Impaired potency of neutralizing antibodies against cell-cell fusion mediated by SARS-CoV-2

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Abstract

The SARS-CoV-2 Omicron subvariants have dominated the pandemic due to their high transmissibility and immune evasion conferred by the spike mutations. The Omicron subvariants can spread by cell-free virus infection and cell-cell fusion, the latter of which is more effective but has not been extensively investigated. In this study, we developed a simple and high-throughput assay that provides a rapid readout to quantify cell-cell fusion mediated by the SARS-CoV-2 spike proteins without using live or pseudotyped virus. This assay can be used to identify variants of concern and to screen for prophylactic and therapeutic agents. We further evaluated a panel of monoclonal antibodies (mAbs) and vaccinee sera against D614G and Omicron subvariants, finding that cell-cell fusion is substantially more resistant to mAb and serum inhibition than cell-free virus infection. Such results have important implications for the development of vaccines and antiviral antibody drugs against cell-cell fusion induced by SARS-CoV-2 spikes.

Keywords: SARS-CoV-2, Omicron subvariants, spike protein, cell-cell fusion, neutralization, antibody evasion

Main text

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel beta-coronavirus that causes the global coronavirus disease 2019 (COVID-19) pandemic. The spike protein on the viral particles of SARS-CoV-2 mediates cell entry by binding to the human angiotensin-converting enzyme 2 (hACE2) receptor, which triggers the fusion of viral and cellular membranes (1). The spike protein is also the target for neutralizing antibodies, which are critical for the host's immunity to control viral infection (2, 3).

SARS-CoV-2 can spread by cell-free particles and through cell-cell interactions (4). Cell-free virus infection of Omicron subvariants, with additional spike mutations, is more resistant to antibody neutralization relative to the ancestral strain D614G (5). Whether cell-cell fusion mediated by Omicron subvariants has such antibody evasion properties, however, is not fully understood. Herein, we developed a simple, rapid, and high-throughput assay to quantitatively analyze cell-cell fusion. We further showed that cell-cell fusion induced by SARS-CoV-2 spike protein is largely resistant to the inhibition by monoclonal antibodies (mAbs) and vaccinee sera, as compared to the cell-free virus infection.

The experimental design of the cell-cell fusion assay is illustrated in **Figure S1A.** The TZM-bl-hACE2 target cell line was generated from parental TZM-bl cell line through stable transduction of hACE2 using a lentiviral system, followed by successive rounds of fluorescence-activated cell sorting (FACS) and selection of transduced cells with puromycin. Cell surface staining data revealed that TZM-bl-hACE2 cells displayed elevated levels of hACE2 compared to TZM-bl cells and Vero-E6 cells (**Figure S1B**), which are commonly used as target cells in pseudovirus assays for human immunodeficiency virus (HIV)-1 and SARS-CoV-2, respectively. The donor 293T cells

were transiently transfected with HIV-1 Trans-Activator of Transcription (Tat) and spike genes, before being co-cultured with the TZM-bl-hACE2 target cells at a ratio of 2:1 for 16 hours. Cell-cell fusion could be evaluated both qualitatively, via observation with a light microscope (**Figure S1C**), and quantitatively, through a luciferase reporter assay (**Figure S1D**). Cell-cell fusion activity mediated by D614G and BA.1 variants was determined at various time points following co-culture and was found to reach high and comparable levels at a co-culture time of 16 hours for both variants (**Figure S1D**).

We initially assessed the susceptibility of TZM-bl-hACE2 cells to SARS-CoV-2 infection using vesicular stomatitis virus (VSV)-pseudotypes. The results showed a dose-dependent infection of TZM-bl-hACE2 cells by the wild-type SARS-CoV-2 D614G, while TZM-bl cells were not infected, suggesting that hACE2 receptors were necessary for virus entry (**Figure S2A**). Furthermore, we tested the infectivity of multiple Omicron subvariants and found that they were all capable of infecting TZM-bl-hACE2 cells, although with varying levels of infectivity (**Figure S2B**). Through these evaluations, a rapid and reliable platform was developed and validated, allowing for the comparison of SARS-CoV-2 variant spikes in mediating cell-free virus infection and cell-cell fusion, as well as for testing the inhibitory effects of mAbs and vaccinee sera on these processes.

We then assessed the efficacy of mAbs and human ACE2 (hACE2) in blocking cell-free pseudovirus infection and cell-to-cell fusion mediated by the spikes of SARS-CoV-2 variants. The mAbs used in this study included CAB-A17 (6), Omi-3 (7), XGv347 (8), XGv282 (8), LY-CoV1404 (9), and COV2-2130 (10), which could neutralize SARS-CoV-2 by recognizing the receptor binding domain (RBD) on the spike and preventing hACE2 binding. **Figure 1A** displays the structural modeling of the binding of these RBD-directed mAbs and hACE2 to the RBD of D614G spike. The neutralization activity of aforementioned mAbs was evaluated with VSV-pseudotyped SARS-CoV-2 on Vero-E6 cells (**Figure S3**) and TZM-bl-hACE2 cells (**Figures 1B and S4**). Our results indicated that all mAbs effectively inhibited cell-free infection of D614G, with IC_{50} values less than 0.3 μg/mL on TZM-bl-hACE2 cells and less than 0.1 μg/mL on Vero-E6 cells. Although the neutralizing efficacy of some mAbs was significantly impaired by Omicron, such as XGv347 against BA.4/5 and COV2-2130 against BA.1, most mAbs remained potent against the cell-free virus infection of multiple Omicron subvariants. The IC_{50} values on TZM-bl-hACE2 cells were higher than those on Vero-E6 cells, likely because TZM-bl-hACE2 cells had a higher level of hACE2 expression, which hinders the inhibition of virus entry (**Figure S1B**). In contrast, our results showed that none of the mAbs tested could potently and broadly inhibit cell-cell fusion mediated by SARS-CoV-2 spikes (**Figures 1C and S3B**). They retained weak potency against some of the Omicron subvariants we tested. Among the variants tested, cell-cell fusion induced by D614G was the most resistant to mAb inhibition, followed by BA.4/5, while BA.2 was the most sensitive. Overall, our results demonstrated that cell-to-cell fusion mediated by SARS-CoV-2 is more resistant to mAb inhibition than cell-free virus infection.

We further profiled the inhibition efficiency of serum samples in cell-free virus infection

and cell-cell fusion mediated by SARS-CoV-2 spikes. Sera were collected from a study cohort in which each subject had received three doses of mRNA-based SARS-CoV-2 vaccines. The clinical information of this study cohort is provided in **Table S1**. In both Vero-E6 cells and TZM-bl-hACE2 cells, VSV-pseudotyped SARS-CoV-2 variants were sensitive to serum neutralization, with ID_{50} titers ranging from 525 to 2074 on Vero-E6 cells, and from 403 to 1017 on TZM-bl-hACE2 cells (**Figures 1D and S5**). The ID_{50} titers on the TZM-bl-hACE2 cells were slightly lower than those on the Vero-E6 cells, which is consistent with what was observed in the pseudovirus neutralization assay to mAbs. However, in the cell-cell fusion inhibition assay on TZM-bl-hACE2 cells, the ID50 titers to serum inhibition were more than 3-fold lower than those obtained from the cell-free virus neutralization assay on the same cell line. Our data suggest that cell-cell fusion induced by SARS-CoV-2 spike protein is more resistant to inhibition by vaccinee sera, compared to cell-free virus infection mediated by the spike protein on viral particles. Among the Omicron subvariants we tested, the cell-cell fusion mediated by BA.4/5 showed the strongest evasion to vaccinee sera.

It was previously shown that cell-cell transmission of SARS-CoV-2 is more efficient than cell-free infection, because the tight cell-cell contact allows for a direct transfer of dense viral particles from an infected cell to its neighboring cells (4). In this study, we further demonstrate that cell-cell transmission of SARS-CoV-2 is resistant to inhibition by mAbs (**Figure 1C**) and vaccinee sera (**Figure 1D**). Future prophylactic or therapeutic agents should consider strategies to suppress the cell-cell fusion of SARS-CoV-2.

It should be noted that all experiments in this study were performed *in vitro*, and it is possible that the results may differ from *in vivo* outcomes. In addition, only humoral immunity against cell-cell transmission of SARS-CoV-2 was evaluated, and the role of cellular immunity requires further investigation. Lastly, cell-cell transmission was not measured directly, but indirectly through a cell-cell fusion assay. If SARS-CoV-2 triggers intense cell-cell fusion, giant syncytia may form and result in cell death, which in turn lessens cell-to-cell transmission.

In summary, we designed and validated a rapid and reliable cell-cell fusion assay for SARS-CoV-2. Using this assay, we demonstrated that mAbs and vaccinee sera could not effectively inhibit the cell-cell fusion mediated by SARS-CoV-2 spikes, in contrast to cell-free infection. Such results suggest that developing vaccines and antiviral antibody drugs that target the cell-cell fusion of SARS-CoV-2 may be a promising approach.

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Disclosure statement

J.Y., L.L., and D.D.H. are inventors on patent applications (WO2021236998) or provisional patent applications (63/271,627) filed by Columbia University. D.D.H. is a

co-founder of TaiMed Biologics and RenBio, consultant to WuXi Biologics and Brii Biosciences, and board director for Vicarious Surgical.

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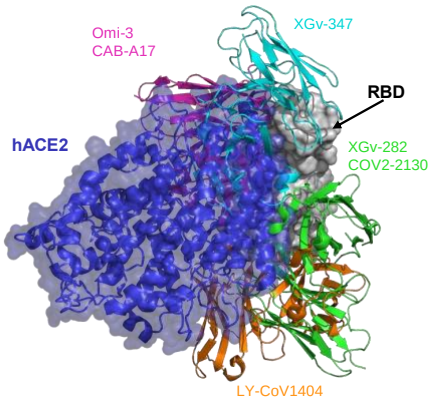
A



B

Pesudovirus inhibition on TZM-bl-hACE2 cells						
IC_{50} (μg/mL)	D614G	BA.1	BA.2	BA.2.12.1	BA.4/5	BA.2.75
hACE2-Fc	1.097	0.206	0.160	0.196	0.222	0.168
CAB-A17	0.042	0.051	0.069	0.043	0.043	1.977
Omi3	0.030	0.026	0.048	0.050	0.106	0.122
XGv347	0.009	0.005	0.005	0.005	>10	0.001
XGv282	0.0005	1.877	0.001	0.003	0.002	0.242
LY-CoV1404	0.005	0.004	0.002	0.002	0.003	0.020
COV2-2130	0.013	>10	0.016	0.035	0.604	0.498

C

Cell-cell fusion inhibition on TZM-bl-hACE2 cells						
IC_{50} (μg/mL)	D614G	BA.1	BA.2	BA.2.12.1	BA.4/5	BA.2.75
hACE2-Fc	>50	>50	>50	>50	>50	>50
CAB-A17	>50	>50	>50	>50	>50	>50
Omi-3	>50	24.64	30.66	>50	>50	5.069
XGv347	>50	8.702	14.18	10.82	>50	0.782
XGv282	>50	>50	0.494	42.89	33.46	>50
LY-CoV1404	>50	20.71	4.931	7.463	13.30	>50
COV2-2130	>50	>50	5.738	>50	>50	>50

D

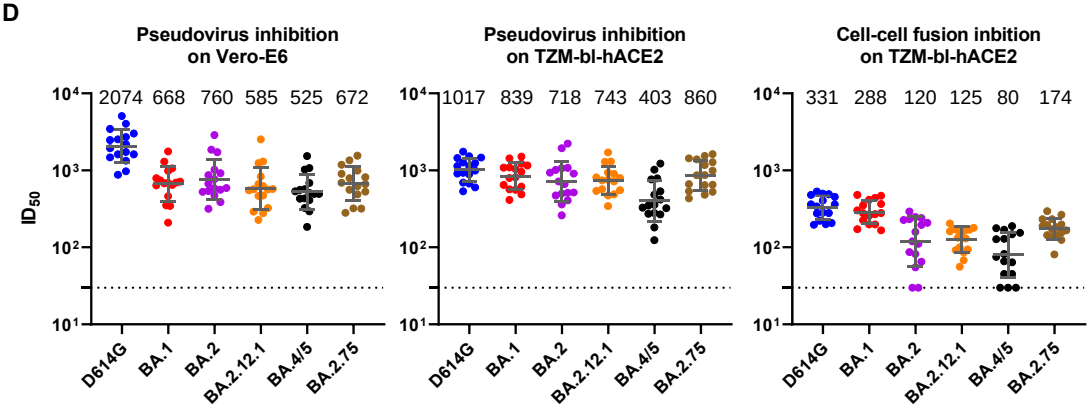


Figure 1. Resistance of cell-cell fusion induced by SARS-CoV-2 to monoclonal antibody and serum neutralisation. (**A**) Structural modelling of RBD, hACE2, and mAbs. Different colors indicate different classes of mAbs, as grouped by their epitopes. (**B**) Pseudovirus neutralizationIC_{50} titres for mAbs against D614G and Omicron subvariants on TZM-bl cells. (**C**) Cell-cell fusion inhibition IC_{50} titres for mAbs against D614G and Omicron subvariants. (**D**) Pseudovirus neutralizationand cell-cell fusion inhibition ID_{50} titres for sera against D614G and Omicron subvariants. Each point represents a serum sample. Horizontal solid bar indicates the geometric mean of ID_{50} titres. Horizontal dotted line denotes the limit of detection (LOD) at 30.

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