SARS-CoV-2 Omicron BA.2 Variant Evades Neutralization by Therapeutic Monoclonal Antibodies

Hao Zhou¹.*, Takuya Tada¹.*, Belinda M. Dcosta¹.* and Nathaniel R. Landau¹.#

Affiliation:

¹Department of Microbiology, NYU Grossman School of Medicine, New York, NY, USA.

*These authors contributed equally.

#Corresponding author:

Nathaniel R. Landau, Ph.D.
NYU Grossman School of Medicine
430 East 29th Street, Alexandria West Building, Rm 509, New York, NY 10016
Email: nathaniel.landau@med.nyu.edu
Phone: (212) 263-9197
Keywords: SARS-CoV-2 variants, Omicron BA.2, monoclonal antibodies, spike protein, Sotrovimab, Evusheld.
Abstract

Monoclonal antibody therapy for the treatment of SARS-CoV-2 infection has been highly successful in decreasing disease severity; however, the recent emergence of the heavily mutated Omicron variant has posed a challenge to this treatment strategy. The Omicron variant BA.1 has been found to evade neutralization by the Regeneron and Eli Lilly therapeutic monoclonal antibodies, while Sotrovimab and the Evusheld monoclonal antibody cocktail retain significant neutralizing activity. A newly emerged variant, Omicron BA.2, containing the BA.1 mutations plus an additional 6 mutations and 3 deletions, 3 of which lie in the receptor binding domain, has been found to be spreading with increased transmissibility. We report here, using a spike protein-pseudotyped lentivirus assay, that Omicron BA.2 is not neutralized with detectable titer by any of the therapeutic monoclonal antibodies, including Sotrovimab and the Evusheld monoclonal antibodies. The results demonstrate the difficulty of identifying broadly neutralizing monoclonal antibodies against SARS-CoV-2 and the importance of the T cell response from which immunoevasion is more difficult.
Main text

The BA.2 variant of the highly mutated Omicron SARS-CoV-2 variant identified in late 2021 in patients in countries including Denmark, South Africa and India (1) and since then, in more countries including the United States. BA.2 has been found to be 1.5-fold more transmissible than the already highly transmissible BA.1 variant and is thus expected to continue to increase in prevalence (2). The BA.2 spike protein has all of the mutations of BA.1 plus an additional 6 mutations and 3 deletions, three of which lie in the receptor binding domain (3) (Supplemental Figure 1A). Of the monoclonal antibodies authorized by the Food and Drug Administration for emergency use (4), several were found to be largely inactive against BA.1 while the GlaxoSmithKline/Vir monoclonal antibody Vir-7831 (Sotrovimab) (5) and AstraZeneca Evusheld cocktail consisting of monoclonal antibodies AZD8895 (Tixagevimab) and AZD1061 (Cilgavimab) retain neutralizing titer, albeit with a one to two log decrease in neutralizing titer as compared to D614G (6-8). Evusheld (Tixagevimab/Cilgavimab) is formulated for slow release to be used prophylactically primarily in immunocompromised individuals (9).

In this report, we tested the ability of the therapeutic monoclonal antibodies to neutralize the BA.2 variant, with particular interest in Vir-7831 (Sotrovimab) and Evusheld (Tixagevimab/Cilgavimab), given their ability to neutralize BA.1. Neutralizing antibody titers against the parental D614G variant, Delta, BA.1 and BA.2 were determined using spike protein-pseudotyped lentiviruses. The lentiviral pseudotype assay has been found to yield neutralizing antibody titers that are in closely agreement with those
determined with the live virus plaque reduction neutralization test (PRNT) (10). To understand the mechanism by which the variants evade monoclonal antibody neutralization, we tested viruses with spike proteins containing the individual RBD point mutations of BA.2 for their effect on neutralization by each antibody.

Antibody neutralization curves and the IC50s calculated from the curves for D614G, Delta, BA.1 and BA.2 pseudotyped viruses are shown in Figures 1A and B. All of the monoclonal antibodies potently neutralized the D614G virus. The monoclonal antibodies also neutralized the Delta variant except for LY-CoV555 (Bamlanivimab) for which the neutralizing titer was significantly decreased (39-fold) compared to D614G, consistent with previous reports (11). REGN10933 (Casirivimab), REGN10987 (Imdevimab), REGN-CoV-2 (Casirivimab/Imdevimab), LY-CoV555 (Bamlanivimab), LY-CoV016 (Etesevimab), LY-CoV555 + LY-CoV016 (Bamlanivimab/Etesevimab), AZD8895 (Tixagevimab), AZD1061 (Cilgavimab) all lacked detectable neutralizing titer against BA.1. The Evusheld cocktail and Vir-7831 (Sotrovimab) monoclonal antibody had neutralizing titers were decreased by 14-fold and 108-fold compared to D614G, consistent with previous reports. Strikingly, none of the monoclonal antibodies detectably neutralized BA.2 (Figure 1A and B).

Analysis of viruses pseudotyped by spike proteins with the individual novel RBD mutations of BA.2 provided insight into the cause of the decrease in neutralization titers by the monoclonal antibodies (Supplemental Figure 1B and C). The structural models of the spike protein:antibody complexes are shown with mutations previously found to
cause >5-fold decrease in titer against BA.1 together with the BA.2 mutations identified here that cause >2-fold decreased in titer. For the most part, the active mutations in BA.1 map to the sites of interaction with the antibodies while the additional active mutations in BA.2 (T376A, D405N and R408S) lie distal to the interaction sites. Presumably, these mutations act at a distance by affecting the conformation of the RBD spike.

**Discussion**

We report here that none of the therapeutic monoclonal antibodies tested here neutralized the Omicron BA.2 variant with titers detectable in a highly sensitive assay. Vir-7831 (Sotrovimab) and the monoclonal antibodies that constitute Evusheld (Tixagevimab/Cilgavimab), which have good significant titers against BA.1, failed to detectably neutralize BA.2 spike protein-pseudotyped virus. Consistent with previous reports, BA.1 was resistant to neutralization by Regeneron and Eli Lilly antibody cocktails but partially susceptible to neutralization by Vir-7831 (Sotrovimab) and the Evusheld (Tixagevimab/Cilgavimab) monoclonal antibody cocktail (6-8). Evasion of BA.2 from antibody neutralization is caused in part by the additional mutations in the RBD in combination with the additional N-terminal domain mutations (not tested here).

Since the identification of the Omicron BA.2 variant in several countries including Denmark, South Africa and India, the variant has been identified in infected individuals in additional countries and mathematical models predict that its increased transmissibility will result in its continued increase in prevalence (2). While monoclonal
antibody therapy has been highly effective at preventing hospitalization and death, the emergence of the Omicron variant poses a major threat to the efficacy of current treatments. As BA.2 prevalence increases, current monoclonal antibodies may become less effective for the treatment of COVID-19. Therapies that target BA.2 and potential future variants that may emerge are therefore of great importance. The findings presented here demonstrate the difficulty of finding a pan-neutralizing monoclonal antibody against SARS-CoV-2. Our findings support the importance of small molecule drugs such as Molnupiravir (12) and Nirmatrelvir (13) that act on viral targets outside of the highly mutable spike protein (14). They also provide further rationale to protect against severe disease by vaccination which induces cross-reactive antibodies and T cell responses against spike protein epitopes that are not mutated in the Omicron variants.
Material and Methods

Plasmids
The SARS-CoV-2 spike expression vector (Omicron BA.2) was generated by overlap extension PCR with the two fragments amplified with external primers containing a Kpn-I and Xho-I sites and cloned into pcDNA6. Spike expression vectors with the individual mutations of the Omicron BA.2 RBD were generated by overlap PCR mutagenesis using the D614G spike expression vector pcCOV2.Δ19.D614G as a template.

Cells
293T, ACE2.293T and Vero cells were grown in Dulbecco’s Modified Eagle’s Medium with 10% fetal bovine serum at 37°C.

SARS-CoV-2 spike proteins lentiviral pseudotypes
SARS-CoV-2 spike protein pseudotyped lentivirus stocks were produced by cotransfection of 293T cells with pMDL Gag/Pol vector, plenti.GFP.nLuc and spike protein expression vectors which deleted 19 amino acid from cytoplasmic tail. After 2 days transfection, supernatants were harvested and concentrated by ultracentrifugation. The viruses were normalized for reverse transcriptase (RT) activity.

Antibody neutralization assay
Monoclonal antibodies were serially diluted (two-fold) and incubated with pseudotypes (MOI=0.2 on target cells). After 30 minutes incubation, the virus was added to target cells in a 96 well culture dish. After 2 days of infection, infectivity was developed by
Nano Glo substrate and luminescence was read in an Envision 2103 microplate luminometer.

**Data analysis**

All samples were tested in duplicate. Data were analyzed using GraphPad Prism 8 software. Analyses of the structures of the SARS-CoV-2 spike protein with antibody Fabs was performed with the PyMOL Molecular Graphics System, v2.1.1 (Schrödinger, LLC).

**Acknowledgements**

The work was funded by grants from the NIH to N.R.L. (DA046100, AI122390 and AI120898).

**Declaration of interests**

The authors declare no conflicting financial interests.
Figure legends

Figure 1. Lack of detectable neutralization of BA.2 by therapeutic monoclonal antibodies.

(A) Lentiviral pseudotyped viruses were generated as previously described using codon-optimized 19 amino acid deleted spike proteins (15). A fixed amount of virus, normalized for reverse transcriptase activity, was treated with 5-fold serially diluted monoclonal antibody, in duplicate, for 30 minutes and then used to infect ACE2.293T cells. Luciferase activity was measured after 24 hours. Neutralization curves are shown.

(B) The IC50 was calculated from the neutralization curves using GraphPad Prism 8 software.

(C) The location of mutations found to affect monoclonal antibody binding is shown on antibody:spike protein complexes. Complexes are visualized with PyMOL Molecular Graphics System, v2.1.1 (Schrödinger, LLC) software. Mutations in BA.1 previously reported to have >5-fold effect on neutralizing titer are shown in red. Mutations identified in this study with >2-fold effect are shown in purple.

Supplementary Figure 1. Neutralization of single point mutated virus (BA.2) by therapeutic monoclonal antibodies.

(A) The structure of the SARS-CoV-2 Omicron BA.2 spike is indicated. NTD, N-terminal domain; RBD, receptor-binding domain; RBM, receptor-binding motif; SD1 subdomain 1; SD2, subdomain 2; CS, cleavage site; FP, fusion peptide; HR1, heptad repeat 1; HR2, heptad repeat 2; TM, transmembrane region; IC, intracellular domain. Nobel mutation
found in BA.2 were shown in red. The mutations which were specific to BA.1 were shown in blue.

(B) Individual point mutated viruses were treated with 5-fold serially diluted monoclonal antibodies and then used to infect ACE2.293T cells. Luciferase activity was measured after 24 hours.

(C) The location of the point mutations found to cause >5-fold decrease in neutralizing titer is shown in red. The location of the point mutations found to cause >2-fold decrease in neutralizing titer is shown in blue.
References

1. CDC. Omicron Variant: What You Need to Know. 2022.


Figure 1

**Panel A**

Graphs showing the inhibition of different antibodies (REGN10933, REGN10987, Casirivimab, Ly-CoV555, Ly-CoV016, Bamlayvimab, Azd8985, Tixagevimab, and Vir-7831) against SARS-CoV-2 variants (D614G, Delta, Omicron BA.1, and Omicron BA.2). Each graph plots the percentage of virus inhibition against varying concentrations of antibodies.

**Panel B**

Table showing IC50 values for different antibodies against SARS-CoV-2 variants. The table includes columns for D614G, Delta, Omicron BA.1, and Omicron BA.2.

**Panel C**

Structural models of the virus binding sites for different antibodies.