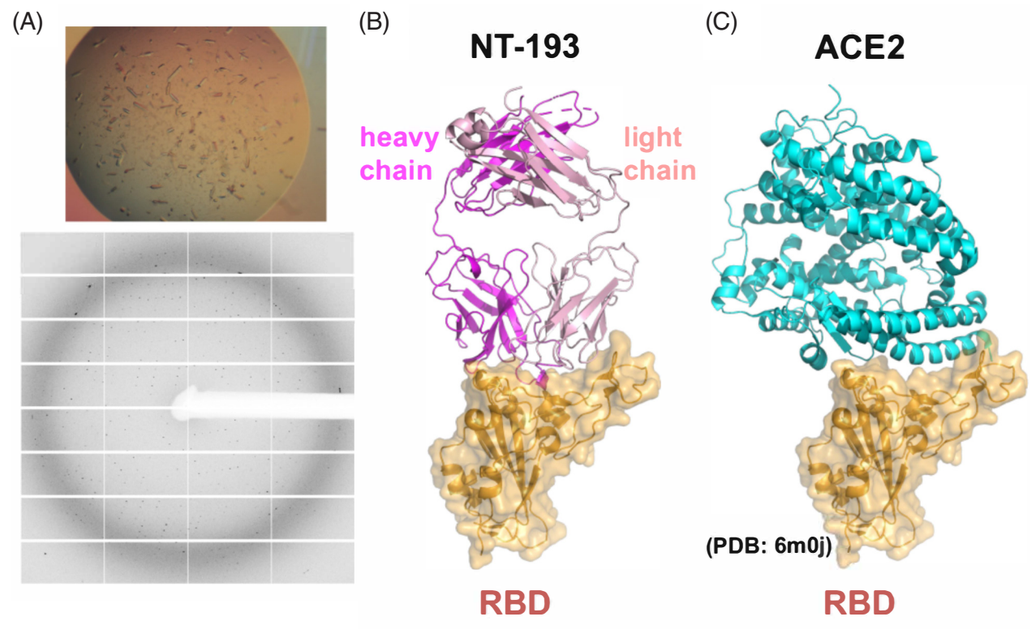

Figure 1: (A) Crystal picture (top) and X-ray diffraction image (bottom) of NT-193 and RBD complex. **(B)** Overall structure of NT-193 and RBD complex. **(C)** Overall structure of ACE and RBD complex (PDB ID: 6m0j) in the same orientation as **(B)**.

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BEAMLINES

BL-17A and BL-1A

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