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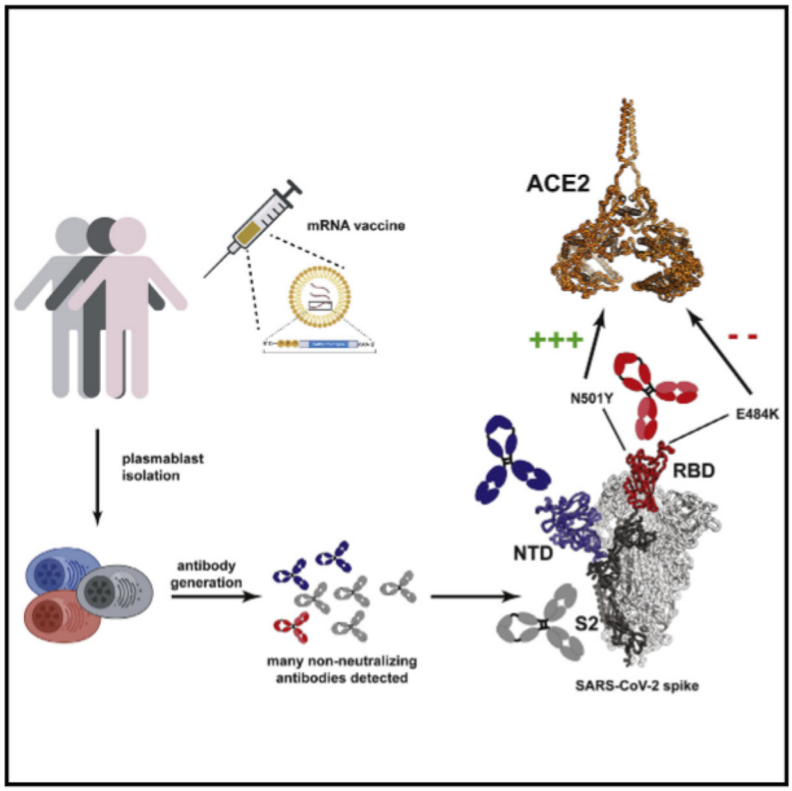
SARS-CoV-2 mRNA vaccination induces functionally diverse antibodies to NTD, RBD, and S2

Graphical abstract



Authors

Fatima Amanat, Mahima Thapa, Tinting Lei, ..., Viviana Simon, Ali H. Ellebedy, Florian Krammer

Correspondence

goran.bajic@mssm.edu (G.B.), viviana.simon@mssm.edu (V.S.), ellebedy@wustl.edu (A.H.E.), florian.krammer@mssm.edu (F.K.)

In brief

An analysis of mRNA vaccine-induced polyclonal antibodies and plasmablast-derived monoclonal antibodies from individuals vaccinated against SARS-CoV-2 identifies a high proportion of non-neutralizing antibodies and the induction of cross-reactive antibodies to seasonal coronaviruses and also maps the regions in the spike protein that are targeted, even among viral variants.

Highlights

- Antibody responses after SARS-CoV-2 mRNA vaccination target RBD, NTD, and S2
- SARS-CoV-2 mRNA vaccination induces a high rate of non-neutralizing antibodies
- Crossreactive antibodies to seasonal β-coronaviruses are induced by vaccination
- Variant mutation N501Y enhances affinity to human ACE2 while E484K reduces it

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SARS-CoV-2 mRNA vaccination induces functionally diverse antibodies to NTD, RBD, and S2

Fatima Amanat,[1,2] Mahima Thapa,[3] Tinting Lei,[3] Shaza M. Sayed Ahmed,[3] Daniel C. Adelsberg,[1] Juan Manuel Carreño,[1] Shirin Strohmeier,[1] Aaron J. Schmitz,[3] Sarah Zafar,[1] Julian Q. Zhou,[4] Willemijn Rijnink,[1] Hala Alshammary,[1] Nicholas Borcherding,[3] Ana Gonzalez Reiche,[5] Komal Srivastava,[1] Emilia Mia Sordillo,[6] Harm van Bakel,[5,7] The Personalized Virology Initiative, Jackson S. Turner,[3] Goran Bajic,[1,*] Viviana Simon,[1,8,9,*] Ali H. Ellebedy,[3,10,11,*] and Florian Krammer[1,12,*]

[1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[2]Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[3]Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA
[4]AbCellera Biologics Inc., Vancouver, BC V5Y 0A1, Canada
[5]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[6]Department of Pathology, Molecular and Cell Based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[7]Icahn Institute for Data Science and Genomic Technology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[8]Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[9]The Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[10]Andrew M. and Jane M. Bursky Center for Human Immunology and Immunotherapy Programs, Washington University School of Medicine, Saint Louis, MO 63110, USA
[11]Center for Vaccines and Immunity to Microbial Pathogens, Washington University School of Medicine, Saint Louis, MO 63110, USA
[12]Lead contact
*Correspondence: goran.bajic@mssm.edu (G.B.), viviana.simon@mssm.edu (V.S.), ellebedy@wustl.edu (A.H.E.), florian.krammer@mssm.edu (F.K.)
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SUMMARY

In this study we profiled vaccine-induced polyclonal antibodies as well as plasmablast-derived mAbs from individuals who received SARS-CoV-2 spike mRNA vaccine. Polyclonal antibody responses in vaccinees were robust and comparable to or exceeded those seen after natural infection. However, the ratio of binding to neutralizing antibodies after vaccination was greater than that after natural infection and, at the monoclonal level, we found that the majority of vaccine-induced antibodies did not have neutralizing activity. We also found a co-dominance of mAbs targeting the NTD and RBD of SARS-CoV-2 spike and an original antigenic-sin like backboost to spikes of seasonal human coronaviruses OC43 and HKU1. Neutralizing activity of NTD mAbs but not RBD mAbs against a clinical viral isolate carrying E484K as well as extensive changes in the NTD was abolished, suggesting that a proportion of vaccine-induced RBD binding antibodies may provide substantial protection against viral variants carrying single E484K RBD mutations.

INTRODUCTION

Understanding of the innate and adaptive immune responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has progressed rapidly since the beginning of the coronavirus disease 2019 (COVID-19) pandemic (Carvalho et al., 2021). Polyclonal antibody responses against the spike protein of the virus in serum, and to a lesser degree also at mucosal surfaces, have been well characterized with respect to their kinetics, binding capacity, and functionality (Grandjean et al., 2020; Isho et al., 2020; Iyer et al., 2020; Ripperger et al., 2020; Seow et al., 2020; Wajnberg et al., 2020). Similarly, encouraging data have been published on both the plasmablast response and the memory B cell response induced by SARS-CoV-2 infection (Dan et al., 2021; Gaebler et al., 2020; Guthmiller et al., 2021; Huang et al., 2021; Robbiani et al., 2020; Rodda et al., 2021; Wilson et al., 2020). The immune responses to SARS-CoV-2 vaccination, including to mRNA-based vaccines, are less well studied since these vaccines only became available in the last months of 2020 (Baden et al., 2020; Polack et al., 2020). However, understanding vaccine-induced immunity is of high importance given the goal to achieve immunity for most people through vaccination, rather than as a consequence of infection.

The receptor binding domain (RBD) of the SARS-CoV-2 spike is an important target for serological and B cell studies because it directly interacts with the cellular receptor angiotensin converting enzyme 2 (ACE2) which mediates host cell entry (Letko et al., 2020; Wrapp et al., 2020). Antibodies binding to the RBD can potently block attachment of the virus to ACE2 and thereby neutralize the virus (Barnes et al., 2020). As a consequence,

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RBD-based vaccines are in development in addition to full-length spike-based vaccines (Krammer, 2020). Analyses of the B cell responses to the spike generally focus on the RBD and on cells sorted with RBD baits introducing an inherent bias by omitting non-RBD targets (Cao et al., 2020; Gaebler et al., 2020; Robbiani et al., 2020; Weisblum et al., 2020). This is also true for B cells and monoclonal antibodies (mAbs) isolated from vaccinated individuals (Wang et al., 2021). However, other epitopes within the spike protein, notably the N-terminal domain (NTD) but also S2, do harbor neutralizing epitopes (Chi et al., 2020; Liu et al., 2020; McCallum et al., 2021; Song et al., 2020). In fact, the NTD is heavily mutated in the three most prominent variants of concern (VOCs) (B.1.1.7, B.1.351, and P.1) (Davies et al., 2021; Faria et al., 2021; Tegally et al., 2020). Here, we studied the unbiased plasmablast response to SARS-CoV-2 mRNA-based vaccination and report several new findings. First, we document that RBD and NTD co-dominate as B cell targets on the viral spike protein, highlighting the importance of the NTD. We also report the first vaccine-induced NTD mAbs. In addition, we show that the majority of mAbs isolated are non-neutralizing, which is reflective of the higher binding to neutralization ratios found in serum after vaccination compared to natural infection. Finally, data from plasmablasts suggest that, at least some of the vaccine-induced response is biased by pre-existing immunity to human β-coronaviruses.

RESULTS

The polyclonal antibody response to mRNA vaccination exceeds titers seen in convalescent individuals but is characterized by a high ratio of non-neutralizing antibodies

In late 2020, six adult participants of an ongoing observational study received mRNA-based SARS-CoV-2 vaccines (Table S1). Blood from these individuals (termed V1–V6) was collected at several time points including before vaccination (for 4/6), after the first vaccination and at several time points after the second vaccination. We examined their immune responses to recombinant spike protein and RBD in enzyme-linked immunosorbent assays (ELISA), in comparison to those of 30 COVID-19 survivors (Figures 1A and 1B, Table S1). The sera from convalescent individuals were selected based on their anti-spike titers and grouped into three groups (low +: n = 8; moderate ++: n = 11; and high +++: n = 11, based on the antibody titer measured in the Mount Sinai's CLIA laboratory (Wajnberg et al., 2020), taken 111–273 days after symptom onset), in order to facilitate identifying different features that may track with the strength of the antibody response. Five out of six vaccinees produced anti-spike and anti-RBD responses that were, at the peak, markedly higher than responses observed even in the high titer convalescent group while one vaccinee (V4) produced titers comparable to the high titer group. Notably, the antibody response peaked 1 week after the second vaccine dose, followed by a decline in titers over the following weeks as expected from an antibody response to vaccination. Interestingly, anti-RBD antibody titers seemed to decline faster than anti-spike antibody titers, which appeared to be more stable over time. We also measured neutralizing antibody titers using authentic SARS-CoV-2 and found a similar trend with all vaccinees displaying high titers, even though V4 responded with delayed kinetics (Figure 1C). Importantly, although at the peak response, the vaccine group mounted neutralization titers that fell in the upper range for the high convalescent group, they did not exceed that group markedly. This finding prompted us to calculate the proportions of spike binding to neutralizing antibodies. For the convalescent group, we found that individuals with lower titers had a higher proportion of binding to neutralizing antibodies than high-responding convalescent individuals (Figure 1D). When determined at the time of peak response, the vaccinees had the highest proportion of binding to neutralizing antibody titers, indicating an immune response more focused on non-neutralizing antibodies or an induction of less potent neutralizing antibodies in general (or both). These proportions remained stable over time with the ratio of binding to neutralizing antibodies in vaccinated individuals being significantly higher than those observed for any of the three convalescent groups ($p = 0.0004$, 0.0002 and 0.0041 for the three groups respectively; Figure S1). We also investigated the spike binding to RBD binding ratio and found no difference to convalescent individuals except a general trend toward proportionally less RBD binding over time in the vaccinees (Figure S1).

mRNA vaccination induces a modest but measurable immune response to seasonal β-coronavirus spike proteins

It has been reported that SARS-CoV-2 infection induces an original antigenic sin-type immune response against human coronaviruses (hCoVs) to which the majority of the human population has pre-existing immunity (Aydillo et al., 2021; Song et al., 2020). Here, we explored whether this phenomenon is also induced by SARS-CoV-2 mRNA vaccination. Antibody titers in four vaccinees against spike protein from α-coronaviruses 229E and NL63 were detectable at the pre-vaccination time point but did not increase substantially after vaccination (Figures 1E and 1F; for V5 and V6 no pre-vaccination serum was available). However, titers against the spike proteins of β-coronaviruses OC43 and HKU1 increased substantially in these four vaccinees after vaccination (Figures 1G and 1H). Thus, vaccination with mRNA SARS-CoV-2 spike also boosts immune responses against seasonal β-coronavirus spike proteins in a manner reminiscent of that reported for natural infection with SARS-CoV-2.

The plasmablast response to SARS-CoV-2 mRNA vaccination targets both the RBD and the NTD

In order to characterize the B cell response to vaccination in an unbiased manner, plasmablasts were single cell sorted from blood specimens obtained from three individuals (V3, V5, and V6) 6 days after the booster immunization (Figure S2). All mAbs were generated from single-cell sorted plasmablasts and probed for binding to recombinant SARS-CoV-2 spike protein. A total of 21 (40 mAbs were screened, with 28 being clonally unique, Table S2) spike-reactive mAbs were isolated from V3, 6 (82 screened, 20 unique) from V5, and 15 (84 screened, 24 unique) from V6 (Figure 2A). Using recombinant spike, RBD, NTD, and S2 proteins, we mapped the domains to which these mAbs bind. Interestingly, only a minority of these antibodies recognized RBD

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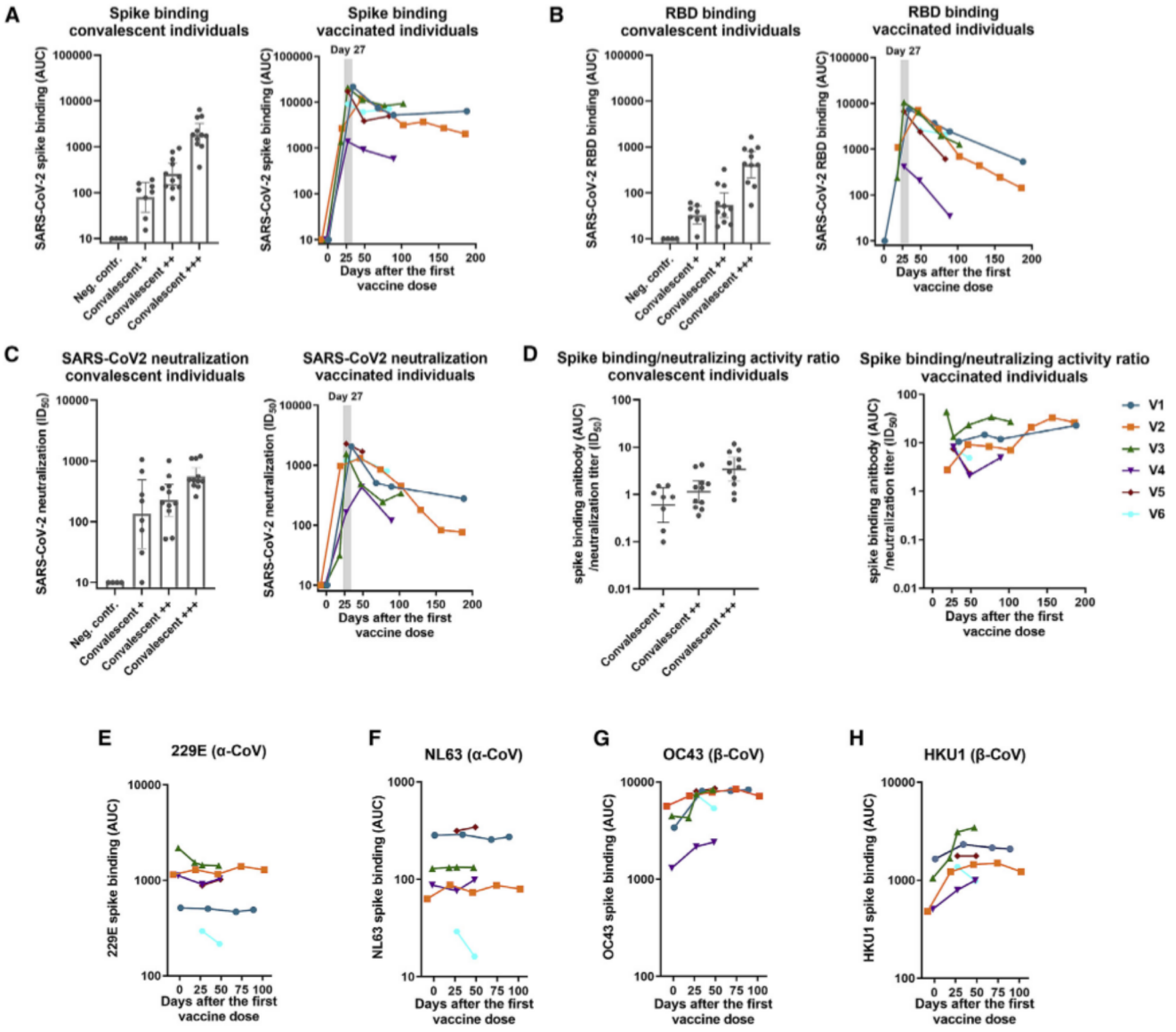

Figure 1. Antibody responses in individuals vaccinated with mRNA-based SARS-CoV-2 vaccines

(A–C) Antibody responses of convalescent individuals and vaccinees to full-length spike protein (A) and RBD (B) as measured by ELISA and neutralizing activity of the sera of the same individuals in a microneutralization assay against authentic SARS-CoV-2 (C). Convalescent individuals were grouped based on their initial antibody response (measured in a CLIA laboratory) to spike protein into +, ++, and +++.

(D) Ratios between binding and neutralizing antibody levels in vaccinees and convalescent individuals. Higher ratios indicate a bias toward non-neutralizing antibodies.

(E–H) show antibody responses against α-coronavirus 229E and NL63 and β-coronavirus OC43 and HKU1 spike proteins over time. Bars represent the geometric mean, error bars represent the 95% confidence intervals.

(24% for V3, 47% for V6, and no RBD binders were identified for V5) (Figures 2B and 2E). A substantial number of the isolated mAbs bound to NTD including 14% for V3, 33% for V5, and 33% for V6 (Figures 2C and 2E). These data indicate that RBD and NTD are co-dominant in the context of the mRNA-induced plasmablast response. The epitopes for the majority of the remaining spike binding mAbs, 52% for V3, 50% for V5, and 20% for V6, mapped to S2 (Figures 2D and 2E). Only three mAbs were not accounted for in terms of binding target (two for V3 and one for V5; Figure 2E).

The majority of isolated mAbs from SARS-CoV2 vaccinees are non-neutralizing

All antibodies were tested for neutralizing activity against the USA-WA1/2020 strain of SARS-CoV-2. Only a minority of the binding antibodies, even those targeting the RBD, showed neutralizing activity (Figures 2F and 2G). For V3, only one (an RBD binder) out of 21 mAbs (5%) displayed neutralizing activity (Figure 2G). For V5, a single NTD antibody neutralized authentic SARS-CoV-2 (17%) (Figure 2G). The highest frequency of neutralizing antibodies was found in V6 (33%) with one RBD

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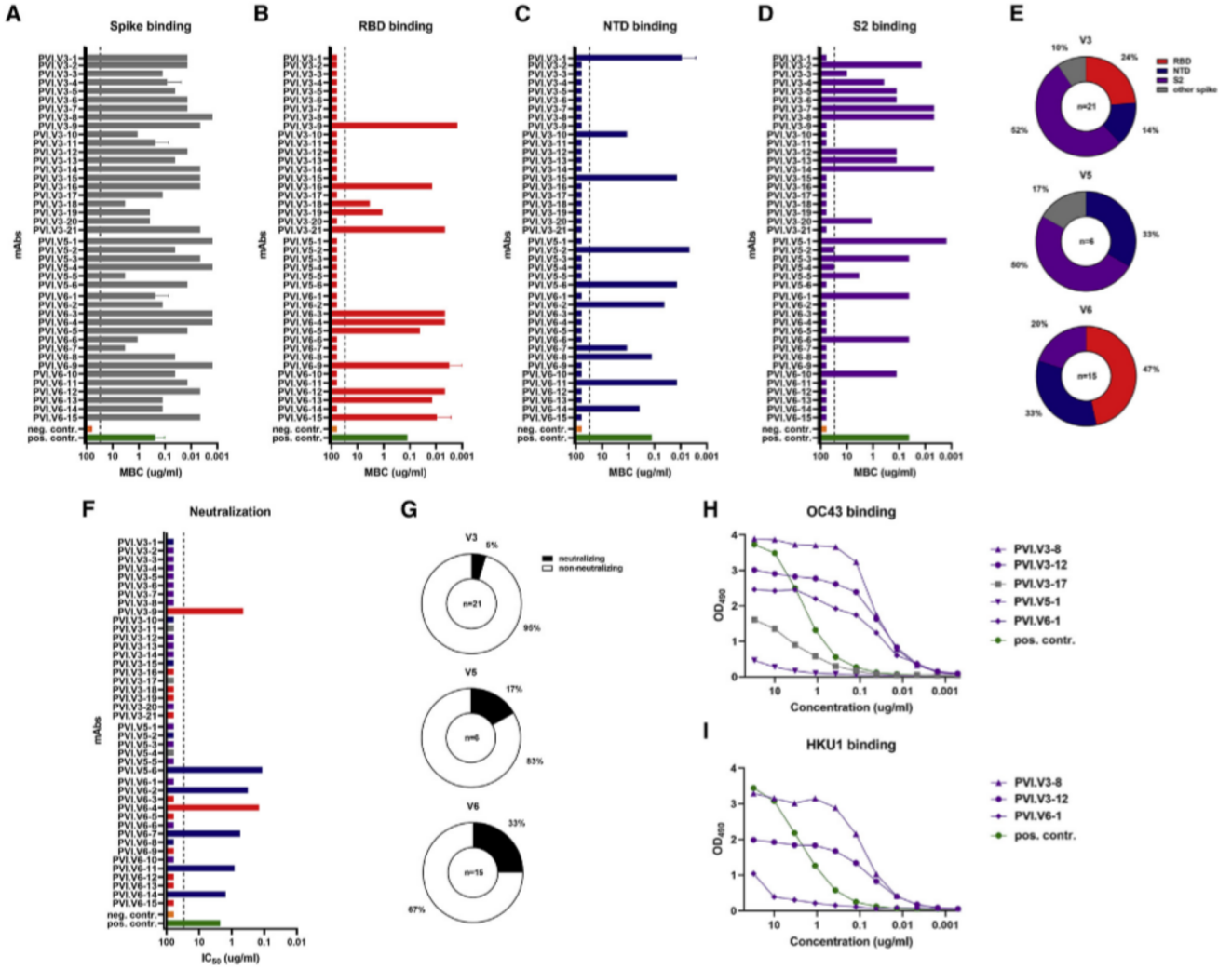

Figure 2. Characterization of mAbs derived from vaccine plasmablasts

(A–D) Binding of plasmablasts derived from three vaccinees (V3, V5, and V6) against full-length spike (A), RBD (B), NTD (C), and S2 (D).

(E) The percentages of the respective antibodies per subject.

(F and G) Neutralizing activity of the mAbs against authentic SARS-CoV-2 (F) and the proportion of neutralizing antibodies per subject is shown in (G).

(H and I) Reactivity of mAbs to spike protein of human β-coronaviruses OC43 and HKU1.

MBC, minimal binding concentration. All experiments except data shown in (H) and (I) were performed in duplicates and the mean of the duplicates is shown with standard deviation. For (H) and (I), a representative dataset from a singlet ELISA run is shown.

neutralizer and four NTD neutralizers (Figure 2G). Interestingly, the highest neutralizing potency was found for mAb PVI.V5-6, an NTD binder followed by PVI.V6-4, an RBD binder.

We also tested all mAbs for reactivity to the spike proteins of the four hCoVs: 229E, NL63, HKU1, and OC43. No antibody binding to the spike proteins of α-coronaviruses 229E and NL63 was found but we identified five mAbs (including three from V3, one from V5, and one from V6) that bound, to varying degrees, to the spike of OC43, which, like SARS-CoV-2, is a β-coronavirus (Figure 2H). Three mAbs showed strong binding (PVI.V3-8, PVI.V3-12, and PVI.V6-1), while PVI.V3-17 showed an intermediate binding phenotype and PVI-V5-1 bound very weakly. Three of these mAbs also showed binding to the spike of HKU1, another β-coronavirus. Of these, PVI.V6-1 showed only very weak binding while PVI.3-8 and PVI.3-12 had low minimal binding concentrations (MBCs) indicating higher affinity (Figure 2I).

The spike-reactive plasmablast response is dominated by IgG1$^+$ cells and is comprised of a mixture of cells with low and high levels of somatic hypermutation (SHM)

Single-cell RNA sequencing (scRNA-seq) was performed on bulk sorted plasmablasts from the three vaccinees (V3, V5, V6) to comprehensively examine the transcriptional profile, isotype distribution, and somatic hypermutation (SHM) of vaccine-induced plasmablasts. We analyzed 4,584, 3,523, and 4,461 single cells from subjects V3, V5, and V6, respectively. We first verified the identity of sequenced cells as plasmablasts through

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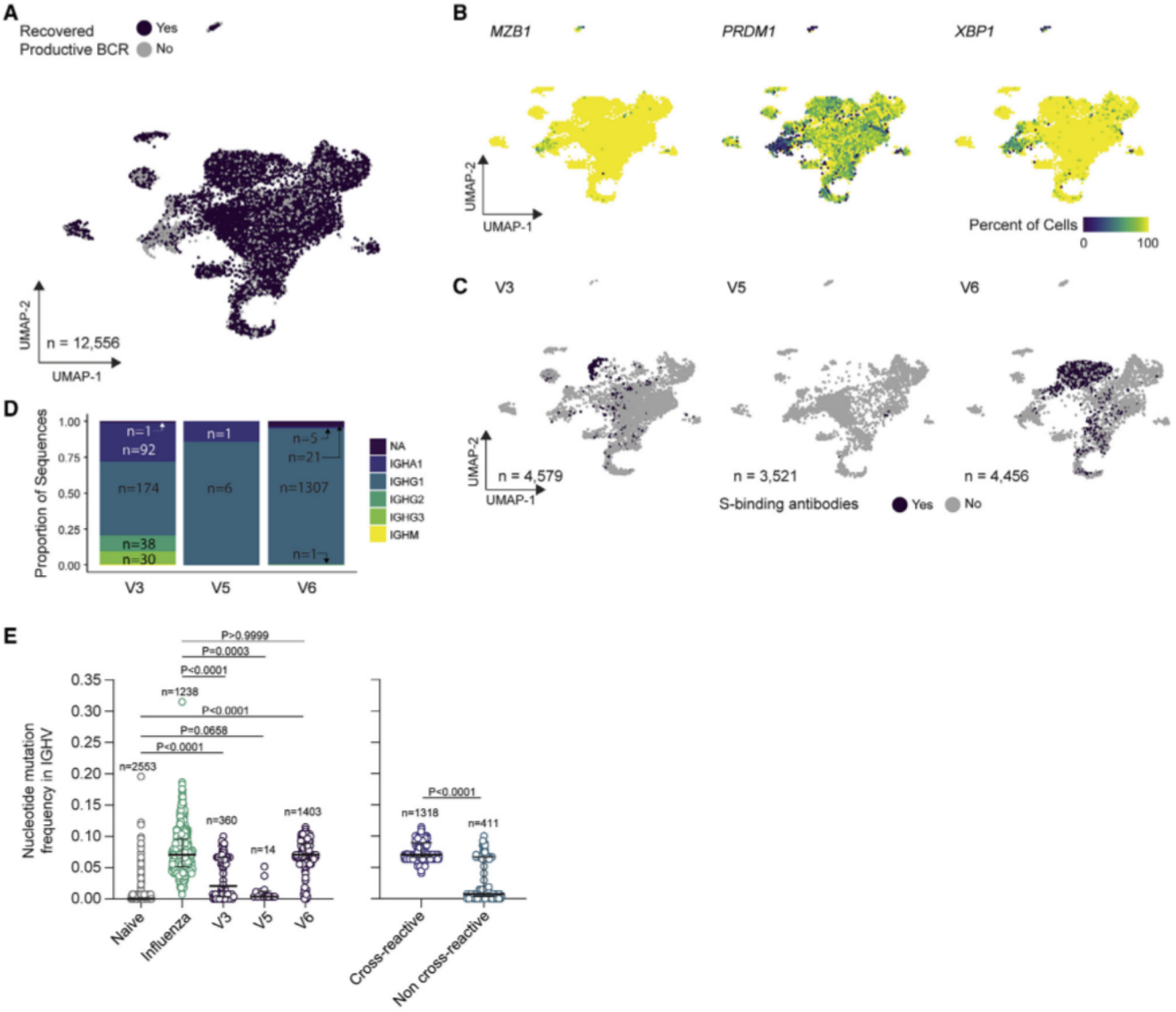

Figure 3. Characterization of bulk sorted plasmablasts via single-cell RNA sequencing

(A) Uniform manifold approximation and projection (UMAP) of scRNA-seq from bulk plasmablast with recovered BCR sequences (purple) or unrecovered (gray).

(B) UMAP overlay of percent of cellular population expressing *MZB1*, *PRDM1*, and *XPB1*. Hexbin equals 80 individual cells.

(C) UMAP overlay of BCR sequences with confirmed spike binding activity.

(D) Proportional composition of heavy chains genes in the spike binding sequences broken down by sample.

(E) Comparison of nucleotide-level mutation frequency in immunoglobulin heavy chain variable (IGHV) genes between plasmablasts clonally related to spike-binding mAbs from SARS-CoV-2 vaccinees, plasmablasts sorted from PBMCs 1 week after seasonal influenza vaccination and found in vaccine-responding B cell clones, and naive B cells found in blood of an influenza vaccinee (left); and between plasmablasts from SARS-CoV-2 vaccinees found to be clonally related to spike-binding mAbs that were, respectively, cross-reactive and non-cross-reactive to human β-coronaviruses spike proteins (right). p values were generated using a two-sided Kruskal-Wallis test with Dunn's post-test (left) or a Mann-Whitney U test (right).

the combined expression of B cell receptors (BCRs) (Figure 3A) and that of the canonical transcription as well as other factors essential for plasma cell differentiation, such as *PRDM1*, *XBP1*, and *MZB1* (Figure 3B). To identify vaccine-responding B cell clones among the analyzed plasmablasts, we used scRNA-seq to also analyze gene expression and V(D)J libraries from the sorted plasmablasts and clonally matched the BCR sequences to those from which spike-specific mAbs had been made. Using this method, we recovered 332, 7, and 1,384 BCR sequences from the scRNA-seq data that are clonally related to the spike-binding mAbs derived from subjects V3, V5, and V6, respectively (Figure 3C). It is important to note here that we were not able to recover clonally related sequences for all of the mAbs that we cloned and expressed from each of the three vaccinees.

We next examined the isotype and IgG subclass distribution among the recovered sequences. IgG1 was by far the most

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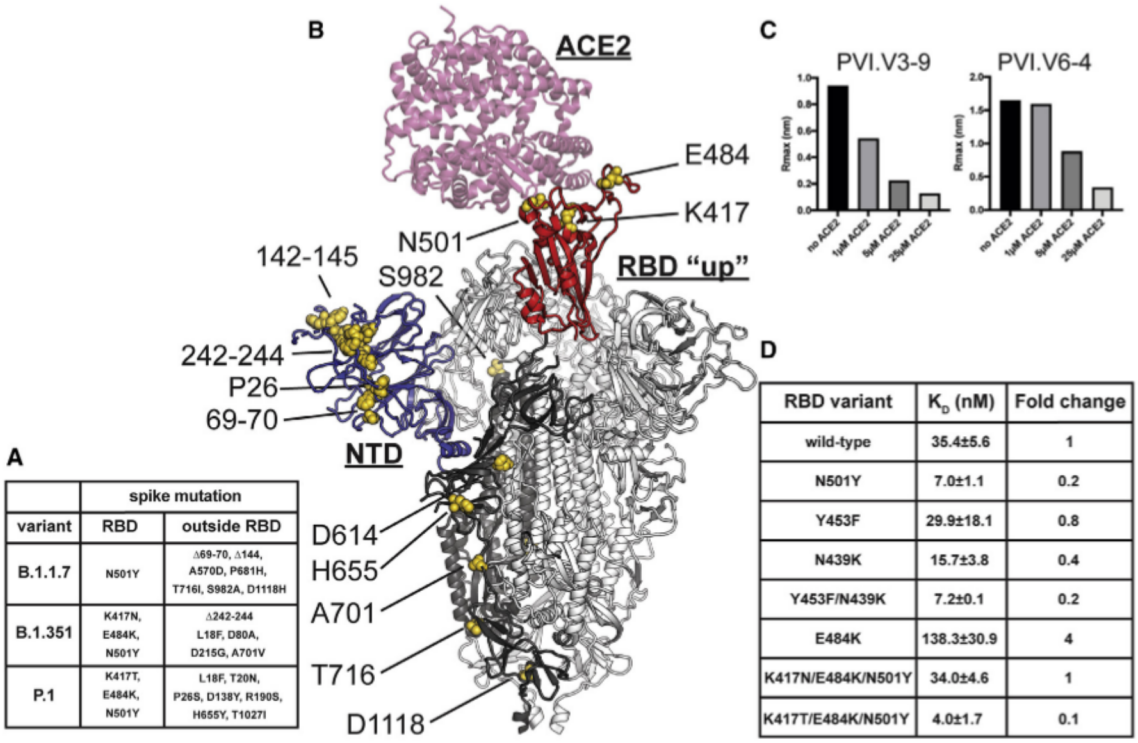

A

	spike mutation	
variant	RBD	outside RBD
B.1.1.7	N501Y	Δ69-70, Δ144, A570D, P681H, T716I, S982A, D1118H
B.1.351	K417N, E484K, N501Y	Δ242-244 L18F, D80A, D215G, A701V
P.1	K417T, E484K, N501Y	L18F, T20N, P26S, D138Y, R190S, H655Y, T1027I

D

RBD variant	K_D (nM)	Fold change
wild-type	35.4±5.6	1
N501Y	7.0±1.1	0.2
Y453F	29.9±18.1	0.8
N439K	15.7±3.8	0.4
Y453F/N439K	7.2±0.1	0.2
E484K	138.3±30.9	4
K417N/E484K/N501Y	34.0±4.6	1
K417T/E484K/N501Y	4.0±1.7	0.1

Figure 4. Mapping of the amino acid substitutions and deletions onto the structure of the SARS-CoV-2 spike glycoprotein

(A) Mutations of the three major variants of concern B.1.1.7, B.1.315, and P.1.

(B) These mutations mapped onto the structure of the spike glycoprotein (model generated by superposition of PDB: 6M0J and 7C2L) (Chi et al., 2020; Lan et al., 2020). One RBD in the up conformation (red) is bound with ACE2 receptor (pink). The NTD is colored blue and the various amino acid substitutions are shown as yellow spheres. One spike protomer is shown in bold colors while the other two are colored white.

(C) Competition between ACE2 and neutralizing RBD targeting mAbs PVI.V3-9 and PVI.V6-4 for binding to RBD.

(D) BLI-measured binding affinities of the RBD mutants to ACE2, as well as the calculated fold change compared to wild type, are shown in the table on the right.

dominant isotype in the three vaccinees (Figure 3D). Finally, we assessed the level of somatic hypermutation (SHM) among the mAbs-related sequences from the three subjects. We used the SHM levels observed in human naive B cells and seasonal influenza virus vaccination-induced plasmablasts that were previously published for comparison (Turner et al., 2020). Spike-reactive plasmablasts from V3 and V6 but not V5 had accumulated SHM at levels that are significantly greater than those observed with naive B cells (Figure 3E, left). Strikingly, the SHM level among V6 plasmablasts was equivalent to those observed after seasonal influenza virus vaccination (Figure 3E, left). We reasoned that the high level of SHM among spike-reactive plasmablasts may be derived from those targeting conserved epitopes that are shared with human β-coronaviruses. Indeed, we found that the SHM level among clones that are related to cross-reactive mAbs was significantly higher than their non-cross-reactive counterparts (Figure 3E, right).

Competition of RBD binding neutralizing mAbs with ACE2 and affinity of variant RBDs for human ACE2

Two mAbs were identified as neutralizing and binding to RBD. We wanted, therefore, to test whether they competed with ACE2 for RBD binding. Concentration-dependent competition was indeed observed for both mAbs demonstrating that inhibition of ACE2 binding is the mechanism of action of the two mAbs (Figure 4). Since we prepared RBD proteins of viral variants of concern for analysis of antibody binding (see below), we also wanted to assess the affinity of each variant RBD for human ACE2. Using biolayer interferometry (BLI), we measured association and dissociation rates of the N501Y RBD mutant (B.1.1.7 carries that mutation as its sole RBD mutation), Y453F, as found in mink isolates (Larsen et al., 2021), N439K, which is found in some European clades (Thomson et al., 2021), a combination of Y453F and N439K, E484K (part of B.1.351 and P.1) as well as for the B.1.351 and the P.1 RBDs for a recombinant version of human ACE2 (Figures 4A, 4B, and 4D). Almost all of the single and double mutations in RBD tested increased affinity to human ACE2. Specifically, N501Y and Y453F combined with N439K increased affinity for human ACE2 by 5-fold (Figures 4D and S3). In contrast, E484K on its own decreased affinity by 4-fold. Of note, the B.1.351 RBD affinity for ACE2 was comparable to that of the wild-type RBD. These data were confirmed using an ELISA-based method which showed the same trends (Figure S4).

Binding profiles of polyclonal serum and mAbs to RBDs carrying mutations found in viral variants of concern

Next, we assessed binding of sera from vaccinated individuals, COVID-19 survivors, and mAbs derived from plasmablasts to