SARS-COV-2 SPIKE EXPRESSION AT THE SURFACE OF INFECTED HUMAN AIRWAY EPITHELIAL CELLS

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BACKGROUND

Very shortly after SARS-CoV-2 was declared a pandemic by the World Health Organization, major efforts to understand humoral responses against this new virus were undertaken. A myriad of different assays were deployed around the globe, including soluble recombinant forms of the Spike, its receptor-binding domain (RBD), cell-based assays expressing different forms of the Spike, pseudoviral assays, and assays using authentic SARS-CoV-2 and infected cells. These assays were then used to study vaccine-elicited humoral responses and to compare these with those elicited by natural infection.

Antibody-mediated neutralization is a likely correlate of protection; emerging evidence points to the potential benefits of antibody-mediated effector functions. Some of these Fc-mediated effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP), require recognition of the antigen at the surface of infected cells. This raises an important question about SARS-CoV-2 Spike expression at the surface of primary human airway epithelial cells (pAECs) infected with authentic virus.

METHODS

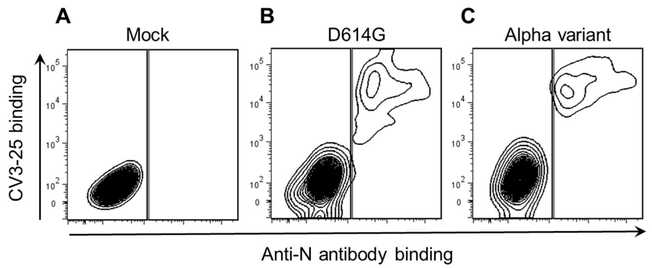
1. We developed a FACS-based assay combining the cell surface detection of the Spike and the intracellular detection of the nucleocapsid (N) in pAECs infected with authentic SARS-CoV-2. This assay allows for the distinction between infected (N+) and uninfected (N-) cells.

2. Flow Cytometry analysis of cell-surface staining with plasma from a cohort of individuals who were SARS-CoV-2 naïve and vaccinated and of those who were previously infected.

3. Correlations between Spike recognition at the surface of infected pAECs and its detection by commonly used serological assays, which include detection at the surface of transfected or transduced cells, pseudoviral neutralization, and Fc-mediated effector functions such as ADCC.

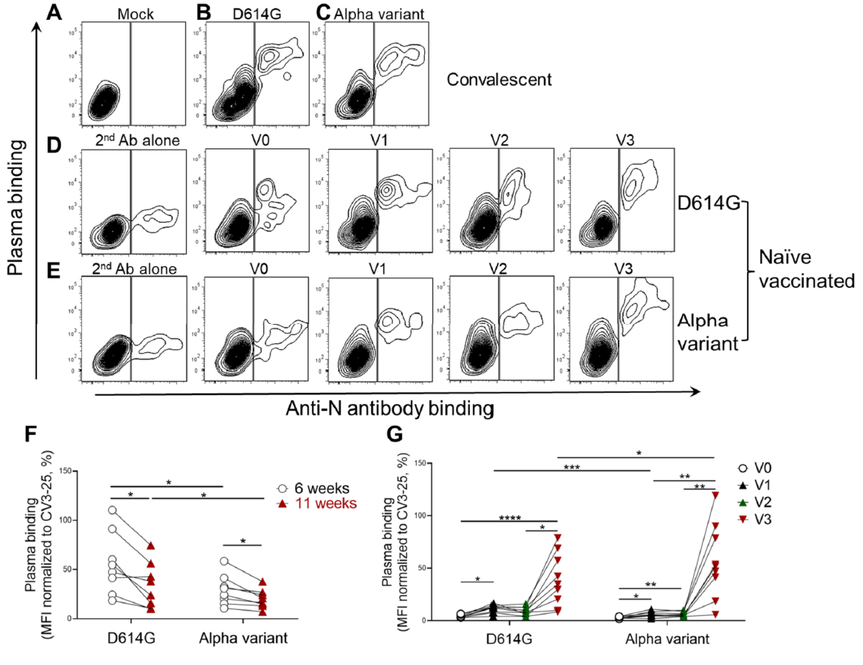
RESULTS

1. Spike recognition at the surface of infected pAEC cells.



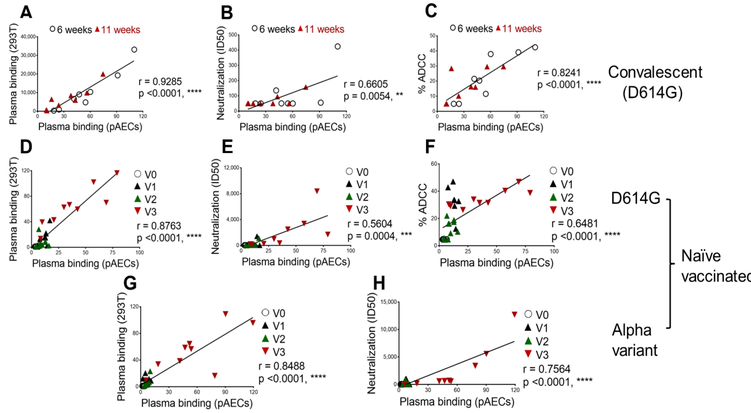
pAECs were either (**A**) mock infected or (**B**) infected with authentic SARS-CoV-2 D614G or (**C**) infected with Alpha VOC. concern. Cells were stained intracellularly with Alexa Fluor 488 pre-coupled anti-nucleocapsid (anti-N) Ab (clone mBG17) for the identification.

2. Recognition of infected human pAEC cells by plasma from individuals who were previously infected, or SARS-CoV-2 naïve and vaccinated.



pAECs were either (**A**) mock-infected or (**B**,**D**,**F**,**G**) infected with authentic SARS-CoV-2 D614G or (**C**,**E**,**F**,**G**) the Alpha variants. In (**A**–**C**,**F**), plasma recovered from eight convalescent individuals was used to stain infected pAECs; In **(D,E,G),** plasma from nine individuals who were SARS-CoV-2 naïve and vaccinated obtained before vaccination, 3 weeks, 12 weeks after first mRNA dose, or 3 weeks after the second dose were used to detect infected pAECs.

3. Recognition of SARS-CoV-2 infected cells correlates with recognition of 293T cells expressing Spike, pseudoviral neutralization and ADCC



Recognition of SARS-CoV-2 D614G (**A**–**F**) or Alpha (**G**,**H**) variants infected (N+) pAECs by plasma from convalescent (**A**–**C**) or vaccinated individuals (**D**–**H**) correlates with (**A**,**D**,**G**) binding to 293T Spike expressing cells, (**B**,**E**,**H**) pseudovirus neutralization, and (**C**,**F**) ADCC activity using CEM-NKr cells stably expressing SARS-CoV-2 Spike. Panels (**A**)–(**C**) show results generated using plasma from convalescent donors. Panels (**D**)–(**H**) show data generated using plasma from nine individuals who were SARS-CoV-2 naïve and vaccinated obtained before vaccination, at 3 weeks and 12 weeks after the first mRNA dose, or 3 weeks after the second dose.

CONCLUSIONS

We found that the SARS-CoV-2 Spike is abundantly expressed at the surface of infected human pAECs. This confirms that SARS-CoV-2-infected cells represent a potential target for Fc effector responses. Moreover, it suggests that the Spike present at the surface of infected pAECs displays a conformation that is detected by many widely used serological assays involving expression of the native full-length Spike.

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