The plasmablast response to SARS-CoV-2 mRNA vaccination is dominated by non-neutralizing antibodies that target both the NTD and the RBD

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Summary

In this study we profiled vaccine-induced polyclonal antibodies as well as plasmablast derived mAbs from subjects 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, that 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 seasonal human coronaviruses OC43 and HKU1 spike proteins. 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 E484K.
Introduction

Understanding of the innate and adaptive immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has progressed rapidly since the beginning of the coronavirus disease 2019 (COVID-19) pandemic. 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, much encouraging data has been published about 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; 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 have only become 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) mediating 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, 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 (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 all three current 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 the co-dominance of RBD and NTD as B-cell targets on the viral spike protein.
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 adults (Supplemental Table 1) who were participating in an ongoing observational study received the mRNA-based SARS-CoV-2 vaccines. Blood from these individuals (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 (Figure 1A and B). The sera from convalescent individuals were selected based on their anti-spike titers (low +: n=8; moderate ++: n=11; and high +++: n=11). Five out of six vaccinees produced anti-spike and anti-RBD responses that were 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 one week after second vaccine dose, followed by a decline in titers over the following weeks as expected from an antibody response to vaccination. 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 focused on non-neutralizing antibodies or an induction of less potent neutralizing antibodies in general (or both).

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., 2020; 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 post-vaccination (Figure 1E-F; 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 (Figure 1G-H). Thus, vaccination with mRNA SARS-CoV-2 spike also boosts seasonal β-coronaviruses immune responses in a manner reminiscent of that reported for natural infection with SARS-CoV-2.

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) one week after the booster immunization (Suppl. Figure 1). All mAbs were generated from single-cell sorted plasmablasts and probed for binding to recombinant SARS-CoV-2 spike protein. Twenty-one (40 mAbs
were screened, with 28 being clonally unique) spike-reactive mAbs were isolated from V3, six (82 screened, 20 unique) from V5 and fifteen (84 screened, 24 unique) from V6 (Figure 2A). Interestingly, only a minority of these antibodies recognized RBD (24% for V3, 47% for V6 and no RBD binders were identified for V5) (Figure 2B and D). A substantial number of the isolated mAbs bound to NTD including 14% for V3, 33% for V5 and 33% for V6 (Figure 2C and D). These data indicate that RBD and NTD are co-dominant in the context of mRNA-induced plasmablast response. The epitopes for the remaining spike binding mAbs, 62% for V3, 67% for V5 and 20% for V6, likely bind to epitopes outside of the NTD and RBD.

The majority of isolated mAbs 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 (Figure 2E and F). For V3, only one (an RBD binder) out of 21 mAbs (5%) displayed neutralizing activity (Figure 2F). For V5, a single NTD antibody neutralized authentic SARS-CoV-2 (17%) (Figure 2F). The highest frequency of neutralizing antibodies was found in V6 (34%) with one RBD neutralizer and four NTD neutralizers (Figure 2F). Interestingly, the highest neutralizing potency was found in mAb PVI.V5-6, an NTD binder followed by PVI.V6-4, an RBD binder.

We also tested all antibodies for reactivity to the spike proteins of 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 variable degrees to the spike of OC43, which, like SARS-CoV-2, is a β-coronavirus (Figure 2G). 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 2H).

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 (scRNAseq) 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 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 scRNAseq 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 1384 BCR sequences from the scRNAseq 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 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 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 naïve 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 naïve B cells (Figure 3E, left panel). Strikingly, the SHM among V6 plasmablasts was equivalent to those observed after seasonal influenza virus vaccination (Figure 3E, left panel). 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 panel).

**Affinity of variant RBDS for human ACE2**

Since we were prepared RBD proteins of viral variants of concern for analysis of antibody binding, we also wanted to assess the affinity of each variant RBD for human ACE2. Using biolayer interferometry (BLI), we measured rates of association and dissociation 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 RBDS for a recombinant version of human ACE2. Almost all 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 (Figure 4, Suppl. Figure 2). 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.

**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 variant RBDS. Our panel of RBDS includes published mAb escape mutants, RBD mutants detected by the Mount Sinai Hospital’s Pathogen Surveillance Program in patients seeking care at the Mount Sinai Health System in NYC as well as mutations found in viral variants of interest and variants of concern (Baum et al., 2020; Greaney et al., 2021; Larsen et al., 2021; Thomson et al., 2021; Weisblum et al., 2020). Serum from convalescent individuals showed strong fluctuations depending on the variant (Figure 5A). In general, single mutants E406Q, E484K and F490K exerted the biggest impact on binding. However, complete loss of binding was rare and 2-4-fold reduction in binding was more common. Interestingly, almost all sera bound better to N501Y RBD (B.1.1.7) than to wild type (average 129% compared to wild type). Conversely, the B.1.351 RBD caused on average a 39% reduction in binding. The impact was slightly lower for the P.1 RBD (average 70% binding compared to wild type). For sera from the six vaccinated individuals, however, the highest reduction seen was only two-fold for E406Q, N440K, E484K and F490K (Figure 5B). The highest reduction observed for E484K, F484A, B.1.351 and P.1 were also approximately two-fold but this did not apply to all six vaccinees. Some vaccinees maintained binding levels against these RBDS at levels comparable to wild type RBD.

RBD binding mAbs were also tested for binding to the same variants. In general, mAbs maintained binding levels within 2-fold of the binding seen with the wild type RBD with some exceptions. In fact, for most mAbs, no impact on binding was observed (Figure 5C) with the exception of PVI.V3-9, which lost binding to the RBD carrying F486A. Although there was a negative impact on binding of several mAbs to
the B.1.351 variant, binding was almost unaffected by the mutations in the P.1 variant RBD. Only one mAb, PVI.V6-4, showed a drop in binding against P.1.

**Escape of an NTD and E484K mutant virus from polyclonal post-vaccination serum is negligible but NTD mutations significantly impact the neutralizing activity of NTD binding mAbs**

Through the Mount Sinai Hospital’s Pathogen Surveillance Program, we had access to the SARS-CoV-2 isolate PV14252 (Clade 20C, Pango lineage B.1) that featured two mutations (W64R, L141Y) and one deletion (Δ142-145) in the NTD as well as the E484K mutation in the RBD (strain, **Figure 5D**). To determine the susceptibility of this virus variant to neutralization by post-vaccination serum, we performed microneutralization assays. Wild type SARS-CoV-2 and PV14252 were tested in parallel with the antiviral drug remdesivir serving as control to ensure that the assay setup for both viruses allowed comparison. The activity of remdesivir on both viruses was comparable (**Figure 5E**). We found a relatively minor impact when testing polyclonal sera from vaccinees for neutralizing activity (**Figure 5F**). The activity of sera from V2, V5 and V6 slightly increased while the activity for V1, V3 and V4 decreased. Next, we tested the seven neutralizing mAbs that we isolated from plasmablasts. Consistent with their binding profiles in the variant RBD ELISA, the two RBD mAbs neutralized both viruses with comparable efficiency. In fact, the activity of PVI.V3-9 increased slightly (**Figure 5G**). In stark contrast, all five anti-NTD antibodies completely lost neutralizing activity against PV14252 due to mutations present in the NTD of this viral isolate.
Discussion

Our knowledge of B-cell responses to SARS-CoV-2 mRNA vaccination remains incomplete. We urgently need information about the nature of polyclonal vaccine-induced responses as well as unbiased, in depth analyses of plasmablast responses. Our data provide important new insights into these responses in comparison with immune responses to natural infection. Indeed, SARS-CoV-2 infection results in a very heterogeneous antibody response to the spike protein in terms of antibody quantity. In contrast, mRNA vaccination appears to induce a high antibody response of relatively homogenous titers. However, we also found that vaccinees generate more non-neutralizing antibodies than COVID-19 survivors resulting in a worse ratio of neutralizing to binding antibodies. These data were already apparent in the early phase clinical trials but remained unrecognized at the time (Walsh et al., 2020). Interestingly, low titer convalescent serum had the highest relative amount of neutralizing antibodies, whereas the proportion of binding antibodies was increased in sera with higher measured antibody titers. The majority of plasmablasts sampled after vaccination do, in fact, produce non-neutralizing antibodies. Future studies are needed to reveal the role of non-neutralizing antibodies in SARS-CoV-2 immune protection. Indeed, antibody functions other than neutralization have been shown to correlate with protection (Bartsch et al., 2021; Gorman et al., 2021; Schäfer et al., 2021). The importance of absolute antibody titers and not ratios is underscored by the fact that post-vaccination neutralization titers were equal to or exceeded the titers found in the high responder convalescent group.

Of the four seasonal CoVs are widely circulating in humans but OC43 and HKU1 have higher homology to SARS-CoV-2 spike. Vaccinated individuals mounted a response to spike proteins from β-coronaviruses OC43 and HKU1 but not to α-coronaviruses 229E and NL63. This phenomenon resembles the immune imprinting described in influenza virus immunology and has already been described for natural infection with SARS-CoV-2 (Aydillo et al., 2020; Song et al., 2020). A few of the mAbs isolated in our study also had such cross-reactive phenotype. It remains unclear whether these antibodies, which target epitopes outside of the NTD and RBD, contribute to protection against SARS-CoV-2, OC43 or HKU1 infection. However, the cross-reactive epitopes of mAbs that do bind SARS-CoV-2, HKU1 and OC43 spikes could provide the basis for future pan-β-coronavirus vaccines.

Another interesting point we noted is the co-dominance of RBD and NTD. Previous analyses of B-cell responses to SARS-CoV-2 mRNA vaccination focused on cells baited by labeled RBD (Wang et al., 2021). We, in contrast, took an unbiased approach to sort and clone plasmablasts in an antigen-agnostic manner. We found similar levels of NTD and RBD binders with many mAbs binding to epitopes outside the RBD and the NTD. In one vaccinee not a single RBD binding mAbs was isolated with the caveats that the overall number of mAbs derived from that individual were low and their polyclonal serum antibody responses included RBD recognition. These data suggest that the NTD, which also harbors neutralizing epitopes, is - at least - as important as the RBD and warrants as much attention. In fact, five out of seven neutralizing antibodies isolated in this study bound to the NTD and only two targeted the RBD. Thus, a re-evaluation of the B cell responses to natural SARS-CoV-2 infection with unbiased approaches is needed to understand if the co-dominance of NTD and RBD is vaccine specific or also seen upon natural infection. Further characterization of the mAbs obtained in this study showed a complete loss of neutralization against an authentic, replication-competent variant virus that harbored extensive changes in the NTD. These observations may explain why a reduction in neutralization against the viral variant of concern B.1.17 is seen in some studies despite the fact the N501Y substitution in the RBD of this variant does not significantly impact binding and neutralizing activity (Emary et al., 2021).
We also assessed the impact of different RBD mutations on affinity towards human ACE2. Interestingly, N501Y increased the affinity by five-fold. This increase in receptor binding affinity may contribute to the higher infectivity of B.1.1.7, which carries this mutation in its RBD. In contrast, introduction of E484K reduced the affinity by 4-fold which may explain why virus variants carrying only the E484K mutation have rarely spread efficiently, although viruses carrying E484K have been detected since the fall of 2020 in a handful of patients receiving care at the Mount Sinai Health System and have also been reported in immunocompromised patients (Choi et al., 2020). It is tempting to speculate that the N501Y mutation enables the acquisition of E484K without a fitness loss. In fact, the B.1.351 RBD, which carries N501Y and E484K (as well as N417K) showed binding to hACE2 that was similar to wild type RBD. Recently, B.1.1.7 variant strains carrying E484K, in addition to N501Y, have been isolated in the UK (PHE, 2021), providing evidence for the hypothesis that N501Y enables acquisition of mutations in the RBD that may be detrimental to receptor binding. However, recent expansion of B.1.526, a lineage also featuring E484K but without N501Y in New York City, suggests that this fitness loss may be overcome by other, yet uncharacterized, changes in the virus as well (Annavajhala et al., 2021; Lasek-Nesselquist et al., 2021). Interestingly, binding of convalescent sera to the N501Y RBD was also increased, suggesting that changes that increase affinity for the receptor may also increase affinity of a set of antibodies that may mimic the receptor.

We also noted that the two neutralizing antibodies against the RBD showed some reduced binding to a mutant RBD carrying the E484K mutation while having similar or even increased neutralizing potency against a variant virus carrying the E484K mutation as the only change in its RBD. The reduced affinity of the E484K variant RBD for hACE2 could render the virus more susceptible to RBD binding mAbs. Thus, an antibody binding to the RBD may just be more effective in interfering with a low affinity as compared to a high affinity RBD-hACE2 interaction. Increased affinity as an escape mechanism for viruses has been described in the past (Hensley et al., 2009; O’Donnell et al., 2012) and the converse mechanism could be at play here.

Whether or not the current vaccines will provide effective protection against circulating and emerging viral variants of concern is an important question which has gathered a lot of attention in early 2021. Our data indicate that reduction in binding to the E484K and B.1.351 variant RBDs was minor (often only 2-fold) compared to reported reduction in neutralization (which ranges from 6-8 fold to complete loss of neutralization (Cele et al., 2021; Wibmer et al., 2021; Wu et al., 2021)). Although not tested here, it is likely that the reduction in binding to full length spike is even lower, given the many epitopes on the spike other than NTD and RBD. The maintenance of binding to a large degree observed in this study suggests that viral variants will have a minor impact on serological assays which are currently in wide use for medical, scientific and public health reasons. Binding, non-neutralizing antibodies have also been shown to have a protective effect in many viral infections (Asthagiri Arunkumar et al., 2019; Dilillo et al., 2014; Saphire et al., 2018) and may be a factor in the substantial residual protection seen in the Johnson &Johnson and Novavax vaccine trials against B.1.351 in South Africa (Shinde et al., 2021). Production of non-neutralizing antibodies may also play a role in protection by mRNA vaccines after the first dose, as it is substantial and occurs during a time when neutralizing antibody titers are either very low or absent (Baden et al., 2020; Dagan et al., 2021; Polack et al., 2020). Finally, although some antibodies may lose neutralizing activity due to reduced affinity, they do still bind. Furthermore, B cells with these specificities potentially could undergo affinity maturation after exposure to a variant virus or a variant spike-containing vaccine, leading to high affinity antibodies to variant viruses of concern.
In summary, we demonstrate that the antibody responses to SARS-CoV-2 mRNA vaccination comprise a large proportion of non-neutralizing antibodies and are co-dominated by NTD and RBD antibodies. The NTD portion of the spike represents, thus, an important vaccine target. Since all viral variants of concern are heavily mutated in this region, these observations warrant further attention to optimize SARS-CoV-2 vaccines. Finally, broadly cross-reactive mAbs to β-coronavirus spike proteins are induced after vaccination, and suggest a potential development path for a pan-β-coronavirus vaccine.

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Conflict of interest statement

The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines which list Florian Krammer as co-inventor. Viviana Simon is also listed on the serological assay patent application as co-inventors. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. Florian Krammer has consulted for Merck and Pfizer (before 2020), and is currently consulting for Pfizer, Seqirus and Avimex. The Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2. Ali Ellebedy has consulted for InBios and Fimbrion Therapeutics (before 2021) and is currently a consultant for Mubadala Investment Company. The Ellebedy laboratory received funding under sponsored research agreements that are unrelated to the data presented in the current study from Emergent BioSolutions and from AbbVie.
Materials and methods.

Human subjects and specimen collection. The study protocols for the collection of clinical specimens from individuals with and without SARS-CoV-2 infection by the Personalized Virology Initiative were reviewed and approved by the Mount Sinai Hospital Institutional Review Board (IRB-16-16772; IRB-16-00791; IRB-20-03374). All participants provided written informed consent prior to collection of specimen and clinical information. All specimens were coded prior to processing and analysis. An overview of the characteristics of the vaccinees as well as the study participants with and without COVID-19 is provided in Suppl. Table 1. The vaccinees received two doses of the Pfizer mRNA vaccine.

Whole blood was collected via phlebotomy in serum separator tubes (SST) or ethylenediaminetetraacetic acid (EDTA) tubes. Serum was collected after centrifugation as per manufacturers’ instructions. Peripheral blood mononuclear cells (PBMCs) isolation was performed by density gradient centrifugation using SepMate tubes ( Stemcell) according to manufacturers’ instructions. PBMCs were cryo-preserved and stored in liquid nitrogen until analysis.

Recombinant proteins. All recombinant proteins were produced using Expi293F cells (Life Technologies). Receptor binding domain (RBD) and spike protein of SARS-CoV-2 (GenBank: MN908947.3) was cloned into a mammalian expression vector, pCAGGS as described earlier (Amanat et al., 2020b; Stadlbauer et al., 2020). RBD mutants were generated in the pCAGGS RBD construct by changing single residues using mutagenesis primers. All proteins were purified after transient transfections with each respective plasmid. Six-hundred million Expi293F cells were transfected using the ExpiFectamine 293 Transfection Kit and purified DNA. Supernatants were collected on day four post transfection, centrifuged at 4,000 g for 20 minutes and finally, the supernatant was filtered using a 0.22 um filter. Ni-NTA agarose (Qiagen) was used to purify the protein via gravity flow and proteins were eluted as previously described (Amanat et al., 2020b; Stadlbauer et al., 2020). The buffer was exchanged using Amicon centrifugal units (EMD Millipore) and all recombinant proteins were finally re-suspended in phosphate buffered saline (PBS). Proteins were also run on a sodium dodecyl sulphate (SDS) polyacrylamide gels (5–20% gradient; Bio-Rad) to check for purity (Amanat et al., 2018; Margine et al., 2013). Plasmids to express recombinant spike proteins of 229E, HKU1, NL63 and OC43 were generously provided by Dr. Barney Graham (Pallesen et al., 2017).

ELISA. Ninety-six well plates (Immulon 4 HBX; Thermo Scientific) were coated overnight at 4°C with recombinant proteins at a concentration of 2 ug/ml in PBS (Gibco; Life Technologies) and 50 uls/well. The next day, the coating solution was discarded. One hundred uls per well of 3% non-fat milk prepared in PBS (Life Technologies) containing 0.01% Tween-20 (TPBS; Fisher Scientific) was added to the plates to block the plates for 1 hour at room temperature (RT). All serum dilutions were prepared in 1% non-fat milk prepared in TPBS. All serum samples were diluted 3-fold starting at a dilution of 1:50. After the blocking step, serum dilutions were added to the respective plates for two hours at RT. Next, plates were washed thrice with 250 uls/well of TPBS to remove any residual primary antibody. Secondary antibody solution was prepared in 1% non-fat milk in TPBS as well and 100 uls/well was added to the plates for 1 hour at RT. For human samples, anti-human IgG conjugated to horseradish peroxidase (HRP) was used at a dilution of 1:3000 (Millipore Sigma; catalog #A0293). For mouse samples, anti-mouse IgG conjugated to HRP was used at the same dilution (Rockland antibodies and assays; catalog #610-4302). Specifically, a mouse anti-histidine antibody (Takara; catalog #631212) was used as a positive control to detect proteins with a hexa-histidine tag. Once the secondary incubation was done, plates were again washed thrice with
250 uls/well of TPBS. Developing solution was made in 0.05M phosphate-citrate buffer at pH 5 using o-phenylenediamine dihydrochloride tablets (Sigma-Aldrich; OPD) at a final concentration of 0.04 mg/ml. One hundred uls/well of developing solution was added to each plate for exactly 10 minutes after which the reaction was halted with addition of 50 uls/well of 3M hydrochloric acid (HCl). Plates were read at an optical density of 490 nanometers using a Synergy 4 (BioTek) plate reader. Eight wells on each plate received no primary antibody (blank wells) and the optical density in those wells was used to assess background. Area under the curve was calculated by deducting the average of blank values plus 3 times standard deviation of the blank values. All data was analyzed in Graphpad Prism 7. This protocol has been described in detail earlier (Bailey et al., 2019; Wohlbold et al., 2015).

Purified monoclonal antibodies were used at a concentration of 30 ug/ml and then subsequently diluted 3-fold. Purified monoclonal antibodies were only incubated on the coated plates for an hour. The remaining part of the protocol was the same as above (Amanat et al., 2020a; Wohlbold et al., 2016).

**Bio-layer Interferometry Binding Experiments**

Bio-layer Interferometry (BLI) experiments were performed using the BLItz system (fortéBIO, Pall Corporation). Recombinant human Fc fusion ACE2 (SinoBiological) was immobilized on an anti-human IgG Fc biosensor, and RBDs were then applied to obtain binding affinities. Single-hit concentrations were tested at 5.8 μM for binding. All measurements were repeated in subsequent independent experiments. K_D values were obtained through local fit of the curves by applying a 1:1 binding isotherm model using vendor-supplied software. All experiments were performed in PBS pH 7.4 and at room temperature.

**Viruses and cells.** Vero.E6 cells (ATCC #CRL-1586) cells were maintained in culture using Dulbecco’s Modified Eagles Medium (DMEM, Gibco) which was supplemented with 10% fetal bovine serum (FBS, Corning) and antibiotics solution containing 10,000 units/mL of penicillin and 10,000 µg/mL of streptomycin (Pen Strep, Gibco)(10). Wild type SARS-CoV-2 (isolate USA-WA1/2020) was grown in cells for 3 days, the supernatant was clarified by centrifugation at 4,000 g for 5 minutes and aliquots were frozen at -80°C for long term use. A primary virus isolate, PV14252, bearing mutations and deletions in the spike was obtained by incubating 200 uls of viral transport media from the nasopharyngeal swab with Vero.E6 cells. The sequence of the passage 2 viral isolate was identical to the sequence obtained directly from the clinical specimen. Sequencing was performed on the Illumina platform as described previously (Gonzalez-Reiche et al., 2020). Both replication competent viruses were used to test serum from study participants and antibodies for neutralization activity.

**Neutralization assay.** Twenty-thousand cells in 100 uls per well were seeded on sterile 96-well cell culture plates one day prior to the neutralization assay. In general, cells were used at 90% confluency to perform the assay. All serum samples were heat-inactivated to eliminate any complement activity. Serial dilutions of serum samples were made in 1X minimal essential medium (MEM; Life Technologies) starting at a dilution of 1:20. All work with authentic SARS-CoV-2 (isolate USA-WA1/2020 and PV14252) was done in a biosafety level 3 (BSL3) laboratory following institutional biosafety guidelines and has been described in much greater detail earlier (Amanat et al., 2020b; Amanat et al., 2020c). Six hundred median cell culture infectious doses (TCID_{50}s) of authentic virus (USA-WA1/2020 and PV14252) was added to each serum dilution and virus-serum mixture was incubated together for 1 hour inside the biosafety cabinet. Media from the cells was removed and 120 uls of the virus-serum mixture was added onto the cells for 1 hour at 37°C. After one hour, the virus-serum mixture was removed and 100 uls of each corresponding dilution was added to every well. In addition, 100uls of 1X MEM was also added to every well. Cells were incubated
for 48 hours at 37°C after which the media was removed and 150 uls of 10% formaldehyde (Polysciences) was added to inactivate the virus. For assay control, remdesivir was used against both the wild type virus as well as the patient isolate. After 24 hours, cells were permeabilized and stained using an anti-nucleoprotein antibody 1C7 as discussed in detail earlier (Amanat et al., 2020b; Sun et al., 2020).

Cell sorting and flow cytometry. Staining for sorting was performed using cryo-preserved PBMCs in 2% FBS and 2 mM ethylenediaminetetraacetic acid (EDTA) in PBS (P2). Cells were stained for 30 min on ice with CD20-Pacific Blue (2H7, 1:400), Zombie Aqua, CD71-FITC (CY1G4, 1:200), IgD-PerCP-Cy5.5 (JAG-2, 1:200), CD19-PE (HIB19, 1:200), CD38-PE-Cy7 (HIT2, 1:200), and CD3-Alexa 700 (HIT3a, 1:200), all BioLegend. Cells were washed twice, and single plasmablasts (live singlet CD19+ CD3- IgDlo CD38+ CD20- CD71+) were sorted using a FACSaria II into 96-well plates containing 2 µL Lysis Buffer (Clontech) supplemented with 1 U/µL RNase inhibitor (NEB) and immediately frozen on dry ice, or bulk sorted into PBS supplemented with 0.05% BSA and processed for single cell RNAseq.

Monoclonal antibody (mAb) generation. Antibodies were cloned as described previously (Wrammert et al., 2011). Briefly, VH, Vκ, and Vλ genes were amplified by reverse transcription-PCR and nested PCR reactions from singly sorted plasmablasts using primer combinations specific for IgG, IgM/A, Igκ, and Igλ from previously described primer sets (Smith et al., 2009) and then sequenced. To generate recombinant antibodies, restriction sites were incorporated via PCR with primers to the corresponding heavy and light chain V and J genes. The amplified VH, Vκ, and Vλ genes were cloned into IgG1 and Igκ expression vectors, respectively, as described previously (Nachbagauer et al., 2018; Wrammert et al., 2008). Heavy and light chain plasmids were co-transfected into Expi293F cells (Gibco) for expression, and antibody was purified with protein A agarose (Invitrogen).

Single-cell RNAseq library preparation and sequencing. Bulk-sorted plasmablasts were processed using the following 10× Genomics kits: Chromium Next GEM Single Cell 5′ Kit v2 (PN-1000263); Library Construction Kit (PN-1000190); Chromium Next GEM Chip K Single Cell Kit (PN-1000286); Chromium Single Cell Human BCR Amplification Kit (PN-1000253), and Dual Index Kit TT Set A (PN-1000215). The cDNAs were prepared after GEM generation and barcoding, followed by GEM RT reaction and bead cleanup steps. Purified cDNA was amplified for 10–14 cycles before cleaning with SPRIselect beads. Then, samples were evaluated on a 4200 TapeStation (Agilent) to determine cDNA concentration. B-cell receptor (BCR) target enrichments were performed on full-length cDNA. Gene expression and enriched BCR libraries were prepared as recommended by the Chromium Next GEM Single Cell 5′ Reagent Kits v2 (Dual Index) user guide, with appropriate modifications to the PCR cycles based on the calculated cDNA concentration. The cDNA libraries were sequenced on Novaseq S4 (Illumina), targeting a median sequencing depth of 50,000 and 5,000 read pairs per cell for gene expression and BCR libraries, respectively.

Single cell RNAseq analysis. Single-cell RNA sequencing and BCR sequencing data was processed using Cell Ranger v5.0 and the GRCh38-2020 version of the human genome provided by the manufacturer. Total recovered cells by RNA sequencing were V3: 6,608, V5: 5,256, and V6: 6,325 with a mean of 90.64% read mapped to the genome. Count matrices were processed in R (v4.0.2) using the Seurat (v3.2.2) R package (Stuart et al., 2019). Cells were filtered for percentage of mitochondrial genes less than 15% and number features less than 4,000. The three specimen sequencing runs were integrated using log-normalized count values and canonical correlation approach (Stuart et al., 2019) with 2,000 variable features. The resulting single-cell object underwent principal component analysis and the top 30 principal components were used for uniform manifold approximation and projection and identifying neighbors. Clustering was performed using a resolution of 0.6. The integrated RNA sequencing object included 12,568 cells with V3: 4,584, V5: 3,523, and V6: 4,461 cells. The filtered contig annotation output of Cell Ranger vdj were loaded into R and
processed using the scRepertoire (v1.1.3) R package (Borcherding et al., 2020). Clonotypes were assigned using igraph (v1.2.6) network analysis of components generated from CDR3 sequences greater than or equal to 0.85 normalized Levenshtein distance. Percent of cells expressing genes along the UMAP embedding was visualized using the schex (v1.3.0) R package. For mutation analysis, heavy chains of mAbs and single-cell BCRs first underwent V(D)J gene annotation using IgBLAST (v1.14.0) (Ye et al., 2013) with human reference (release 201931-4) from the international ImMunoGeneTics information system (IMGT) (Giudicelli et al., 2005) and then parsing using Change-O (v0.4.6) (Gupta et al., 2015). Mutation frequency was calculated, as described in (Turner et al., 2020), using the "calcObservedMutations" function from SHazaM (v.1.0.2) (Gupta et al., 2015) and by counting the number of nucleotide mismatches from the germline sequence in the heavy chain variable segment leading up to the complementary-determining region 3 (CDR3), while excluding the first 18 positions that could be error-prone due to the primers used for generating the mAb sequences.

Structure visualization. Structural figures were modeled and rendered in Pymol (The PyMOL Molecular Graphics System, Version 2.4 Schrödinger, LLC).

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Figure Legends

**Figure 1**: Antibody responses in individuals vaccinated with mRNA-based SARS-CoV-2 vaccines. 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 antibody response to spike protein into +, ++, and ++++. D shows ratios between binding and neutralizing antibody levels in vaccinees and convalescent individuals. Higher ratios indicate a bias towards non-neutralizing antibodies. E, F, G and H show antibody responses against α-coronavirus 229E and NL63 and β-coronaviruses OC43 and HKU1 spike proteins over time.

**Figure 2**: Characterization of mAbs derived from vaccine plasmablasts. Binding of plasmablasts derived from three vaccinees (V3, V5 and V6) against full length spike (A), RBD (B) and NTD (C). D shows the percentages of the respective antibodies per subject. E shows neutralizing activity of the mAbs against authentic SARS-CoV-2 and the proportion of neutralizing antibodies per subject is shown in F. G and H show reactivity of mAbs to spike protein of human β-coronaviruses OC43 and HKU1.

**Figure 3**: Characterization of bulk sorted plasmablasts via single-cell RNA sequencing. (A) Uniform manifold approximation and projection (UMAP) of scRNAseq from bulk plasmablast with recovered BCR sequences (purple) or unrecovered (grey). (B) UMAP overlay of percent of cellular population expressing MZB1, PRDM1, and XBP1. 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 one 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 panel); 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 panel).

**Figure 4**: Mapping of the amino-acid substitutions and deletions onto the structure of the SARS-CoV-2 spike glycoprotein. Mutations of the three major variants of concern B.1.17, B.1.315 and P.1 show in the table on the left are 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). 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. BLI- measured binding affinities of the RBD mutants to ACE2, as well as the calculated fold change, are shown in the table on the right.

Figure 5. Binding and neutralization of SARS-CoV-2 variants. Binding of serum samples from convalescent individuals, vaccinees and vaccine derived mAbs to a panel of RBD mutants is shown in A, B and C respectively. The red line in A indicates the average reduction. Dotted lines in A and B indicate 100%, the line with smaller dots in C indicated reactivity of the anti-his coating control. D shows the spike mutations of virus isolate PV14252 modelled on a co-crystal structure of the SARS-CoV-2 spike protein with ACE2 (model generated by superposition of PDB 6M0j and 7C2L (Chi et al., 2020; Lan et al., 2020)). E shows the inhibitory effect of remdesivir on growth of wild type SARS-CoV-2 and PV14252. Remdesivir was used as control here to show that both viruses when run side by side in a neutralization assay are inhibited to a similar extent. F and G show the inhibitory effect of vaccine serum and vaccine derived neutralizing antibodies on both wild type SARS-CoV-2 and PV14252. Of note, these assays were performed side by side but by different operators and on a different Vero cell clone as the neutralization assays shown in Figure 2.

Supplemental Table 1

| Vaccinees | Spike IgG response | Sex | Age group (yrs) | Specimen tested |
|-----------|--------------------|-----|----------------|-----------------|
| V1        | Strong positive    | F   | >60            | several longitudinal time points |
| V2        | Strong positive    | M   | 30-40          | several longitudinal time points |
| V3        | Strong positive    | F   | 50-60          | several longitudinal time points |
| V4        | Strong positive    | M   | >60            | several longitudinal time points |
| V5        | Strong positive    | F   | 40-50          | several longitudinal time points |
| V6        | Strong positive    | F   | 30-40          | several longitudinal time points |

Seronegative, post pandemic

| Sex | Age group (yrs) | Days from last negative serology test |
|-----|----------------|---------------------------------------|
| F   | 40-50          | 23                                    |
| F   | 20-29          | 24                                    |
| F   | 20-29          | 23                                    |
| F   | 30-35          | 22                                    |
| Seropositive, natural infection | Sex | Age group (yrs) | Days post onset of COVID 19 symptoms |
|-------------------------------|-----|----------------|-------------------------------------|
| P1 Weak positive M 20-29      |     |                | 260                                 |
| P2 Weak positive M 50-59      |     |                | no data available                   |
| P3 Weak positive F 30-39      |     |                | 111                                 |
| P4 Weak positive F 30-39      |     |                | 221                                 |
| P5 Weak positive F 30-39      |     |                | 254                                 |
| P6 Weak positive F 20-29      |     |                | 247                                 |
| P7 Weak positive M 30-39      |     |                | 220                                 |
| P8 Weak positive F 20-29      |     |                | Asymptomatic                        |
| P9 Moderate positive M 30-39  |     |                | no data available                   |
| P10 Moderate positive F 30-39 |     |                | 197                                 |
| P11 Moderate positive F 50-59 |     |                | Asymptomatic                        |
| P12 Moderate positive F 30-39 |     |                | Asymptomatic                        |
| P13 Moderate positive M 30-39 |     |                | 234                                 |
| P14 Moderate positive F 20-29 |     |                | 273                                 |
| P15 Moderate positive M 30-39 |     |                | Asymptomatic                        |
| P16 Moderate positive F 20-29 |     |                | 258                                 |
| P17 Moderate positive F 20-29 |     |                | 246                                 |
| P18 Moderate positive M 20-29 |     |                | Asymptomatic                        |
| P19 Moderate positive F 50-59 |     |                | 204                                 |
| P20 Strong positive F 50-59   |     |                | no data available                   |
| P21 Strong positive F 30-39   |     |                | 245                                 |
| P22 Strong positive M NA      |     |                | 170                                 |
| P23 Strong positive F >60     |     |                | Asymptomatic                        |
| P24 Strong positive F 40-49   |     |                | no data available                   |
| P25 Strong positive F 50-59   |     |                | 191                                 |
| P26 Strong positive F 30-39   |     |                | no data available                   |
| P27 Strong positive F 50-59   |     |                | 113                                 |
| P28 Strong positive M >60     |     |                | Asymptomatic                        |
| P29 Strong positive M 18-19   |     |                | 218                                 |
| P30 Strong positive M 50-59   |     |                | 219                                 |

Supplementary Figure 1. Gating strategy for sorting plasmablasts from total PBMCs isolated one week after second immunization.
Supplementary Figure 2. Representative Biolayer Interferometry binding isotherms from two independent experiments. The raw data are shown in pink and the Langmuir 1:1 kinetics fit is shown in black.
The figure illustrates the interaction between the Spike protein and the ACE2 receptor. The Spike protein is shown in various orientations, highlighting key regions such as the N-terminal domain (NTD), the RBD, and the RBD "up" state. Mutations in the Spike protein are denoted at specific residues, including N501Y, K417N, E484K, and N501Y.

The table provides K0 values for different RBD variants and their fold changes compared to the wild-type. The variants include:
- Wild-type: 35.4±5.6 nM, Fold change: 1
- N501Y: 7.0±1.1 nM, Fold change: 0.2
- Y453F: 29.9±18.1 nM, Fold change: 0.8
- N439K: 15.7±3.8 nM, Fold change: 0.4
- Y453F/N439K: 7.2±0.1 nM, Fold change: 0.2
- E484K: 138.3±30.9 nM, Fold change: 4
- K417N/E484K/N501Y: 34.0±4.6 nM, Fold change: 1
Convalescent sera vs variant RBD binding

% binding compared to wt RBD

- wt RBD
- E484K
- Δ142-145
- V64R
- L141Y
- NTD
- ACE2

- PI4.V3-16
- PI4.V3-9 (neut+)
- PI4.V3-18
- PI4.V3-19
- PI4.V3-21
- PI4.V6-3
- PI4.V6-4 (neut+)
- PI4.V6-5
- PI4.V6-9
- PI4.V6-12
- PI4.V6-13
- Anti-his (coating contr.)
- Negative

Remdesivir (assay control)

% inhibition vs concentration (μM)

- wt
- PI4.V1252
- (W64R, L141Y, E484K, D614G, Δ142-145)

Vaccinee serum wt versus PI4.V1252

Neutralization

ID_{50}

- V1
- V2
- V3
- V4
- V5
- V6

PV14252 spike

mAb wt versus PI4.V1252

Neutralization

IC_{50} (μg/ml)

- PI4.V3-9
- PI4.V5-6
- PI4.V6-2
- PI4.V6-4
- PI4.V6-7
- PI4.V6-11
- PI4.V6-14
