mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection

Leonidas Stamatatos1,2,*, Julie Czartoski1, Yu-Hsin Wan1, Leah J. Homad1, Vanessa Rubin1, Hayley Giantz1, Moni Neradilek1, Emilie Seydoux1, Madeleine F. Jennewein2, Anna J. MacCamy1, Junli Feng1, Gregory Mize1, Stephen C. De Rosa1,3, Andrés Finzi4,5,6, Maria P. Lemos1, and previously infected persons to elicit cross-variant neutralizing antibodies.

We examined whether sera from recovered and naïve donors, collected before and after immunizations with existing messenger RNA (mRNA) vaccines, could neutralize the Wuhan-Hu-1 and B.1.351 variants. Prevaccination sera from recovered donors neutralized Wuhan-Hu-1 and sporadically neutralized B.1.351, but a single immunization boosted neutralizing titers against all variants and SARS-CoV-1 by up to 1000-fold. Neutralization was a result of antibodies targeting the receptor binding domain and was not boosted by a second immunization. Immunization of naïve donors also elicited cross-neutralizing responses but at lower titers. Our study highlights the importance of vaccinating both uninfected and previously infected persons to elicit cross-variant neutralizing antibodies.

Emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants have raised concerns about resistance to neutralizing antibodies elicited by previous infection or vaccination. We examined whether sera from recovered and naïve donors, collected before and after immunizations with existing messenger RNA (mRNA) vaccines, could neutralize the Wuhan-Hu-1 and B.1.351 variants. Prevaccination sera from recovered donors neutralized Wuhan-Hu-1 and sporadically neutralized B.1.351, but a single immunization boosted neutralizing titers against all variants and SARS-CoV-1 by up to 1000-fold. Neutralization was a result of antibodies targeting the receptor binding domain and was not boosted by a second immunization. Immunization of naïve donors also elicited cross-neutralizing responses but at lower titers. Our study highlights the importance of vaccinating both uninfected and previously infected persons to elicit cross-variant neutralizing antibodies.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) betacoronavirus first emerged in the Hubei Province of China in late 2019 and has since infected more than 115 million people and caused more than 2.5 million deaths in 192 countries (1–3). Infection is mediated by the viral spike protein (S), which is composed of an S1 domain that contains an N-terminal domain (NTD), a C-terminal domain (CTD), and a receptor binding domain (RBD) that mediates attachment to the entry receptor angiotensin-converting enzyme 2 (ACE2) as well as an S2 domain that contains the fusion machinery (4–6).

Preexisting immunity to SARS-CoV-2 is associated with protection against reinfection in humans (9–11) and in nonhuman primates (12, 13). Although the correlates of protection in humans against repeat infection or after vaccination have not been firmly established, neutralizing antibodies (nAbs) are thought to be an important component of a protective immune response against SARS-CoV-2 (14, 15). In support of this, passive transfer of nAbs limits respiratory tract infection and protects against infection in animal models (16–20), and nAbs may contribute to protection against infection in humans (9). SARS-CoV-2 infection rapidly elicits nAbs (16, 21–24) that decline, but remain detectable, over several months (25–29).

Most serum nAbs responses elicited during natural infection are directed at the RBD (21, 23, 30, 31). Numerous neutralizing anti-RBD monoclonal antibodies (mAbs) have been characterized, the most potent of which block the RBD-ACE2 interaction (16, 17, 22–24, 32–37). Neutralizing mAbs that bind regions of the viral spike have also been identified (24, 33, 38–42).

Two mRNA-based vaccines (Pfizer-BioNTech BNT162b2 and Moderna mRNA-1273) have received emergency use authorization in several countries. Both vaccines encode a stabilized ectodomain version of the S protein derived from the Wuhan-Hu-1 variant isolated in December 2019 (43), show >94% efficacy at preventing COVID-19 illness (44–47), and elicit nAbs (48, 49).

Because of the high global burden of SARS-CoV-2 transmission, viral evolution is occurring. Recently, viral variants of concern have emerged in the UK (B.1.1.7), South Africa (B.1.351), and Brazil (P.1) that harbor specific mutations in their S proteins that may be associated with increased transmissibility (50–55).

Of particular concern are mutations found in the B.1.351 lineage, which is defined by the D614G mutation, which increases virion spike density, infectivity, and transmissibility (39, 60). The B.1.351 and P.1 lineages also share the E484K mutation in the RBD, and both variants are mutated at position 417 (K417T in P.1).

Mutations found in emergent S variants decrease sensitivity to neutralization by mAbs, convalescent plasma, and sera from vaccinated individuals (27, 37, 58, 61–70). As a result, there is concern that these and other emerging variants can evade nAB responses generated during infection with variants that were circulating earlier in the pandemic and also nAb responses elicited by vaccines based on the S protein of the Wuhan-Hu-1 variant. There is concern that these mutations are responsible for the reduced efficacy observed in ongoing trials of SARS-CoV-2 vaccines in South Africa (71, 72).

Here, we evaluated the neutralization susceptibility of spike variants harboring lineage-defining and prevalent B.1.351 mutations to sera from two groups. Sera were collected from 15 donors with previously confirmed SARS-CoV-2 infection (referred to as previously infected donors (PIDs)) before and after one or two immunizations with either mRNA vaccine and from 13 uninfected donors who received two doses of the above vaccines (referred to as naïve donors (NDs); tables S1 and S2).

Antibody neutralization experiments were performed with pseudoviruses expressing either the full-length Wuhan-Hu-1 S or either of two versions of the B.1.351 lineage S—one herein referred to as B.1.351, containing the lineage-defining S mutations D80A, D614G, K417N, E484K, N501Y, and D614G and the A701V mutation that is highly prevalent in this lineage, and a second variant that also includes a ΔS242–243 deletion (B.1.351–ΔS242-243). The viral stocks were appropriately diluted to achieve comparable entry levels during the neutralization experiments (fig. S1).

We first evaluated the neutralizing potency of several mAbs isolated from nonvaccinated patients infected early in the pandemic. These mAbs target different epitopes: three against the RBD (CV30, CV3-1, and CV2-75) and one against the NTD (CV1) (fig. S2). CV30 is a member of the VH3-53 class of antibodies that bind to the receptor binding motif (RBM) (22, 22, 73–78). It makes direct contact with the K417 and
N501 residues in the RBM that are mutated in the B.1.351 and P.1 lineages; however, unlike other known VH3-53 mAbs, it does not contact E484 (78). The neutralization potency of this mAb was ~10-fold weaker toward both tact E484 (other known VH3-53 mAbs, it does not con-
in the B.1.351 and P.1 lineages; however, unlike
N501 residues in the RBM that are mutated
in the Wuhan-Hu-1 variant. We also included SARS-CoV-1 pseudoviruses in this analysis as a represen-
tative of two independent experiments.

We next evaluated the ability of sera collected before and after immunization in NDs and PIDs to neutralize the more resistant B.1.351 and B.1.351Δ242-243 pseudoviruses. These variants are 0.5 and 0.7% divergent from the Wuhan-
Hu-1 variant. We also included SARS-CoV-1 pseudoviruses in this analysis as a representa-
tive of two independent experiments.

Before vaccination, 5 of 15 sera from PIDs neutralized B.1.351, and only three had ID50 titers above 100 (Fig. 3A, B, and E, and fig. S4); 7 of 15 neutralized B.1.351Δ242-243, and only one had titers above 100 (Fig. 3A, C, and E, and fig. S4). Only two prevaccine PID sera achieved 80% neutralization of B.1.351, and only one achieved 80% neutralization of B.1.351Δ242-243.
The median ID_{50} of the prevaccine sera against the Wuhan-Hu-1 variant was significantly higher than that against B.1.351 or B.1.351–Δ242-243 (Fig. 3E). Consistent with the high level of sequence disparity, sera from only one PID showed very weak neutralizing activity toward SARS-CoV-1 before vaccination (Fig. 3, D and E, and fig. S7).

A single immunization boosted the nAb titers against all three SARS-CoV-2 variants and SARS-CoV-1 in 13 of 15 PIDs (Fig. 3, A to D); however, the median ID_{50} titers were ~3-fold lower against B.1.351, ~10-fold lower against B.1.351–Δ242-243, and 100-fold lower against SARS-CoV-1 than against Wuhan-Hu-1 (Fig. 3E). A single immunization did not elicit nAbs against the B.1.351 variants or SARS-CoV-1 in the two asymptomatic donors who lacked RBD-specific IgG memory (donor L and M; Fig. 3, A to D, and Fig. 3E, open circles). The median ID_{80} values were also lower for the B.1.351 and B.1.351–Δ242-243 variants compared with the Wuhan-Hu-1 variant (fig. S7A).

The neutralizing titers elicited by a single immunization in PIDs were significantly higher than those elicited by two immunizations in NDs against all pseudoviruses tested—10-fold higher against Wuhan-Hu-1 (Fig. 3A), 20-fold higher against B.1.351 (Fig. 3B), 30-fold higher against B.1.351–Δ242-243 (Fig. 3C), and 7-fold higher against SARS-CoV-1 (Fig. 3D). Only 8 of 13 vaccinated NDs were able to achieve 80% neutralization of B.1.351–Δ242-243, and none could achieve 80% neutralization of SARS-CoV-1 (fig. S7B).

The B.1.351 and B.1.351–Δ242-243 variants contain three RBD mutations that affect the neutralization potency of anti-RBD mAbs (Fig. 1). Moreover, preexisting anti-RBD IgG memory appears to be important for a robust recall response to vaccination. To determine the relative contribution of anti-RBD antibodies to serum neutralization, we depleted RBD-specific antibodies from the sera of 10 PIDs after one vaccination and from nine NDs after two vaccinations. This approach efficiently removed RBD-specific (Fig. 4, A and C) but not anti-S2P-specific antibodies from sera, as measured by enzyme-linked.

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**Fig. 2.** A single dose of a spike-derived mRNA vaccine elicits a strong recall response. (A to C) IgG (A), IgA (B), and IgM (C) end-point antibody titers specific to the RBD of the Wuhan-Hu-1 variant were measured in serum collected from PIDs before and after one or two immunizations with the Pfizer-BioNTech or Moderna mRNA vaccines by ELISA, as indicated. End-point titers measured in sera from NDs after two vaccine doses are shown for comparison (gray dots). (D) Frequency of Wuhan-Hu-1 RBD-specific IgG⁺ memory B cells (live, IgD⁻, CD19⁺, CD20⁺, CD3⁻, CD14, CD56⁻, singlet, and lymphocytes) in peripheral blood mononuclear cells (PBMCs) from PIDs was measured before and after one or two immunizations. (E and F) The frequency of S6P-specific IgG⁺ (E) and IgA⁺ (F) memory B cells in PBMCs from PIDs was measured before and after one or two immunizations. The frequencies of memory B cells from NDs after two vaccine doses are shown for comparison in (D) to (F) (gray dots). (G) The frequency of S-specific CD4⁺ T cells expressing interferon-γ (IFN-γ) and/or interleukin-2 (IL-2) and/or CD40L in PBMCs from PIDs was measured before and after one or two immunizations. The frequencies of S-specific CD4⁺ T cells in PBMCs from uninfected donors after two vaccine doses are shown for comparison (gray dots). Experiments were performed once. Significant differences in infected donors before or after vaccination [(A) to (G)] were determined using a Wilcoxon signed rank test (n.s., not significant; *P < 0.05; **P < 0.01; and ***P < 0.001). Significant differences between previously infected and uninfected donors [(A) to (G)] were determined using a Wilcoxon rank sum test (*P < 0.05; **P < 0.01; and ***P < 0.001).
Fig. 3. Preexisting SARS-CoV-2 nAb responses are boosted by a single dose of a spike-derived mRNA vaccine. (A to D) The serum dilution resulting in 50% neutralization (ID$_{50}$) of Wuhan-Hu-1 (A), B.1.351 (B), B.1.351Δ242-243 (C), and SARS-CoV-1 (D) pseudoviruses was measured in PIDs before and after one or two immunizations with the Pfizer-BioNTech or Moderna vaccines and in NDs after two vaccine doses, as indicated. Data points between PIDs who were symptomatic and asymptomatic are connected by solid and dashed lines, respectively, in (A) to (D). (E) Serum dilution resulting in 50% neutralization (ID$_{50}$) from PIDs before (squares) and after (circles) a single immunization with the Pfizer-BioNTech or Moderna vaccines against Wuhan-Hu-1, B.1.351, B.1.351Δ242-243, and SARS-CoV-1 pseudoviruses, as indicated. PIDs who were asymptomatic and negative for anti-IgG RBD antibodies and RBD-specific IgG$^+$ memory B cells before vaccination are shown as open circles. (F) Neutralizing potency (ID$_{50}$) of serum from NDs after two immunizations with the Pfizer-BioNTech or Moderna vaccines against the indicated pseudoviruses. Each data point represents a different donor, and the horizontal bars represent the medians in (E) and (F). The dashed lines demarcate the lowest serum dilutions tested. Experiments were performed once. Significant differences in infected donors before or after vaccination, or from the same time point against different variants, were determined using a Wilcoxon signed rank test ($^*$P < 0.05; **P < 0.01; and ***P < 0.001). Significant differences between previously infected and uninfected donors were determined using a Wilcoxon rank sum test ($^*$P < 0.05; **P < 0.01; and ***P < 0.001).
sensitivity of the authentic and pseudovirus assays may differ, we anticipate that the relative differences we report here will not vary between the two.

Although the correlates of protection for SARS-CoV-2 vaccines have not been established, studies in nonhuman primates indicate that even low titers of nAbs are sufficient to prevent experimental SARS-CoV-2 infection, particularly if CD8+ T cell responses are mounted (18). Our study suggests that most previously infected subjects will benefit from a single immunization with either the Pfizer-BioNTech or Moderna vaccines, as it will lead to significant increases in serum nAb responses against vaccine-matched and emerging variants. The observation that a second dose administered 3 to 4 weeks after the first did not further boost neutralizing titers in PIDs who have clear evidence of RBD-directed immunological memory before vaccination suggests that the second dose of an mRNA vaccine could be delayed in some persons who have previously been infected with SARS-CoV-2. Longitudinal monitoring of the nAb titers before and after the first dose should be used to determine the necessity or optimal timing of the second dose in the context of previous infection.

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