Antibody Evolution after SARS-CoV-2 mRNA Vaccination

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Summary
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection produces B-cell responses that continue to evolve for at least one year. During that time, memory B cells express increasingly broad and potent antibodies that are resistant to mutations found in variants of concern. As a result, vaccination of coronavirus disease 2019 (COVID-19) convalescent individuals with currently available mRNA vaccines produces high levels of plasma neutralizing activity against all variants tested. Here, we examine memory B cell evolution 5 months after vaccination with either Moderna (mRNA-1273) or Pfizer-BioNTech (BNT162b2) mRNA vaccines in a cohort of SARS-CoV-2 naïve individuals. Between prime and boost, memory B cells produce antibodies that evolve increased neutralizing activity, but there is no further increase in potency or breadth thereafter. Instead, memory B cells that emerge 5 months after vaccination of naïve individuals express antibodies that are equivalent to those that dominate the initial response. We conclude that memory antibodies selected over time by natural infection have greater potency and breadth than antibodies elicited by vaccination. These results suggest that boosting vaccinated individuals with currently available mRNA vaccines would produce a quantitative increase in plasma neutralizing activity but not the qualitative advantage against variants obtained by vaccinating convalescent individuals.

Between January 21 and June 23, 2021, we recruited 32 volunteers with no history of prior SARS-CoV-2 infection receiving either Moderna (mRNA-1273; n=8) or Pfizer-BioNTech (BNT162b2; n=24) mRNA vaccines for sequential blood donation. Matched samples were obtained an average of 2.5 and 5 weeks after the prime and boost, respectively, and an additional
set of matched samples an average of 2 and 5 months after the boost. The volunteers ranged in age from 23-78 years (median 34.5 years), 53% were male and 47% female (for details see Methods and Supplementary Tables 1 and 2).

**Plasma binding and neutralization assays**

Plasma IgM, IgG, and IgA responses to SARS-CoV-2 receptor binding domain (RBD) were measured by enzyme linked immunosorbent assay (ELISA). As reported by others there was a significant increase in IgG reactivity to RBD between prime and boost (p<0.0001, Fig. 1a). IgM and IgA titers were lower than IgG titers and remained low after the second vaccine dose (Extended data Fig. 1a and b). The magnitude of the response was inversely correlated with age after the prime (r=-0.54, p=0.005), but the difference was no longer significant after the boost (Fig. 1b). Between 2 and 5 months after the boost, anti-RBD titers of all isotypes decreased significantly. IgG titers decreased by an average of 5.2-fold (range: 2.1- to 10.2-fold) and the loss of activity was directly correlated to the time after vaccination (p<0.0001, Fig. 1c and d).

Neutralizing activity was measured using HIV-1 pseudotyped with the SARS-CoV-2 spike. Naïve individuals showed variable responses to the initial vaccine dose with a geometric mean half-maximal neutralizing titer (NT$_{50}$) of 171 (Fig. 1e and Supplementary Table 2). The magnitude of the neutralizing responses to the initial vaccine dose in naïve volunteers was inversely correlated with age (r=-0.39, p=0.05, Fig. 1f). Both binding and neutralizing responses to the second vaccine dose were correlated to the prime (r=0.46, p=0.02, Extended data. Fig. 1e; r=0.54, p=0.003, Extended data Fig. 1f) and produced a nearly 15-fold increase in the geometric
mean neutralizing response that was similar in males and females and eliminated the age-related
difference in neutralizing activity (Extended data 2g and Fig. 1f). After the boost naïve vaccinees
had 6.2-fold higher neutralizing titers than a cohort of infected individuals measured 1.3 months
after symptom onset ($p<0.0001$, Fig. 1e). Neutralizing responses were directly correlated to IgG
anti-RBD titers ($r=0.96$, $p<0.0001$, Fig. 1g). Thus, the data obtained from this cohort agree with
prior observations showing a significant increase in plasma neutralizing activity that are
correlated with improved vaccine efficacy in naïve individuals that receive the second dose of
mRNA vaccine$^{2,6,9,10}$.

The 15 individuals assayed 5 months after vaccination had an average 4.7-fold decrease in
geometric mean neutralizing activity from their 2-month measurement ($p=0.04$, Fig. 1h), with a
range of 1.4- to 27-fold (Fig. 1i). Neutralizing activity was inversely correlated with the time
from vaccination ($r=-0.75$, $p<0.0001$, Fig. 1j), and directly correlated to IgG anti-RBD binding
titers when assessed 5 months after vaccination (Extended data. Fig. 1h).

We and others showed that the neutralizing responses elicited by mRNA vaccination are more
potent against the original Wuhan Hu-1 strain than for some of the currently circulating variants
of concern$^{2,11-13}$. To confirm these observations, we measured the neutralizing activity of the
paired plasmas from naive individuals 2 and 5 months after the second vaccine dose against
B.1.1.7 (alpha variant), B.1.351 (beta variant), B.1.526 (first isolated in New York City), P.1
(gamma variant) and B.1.617.2 (delta variant). Consistent with previous reports$^{12,14-16}$ the
neutralizing activity against the variants was lower than against the original Wuhan Hu-1 strain
(Fig. 1k, Supplementary Table 3). Initial geometric mean neutralizing titers at 2 months against
B.1.351, B.1.1.7, B.1.526, P.1 and B.1.617.2 were 5.7, 1.8, 1.1, 1.4 and 2.7-fold lower than against Wuhan-Hu respectively (Fig. 1k). In the months following vaccination there was a decrease in neutralizing activity against all variants that paralleled the drop in activity against Wuhan Hu-1 (R683G) with geometric mean neutralizing titers for WT, B.1.351, B.1.1.7, B.1.526, P.1 and B.1.617.2 decreasing by 2.9-, 1.8-, 2.3-, 2.9-, 2.4- and 2.6-fold, respectively (Fig. 1k and Supplementary Table 3). Nevertheless, vaccine-induced neutralizing activity at 5 months against all variants exceeds Wuhan-Hu plasma neutralization of convalescent individuals after 6.2 months.

Monoclonal Antibodies

Circulating antibodies produced by plasma cells can prevent infection if present at sufficiently high concentrations at the time of exposure. In contrast, the memory B cell compartment contains long lived antigen-specific B cells that mediate rapid recall responses that contribute to long term protection[17]. To examine the nature of the memory compartment elicited by one or two mRNA vaccine doses and its evolution after 5 months we used flow cytometry to enumerate B cells expressing receptors that bind to Wuhan Hu-1 (wild type) and the B.1.351 K417N/E484K/N501Y (KEN) variant RBDs (Fig. 2a and b, and Extended data Fig. 2). Wuhan-Hu RBD-specific memory B cells developed after the prime in all volunteers examined and their numbers increased for up to 5 months after vaccination (Fig. 2a). Memory B cells binding to the B.1.351 RBD were detectable but in lower numbers than wild type RBD-binding B cells in all samples examined (Fig. 2b). Whereas IgG memory cells increased after the boost, IgM-expressing memory B cells that made up 23% of the memory compartment after the prime were
nearly absent after boosting (Fig. 2c). Finally, circulating RBD-specific plasmablasts were readily detected after the prime but were infrequent after the boost (Fig. 2d, and Extended data Fig. 2d).

The memory compartment continues to evolve up to one year after natural infection with selective enrichment of cells producing broad and potent neutralizing antibodies. To determine how the memory compartment evolves after vaccination, we obtained 1524 paired antibody sequences from 6 individuals sampled at 2.5 weeks after prime and 5 weeks or 5 months after boost, and an additional 804 paired antibody sequences from 5 individuals sampled after 2- or 5-months after boost (Fig. 2e and f, Extended Data Fig 3, Supplementary Table 4). As expected IGHV3-30 and IGHV3-53 were over-represented after the first and second vaccine dose and remained over-represented 5 months after vaccination (Extended data Fig. 4).

All individuals examined showed expanded clones of memory B cells that expressed closely related IGHV and IGHL genes (Fig. 2e and f, Extended data Fig. 4). Paired prime and boost samples showed expanded clones of memory B cells some of which were shared across plasmablast, IgM and IgG prime, and IgG boost memory cells (Extended data Fig. 3 and 5). Thus, the cell fate decision controlling the germinal center versus plasmablast decision is not entirely affinity dependent since cells with the same initial affinity can enter both compartments.

The relative fraction of memory cells found in expanded clones varied between prime and boost and between individuals (Fig. 2e). However, new clones that develop after the boost represent a
greater fraction (80%) of the total clones than the conserved clones in all individuals (Fig. 2e).

Finally, memory B cells emerging after the boost showed significantly higher levels of somatic mutations than plasmablasts or memory B cells isolated after the prime (Extended data Fig. 3b).

After 5 months, there was an overall decrease in the percentage of clones in the RBD-binding memory compartment (Fig. 2g). Nevertheless, clones of memory B cells continued to evolve for up to 5 months in vaccinated individuals as evidenced by the dominance of newly emerging clones (86%, Fig. 2e and f) and the significant increase in somatic mutation between the time points (p<0.0001, Fig. 2h, Extended data Fig. 3c). The number of mutations in antibody genes was comparable between vaccinated and convalescent individuals after 5 months (Fig. 2h, Extended data Fig. 3c). In conclusion the memory B cell compartment continues to evolve for up to 5 months after mRNA vaccination.

Neutralizing Activity of Monoclonal Antibodies

We performed ELISAs to confirm that the antibodies isolated from memory B cells bind to RBD (Extended data Fig. 6). 403 antibodies were tested by ELISA including: 86 isolated after the first vaccine dose; 92 isolated after the second vaccine dose; 111 isolated 2 months after the second vaccine dose and 114 isolated from individuals that had been fully vaccinated 5 months earlier. Among the 403 antibodies tested 381 (95%) bound to the Wuhan Hu-1 RBD indicating that the method used to isolate RBD-specific memory B cells was highly efficient (Supplementary Table 5-7). The geometric mean ELISA half-maximal concentration (EC_{50}) of the antibodies obtained after prime, boost, 2 months and 5 months was 3.9, 2.6, 3.2 and 2.8 ng/ml respectively, suggestive of no change in binding over time after vaccination (Extended data Fig. 6 and
Supplementary Table 5). Binding among all antibodies did not improve between prime and boost, or 2 and 5 months (Extended data Fig. 6). However, a slight improvement was observed after 5 months among clones (Extended data Fig. 6).

381 RBD-binding antibodies were tested for neutralizing activity using HIV-1 pseudotyped with the SARS-CoV-2 spike^{3,8}. The geometric mean half-maximal inhibitory concentration (IC_{50}) of the RBD-specific memory antibodies improved from 380 ng/ml to 170 ng/ml between the first and second vaccine dose (p=0.005, Fig. 3a). The improvement was reflected in all clones (IC_{50} 370 vs. 182 ng/ml, p=0.03 Fig. 3b), newly arising clones (IC_{50} 430 vs. 165 ng/ml, p=0.02 Fig. 3c), single antibodies (IC_{50} 357 vs. 131 ng/ml, Fig. 3d) and conserved clones (IC_{50} 314 vs. 208 ng/ml, Fig. 3e, Supplementary Table 6). The increase in neutralizing activity between the first and second vaccine dose was associated with a decrease in the percentage of non-neutralizing antibodies (defined as IC_{50}>1000 ng/ml) and increased representation of neutralizing antibodies (p= 0.03, Fig. 3a). In conclusion, memory B cells recruited after the boost account for most of the improvement in neutralizing activity in this compartment between the 2 vaccine doses. Thus, in addition to the quantitative improvement in serum neutralizing activity there is a qualitative improvement in the memory compartment after boosting.

There was no further improvement in neutralizing activity of the monoclonal antibodies obtained between 2 and 5 months after vaccination (IC_{50} 140 vs. 144 ng/ml, Fig. 3f). This was true when considering all B cell clones, newly arising clones or single memory B cells separately (Fig. 3g-j, Supplementary table 7). In contrast, memory antibodies obtained from convalescent individuals showed improved neutralizing activity between 1.3^{3} and 6.2 months^{7} with IC_{50} of 171 ng/ml to
116 ng/ml (Fig. 3f), which improved further after 1 year\(^1\). This improvement was due to
increased neutralizing activity among persisting clones (p=0.003, Fig. 3h).

**Affinity, Epitopes and Neutralization Breadth**

To examine affinity maturation after vaccination, we performed biolayer interferometry (BLI)
experiments using the Wuhan Hu-1 RBD\(^2\). 60 randomly selected antibodies were assayed from
the prime-boost cohort, 30 obtained after the prime and 30 after the boost, with similar overall
geometric mean IC\(_{50}\)s ranging from <10 ng/ml to >1000 ng/ml (Extended data Fig. 7a). There
was no significant difference in affinity between the antibodies obtained after the prime and
boost, and no correlation between affinity and neutralizing activity (Fig. 4a and Extended Data
Fig. 7 b and c). Similar experiments were performed on 31 and 30 antibodies obtained from the
2- and 5-month time points from vaccinated individuals, respectively. Despite similar levels of
neutralizing activity (Extended data Fig. 7d), there was an average 2.6-fold increase in the
affinity of the antibodies obtained between 2 and 5 months after vaccination (p=0.02, Fig. 4b and
Extended Data Fig. 7e and f).

We also compared the affinities of pairs of antibodies obtained from conserved clones between 2
and 5 months after vaccination. Conserved clones obtained at 2 and 5 months from vaccinated
individuals showed an average 1.9-fold increase in affinity (p=0.03, Fig. 4c). In contrast, a
comparable group of conserved clonal antibodies obtained from convalescent individuals 1.3 and
6.2 months after infection showed an average 24-fold increase in affinity (p=0.002, Fig. 4c).
In addition to the increase in potency, the neutralizing breath of memory antibodies obtained from conserved clones from convalescent individuals increases with time after infection\textsuperscript{1,7,22}. To determine whether there is a similar increase in breadth with time after vaccination, we selected 20 random antibodies from the prime or boost, with representative levels of activity against the original Wuhan Hu-1 strain, and measured their neutralization potency against a panel of pseudotypes encoding RBD mutations associated with circulating variants of concern (Extended data. Fig. 8). There was little change in breadth between prime and boost, with only a small increase in resilience to K417N and A475V substitutions (Extended data Fig. 8, Supplementary Table 8).

In addition, we assayed 10 conserved pairs of neutralizing antibodies obtained 2 and 5 months after vaccination against the same RBD mutant pseudotypes (Fig. 4d and Supplementary Table 9). They were compared to 6 previously reported\textsuperscript{22}, plus 10 additional pairs of antibodies obtained from convalescent individuals at 1.3- and 6.2-month time points (Fig. 4e and Supplementary Table 9). Whereas only 4 of 10 of the vaccine antibodies showed improved breadth, 15 of the 16 convalescent pairs developed increased breadth. Moreover, only 1 of the 10 vaccine antibody pairs showed improved potency against pseudotypes carrying B.1.617.2 (delta variant)-specific RBD amino acid substitutions (L452R/T478K), while 11 out of 16 convalescent antibody pairs showed improved activity against this virus (Fig. 4d and e). We conclude that antibody evolution differs in convalescent and vaccinated individuals in that there is less affinity maturation and little increase in breadth between 2 and 5 months after mRNA vaccination.
Circulating antibodies are produced by plasma cells with variable longevity\(^23-26\). SARS-CoV-2 infection or mRNA vaccination produces an early peak antibody response that decreases by 5-10-fold after 5 months\(^27-31\). Notably, peak neutralization titres after vaccination exceed those of COVID-19 recovered individuals. Nevertheless, neutralizing potency against variants is significantly lower than against Wuhan Hu-1, with up to 5-10-fold reduced activity against the B.1.351 variant\(^5,6,12,13,32\). Taken together with the overall decay in neutralizing activity there can be 1-2 orders of magnitude decrease in serum neutralizing activity after 5 or 6 months against variants when compared to the peak of neutralizing activity against Wuhan Hu-1. Thus, antibody mediated protection against variants is expected to wane significantly over a period of months, consistent with reports of reinfections in convalescent individuals and breakthrough infection by variants in fully vaccinated individuals\(^33-35\).

In contrast to circulating antibodies, memory B cells are responsible for rapid recall responses\(^36-39\), and this compartment is relatively stable over the first 6 months after natural infection\(^7,40\) or mRNA vaccination. In both cases memory B cells continue to evolve for up to 5 months as evidenced by increasing levels of somatic mutation and emergence of newly expanded clones.

The memory response would be expected to protect individuals that suffer breakthrough infection from developing serious disease. However, memory B cell evolution differs in important ways between infection and mRNA vaccination. Both natural infection and mRNA vaccination produce memory antibodies that evolve increased affinity, but the increase in affinity is more modest after vaccination. This difference is consistent with the observation that vaccine-
elicited memory antibodies fail to show the increased neutralizing breadth that developed after natural infection\textsuperscript{1,7}.

There are innumerable differences between natural infection and mRNA vaccination that could account for the differences in antibody evolution over time. These include but are not limited to:

1. Route of antigen delivery, respiratory tract vs. intra-muscular injection\textsuperscript{41,42}; 2. The physical nature of the antigen, intact virus vs. S protein\textsuperscript{43}; 3. Antigen persistence, weeks in the case of natural infection\textsuperscript{7} vs. hours to days for mRNA\textsuperscript{44}. Each of these could impact on B cell evolution and selection directly, and indirectly through differential T cell recruitment.

The increase in potency and breadth in the memory compartment that develops after natural infection accounts for the exceptional responses to Wuhan Hu-1 and its variants that convalescent individuals develop when boosted with mRNA vaccines\textsuperscript{1,5}. The expanded memory B cell compartment in mRNA vaccinees should also produce high titers of neutralizing antibodies but with decreased breath in comparison to natural infection when vaccinees are boosted or when they are re-exposed to the virus\textsuperscript{45}. Thus, boosting vaccinated individuals with currently available mRNA vaccines will produce strong responses that mirror their initial vaccine responses to Wuhan-Hu with similarly decreased coverage against variants. Finally, timing a boost for optimal responses will depend on whether the objective is to prevent viral acquisition or disease\textsuperscript{46}. Given the rapid emergence of variants, in the former, boosting would be needed on a far shorter time scale than the latter. The optimal timing for boosting to prevent serious disease will depend on the stability and further evolution of the memory B cell compartment.
**Figures**

**Fig. 1: Plasma ELISAs and neutralizing activity.**

*Figures a-j.*

- **a.** Graph shows area under the curve (AUC, Y-axis) for plasma IgG antibody binding to SARS-CoV-2 RBD after prime and boost for paired samples. (n=26)
- **b.** Graph shows plasma IgG antibody binding (AUC, Y-axis) plotted against age (X-axis) after prime (black) and boost (orange).
- **c.** Graph shows AUC (Y-axis) for plasma IgG antibody binding to SARS-CoV-2 RBD for paired samples obtained 2 and 5 months after the boost. (n=15)
- **d.** Graph shows AUC values from a, and b, (Y-axis) plotted against time after vaccination (X-axis).
- **e.** NT50 in convalescent individuals 1.3m after infection and in vaccinated individuals after 1 dose (prime) or 2 doses (boost) of an mRNA vaccine.
- **f.** NT50 values (Y-axis) vs. age (years X-axis) in individuals receiving 1 dose (prime, black) or two doses (boost, orange) of an mRNA vaccine.
- **g.** NT50 values (Y-axis) vs. IgG binding (AUC, Y-axis) plotted against age (X-axis) for plasma IgG antibody binding to SARS-CoV-2 RBD after prime and boost for paired samples. (n=26)
antibody binding (AUC, X-axis) after boost in individuals receiving two doses of an mRNA vaccine. h, NT50 values in convalescent individuals 1.3m and 6.2m after infection and in vaccinated individuals 2-12 and 5-months (m) after receiving 2 doses of an mRNA vaccine. i, NT50 values in vaccinated individuals 2-12 and 5-months after receiving 2 doses of an mRNA vaccine. Lines connect paired longitudinal samples from the same individual. j, Graph shows NT50 values (Y-axis) vs. days (X-axis) after boost in individuals receiving two doses of an mRNA vaccine. k, Plasma neutralizing activity against indicated SARS-CoV-2 variants of concern (n=15 paired samples at 2- and 5-months after full vaccination). Refer to Methods for a list of all substitutions/deletions/insertions in the spike variants. All experiments were performed at least in duplicate. NT50 values for Moderna mRNA-1273 and Pfizer-BioNTech BNT162b2 in d, e, h-j are shown in black and red, respectively. Red bars and values in e, h, and k represent geometric mean NT50 values. Statistical significance in a, c, i and k was determined by Wilcoxon test, in b, d, f, g and j by spearman correlation test and in e and h by Kruskal-Wallis test with subsequent Dunn’s multiple comparisons.
**Fig. 2:** Anti-SARS-CoV-2 RBD B cells after vaccination. a-d, Graphs summarizing a, the number of Wuhan-Hu RBD (WT)-specific memory B cells per 10 million B cells for vaccinees after prime or boost (n=26) or at follow-up of 2-12 and 5-months after full vaccination (n=9), including 3 individuals who were sampled at prime, boost, and again 5 months post-vaccination (illustrated by red dots). b, the number of antigen-specific memory B cells cross-reactive with both WT and K417N/E484K/N501Y (KEN) RBD mutant per 10 million B cells after prime or boost.
The frequency of IgM, IgG, or IgA isotype expression by Wuhan-Hu RBD-specific memory B cells after prime or boost (n=10), and d, number of Wuhan-Hu RBD-binding plasmablasts per 10 million B cells (n=26) after prime or boost. Red numbers indicate geometric means. Gating strategy is in Extended Data Fig. 2. e-f, Pie charts show the distribution of IgG antibody sequences obtained from memory B cells from 11 individuals after e, prime or boost or 5-months, and f, 2- or 5- months. The number inside the circle indicates the number of sequences analyzed for the individual denoted above the circle, with Pfizer vaccinees indicated by (P) and Moderna by (M). Individuals in red text indicate those that were sampled at prime, boost, and again 5 months post-vaccination. Pie slice size is proportional to the number of clonally related sequences. The black outline and associated numbers indicate the percentage of clonally expanded sequences detected at each time point. Colored slices indicate persisting clones (same IGHV and IGLV genes, with highly similar CDR3s) found at more than one timepoint within the same individual. Grey slices indicate clones unique to the timepoint. White slices indicate repeating sequences isolated only once per time point. g, Graph shows the relative percentage of expanded clonal sequences at each time point in e and f. The red numbers indicate the geometric means. h, Number of nucleotide mutations in the IGVH and IGVL combined (also Supplementary Table 4) in the antibodies illustrated in e and f, compared to the number of mutations obtained after 1.3 or 6.2 months after infection (illustrated by grey dots). Red horizontal bars and numbers indicate mean number of nucleotide mutations at each time point. Statistical significance in a-d and g was determined using Wilcoxon matched-pairs signed rank test. Statistical significance in h was determined by Kruskal Wallis test with subsequent Dunn’s multiple comparisons.
Fig. 3: Anti-SARS-CoV-2 RBD monoclonal antibodies. a-j, Graphs show anti-SARS-CoV-2 neutralizing activity of monoclonal antibodies measured by a SARS-CoV-2 pseudotype virus neutralization assay using wild-type (Wuhan Hu-1) SARS-CoV-2 pseudovirus\(^3,8\). a-e, Half-maximal inhibitory concentration (IC\(_{50}\)) values for all antibodies (a), all clones (b), non-persisting clones (c), singlets (d) and shared clones (e) isolated after prime or boost. f-j, Half-maximal inhibitory concentration (IC\(_{50}\)) values for all antibodies (f), all clones (g), shared clones (h), non-persisting clones (i) and singlets (j) isolated from COVID-19 convalescent individuals 1.3\(^2\) and 6.2\(^7\) months after infection or from vaccinated individuals 2m\(^1\) or 5m after vaccination. Each dot represents one antibody. Pie charts illustrate the fraction of non-neutralizing (IC\(_{50} > 1000\) ng/ml) antibodies (grey slices), inner circle shows the number of antibodies tested per group. Horizontal bars and red numbers indicate geometric mean values. Statistical significance in a-d, f, g, i, j was
determined by Mann-Whitney test and in \( e \) and \( h \) by Wilcoxon test. Statistical significance for ring plots was determined using Fisher’s exact test. All experiments were performed at least twice.
**Fig. 4: Affinity and Breadth.** a-c, Graphs show antibody \( K_{D} \) for Wuhan-Hu RBD measured by BLI. a, antibodies isolated from vaccinees after prime (red, n=30) or boost (green, n=30). b, same as a, but from vaccinees after 2- (red, n=31) and 5-months (green, n=30). c, Clonal-paired antibodies isolated from convalescents 1.3\(^3\) and 6\(^7\)-months after infection (n=15) or vaccinated individuals 2- and 5-months after full vaccination (n=16). Black horizontal bars and numbers indicate geometric mean values. Statistical significance was determined using two-tailed unpaired
t-test (a, b) or two tailed Kruskal Wallis test with subsequent Dunn’s multiple comparisons (c).

BLI traces can be found in Extended Data Fig 7. d-e, Heat-maps show inhibitory concentrations of antibodies isolated 5m after vaccination (d) or 6.2 months\(^7\) after infection (e) normalized to their shared clone isolated 2m after vaccination (d) or 1.3 months\(^3\) after infection (e), expressed as %IC50, against indicated mutant SARS-CoV-2 pseudoviruses (Supplementary Table 9). Antibodies with improved (<30%) IC50 compared to their clonal relative isolated at an earlier timepoint are colored in shades of green with most improved antibodies in darkest green. Antibodies with worse (>300%) IC50 than their clonal relative isolated at an earlier timepoint are colored in red with the most worsened antibodies in dark red. Antibodies that did not change their IC50 by more than ~3-fold are shown in yellow.
METHODS

Study participants.

Participants were healthy volunteers receiving either the Moderna (mRNA-1273) or Pfizer-BioNTech (BNT162b2) mRNA vaccines against SARS-CoV-2 who were recruited for serial blood donations at Rockefeller University Hospital in New York between January 21 and June 23, 2021. Participants indicated as “Prime/Boost” were individuals who were de novo recruited for this study, while a subgroup of individuals (indicated as “2m/5m”) were from a long-term study cohort. Eligible participants were healthy adults with no history of infection with SARS-CoV-2, as determined by clinical history and confirmed through serology testing, receiving one of the two Moderna (mRNA-1273) or Pfizer-BioNTech (BNT162b2), according to current dosing and interval guidelines. Exclusion criteria included incomplete vaccination status, presence of clinical signs and symptoms suggestive of acute infection with or a positive RT-PCR results for SARS-CoV-2 in saliva, or a positive COVID-19 serology. Seronegativity for COVID-19 was established through the absence of serological activity toward the nucleocapsid protein (N) of SARS-CoV-2. Participants presented to the Rockefeller University Hospital for blood sample collection and were asked to provide details of their vaccination regimen, possible side effects, comorbidities and possible COVID-19 history. All participants provided written informed consent before participation in the study and the study was conducted in accordance with Good Clinical Practice. The study was performed in compliance with all relevant ethical regulations and the protocol (DRO-1006) for studies with human participants was approved by the Institutional Review Board of the Rockefeller University. For detailed participant characteristics see Supplementary Tables 1 and 2.
**Blood samples processing and storage.**

Peripheral Blood Mononuclear Cells (PBMCs) obtained from samples collected at Rockefeller University were purified as previously reported by gradient centrifugation and stored in liquid nitrogen in the presence of FCS and DMSO\textsuperscript{3,7}. Heparinized plasma and serum samples were aliquoted and stored at -20°C or less. Prior to experiments, aliquots of plasma samples were heat-inactivated (56°C for 1 hour) and then stored at 4°C.

**ELISAs**

ELISAs\textsuperscript{48,49} to evaluate antibodies binding to SARS-CoV-2 RBD were performed by coating of high-binding 96-half-well plates (Corning 3690) with 50 μl per well of a 1μg/ml protein solution in PBS overnight at 4°C. Plates were washed 6 times with washing buffer (1× PBS with 0.05% Tween-20 (Sigma-Aldrich)) and incubated with 170 μl per well blocking buffer (1× PBS with 2% BSA and 0.05% Tween-20 (Sigma)) for 1 h at room temperature. Immediately after blocking, monoclonal antibodies or plasma samples were added in PBS and incubated for 1 h at room temperature. Plasma samples were assayed at a 1:66 starting dilution and 10 additional threefold serial dilutions. Monoclonal antibodies were tested at 10 μg/ml starting concentration and 10 additional fourfold serial dilutions. Plates were washed 6 times with washing buffer and then incubated with anti-human IgG, IgM or IgA secondary antibody conjugated to horseradish peroxidase (HRP) (Jackson Immuno Research 109-036-088 109-035-129 and Sigma A0295) in blocking buffer at a 1:5,000 dilution (IgM and IgG) or 1:3,000 dilution (IgA). Plates were developed by addition of the HRP substrate, TMB (ThermoFisher) for 10 min (plasma samples) or 4 minutes (monoclonal antibodies). The developing reaction was stopped by adding 50 μl of 1
M H$_2$SO$_4$ and absorbance was measured at 450 nm with an ELISA microplate reader (FluoStar Omega, BMG Labtech) with Omega and Omega MARS software for analysis. For plasma samples, a positive control (plasma from participant COV72, diluted 66.6-fold and ten additional threefold serial dilutions in PBS) was added to every assay plate for validation. The average of its signal was used for normalization of all the other values on the same plate with Excel software before calculating the area under the curve using Prism V9.1 (GraphPad). For monoclonal antibodies, the EC50 was determined using four-parameter nonlinear regression (GraphPad Prism V9.1). EC50s above 2000 ng/mL were considered non-binders.

**Proteins**

Mammalian expression vector encoding the RBD of SARS-CoV-2 (GenBank MN985325.1; S protein residues 319-539) was previously described.$^{50}$

**SARS-CoV-2 pseudotyped reporter virus**

A panel of plasmids expressing RBD-mutant SARS-CoV-2 spike proteins in the context of pSARS-CoV-2-S$_{Δ19}$ has been described.$^{12,22,51}$ Variant pseudoviruses resembling variants of concern B.1.1.7 (first isolated in the UK), B.1.351 (first isolated in South-Africa), B.1.526 (first isolated in New York City), P.1 (first isolated in Brazil) and B.1.617.2 (first isolated in India) were generated by introduction of substitutions using synthetic gene fragments (IDT) or overlap extension PCR mediated mutagenesis and Gibson assembly. Specifically, the variant-specific deletions and substitutions introduced were:

B.1.526: L5F, T95I, D253G, E484K, D614G, A701V.


The E484K, K417N/E484K/N501Y (KEN), L452R/E484Q and L452R/T478K substitution, as well as the deletions/substitutions corresponding to variants of concern listed above were incorporated into a spike protein that also includes the R683G substitution, which disrupts the furin cleavage site and increases particle infectivity. Neutralizing activity against mutant pseudoviruses were compared to a wildtype SARS-CoV-2 spike sequence (NC_045512), carrying R683G where appropriate.

SARS-CoV-2 pseudotyped particles were generated as previously described\(^3\),\(^8\). Briefly, 293T cells were transfected with pNL4-3Env-nanoluc and pSARS-CoV-2-S\(_{Δ19}\), particles were harvested 48 hpt, filtered and stored at -80°C.

**Pseudotyped virus neutralization assay**

Fourfold serially diluted plasma from COVID-19-convalescent individuals or monoclonal antibodies were incubated with SARS-CoV-2 pseudotyped virus for 1 h at 37 °C. The mixture was subsequently incubated with 293T\(_{Ace2}\) cells\(^3\) (for all WT neutralization assays) or HT1080Ace2 cl14 (for all mutant panels and variant neutralization assays) cells\(^1\)\(^2\) for 48h after which cells were washed with PBS and lysed with Luciferase Cell Culture Lysis 5x reagent (Promega). Nanoluc Luciferase activity in lysates was measured using the Nano-Glo Luciferase Assay System (Promega) with the Glomax Navigator (Promega). The relative luminescence units
were normalized to those derived from cells infected with SARS-CoV-2 pseudotyped virus in the absence of plasma or monoclonal antibodies. The half-maximal neutralization titers for plasma (NT$_{50}$) or half-maximal and 90% inhibitory concentrations for monoclonal antibodies (IC$_{50}$ and IC$_{90}$) were determined using four-parameter nonlinear regression (least squares regression method without weighting; constraints: top=1, bottom=0) (GraphPad Prism).

**Biotinylation of viral protein for use in flow cytometry**

Purified and Avi-tagged SARS-CoV-2 RBD or SARS-CoV-2 RBD KEN mutant (K417N, E484K, N501Y) was biotinylated using the Biotin-Protein Ligase-BIRA kit according to manufacturer’s instructions (Avidity) as described before$^3$. Ovalbumin (Sigma, A5503-1G) was biotinylated using the EZ-Link Sulfo-NHS-LC-Biotinylation kit according to the manufacturer’s instructions (Thermo Scientific). Biotinylated ovalbumin was conjugated to streptavidin-BV711 (BD biosciences, 563262) and RBD to streptavidin-PE (BD Biosciences, 554061) and streptavidin-AF647 (Biolegend, 405237)$^3$.

**Flow cytometry and single cell sorting**

Single-cell sorting by flow cytometry was described previously$^3$. Briefly, peripheral blood mononuclear cells were enriched for B cells by negative selection using a pan-B-cell isolation kit according to the manufacturer’s instructions (Miltenyi Biotec, 130-101-638). The enriched B cells were incubated in FACS buffer (1× PBS, 2% FCS, 1 mM EDTA) with the following anti-human antibodies (all at 1:200 dilution): anti-CD20-PECy7 (BD Biosciences, 335793), anti-CD3-APC-eFluro 780 (Invitrogen, 47-0037-41), anti-CD8-APC-eFluor 780 (Invitrogen, 47-0086-42), anti-CD16-APC-eFluor 780 (Invitrogen, 47-0168-41), anti-CD14-APC-eFluor 780
as well as Zombie NIR (BioLegend, 423105) and fluorophore-labelled RBD and ovalbumin (Ova) for 30 min on ice. Single CD3-CR8-CD14-CD16−CD20+Ova−RBD-PE+RBD-AF647+ B cells were sorted into individual wells of 96-well plates containing 4 μl of lysis buffer (0.5× PBS, 10 mM DTT, 3,000 units/ml RNasin Ribonuclease Inhibitors (Promega, N2615) per well using a FACS Aria III and FACSDiva software (Becton Dickinson) for acquisition and FlowJo for analysis. The sorted cells were frozen on dry ice, and then stored at −80 °C or immediately used for subsequent RNA reverse transcription. For plasmablast single-cell sorting, in addition to above antibodies, B cells were also stained with anti-CD19-BV605 (Biolegend, 302244), and single CD3-CR8-CD14-CD16−CD20-Ova-RBD-PE+RBD-AF647+ plasmablasts were sorted as described above. For B cell phenotype analysis, in addition to above antibodies, B cells were also stained with following anti-human antibodies: anti-IgD-BV421 (Biolegend, 348226), anti-CD27-FITC (BD biosciences, 555440), anti-CD19-BV605 (Biolegend, 302244), anti-CD71- PerCP-Cy5.5 (Biolegend, 334114), anti- IgG-PECF594 (BD biosciences, 562538), anti-IgM-AF700 (Biolegend, 314538), anti-IgA-Viogreen (Miltenyi Biotec, 130-113-481).

Antibody sequencing, cloning and expression

Antibodies were identified and sequenced as described previously3,52. In brief, RNA from single cells was reverse-transcribed (SuperScript III Reverse Transcriptase, Invitrogen, 18080-044) and the cDNA was stored at −20 °C or used for subsequent amplification of the variable IGH, IGL and IGK genes by nested PCR and Sanger sequencing. Sequence analysis was performed using MacVector. Amplicons from the first PCR reaction were used as templates for sequence- and
ligation-independent cloning into antibody expression vectors. Recombinant monoclonal antibodies were produced and purified as previously described.

**Biolayer interferometry**

Biolayer interferometry assays were performed as previously described. Briefly, we used the Octet Red instrument (ForteBio) at 30 °C with shaking at 1,000 r.p.m. Affinity measurement of anti-SARS-CoV-2 IgGs binding were corrected by subtracting the signal obtained from traces performed with IgGs in the absence of WT RBD. The kinetic analysis using protein A biosensor (ForteBio 18-5010) was performed as follows: (1) baseline: 60 sec immersion in buffer. (2) loading: 200 sec immersion in a solution with IgGs 10 μg/ml. (3) baseline: 200 sec immersion in buffer. (4) Association: 300 sec immersion in solution with WT RBD at 20, 10 or 5 μg/ml (5) dissociation: 600 sec immersion in buffer. Curve fitting was performed using a fast 1:1 binding model and the Data analysis software (ForteBio). Mean KD values were determined by averaging all binding curves that matched the theoretical fit with an $R^2$ value ≥ 0.8.

**Computational analyses of antibody sequences**

Antibody sequences were trimmed based on quality and annotated using Igblastn v.1.14. with IMGT domain delineation system. Annotation was performed systematically using Change-O toolkit v.0.4.540. Heavy and light chains derived from the same cell were paired, and clonotypes were assigned based on their V and J genes using in-house R and Perl scripts. All scripts and the data used to process antibody sequences are publicly available on GitHub (https://github.com/stratust/igpipeline/tree/igpipeline2_timepoint_v2).
The frequency distributions of human V genes in anti-SARS-CoV-2 antibodies from this study was compared to 131,284,220 IgH and IgL sequences generated by and downloaded from cAb-Rep, a database of human shared BCR clonotypes available at https://cabrep.c2b2.columbia.edu. Based on the 112 distinct V genes that make up the 7936 analyzed sequences from Ig repertoire of the 11 participants present in this study, we selected the IgH and IgL sequences from the database that are partially coded by the same V genes and counted them according to the constant region. The frequencies shown in Extended Data Fig. 4 are relative to the source and isotype analyzed. We used the two-sided binomial test to check whether the number of sequences belonging to a specific IgHV or IgLV gene in the repertoire is different according to the frequency of the same IgV gene in the database. Adjusted p-values were calculated using the false discovery rate (FDR) correction. Significant differences are denoted with stars.

Nucleotide somatic hypermutation and CDR3 length were determined using in-house R and Perl scripts. For somatic hypermutations, IGHV and IGLV nucleotide sequences were aligned against their closest germlines using Igblastn and the number of differences were considered nucleotide mutations. The average number of mutations for V genes was calculated by dividing the sum of all nucleotide mutations across all participants by the number of sequences used for the analysis.

**Data availability statement:** Data are provided in Supplementary Tables 1-9. The raw sequencing data and computer scripts associated with Figure 2 and Extended Data Fig. 3 have been deposited at Github (https://github.com/stratust/igpipeline/tree/igpipeline2_timepoint_v2). This study also uses data from “A Public Database of Memory and Naive B-Cell Receptor
Sequences” (https://doi.org/10.5061/dryad.35ks2), PDB (6VYB and 6NB6) and from “High frequency of shared clonotypes in human B cell receptor repertoires” (https://doi.org/10.1038/s41586-019-0934-8).

Code availability statement: Computer code to process the antibody sequences is available at GitHub (https://github.com/stratust/igpipeline/tree/igpipeline2_timepoint_v2).

Data presentation


Competing interests: The Rockefeller University has filed a provisional patent application in connection with this work on which M.C.N.is an inventor (US patent 63/021,387). The patent has been licensed by Rockefeller University to Bristol Meyers Squib.

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Extended Data Fig 1: Plasma ELISA and neutralization.

**a,b.** Graph shows area under the curve (AUC, Y-axis) for plasma IgM (**a**) or IgA (**b**) antibody binding to SARS-CoV-2 RBD after prime and boost for paired samples.

**c,d.** Graph shows area under the curve (AUC, Y-axis) for plasma IgM (**c**) or IgA (**d**) antibody binding to SARS-CoV-2 RBD of paired samples obtained 2 and 5 months after the boost.

**e,f.** IgG antibody binding (AUC, X-axis) after prime vs. IgG antibody binding (AUC, X-axis) after boost (**e**) and NT50 values after
prime vs. NT50 values after boost (f) in individuals receiving two doses of an mRNA vaccine. g, NT50 values after prime and boost in females and males receiving 2 doses of an mRNA vaccine. h, NT50 values (Y-axis) vs. IgG antibody binding (AUC, X-axis) 5 months after boost in individuals receiving two doses of an mRNA vaccine. All experiments were performed at least in duplicate. Red values or bar in a-d and g represent geometric mean values. Statistical significance in a-d was determined by Wilcoxon test, in e, f, and h by spearman correlation test and in g by Kruskall-Wallis test with subsequent Dunn’s multiple comparisons.
Extended Data Fig. 2: Flow Cytometry. a, Gating strategy for phenotyping. Gating was on singlets that were CD19+ or CD20+ and CD3-CD8-CD16-Ova-. Anti-IgG, IgM, IgA, IgD, CD71 and CD27 antibodies were used for B cell phenotype analysis. Antigen-specific cells were detected based on binding to RBD WT-PE+ and RBD WT/KEN-AF647+. b-d, Flow cytometry plots showing the frequency of b, RBD WT-binding memory B cells, and c, RBD-binding memory B cells cross-reactive with WT and K417N/E484K/N501Y mutant RBD and d, RBD-binding plasmablasts, in 10 selected vaccinees after prime or boost. e, Gating strategy for single-cell sorting for CD20+ memory B cells (top panel) or CD19+CD20- plasmablasts (bottom panel) which were double positive for RBD-PE and RBD-AF647. f-g, Representative flow cytometry plots showing dual AlexaFluor-647-RBD and PE-RBD-binding, single-cell sorted B cells from f, 6 individuals after prime or boost or 5 months post-vaccination and g, 5 individuals from 2- or 5-months post-vaccination. Percentage of RBD-specific B cells is indicated. Individuals in red text indicate those that were sampled at prime, boost, and again 5 months post-vaccination.
Extended Data Fig 3: anti-SARS-CoV-2 RBD-specific plasmablast and MBC responses after vaccination. a, Pie charts show the distribution of antibody sequences from 6 individuals after prime (upper panel) or boost (lower panel). Sequences derived from IgG plasmablast, IgM MBC, and IgG MBC compartments were analyzed after prime, while only IgG MBCs were analyzed after boost, as indicated to the left of the plots. The number inside the circle indicates the number
of sequences analyzed for the individual denoted above the circle. Pie slice size is proportional to
the number of clonally related sequences. The black outline indicates the frequency of clonally
expanded sequences detected in each patient. Colored slices indicate persisting clones (same IGHV
and IGLV genes, with highly similar CDR3s) found in multiple compartments and/or timepoints
within the same patient. Grey slices indicate clones unique to the compartment. White indicates
sequences isolated once. b–c, Number of somatic nucleotide mutations in the IGVH and IGVL,
separately, in antibodies detected in b, different B cell compartments after prime or boost and c,
212 or 5 months post-vaccination compared to convalescent infected individuals after 1.33 and 6.27
months post-infection (also Supplementary Table 4). Red horizontal bars and numbers indicate
mean number of nucleotide mutations in each compartment at each time point. Statistical
significance was determined using a Kruskal Wallis test with subsequent Dunn’s multiple
comparisons.
Extended Data Fig. 4: Frequency distribution of human V genes. a, Circos plot depicting relationship between antibodies that share V and J gene usage in both IgH and IgL when comparing all prime/boost IgG MBC sequences. Purple, green, and grey lines connect related clones, clones and singlets, and singlets to each other, respectively. b, Graph shows relative abundance of human heavy chain IGVH (top), light chain IGVK (middle) or IGVL (bottom) genes comparing Sequence Read Archive accession SRP010970 (orange), and IgG MBCs after prime (blue) or boost (green)
doses of vaccination. Statistical significance was determined by two-sided binomial test. * = p≤0.05, ** = p≤0.01, *** = p≤0.001, **** = p≤0.0001. Color of stars indicates: black - comparing Database versus Prime; blue - comparing Database versus Boost; red - comparing Prime versus Boost. 

c, Circos plot depicting relationship between antibodies that share V and J gene usage in both IgH and IgL when comparing 2 mo/5 mo IgG MBC sequences. Purple, green, and grey lines connect related clones, clones and singlets, and singlets to each other, respectively. 

d, Graph shows relative abundance of human heavy chain IGVH (top), light chain IGVK (middle) or IGVL (bottom) genes comparing Sequence Read Archive accession SRP010970 (orange), and IgG MBCs after 2 months (blue) or 5 months (green) post-vaccination. Statistical significance was determined by two-sided binomial test. * = p≤0.05, ** = p≤0.01, *** = p≤0.001, **** = p≤0.0001. Color of stars indicates: black - comparing Database versus 2 months; blue - comparing Database versus 5 months; red - comparing 2 months versus 5 months.
Extended Data Fig. 5: Somatic hypermutation of anti-SARS-CoV-2 RBD antibody clones after prime or boost. Clonal evolution of RBD-binding B cells from 3 individuals for which plasmablasts, IgM memory B cells, and IgG memory B cells were analyzed after prime, and IgG memory B cells were analyzed after boost (as described in Extended Data Fig. 3). The number of
somatic nucleotide mutations found in shared clonal families found in at least 2 different compartments is graphed to the right of each donut plot. Color of dot plots match the color of pie slices within the donut plot, which indicate persisting clones. nd – clone was Not Detected in the indicated compartment. Black horizontal line indicates median number of SHM.
Extended Data Fig. 6: Anti-SARS-CoV-2 RBD monoclonal antibodies ELISAs. **a-j**, Graphs show anti-SARS-CoV-2 binding activity of monoclonal antibodies measured by ELISA against RBD. **a-e**, ELISA half-maximal concentration (EC$_{50}$) values for all antibodies (**a**), all clones (**b**), non-persisting clones (**c**), singlets (**d**) and shared clones (**e**) isolated after prime or boost. **f-j**, ELISA half-maximal concentration (EC$_{50}$) values for all antibodies (**f**), all clones (**g**), shared clones (**h**), non-persisting clones (**i**) and singlets (**j**) isolated from COVID-19 convalescent individuals 1.3$^3$ and 6.2$^7$ months after infection or from vaccinated individuals 2m$^{12}$ or 5m after vaccination. Each dot represents one antibody. Red horizontal bars and numbers indicate geometric mean values. Statistical significance in **a-d, f, g, i, j** was determined by Mann-Whitney test and in **e** and **h** by Wilcoxon test. All experiments were performed at least twice.
Extended Data Fig. 7. Affinity. Biolayer interferometry measurements. a, IC50 values for randomly selected antibodies isolated from vaccinees after prime (n=30) or boost (n=30). Red horizontal lines and numbers indicate geometric mean. b, Graphs depict affinity measurements of antibodies described in a. c, Graphs show affinities (Y axis) plotted against neutralization activity (X axis) for antibody isolated after vaccination. d-f, same as a-c, but showing monoclonal antibodies isolated from follow-up of 2 and 5 months post-vaccination. d, IC50 values for randomly selected antibodies isolated from vaccinees after 2- (n=31) or 5- (n=30) months post-vaccination. e, Graphs depicting affinity measurements of antibodies described in d. f, Graph shows affinity (Y axis) plotted against neutralization activity (X axis) for antibody isolated after vaccination.
Extended data Fig. 8: Breadth of anti-SARS-CoV-2 RBD antibodies elicited after prime and boost vaccination.  **a**, IC$_{50}$ values for n=40 neutralizing antibodies isolated after prime (a) or boost (b) against indicated mutant SARS-CoV-2 pseudoviruses. Color gradient indicates IC$_{50}$ values ranging from 0 (white) to 1000 ng/ml (red).
REFERENCES


