Enhancement of SARS-CoV-2 infection and growth by an ACE2-specific monoclonal antibody

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Abstract

SARS-CoV-2 causes COVID-19 pandemic and continues to pose a threat to global public health through genetic mutation. In this study, we have found that an ACE2-specific monoclonal antibody at low concentration was able to greatly enhance SARS-CoV-2 infection and growth in cell culture. Strikingly, it promotes SARS-CoV-2 plaque formation, resulting in accurate titration of different SARS-CoV-2 variants, particularly the newly emerged Omicron variants, which otherwise cannot be determined by standard plaque assays. Quantification of infectious titers of the newly emerged variants will facilitate the development and evaluation of vaccines and antiviral drugs against SARS-CoV-2.

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Abstract

SARS-CoV-2 causes COVID-19 pandemic and continues to pose a threat to global public health through genetic mutation. In this study, we have found that an ACE2-specific monoclonal antibody at low concentration was able to greatly enhance SARS-CoV-2 infection and growth in cell culture. Strikingly, it promotes SARS-CoV-2 plaque formation, resulting in accurate titration of different SARS-CoV-2 variants, particularly the newly emerged Omicron variants, which otherwise cannot be determined by standard plaque assays. Quantification of infectious titers of the newly emerged variants will facilitate the development and evaluation of vaccines and antiviral drugs against SARS-CoV-2.

Emerging viral infections continue to pose a major threat to global public health. The coronavirus disease 2019 (COVID-19) pandemic caused by a newly emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has unprecedently resulted in hundreds of millions of infections and millions of deaths worldwide since its outbreak in December 2019 (1). The virus belongs to the family of*Coronaviridae* , consisting of 4 genera: Alpha, Beta, Gamma, and Delta coronaviruses. Seven of them were found to infect humans, including CoV-229E, CoV-NL63, CoV-HKU1, CoV-OC43, SARS-CoV, MERS-CoV, and SARS-CoV-2 (2). Coronaviruses are enveloped RNA viruses with a single-stranded RNA genome in positive polarity. Like other enveloped viruses, SARS-CoV-2 enters the cell through receptor(s)-mediated endocytosis (3, 4), which depends on both angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) (5). Upon internalization, viral RNA genomes replicate in the cytoplasm. The genomic RNA acts as an mRNA for translation of polyprotein 1a/1ab, which encodes nonstructural proteins to form the replication-transcription complex. It also serves as the template for synthesis of a nested set of subgenomic RNAs (sgRNAs) by discontinuous transcription. The minus-strand sgRNAs serve as the templates to produce

subgenomic mRNAs for production of different viral proteins. Nascent RNA genomes are synthesized from the full-length minus-strand RNA and are assembled with viral structural proteins to form progeny virions egressing from infected cells (6).

Various methods have been developed for rapid diagnosis of SARS-CoV-2 infection by detecting viral proteins and genomic RNAs (7). Serological methods have also been used for detection and titration of viral proteins-specific antibodies produced among people infected with SARS-CoV-2. In general, accurate quantification of infectious SARS-CoV-2 is more complex, requiring amplification and titration of viruses in cell culture (8). Both plaque-forming units and the 50% tissue culture infectious dose ($TCID_{50}$), are routinely used for measuring infectious SARS-CoV-2 based on its induction of cytopathic effect (CPE) . However, TCID50 is more qualitative rather than quantitative as a measure of infectious SARS-CoV-2 (9). Most of the recently emerged variants of SARS-CoV-2 are attenuated and do not form clear plaques even using the most sensitive Vero E6 cells that overexpress the SARS-CoV-2 receptors ACE2 and TMPRSS2 (Fig. 2). A reliable method to quantify infectious SARS-CoV-2 is urgently needed for development and evaluation of COVID19 vaccines and antiviral drugs.

Over the course of our studies on SARS-CoV-2 infection, we have found that the growth of SARS-CoV-2 was significantly enhanced by an ACE2-blocking monoclonal antibody(10108-MM37, Sino Biological). This observation is exemplified by a dose-dependent growth enhancement of both SARS-CoV-2 wild type and Omicron BA.2 variant in Vero E6 cells that overexpress both ACE2 and TMPRSS2 (Fig. 1). The levels of viral NP protein of SARS-CoV-2 wild type and BA2 variant were increased to 34 and 78 folds, respectively, by the anti-ACE2 antibody at a concentration of 0.25 μg/mL (Fig. 1A). The infectious titers of wild type SARS-CoV-2 and BA2 variant were also enhanced by 10,000 and 100,000 folds, respectively, at 0.25 μg/mL of the antibody (Fig 1B). Subsequently, we tested the antibody for its enhancement of SARS-CoV-2-induced plaque formation. Strikingly, the anti-ACE2 antibody promoted plaque formation of SARS-CoV-2 wild type and various variants when 0.2 μg/mL of the antibody was added to the agarose overlay, as shown by large plaques with uniform sizes at the bottom half of each 6-well plate (Fig. 2). Both plaque size and numbers were significantly increased, particularly for Omicron variants (BA1, BA2, BA4, BA5, and BF5) that otherwise did not form clear plaques in the absence of the anti-ACE2 antibody (top half of each 6-well plate). Both alpha and delta variants formed smaller and highly heterogeneous plaques in the absence of anti-ACE2 antibody. More significantly, the addition of the ACE2-blocking antibody resulted in a more accurate titration with uniform plaque sizes like that of wild type SARS-CoV-2. The numbers of plaques formed by SARS-CoV-2 variants were much less compared to that formed in the presence of anti-ACE2 antibody. Additionally, the ACE2-blocking antibody induced a more rapid plaque formation, requiring only 2 days instead of routinely 3 days to form large plaques. However, the concentration of anti-ACE2 monoclonal antibody is critical as it inhibits virus growth and plaque formation at higher (> 0.5 μg/mL) concentrations. In general, 0.2 μg/mL of anti-ACE2 antibody resulted in large and uniform plaques of all SARS-CoV-2 variants tested in this study.

It was previously reported that ACE2 autoantibody could be detected among some COVID-19 patients (10). The question arose whether ACE2 autoantibody developed in patients is associated with COVID-19 disease severity by enhancing SARS-CoV-2 infection. This possibility is warranted for futureP investigation. Nevertheless, our *in vitro*study demonstrated that anti-ACE2 antibody can promote SARS-CoV-2 growth and formation of plaques with large, clear, and uniform sizes, resulting in accurate titration of all infectious SARS-CoV-2 isolates, which otherwise cannot be accomplished by standard plaque assays.

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Figure legend

Figure 1 . Growth enhancement of SARS-CoV-2 wild type and Omicron BA.2 variant by an ACE2-blocking monoclonal antibody. Vero E6 cells were seeded in 24-well cell culture plates at a density of 2 x 10^5 cells per well and were infected with SARS-CoV-2 wild type and Omicron BA.2 variant at 0.001 multiplicity of infection. After removing virus and washing with 1xPBS three times, the virus-infected cells were incubated with 1 mL of DMEM containing 2.5% fetal bovine serum (FBS) and varying amounts (indicated on the top of A) of the ACE2-blocking antibody. At 48 hours post-infection, the virus-infected cells were lysed in 100 µl RIPR buffer, which were used for quantification of NP protein by a Western blot analysis (**A**). The supernatants were used for titration of infectious virus (**B**) by a plaque assay same as that in Fig.2. The infectious virus titers are average of triplicates.

Figure. 2 . Anti-ACE2 antibody-induced promotion of plaque formation of SARS-CoV-2 wild type and different variants. Wild type SARS-CoV-2 was previously described (11). SARS-CoV-2 alpha variant (NR-55461), delta variant (NR-55611), Omicron variants BA.1 (NR-56475), BA.2 (NR-56520), BA.4 (NR-56803), BA.5 (NR-58616), and BF.5 (NR-58716) were obtained from Bei Resources and were grown in Calu-3 cells. Vero E6 (NR-54970) was seeded to 6-well cell culture plates at a density of 5 x 10^5 cells per well and were infected with 200 µL of virus diluted serially with 1x PBS containing 1% FBS. Virus dilution factors are indicated beneath each 6-well plate. After 1-hour incubation at 37°C, virus-infected cells were cultured in 2 mL agarose overlay (equal volume of 2 x DMEM, DMEM containing 10% FBS, and 1.5% agarose) with (Anti-ACE2) or without (Control) 0.2 µg/mL of anti-ACE2 monoclonal antibody. After 48 hours, cells were

fixed with 2 mL/well of 7.5% paraformaldehyde for 30 minutes, followed by staining with 0.1% crystal violet solution containing 1% paraformaldehyde.

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