RESEARCH LETTER

Neutralizing Antibodies Against SARS-CoV-2 Variants After Infection and Vaccination

Serum neutralizing antibodies rapidly appear after SARS-CoV-2 infection[1] and vaccination[2] and are maintained for several months.[3,4] The emergence of SARS-CoV-2 variants has raised concerns about the breadth of neutralizing-antibody responses. We compared the neutralizing-antibody response to 4 variants in infected and vaccinated individuals to determine how mutations within the spike protein are associated with virus neutralization.

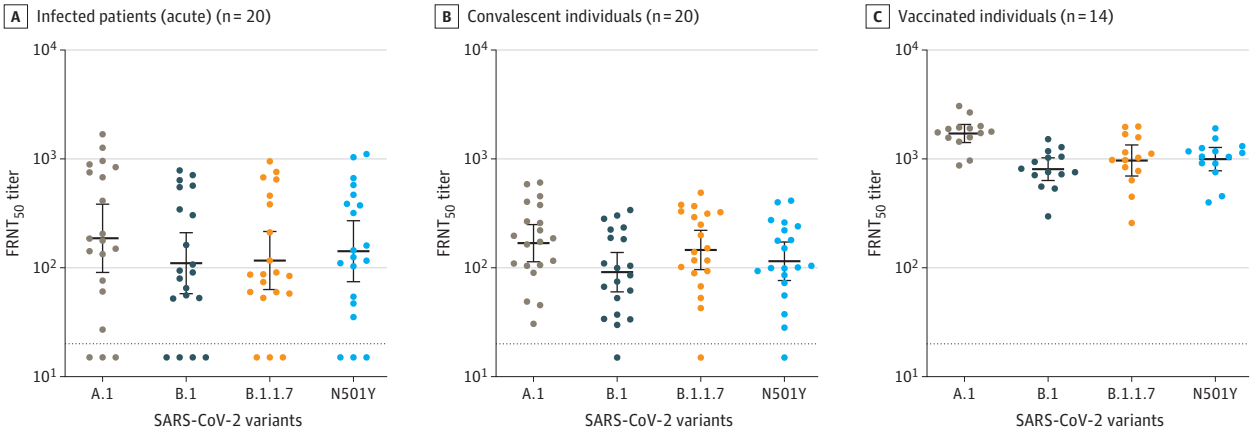
Supplemental content

Methods | Serum samples were obtained from 3 groups of individuals. At Emory University, hospitalized adults with SARS-CoV-2 infection (polymerase chain reaction confirmed) were enrolled 5 to 19 days after symptom onset (July 2020). Infected convalescent individuals (polymerase chain reaction or antigen test confirmed) were enrolled 32 to 94 days after symptom onset (March to August 2020). Deidentified serum samples drawn 14 days after the second dose (100-µg cohort) from individuals in the mRNA-1273 phase 1 clinical trial[2] were obtained from the National Institutes of Health. See the eAppendix in the Supplement for participant details. Institutional review board approval was obtained from Emory University and Advarra; all participants provided written informed consent.

Four variants were examined, chosen to represent the original SARS-CoV-2 strain and emerging variants with mutations in the spike protein. The first variant, nCoV/USA_WA1/2020 (A.1 lineage), closely resembled the original Wuhan strain and the spike used in the mRNA-1273 vaccine, and was propagated from an infectious SARS-CoV-2 clone. The second variant, EHC-083E (B.1 lineage), containing a D614G mutation within the spike, was the predominant circulating strain at the time of the study and was isolated from a residual nasopharyngeal swab from a patient in Atlanta, Georgia, in March 2020 (SARS-CoV-2/human/USA/GA-EHC-083E/2020). The third variant, B.1.1.7 (SARS-CoV-2/human/USA/CA_CDC_5574/2020), was originally identified in the UK and of concern because of increased transmissibility. It contained several spike mutations and was isolated from a residual nasopharyngeal swab from a patient in San Diego, California, in December 2020. The fourth variant, N501Y SARS-CoV-2 virus, containing a mutation in the critical receptor binding domain of the spike that is present across multiple emerging variants, including the B.1.1.7 variant in this study, was generated from an infectious clone as previously described.[5] This virus is not found in nature.

Live-virus focus reduction neutralization tests (FRNTs) were performed as previously described.[6] See the eAppendix in the Supplement for details on the laboratory methods. $FRNT_{50}$ titers, which represent the reciprocal dilution of serum that neutralizes 50% of the input virus, were interpolated with a 4-parameter nonlinear regression, and geometric mean titers (GMTs)

Figure. Neutralizing Antibody Responses Against SARS-CoV-2 Variants



A, Data from 20 patients with acute COVID-19 infection (5-19 days after symptom onset). B, Data from 20 convalescent COVID-19 individuals (32-94 days after symptom onset). C, Data from 14 healthy individuals (aged 18-55 years) who received the Moderna (mRNA-1273) vaccine, 100-µg dose, on day 14 (postsecond dose). The geometric mean titers (GMTs) with 95% CI are shown for samples against the A.1, B.1, B.1.1.7, and N501Y variants. The horizontal dashed lines indicate the limit of detection ($FRNT_{50}$ GMT = 20). Statistical significance was determined with the Kruskal-Wallis test to compare GMTs between the variants, followed by the Dunn's multiple comparison post hoc test. For A (acutely infected patients) and B (convalescent individuals), no comparisons were statistically significant. For C (vaccinated individuals), significant differences were found for variant A.1 vs B.1 ($P < .001$), variant A.1 vs B.1.1.7 ($P = .02$), and variant A.1 vs N501Y ($P = .02$). $FRNT_{50}$ indicates live-virus focus reduction neutralization tests with the reciprocal dilution of serum that neutralizes 50% of the input virus.

were calculated with 95% CI in GraphPad Prism version 8.4.3. Kruskal-Wallis test was used to compare $FRNT_{50}$ GMTs between the variants, followed by Dunn's multiple comparison post hoc test. We determined $P < .05$ (2 sided) to define statistical significance.

Results | Twenty acutely infected COVID-19 patients provided serum samples (mean age, 56.6 years; 50% men). The $FRNT_{50}$ GMT for the A.1 variant was 186 (95% CI, 90-383); for B.1, 110 (95% CI, 57-209); for B.1.1.7, 116 (95% CI, 62-215); and for N501Y, 141 (95% CI, 74-269). Comparison of the $FRNT_{50}$ GMT of the variants was not statistically significant (**Figure**).

Twenty convalescent individuals provided serum samples (mean age, 45 years; 55% men). The $FRNT_{50}$ GMT for the A.1 variant was 168 (95% CI, 113-249); for B.1, 91 (95% CI, 60-138); for B.1.1.7, 145 (95% CI, 96-220); and for N501Y, 145 (95% CI, 76-172). Comparison of the $FRNT_{50}$ GMT of the variants was not statistically significant.

Serum samples were available for 14 mRNA-1273 vaccinated individuals[2] (age range, 18-55 years; 43% men). The $FRNT_{50}$ GMT for the A.1 variant was 1709 (95% CI, 1412-2069); for B.1, 804 (95% CI, 632-1023); for B.1.1.7, 965 (95% CI, 695-1341); and for N501Y, 994 (95% CI, 777-1272). Comparisons of the $FRNT_{50}$ GMT of B.1, B.1.1.7, and the N501Y variant were not statistically significant. The $FRNT_{50}$ GMTs for the B.1 ($P < .001$), B.1.1.7 ($P = .02$), and N501Y ($P = .02$) variants were statistically significantly lower than that for the A.1 variant.

Discussion | This study found neutralizing activity of infection- and vaccine-elicited antibodies against 4 SARS-CoV-2 variants, including B.1, B.1.1.7, and N501Y. Because neutralization studies measure the ability of antibodies to block virus infection, these results suggest that infection- and vaccine-induced immunity may be retained against the B.1.1.7 variant. As additional variants emerge, neutralizing-antibody responses after infection and vaccination should be monitored.

Limitations include the small sample size, possible selection bias, lack of clinical outcomes, and how neutralization titers correlate with protection.

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Concept and design: Edara, Ahmed, Suthar.
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Drafting of the manuscript: Edara, Suthar.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Edara, Hudson, Suthar.
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