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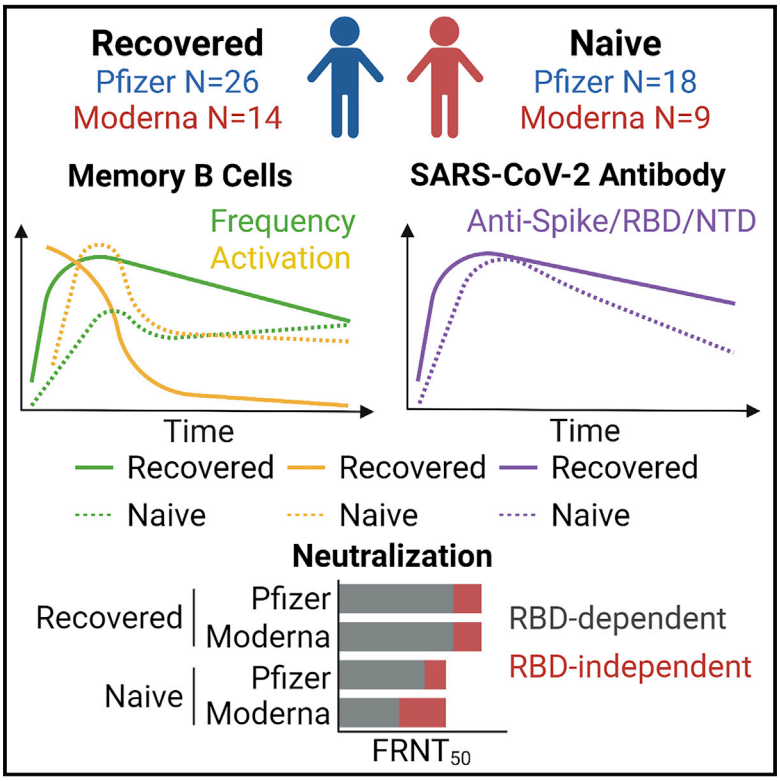
Pre-existing SARS-CoV-2 immunity influences potency, breadth, and durability of the humoral response to SARS-CoV-2 vaccination

Graphical abstract



Highlights

- Single vaccine dose effectively boosts B cell responses in recovered subjects
- SARS-CoV-2-specific MBCs remain activated and increase over time in naive subjects
- Antibody response to vaccination is broader and more durable in recovered versus naive subjects
- Naive vaccinees have higher proportion of non-RBD-specific neutralizing antibodies

Authors

Grace Mantus, Lindsay E. Nyhoff, Venkata-Viswanadh Edara, ..., Rafi Ahmed, Mehul S. Suthar, Jens Wrammert

Correspondence

jwramme@emory.edu

In brief

Mantus et al. find that kinetics, breadth, and durability of humoral immune responses to mRNA vaccination are dependent on pre-existing immunity to SARS-CoV-2. Cellular and serological SARS-CoV-2-specific immunity is detected in both recovered and naive individuals after vaccination, with broader and more durable serological responses detected in recovered individuals.

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Pre-existing SARS-CoV-2 immunity influences potency, breadth, and durability of the humoral response to SARS-CoV-2 vaccination

Grace Mantus,[1,2,3,7] Lindsay E. Nyhoff,[1,2,3,7] Venkata-Viswanadh Edara,[1,2,3,4] Veronika I. Zarnitsyna,[5] Caroline R. Ciric,[1,3] Maria W. Flowers,[1,2,3] Carson Norwood,[1,2,3] Madison Ellis,[2,3,4] Laila Hussaini,[1,3] Kelly E. Manning,[2,3,4] Kathy Stephens,[1,3] Evan J. Anderson,[1,3,6] Rafi Ahmed,[2,5] Mehul S. Suthar,[1,2,3,4,5] and Jens Wrammert[1,2,3,8,*]

[1]Department of Pediatrics, Centers for Childhood Infections and Vaccines, Children's Healthcare of Atlanta and Emory University, Atlanta, GA 30329, USA
[2]Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA 30329, USA
[3]Division of Infectious Diseases, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA
[4]Yerkes National Primate Research Center, Atlanta, GA 30329, USA
[5]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, USA
[6]Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322, USA
[7]These authors contributed equally
[8]Lead contact
*Correspondence: jwramme@emory.edu
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SUMMARY

The ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic highlights the importance of determining the breadth and durability of humoral immunity to SARS-CoV-2 mRNA vaccination. Herein, we characterize the humoral response in 27 naive and 40 recovered vaccinees. SARS-CoV-2-specific antibody and memory B cell (MBC) responses are durable up to 6 months, although antibody half-lives are shorter for naive recipients. The magnitude of the humoral responses to vaccination strongly correlates with responses to initial SARS-CoV-2 infection. Neutralization titers are lower against SARS-CoV-2 variants in both recovered and naive vaccinees, with titers more reduced in naive recipients. While the receptor-binding domain (RBD) is the main neutralizing target of circulating antibodies, Moderna-vaccinated naives show a lesser reliance on RBDs, with >25% neutralization remaining after depletion of RBD-binding antibodies. Overall, we observe that vaccination induces higher peak titers and improves durability in recovered compared with naive vaccinees. These findings have broad implications for current vaccine strategies deployed against the SARS-CoV-2 pandemic.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an ongoing public health crisis with over 450 million infections and 6 million deaths attributed to the virus worldwide 2 years after its emergence.[1] In numerous study cohorts, overwhelming evidence has illustrated the importance of antibodies targeting the trimeric spike (S) protein on the viral surface, especially the receptor-binding domain (RBD), in controlling SARS-CoV-2 infections.[2–6] RBD-specific antibodies in circulation correlate strongly with viral neutralization across infection cohorts,[7,8] and monoclonal antibodies derived from RBD-specific memory B cells generated during infection have been consistently characterized as potent neutralizers with several either approved for use or currently in clinical trials for treatment of coronavirus disease 2019 (COVID-19).[3,5–7] Given the clear importance of RBD-specific antibodies in the protective immune response to infection, eliciting a similar antibody repertoire through vaccination was hypothesized to provide comparable immunity. Several vaccines containing versions of the SARS-CoV-2 S protein were approved at the end of 2020 as effective tools to manage viral spread and disease severity. Two widely available vaccines, Moderna's mRNA-1273 and Pfizer-BioNTech's BNT162b2, utilize an optimized mRNA platform to deliver their immunogen, a pre-fusion stabilized version of the SARS-CoV-2 S protein.[9,10] While both vaccines initially reported similar efficacy in phase III trials, comparisons of vaccine efficacy over time have reported Moderna to be slightly more effective than Pfizer in preventing hospitalizations from COVID-19.[9–11] This difference in efficacy may be due to variability in dose, durability, and the dominant viral variant; however, differences in vaccine-induced immunity may also play a role.

The continuous evolution and emergence of SARS-CoV-2 variants has been an ongoing threat to the pre-existing immunity established within the population from both natural infection and vaccination efforts. Two currently defined variants of concern (VOCs) are the recent Omicron (B.1.1.529) variant and the Delta (B.1.617.2/AY) variant, which remains prevalent globally.[12,13]

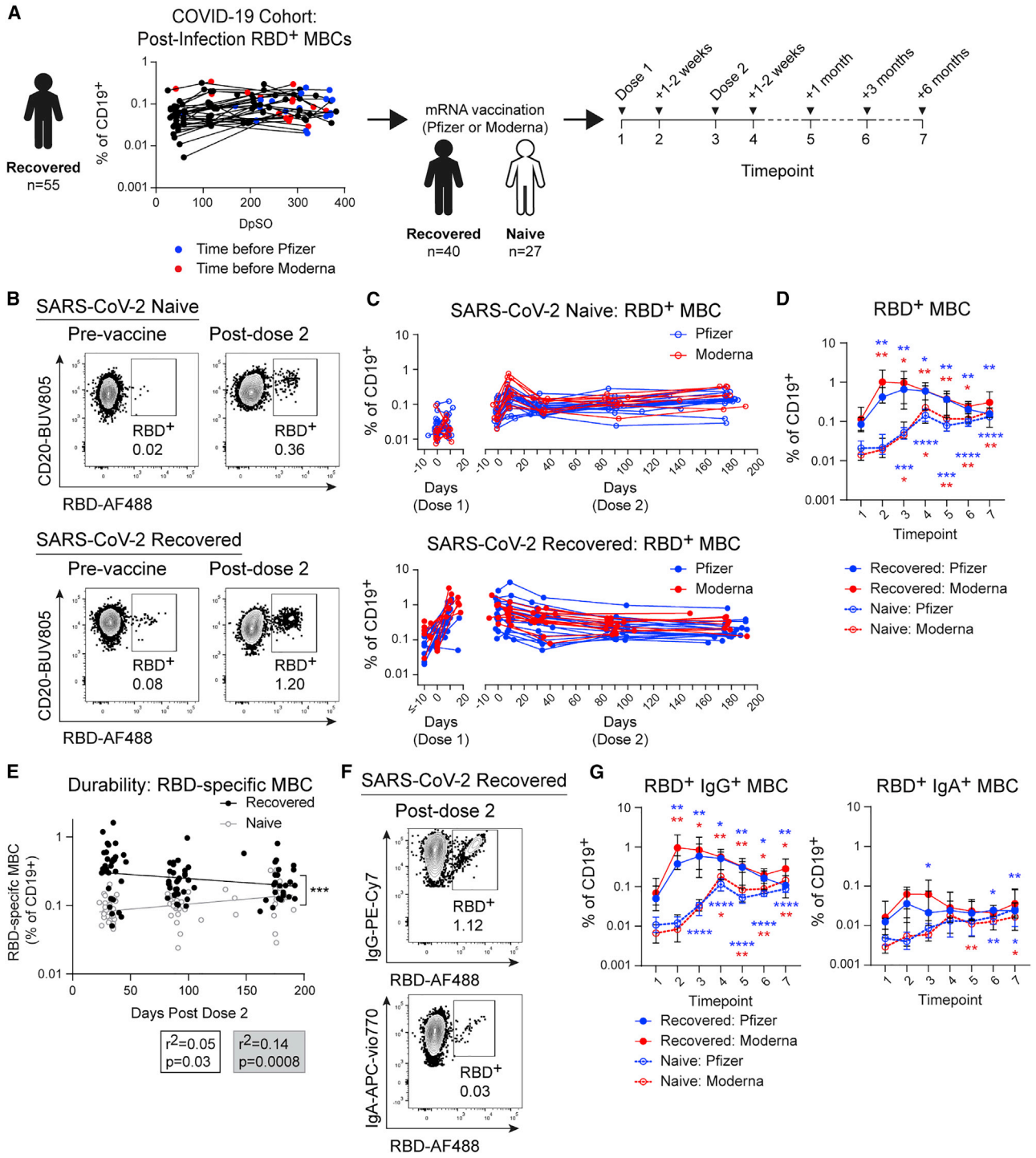

Figure 1. RBD-specific memory B cells expand in naive and recovered subjects following mRNA vaccination

(A) Study design of the Emory SARS-CoV-2 convalescence and vaccination cohort. Frequency of RBD-specific memory B cells (MBCs) in subjects for 1–12 months following confirmed SARS-CoV-2 infection, reported as the percentage of total $CD19^+$ cells. The final visit before vaccination is shown in blue (Pfizer) or red (Moderna).

(B) Gating scheme of RBD-specific MBCs, pre-gated as live single $CD19^+IgD^-CD20^+$ cells, in a naive (top) and recovered (bottom) donor before vaccination (left) and after dose 2 (right).

(C) RBD-specific MBCs as the percentage of $CD19^+$ for individual vaccinated donors by days post-dose 1 and 2.

(D) RBD-specific MBCs as the percentage of $CD19^+$ by time point.

(legend continued on next page)

Initial studies investigating the ability of vaccine-induced responses to combat emerging variants have reported decreased potency against select variants with the most dramatic reductions seen against the Beta (B.1.351) and Omicron (B.1.1.529) variants.[14–17] Understanding which components of vaccine-induced immunity are responsible for durable and cross-reactive responses is critical as countries continue to shift vaccination and treatment strategies, including the recent approval of booster shots for several licensed vaccines.

In this study, we characterize the humoral response to vaccination in a cohort of SARS-CoV-2-recovered and -naive individuals receiving either Moderna's mRNA-1273 or Pfizer's BNT-162b2. We observed striking differences in both the early and long-term kinetics of the cellular and serological responses to vaccination based on the absence or presence of pre-existing immunity to SARS-CoV-2. Interestingly, we find that the half-life of the antibody response is almost double in recovered compared with naive vaccinees between 1- and 6-months post-vaccination. Importantly, the magnitude of the humoral response to SARS-CoV-2 vaccination strongly correlated with the immune responses to initial SARS-CoV-2 infection. Depletion experiments illustrated that naive vaccinees, particularly those receiving Moderna's mRNA-1273, tended to generate a substantial non-RBD neutralizing antibody fraction. These findings highlight potential differences in antibody repertoire breadth generated between recovered and naive individuals vaccinated with the two mRNA vaccines and advances our understanding of potentially variable mechanisms of viral neutralization.

RESULTS

Study cohort

We recruited at total of 67 individuals receiving a SARS-CoV-2 mRNA vaccine. Of these individuals, 39 were previously enrolled in our longitudinal study of COVID-19 immune durability study[18] and had previously been followed for up to 12 months after confirmed SARS-CoV-2 infection (median = 296 days) post-symptom onset (Figure 1A). An additional 28 naive participants were enrolled. These participants reported no known COVID-19 exposure. However, initial pre-screening identified one participant that exhibited serological and cellular evidence of previous infection. This participant was thus moved to the recovered group. These groups were vaccinated with either Pfizer's BNT162b2 (n = 18 naive, n = 26 recovered) or Moderna's mRNA-1273 (n = 9 naive, n = 14 recovered). Gender distribution was similar in both groups, with slightly more female participants (naive = 59%, recovered = 53%) than male. The age of the naive participants trended slightly younger, with a median age of 34 (range: 22–64) compared with the recovered group median of 55 (range: 21–77). Further detailed information on all participants can be found in Table S1. We collected plasma and peripheral blood mononuclear cell (PBMC) specimens from the participants at seven time points, including one pre-vaccination and six follow ups (Figure 1A). Lymphocyte subsets were assessed by flow cytometry over the course of the study and remained stable (Figure S1).

Moderna and Pfizer SARS-CoV-2 vaccines both induce durable RBD-specific memory B cell responses in SARS-CoV-2-naive and -recovered subjects

Antigen-specific memory B cells (MBCs) are known to be strongly induced by vaccination, with significant increases from baseline reported.[19–21] We assessed the magnitude and dynamics of RBD-specific MBCs in SARS-CoV-2-naive and -recovered subjects vaccinated with either the Moderna or Pfizer vaccine. Our gating strategy defined total MBCs as $CD19^+$, immunoglobulin D $(IgD)^-$, $CD20^+$ lymphocytes (Figure S1). RBD specificity was measured by staining with a fluorescently labeled RBD (Figure 1C). Thirty-nine recovered individuals were recruited from a larger group of 55 participants followed longitudinally, as shown in Figure 1A, allowing us to link analyses of SARS-CoV-2-specific immune memory following infection with a subsequent vaccination. Subjects in both the recovered and naive groups were assessed before and after each dose, then 1, 3, and 6 months following full vaccination (Figure 1). Recovered participants responded robustly to either vaccination, with RBD-specific MBCs reaching a median of 0.42% of $CD19^+$ cells in Pfizer-vaccinated subjects and 1.01% of $CD19^+$ in Moderna-vaccinated subjects following the first dose. The second vaccine dose did not cause additional increases in RBD-specific MBCs for either vaccine. In contrast, RBD-specific MBCs in naive participants showed minimal responses after the first vaccine dose, instead peaking following dose 2, reaching medians of 0.14% (Pfizer) and 0.22% (Moderna) of $CD19^+$ ($p < 0.001$ Pfizer, $p = 0.019$ Moderna, compared with baseline) (Figure 1D). Despite minor differences in the median RBD-specific B cells across time points, there were no significant differences between Pfizer and Moderna in either the recovered or naive group. RBD-specific MBC responses were durable in both naive and convalescent vaccinees and were present in all groups 3 to 6 months after immunization (Figure 1D). To compare the durability of the RBD-specific MBC response in recovered and naive individuals, we performed a linear regression analysis on time points collected between 1- and 6-months post-vaccination and compared the slopes of the generated lines (Figure 1E). We observed a significant difference in slopes between recovered and naive groups

(E) RBD-specific MBCs shown for naive (gray, open circles) and recovered (black, closed circles) for 1–6 months following full vaccination. Coefficient of determination (r^2) and significance are determined from linear regression analysis. ***$p < 0.001$.
(F) Gating scheme of RBD-specific IgG (left) or IgA (right) expressing MBCs, shown for a recovered subject post-dose 2.
(G) RBD-specific IgG+ (left) or IgA+ (right) MBCs as percentage of $CD19^+$ by time point.
(A–G) Blue = Pfizer vaccination (recovered n = 18, naive n = 18), red = Moderna (recovered n = 13, naive n = 9), tested in singlets. Open circles are naive subjects, and closed circles are recovered subjects. (D and F) Values are medians ±95% confidence interval (CI). Statistics were calculated using mixed-effects model with Geisser-Greenhouse correction. Blue values indicate Pfizer, red values indicate Moderna, as comparisons with time point 1. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

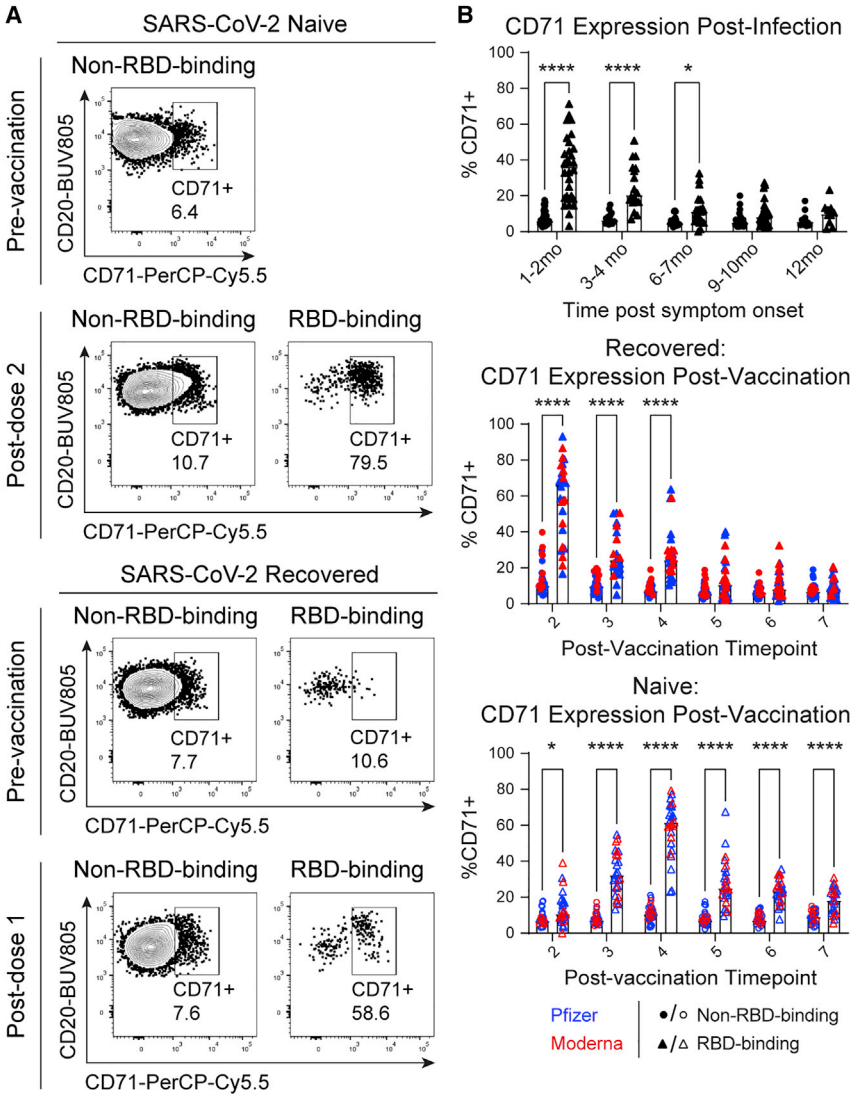

Figure 2. RBD-specific MBCs upregulate CD71 following infection and vaccination

(A) Gating scheme for CD71 expression on non-RBD binding (left) and RBD-binding (right) MBCs.

(B) CD71 expression as the percentage of parent is quantified in RBD-binding (triangles) and non-RBD-binding (circles) following SARS-CoV-2 infection (top) or vaccination of recovered (middle) and naive (bottom) subjects.

Blue = Pfizer vaccination (recovered n = 18, naive n = 18), red = Moderna (recovered n = 13, naive n = 9), tested in singlets. Open symbols are naive subjects, and closed symbols are recovered subjects. Statistics were calculated using mixed-effects model with Geisser-Greenhouse correction. *p < 0.05, ****p < 0.0001.

(p = 0.0002) with RBD-specific MBCs slightly decreasing in recovered individuals over time and naive individuals displaying an increase in RBD-specific MBCs up to 6 months post-vaccination, reaching comparable numbers to recovered individuals at this time point (Figure 1E).These data show that mRNA vaccination induces robust RBD-specific MBC formation in both recovered and naive vaccinees, underlines that one dose may be sufficient for recovered individuals, and shows no significant difference between the two available mRNA-based vaccines.

The RBD-specific MBC response to vaccination is dominated by IgG in both naive and recovered individuals

To further characterize the RBD-specific MBC response, we separated the MBC compartment by expression of IgM, IgG, and IgA. We did not find significant RBD-specific IgM+ MBCs, even at early time points. Though IgM+ MBCs have been shown to form an important part of early immune responses,[22,23] responses to SARS-CoV-2 infection[18] and vaccination[24] are dominated by IgG+ MBCs. RBD-specific IgG+ MBCs formed the bulk of the response, driving the pattern of total RBD-specific MBCs. Recovered individuals robustly responded to the first dose, increasing RBD-specific IgG+ MBCs (p = 0.002 Pfizer, p = 0.008 Moderna), but these did not increase further after dose 2 (Figure 1G). In naive individuals, RBD-specific IgG+ MBCs peaked following dose 2 (p < 0.001 Pfizer, p = 0.017 Moderna) and remained elevated at 6 months after vaccination (p < 0.001 Pfizer, p = 0.004 Moderna, compared with baseline). IgA+ RBD-specific responses were more variable. Pfizer- and Moderna-vaccine responses did not differ significantly from each other in either the naive or recovered group at any time point. Although not observed in Moderna recipients, the recovered Pfizer group exhibited a significant increase in IgA+ RBD-specific MBCs over baseline after the first dose (p = 0.047) and retained a small but significant increase 6 months after vaccination (p = 0.005). Naive vaccinees also generated slight but significant IgA+ RBD-specific MBCs 6 months following vaccination in both Moderna (p = 0.044) and Pfizer (0.015) cohorts (Figure 1G).

RBD-specific MBCs exhibit sustained activation, as measured by expression of CD71

We have recently reported that RBD-specific MBCs upregulate the activation marker CD71 during acute COVID-19.[4] To determine the duration of activation, we assessed CD71 expression in RBD-binding and non-RBD-binding MBCs following SARS-CoV-2 infection and vaccination (Figure 2). One to two months after infection, a significantly higher percentage of RBD-specific MBCs retained expression of CD71 compared with their non-RBD-binding counterparts (p < 0.0001). This difference subsided over time but remained significant until 6–7 months after infection (p = 0.026), suggesting prolonged antigenic

stimulation. After vaccination, RBD-specific MBC activation mirrored the pattern of expansion shown in Figure 1E, with RBD-specific MBCs showing the most CD71 expression post-dose 1 in the recovered ($p < 0.0001$) and post-dose 2 in the naive ($p < 0.0001$) vaccinees (Figure 2B). We did not observe significant differences in CD71 expression between Pfizer- and Moderna-vaccinated groups in either naive or recovered vaccinees. In the recovered group, the second dose did not result in further upregulation of CD71 ($p > 0.999$, time point 3 versus 4), and by 1 month post-vaccination, RBD-specific MBCs trended higher in CD71 expression, compared with non-RBD-specific MBCs, but no longer reached significance ($p = 0.057$). In comparison, RBD-specific MBCs of naive vaccinees remained significantly activated even 6 months after vaccination ($p < 0.0001$).

Plasmablast expansion in peripheral blood is minor following SARS-CoV-2 vaccination

An early measure of the humoral immune response is expansion of peripheral antibody-secreting plasmablasts. Plasmablasts are typically observed early following vaccination[21,25] and have been reported to expand in naive vaccinees following the second dose of the Pfizer vaccine.[26] In Figure 3B, we show plasmablasts as the percentage of $CD19^+$ for each naive (top) and recovered (bottom) subject. Because peripheral plasmablasts are known to increase transiently, we excluded subject visits at time points 2 and 4 that were more than 14 days after the respective dose (Figure 3C). Plasmablast responses observed in all groups were highly variable. Naive vaccinees who received the Pfizer vaccine exhibited significantly expanded plasmablasts at time points 2 ($p = 0.001$) and 4 ($p = 0.007$). Overall, Moderna did not elicit a significant increase in plasmablasts in naive subjects at any time point, but this difference may be due to the high variability of responses, with expansion in Moderna vaccinees ranging from 0.43% to 8.65% of B cells at time point 2 and 0.55% to 4.42% at time point 4, combined with the comparatively lower number of subjects. Recovered subjects did not show significant plasmablast expansion at any time point (Figure 3C).

RBD-specific plasmablasts are detectable in the periphery of naive and recovered subjects following vaccination

Expansion of antigen-specific plasmablasts have been documented after infection[27] and vaccination[21,25] for multiple pathogens and vaccines. We assessed RBD-specific plasmablasts following vaccination of SARS-CoV-2-naive and -recovered individuals, using optimized staining protocols to allow detection of RBD-specific plasmablasts despite low surface-receptor expression. Despite a surprisingly low overall expansion of total plasmablasts, we found that a portion of the plasmablasts present following vaccination did exhibit binding to RBDs. In particular, RBD-specific IgG-expressing plasmablasts were significantly expanded in recovered participants following the first dose of either mRNA vaccine ($p = 0.014$ Pfizer, $p = 0.036$ Moderna). In naive individuals vaccinated with Pfizer, RBD-specific IgG+ plasmablasts significantly expanded following the second dose ($p < 0.001$). Though some individuals vaccinated with Moderna did have RBD-specific plasmablast responses, overall there was no significant response following the second dose (Figure 3F). While the peripheral plasmablast response was generally minor, recovered subject responses occurred mainly after the first dose and naive subjects after the second. In addition, the systemic source of the antibody response to vaccination may not be derived from circulating plasmablasts but may rather be dependent on local germinal center and plasma cell formation, as suggested by a recent report on vaccine responses in draining lymph nodes.[26]

Vaccination induces robust IgG and IgA titers against SARS-CoV-2 S antigens in naive and recovered individuals

We conducted serological analyses using multiplexed antigen panels containing SARS-CoV-2 S antigens (S N-terminal domain (NTD), RBD, and S) in addition to SARS-CoV-2 nucleoprotein (N) and S proteins derived from additional endemic and pathogenic coronaviruses. The kinetics of the antibody response resembled those observed in the RBD-specific MBC response, with antibodies targeting SARS-CoV-2 S antigens peaking in recovered individuals after a single vaccine dose. In contrast, naive individuals required two doses to reach similar antibody levels as the recovered group (Figure 4A). Interestingly, we observed a similar pattern of increase in both antibodies against SARS-CoV-1 and, to a lesser extent, against Middle Eastern respiratory syndrome coronavirus (MERS-CoV) in both recovered and naive individuals after vaccination (Figure S2A). No significant changes were observed in antibodies against S derived from either HKU1 or OC43, suggesting that vaccination has minimal effect on these pre-existing antibody titers (Figure S2A). As expected, titers against N were unaffected throughout vaccination; recovered individuals displayed a higher baseline titer due to their previous exposure to SARS-CoV-2 (Figure S2B).

IgM titers against RBD and S were significantly lower than IgG and IgA titers, rapidly declined, and returned to baseline by 1-month post-vaccination in both recovered and naive groups (Figure S2C). Anti-NTD, -RBD, and -S IgG titers increased rapidly in recovered subjects following the first dose, increasing significantly compared with naive subjects (Figure 4A). Following dose 2, IgG titers in naive individuals were comparable to their recovered counterparts (Table S7). IgG titers against NTD, RBD, and S fell more rapidly in the naive versus recovered group, as shown by significantly higher titers in recovered subjects 1, 3, and 6 months after vaccination (Figure 4A; Table S7). IgA titers followed a similar pattern: recovered groups peaked following one dose while naive groups required two. IgA titers did not significantly differ between recovered and naive individuals post-dose 2 and continued to be comparable in both groups until 6 months post-vaccination (Figure 4A; Table S7). IgG and IgA titers across almost all groups remained significantly higher than baseline out to 6 months post-vaccination (Figure 4A). No difference in antibody titers was observed between the two different vaccines except in recovered individuals after the first dose (Tables S4, S5, and S6). At this point, S-specific IgG titers peaked in the recovered Moderna group compared with the recovered Pfizer group, which continued to increase, reaching comparable titers prior to the second dose (Figure 4A;

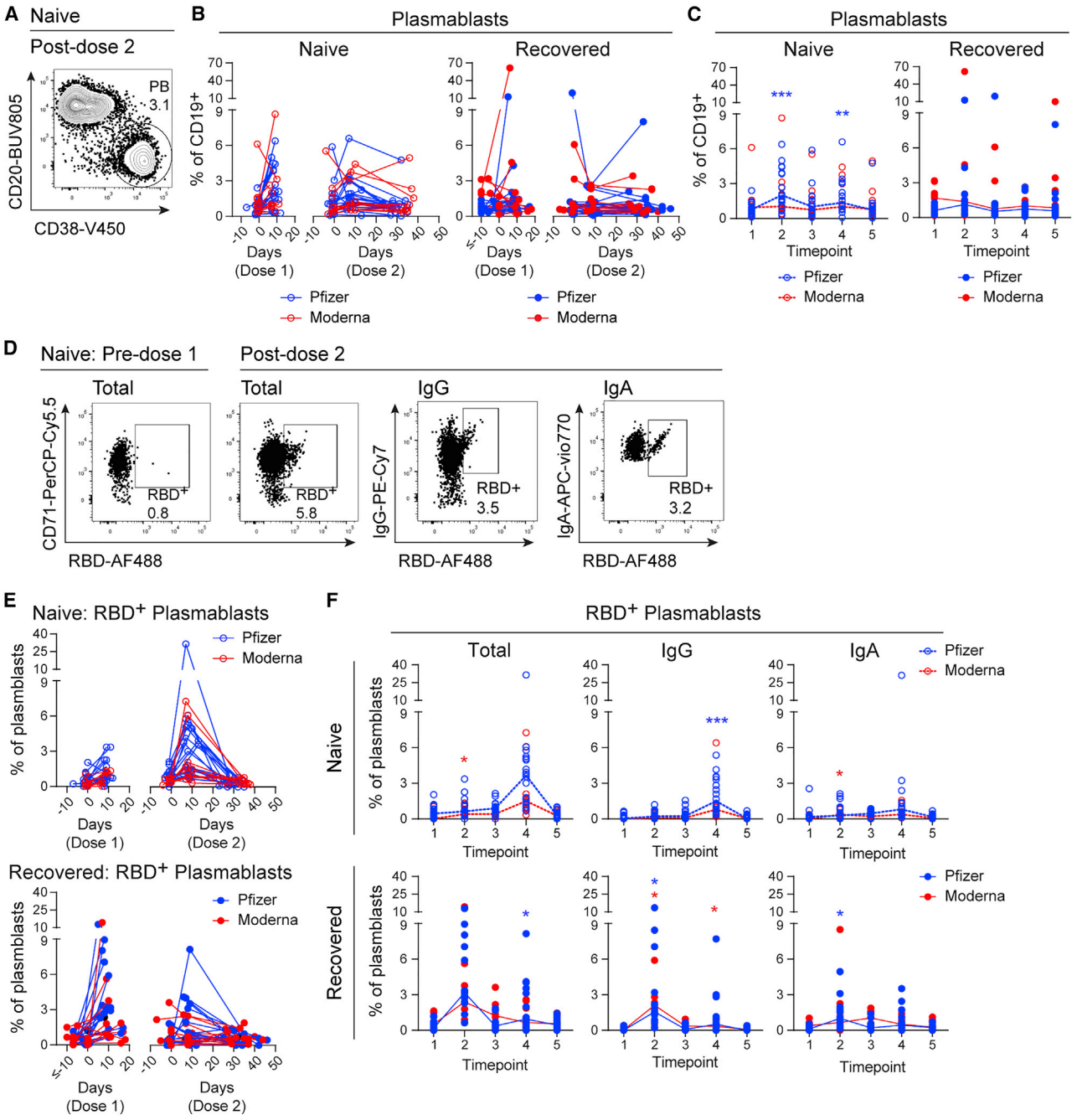

Figure 3. Plasmablast responses in naive and recovered individuals post-SARS-CoV-2 mRNA vaccination

(A) Gating scheme of plasmablasts by CD20 and CD38, pre-gated as $CD19^{+}IgD^{-}$, in a representative naive post-dose 2 (top) or recovered post-dose 1 (bottom).

(B) Plasmablasts as the percentage of CD19 for individual vaccinated donors by days post-dose 1 and 2.

(C) Plasmablasts as the percentage of $CD19^{+}$ by time point in naive (left) and recovered (right) vaccinees.

(D) Gating scheme of RBD-specific plasmablasts pre- and post-vaccination in a naive subject.

(E) RBD-specific total (left), IgG+ (middle), and IgA+ (right) as the percentage of plasmablasts by time point in naive (top) and recovered (bottom) vaccinees.

(A–F) Blue = Pfizer vaccination (recovered n = 18, naive n = 18), red = Moderna (recovered n = 13, naive n = 9), tested in singlets. Open circles are naive subjects, closed circles are recovered. (C and F) Statistics were calculated using mixed-effects model with Geisser-Greenhouse correction. Blue values indicate Pfizer, red values indicate Moderna, as comparisons with time point 1. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

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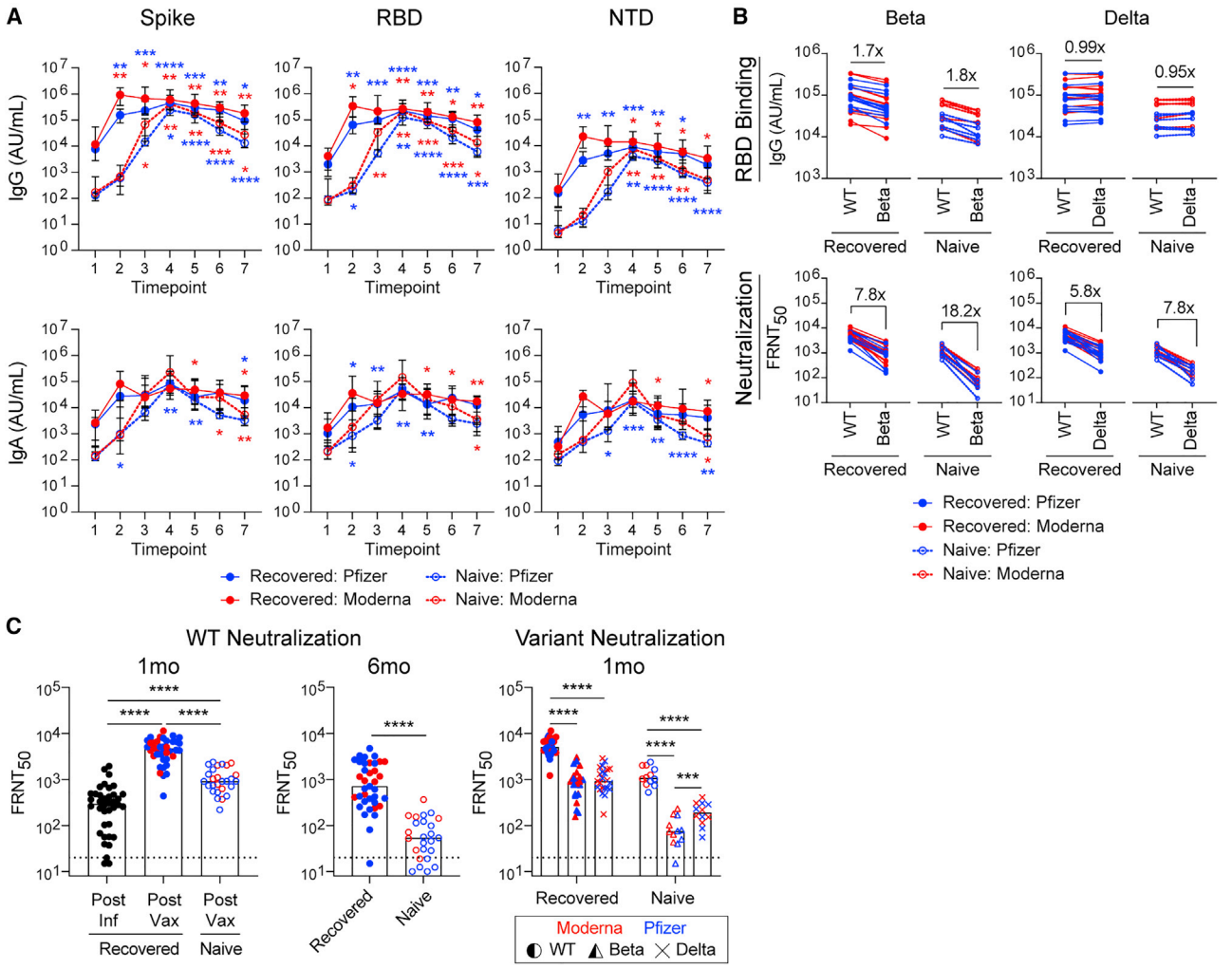


Figure 4. Recovered individuals generate faster and more robust antibody responses to mRNA vaccination against SARS-CoV-2 than naive individuals

(A) IgG and IgA NTD-, RBD-, and S-specific binding titers over the course of vaccination in recovered (Pfizer = 18, Moderna = 10) and naive (Pfizer = 18, Moderna = 9) individuals as determined by MSD-ELICA calculated from reference standard curve.

(B) IgG binding and neutralizing titers against WT, Beta, and Delta RBDs in recovered (Pfizer n = 12, Moderna n = 11) and naive individuals (Pfizer n = 6, Moderna n = 6) at 1-month post-vaccination as determined by MSD-ELICA and *in vivo* neutralization.

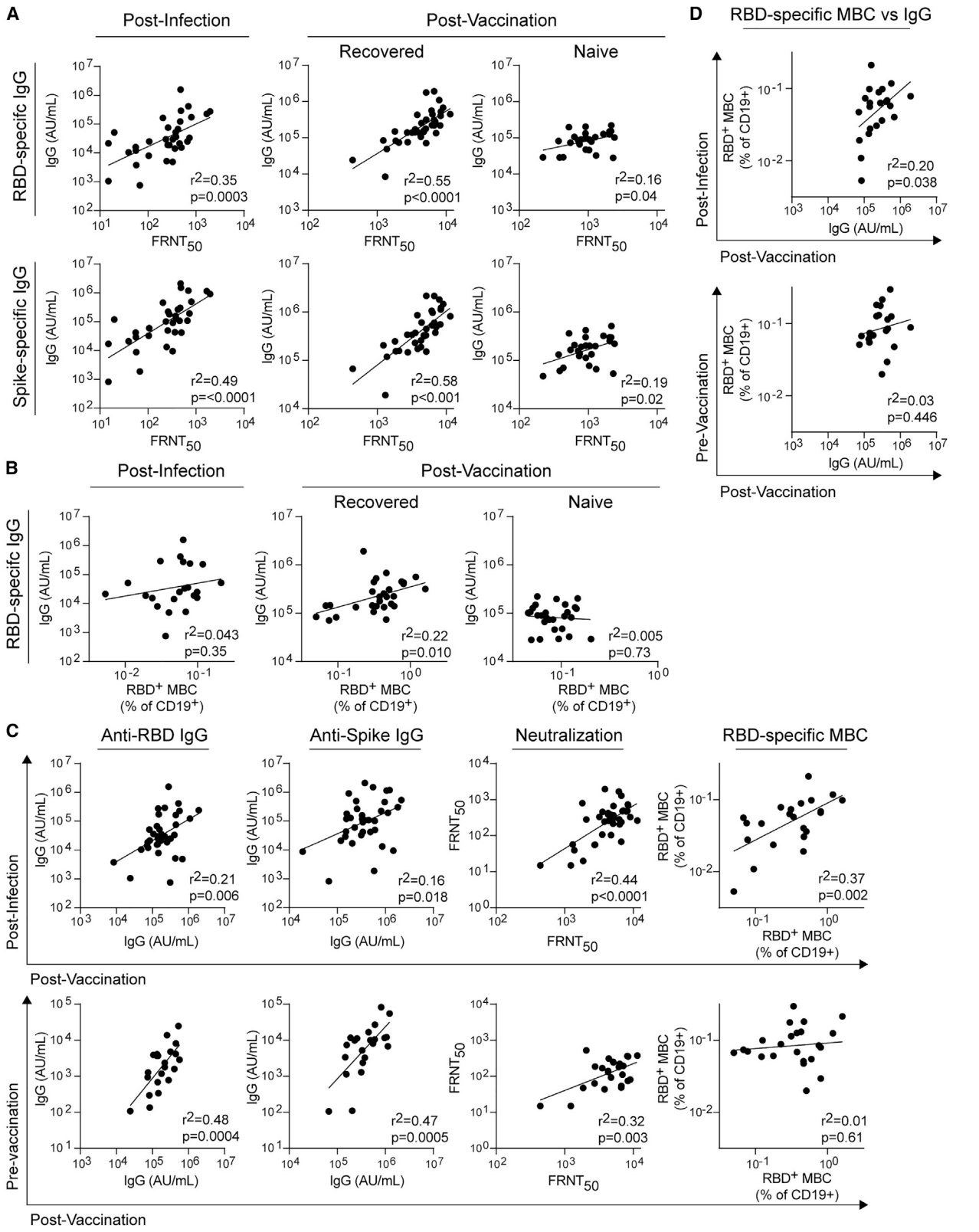
(C) Neutralization titers in recovered (1-month post-infection [n = 39], 1-month post-vaccination [n = 39], 6-month post vaccination [n = 37]) and naive (1-month post-vaccination [n = 27], 6-month post vaccination [n = 25]) individuals against SARS-CoV-2 WT, Beta, or Delta variants.

The 6-month data for the WT virus was previously published in Edara et al.[17] and is shown here for comparative reasons only. MSD-ELICA and neutralization assays were run in duplicate. Significance was determined using either as differences from baseline (T1) using mixed-effects model with Geisser-Greenhouse correction and Tukey's multiple comparison test or using (1) Brown-Forsythe ANOVA and Dunnett's T3 multiple comparison test and (2) two-way ANOVA with Geisser-Greenhouse correction. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Table S4). These data illustrate that, despite differing response kinetics, mRNA vaccination generated robust NTD-, RBD-, and S-specific antibody titers in both recovered and naive individuals.

Neutralizing antibody titers against Beta and Delta variants are reduced in both recovered and naive individuals

In addition to exploring antibody-binding activity, we performed *in vitro* neutralization using a live virus assay on a subset of samples from our cohort taken at either 1-month post-infection (n = 39) or post-vaccination (n = 66). Samples were run against SARS-CoV-2 (WA1\2020). All vaccinated individuals had detectable neutralizing titers against SARS-CoV-2 at 1-month post-vaccination (Figure 4C). Recovered individuals had significantly higher titers than naive individuals with no difference in titers between vaccine brands (Figures 4C and S3B). Neutralizing titers from both recovered and naive individuals were significantly higher than titers from samples collected 1–2 months after initial infection with SARS-CoV-2 (Figure 4C). Additionally, we



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were able to compare neutralization titers between recovered (n = 37) and naive (n = 25) vaccinees at 6 months after vaccination (Figure 4C). These data have recently been published in a study of Omicron neutralization in infected and vaccinated individuals and is shown here for comparative reasons only.[17] We observed that recovered individuals continued to have significantly higher neutralization that naive individuals at this time point but note that the majority of individuals in both groups retain neutralizing titers against wild-type virus even 6 months after initial vaccination (Figure 4C).

We also assessed the binding and neutralizing response to SARS-CoV-2 variants, specifically Beta (B.1.351) and Delta (B.1.617.2/AY). We observed no difference in RBD binding to either variant over time in either naive or recovered individuals (Figure S3A). We conducted additional live virus neutralizing assays on a subset of our cohort (n = 35) at 1-month post-vaccination using Beta and Delta variants of SARS-CoV-2. While we only observed a minimal fold decrease in variant binding at this time point, significant decreases in neutralizing titers were observed between wild-type (WT) and both Beta and Delta variants in recovered and naive individuals regardless of the mRNA vaccine (Figures 4B and 4C). Titers were much lower in naive individuals with an average fold reduction of 18.2 (Beta) and 7.8 (Delta) compared with an average fold reduction of 7.8 (Beta) and 5.8 (Delta) in the recovered group (Figure 4B). Despite decreases in neutralizing capacity against the variants, all individuals retained neutralizing activity above the limit of detection against the Delta variant, and all but one naive individual retained activity against the Beta variant (Figure 4C). Taken together, these data illustrate that neutralizing titers against variants are reduced, but not ablated, in both recovered and naive individuals, with recovered individuals possessing more robust variant-neutralizing fractions than their naive counterparts.

Pre-existing humoral immunity correlates with B cell responses to SARS-CoV-2 vaccination

To determine the correlation between humoral responses to infection and vaccination in our recovered individuals, we compared the relationship between RBD- and S-specific IgG titers at 1-month post-infection and 1-month post-vaccination. In agreement with our prior data[4,8] and that of others,[7] RBD- and S-specific IgG titers correlated (r^2 = 0.35, 0.49) with neutralizing titers post-infection (Figure 5A). A similar correlation was observed between RBD- and S-specific IgG and neutralizing titers post-vaccination in both recovered (r^2 = 0.55, 0.58) and naive (r^2 = 0.16, 0.19) individuals (Figure 5A). We also compared the relationship between RBD-specific MBCs and RBD-specific IgG titers post-infection and -vaccination (Figure 5B). In recovered groups, we observed correlations between levels of RBD-specific MBCs and IgG titers only post-vaccination (r^2 = 0.22) (Figure 5B). RBD^+ MBCs and IgG titers did not correlate in naive vaccinees (p = 0.73) (Figure 5B).

We also sought to determine if levels of binding titers, neutralizing titers, and antigen-specific MBCs prior to vaccination were predictive of the magnitude of response observed after vaccination in recovered individuals. We correlated these metrics between samples taken 1-month post-vaccination and either (1) samples collected 1-month post-infection or (2) samples collected within 1 month prior to vaccination (Figure 5C). We observed positive correlations across all metrics (RBD- and S-IgG, neutralization, and RBD^+ MBCs) between the post-infection and -vaccination samples (Figure 5C). Similar positive correlations were observed between pre-vaccination and post-vaccination binding and neutralizing titers (Figure 5C). We also saw correlation between levels of RBD-binding MBCs following infection and following vaccination (Figure 5D) No correlation was observed between pre-vaccination and post-vaccination levels of RBD^+ MBCs (Figure 5C) or RBD-specific MBCs pre-vaccination and RBD-specific IgG post-vaccination (Figure 5D). We additionally found no correlations between NTD-, RBD-, or S-binding titers or RBD-specific MBCs and either age, gender, or time after infection (data not shown). These data provide evidence that the strength of the initial humoral response to SARS-CoV-2 infection predicts the strength of the response to vaccination in recovered individuals.

Vaccinated individuals display a range of dependency on RBD-binding antibodies for SARS-CoV-2 neutralization

In a previous study, we determined that the majority of the circulating neutralizing activity in acutely infected COVID-19 patients was driven by RBD-specific antibodies.[4] Here, we sought to determine whether the circulating response to vaccination had a similar reliance on RBD-binding antibodies. We depleted RBD-specific antibodies, as previously described,[4] from plasma in a subset of recovered (n = 23) and naive (n = 12) individuals at 1-month post-vaccination and assessed subsequent binding and neutralization activity. We determined the efficacy of the depletion through a comparison of RBD-binding titers pre- and post-depletion (Figures 6A and 6B). RBD-specific IgM, IgG, and IgA titers were reduced significantly in all participants, with average fold changes of 15-, 560-, and 140-fold, respectively (Figures 6A and S4A). The depletion thus resulted in greater than a 97% reduction in RBD-binding IgG, with the majority of individuals exhibiting a 100% reduction in RBD-binding IgG post-depletion (Figure 6A). We then assayed neutralizing activity in pre- and post-depletion samples against SARS-CoV-2 (WA1\2020). Fold change and percentage reduction were calculated using pre- and post-depletion titers

Figure 5. Antigen-specific MBCs and serological responses to SARS-CoV-2 infection correlate with responses to mRNA vaccination

(A) RBD- and S-titers correlate with neutralizing titers 1-month post-infection (left) and 1-month post-vaccination in recovered (middle) and naive (right).

(B) RBD + MBCs (percentage of $CD19^+$) correlated with RBD-specific IgG titers (left) and 1-month post-vaccination in recovered (middle) and naive (right).

(C) RBD- and S-binding titers, neutralizing titers, and RBD + MBCs (percentage of $CD19^+$) correlated between 1-month post-infection and 1-month post-vaccination (top) and 1-month pre-vaccination and 1-month post-vaccination (bottom).

(D) RBD-binding B cells post-infection (top) and pre-vaccination (bottom) are correlated with RBD-specific IgG following vaccination. Coefficient of determination (r^2) and significance determined from linear regression analysis.

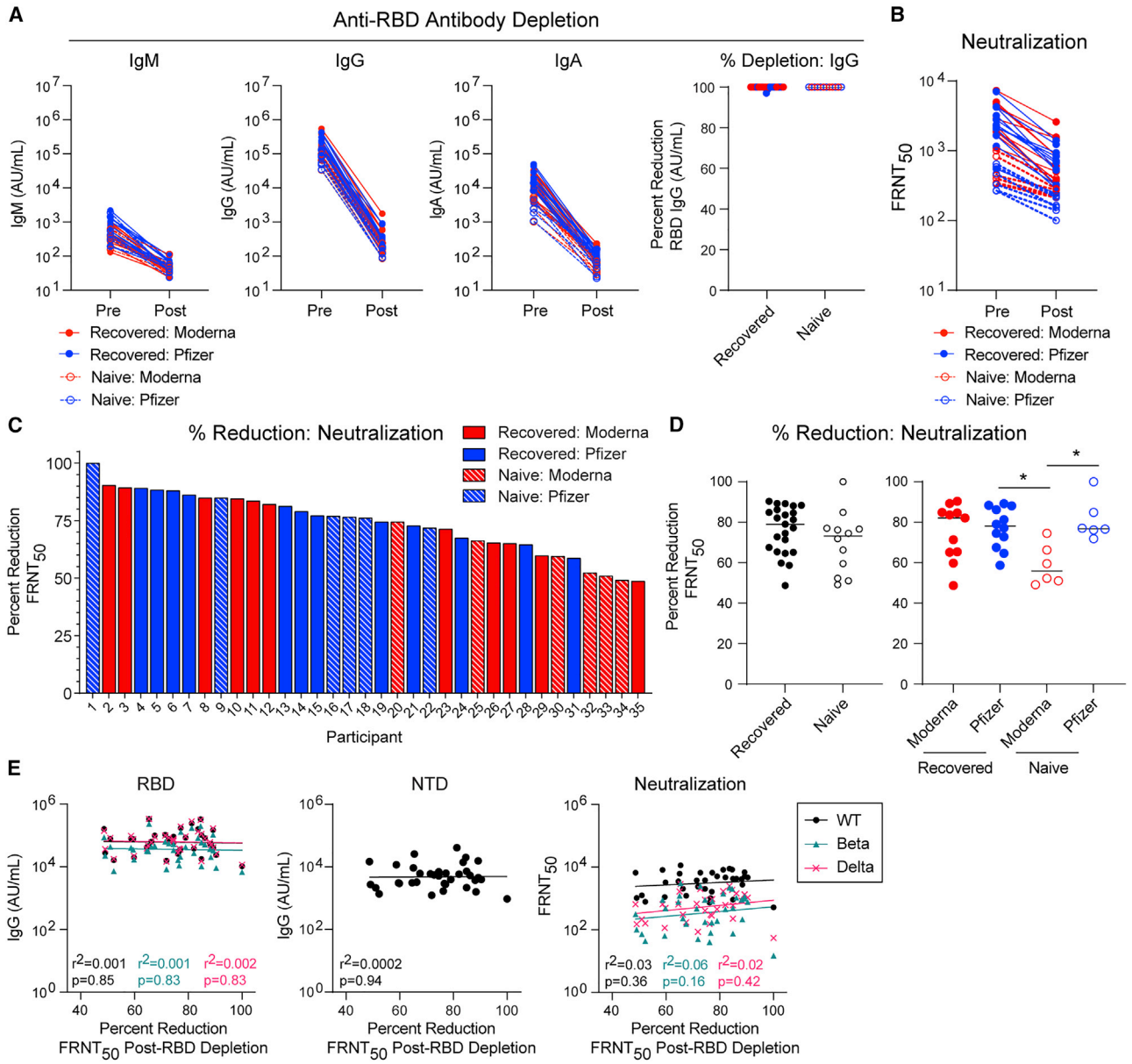

Figure 6. Naive Moderna-vaccinated individuals retain greater neutralizing capacity after depletion of RBD-specific fraction of plasma

(A) MSD-ELICA calculated titers for RBD-specific IgM, IgG, and IgA before (pre) and after (post) RBD depletion (left) and the percentage reduction of RBD-specific IgG post-RBD depletion as calculated from fold change (right).

(B) Neutralization titers against SARS-CoV-2 WT pre- and post-RBD depletion.

(C and D) The percentage reduction of neutralization post RBD-depletion as calculated from fold change.

(E) IgG titers against RBD (WT, Beta, Delta) and neutralization titers (WT, Beta, Delta) correlated the percentage reduction of neutralization post RBD-depletion; coefficient of determination (r^2) and significance determined from linear regression analysis.

Blue = Pfizer vaccination (recovered n = 12, naive n = 6), red = Moderna (recovered n = 11, naive n = 6), tested in duplicate. Open circles are naive subjects, closed circles are recovered. Statistics were calculated by Brown-Forsythe ANOVA and Dunnet's T3 multiple comparisons (A–D). *p < 0.05

after subtracting the LOD. Neutralizing activity was reduced in all post-depletion samples regardless of previous SARS-CoV-2 exposure or vaccine brand (Figure 6B). Prior to factoring in vaccine brand, there was no significant difference in the percentage reduction of neutralization between recovered and naive individuals. However, 82% (10/12) of naive individuals retained >20% neutralizing capacity compared with 52% (12/23) of recovered individuals (Figures 6C and 6D). When recovered and naive individuals were split into subgroups based on the vaccine received, we observed that the naive Moderna

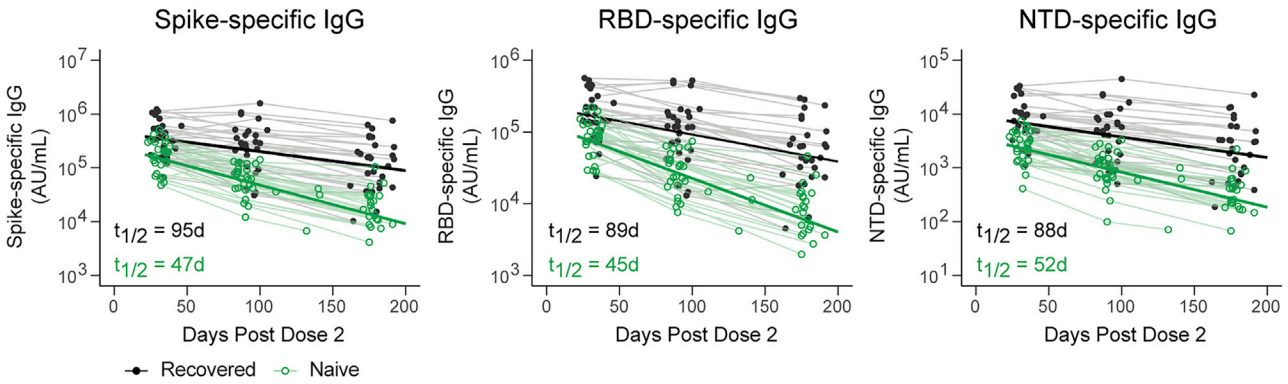

Figure 7. Naive vaccinees exhibit a faster decline in S-, RBD-, and NTD-specific IgG 6 months after vaccination than previously SARS-CoV-2 infected vaccinees

S-specific IgG (left), RBD-specific IgG (middle), and NTD-specific IgG (right) are shown for naive (green, open circles) and recovered (black, closed circles) subjects for 1–6 months following full vaccination. Best-fit lines determined using an exponential decay model and antibody half-lives ($t_{1/2}$) calculated.

group retained greater neutralization activity post-depletion than naive ($p = 0.018$) Pfizer vaccinees, with 66% (4/6) of vaccinees receiving Moderna retaining greater than 40% of neutralizing capacity post-RBD depletion (Figures 6C and 6D). As expected, this difference in RBD dependency did not result in a significant correlation between binding or neutralizing titers against WT, Beta, or Delta variants and the percentage reduction of neutralization (Figures 6E and S4B). In addition, we also assayed binding titers toward the SARS-CoV-2 S1 NTD, another epitope on the S protein shown to elicit neutralizing antibodies,[28,29] and we again observed no significant correlation between IgG titers and the percentage reduction of neutralization (Figure 6E). Further insight into this difference between Moderna and Pfizer vaccine responses in naive vaccinees requires additional work to characterize the repertoire through single-cell analyses. Taken together, this evidence illustrates that pre-existing immunity and vaccine brand both have an effect on the circulating repertoire of neutralizing antibodies produced in response to vaccination.

Pre-existing immunity impacts the durability of SARS-CoV-2 antibody and RBD-specific MBC levels

A key factor in the continuing efficacy of any vaccine is the durability of the immune response it induces. Comparing the vaccination response of naive with recovered individuals may also predict the response to a vaccine booster. We observed that levels of RBD-specific MBCs increase over time in naive individuals and parallel a sustained expression of CD71 on these MBCs (Figures 1G and 2B). This pattern contrasts with recovered vaccinees, whose RBD-specific MBCs decrease over time, with CD71 expression quickly returning to baseline (Figures 1G and 2B). To compare durability of the antibody response between the recovered and naive individuals, we fitted the data to an exponential decay model to determine the half-life of SARS-CoV-2-specific antibodies (S, RBD, and NTD) (Figure 7). We found that recovered individuals had more durable antibody responses after vaccination than naive individuals, with the half-life of antibodies in recovered individuals ($t_{1/2} = 95, 89, 88$) almost double that of their naive counterparts ($t_{1/2} = 47, 45, 52$) (Figure 7).

Overall, these observations illustrate that pre-existing immunity affects the long-term durability of the vaccine-induced immune response.

DISCUSSION

The strength, breadth, and durability of the immune response to SARS-CoV-2 following vaccination is a topic of great importance as variants continue to emerge and regulatory and governmental agencies across the world debate the benefits of booster shots for the general public. Additionally, the comparison of infection- versus vaccine-generated immunity is of public interest. Our study is uniquely suited to assess these factors due to the inclusion of a convalescent cohort followed for up to a year before vaccination as well as a naive group. We have also included both Pfizer and Moderna vaccinees to allow for a direct comparison of the induced immune response. To address the question of humoral and MBC durability, we tracked immune responses in our cohort up to 6 months after the completion of the mRNA vaccine regimen. Both recovered and naive individuals experienced slight declines in RBD- and S-specific IgG and IgA titers from their peak response, with naive individuals declining faster regardless of vaccine brand. Importantly, all individuals retained RBD- and S-specific titers at 6 months after vaccination that were significantly higher than baseline, suggesting that individuals would still have circulating antibodies that are likely protective from SARS-CoV-2 infection or severe COVID-19. We also assessed viral neutralization in both naive and recovered groups. Though recovered individuals did exhibit higher levels of neutralization at 1-month post-vaccination compared with naive individuals, it is important to note that the naive response to vaccination was significantly more neutralizing than that of the recovered group post-infection. This observation highlights that vaccine-generated immunity is as effective or better than the immunity following infection and raises the possibility that a booster in naive individuals may induce a similar increase in neutralizing antibody. In addition, the majority of individuals in both the recovered and naive groups retained neutralizing titers up to

6 months post-vaccination, further highlighting the durability of the vaccine-induced immune response.

Both naive and convalescent individuals generated significant RBD-specific MBC in levels comparable to SARS-CoV-2 infection[18] and influenza vaccination.[19–21] In addition, RBD-specific MBCs in naive individuals continue to increase after vaccination and are significantly elevated 6 months after immunization, reaching levels similar to that of the convalescent group. This increase in RBD-specific MBCs was also observed in individuals after a primary infection with SARS-CoV-2.[18,30] Additionally, we found that a significant portion of those RBD-specific MBCs remain activated ($CD71^+$) out to 6 months. These similar trends in antigen-specific MBC durability and activation are indicative of a sustained immune response months after initial exposure in both the case of primary infection and vaccination. Although further studies are needed to determine if this response is unique to SARS-CoV-2, our findings further highlight that vaccination alone produces MBC and antibody responses that are as good or better than infection alone at a magnitude comparable to other human infections.[19–21] It is likely that these cells will rapidly respond to either the administration of a third vaccine dose or a breakthrough SARS-CoV-2 infection, similarly to RBD-specific MBC expansion in recovered individuals following the first dose of a vaccine. The maintenance of a robust RBD-specific MBC population as circulating antibody titers begin to wane provides a potential explanation for the observation that while vaccinated individuals can be infected with SARS-CoV-2, the disease severity is lower compared with the unvaccinated.[31]

The strength and kinetics of the immune response to initial SARS-CoV-2 vaccination remain important as public health organizations attempt to increase global vaccine access. We observed stronger and more robust antibody responses in recovered individuals immunized with either mRNA vaccine compared with those without a previous exposure, similar to recent reports,[16,32] and provide a more detailed kinetic analysis of the cellular and serological responses during the course of vaccination. When comparing the humoral response between recovered and naive individuals, we found that recovered individuals responded more strongly to the first dose of either mRNA vaccine than their naive counterparts, with peak levels of both RBD-specific MBCs and RBD- and S-specific circulating IgG and IgA seen 1–3 weeks after the first dose. In line with the induction of a potent immune response, we also observed peak levels of CD71 expression, a well-characterized marker of activation on B cells,[33] on RBD-specific MBCs in recovered individuals after the first vaccine dose. These findings support the rapid recruitment and expansion of RBD-specific MBCs in response to vaccination in addition to increased RBD- and S-specific antibody production. Interestingly, we did not observe evidence of an additional immune response in recovered individuals after the second vaccine dose, with no additional peak in either CD71 expression on MBCs or antibody titers. While the second vaccine dose could affect the durability of the vaccine-induced response, the results observed herein suggest that one dose of a current mRNA vaccine is sufficient to boost SARS-CoV-2 immunity in previously infected individuals.

In contrast to their recovered counterparts, naive individuals experienced peak levels in both RBD-specific MBCs and RBD- and S-specific antibody titers 1–2 weeks after the second vaccine dose. This pattern is also evident in CD71 expression on RBD-specific MBCs, which increases progressively before peaking after the second dose. This observation illustrates that the RBD-specific MBCs generated are an active component of the immune response. Our finding that two mRNA vaccine doses are required for peak humoral immunity in naive individuals is well supported by the requirement of a two-dose regimen of both mRNA vaccines to reach robust levels of efficacy. While significant expansion of plasmablasts in peripheral blood has been reported in SARS-CoV-2 infection[4] and did occur in the naive Pfizer vaccinees, mRNA vaccination did not appear to drive a robust RBD-specific plasmablast expansion in either naive or recovered individuals. Though some individuals in both recovered and naive groups had a high frequency of total plasmablasts occurring at 1 month following vaccination, these plasmablasts were not detectably RBD binding and may be due to unrelated immune responses. Given that we observed high levels of RBD- and S-specific titers in circulation in both groups, it is unlikely that antibody-producing cells are absent; rather, vaccination is driving expansion in local sites such as lymph nodes near the site of vaccination, as has been observed in recent studies by Turner et al.[26] Reasons for this lack of peripheral plasmablast expansion are unclear and warrant further examination.

In addition to the humoral durability to WT SARS-CoV-2, we sought to determine whether the immune response to vaccination remained effective against SARS-CoV-2 variants. The Beta and Delta variants of the virus have shown the most resistance to immunity generated from previous infection, with significant declines in neutralization.[34,35] A similar pattern of partial resistance has begun to be reported in studies detailing the immune response to vaccination.[34–36] While we observed no decrease in binding to variant RBDs, we did find that both recovered and naive individuals had significant decreases in neutralization toward both Beta and Delta variants at 1 month after vaccination. Additionally, naive individuals experienced a steeper decline in variant-resistant neutralization with a greater fold change between WT and both variants observed in naive vaccinees compared with recovered vaccinees. These observations suggest that recovered individuals may develop and retain an antibody population that is more resistant to emerging SARS-CoV-2 variants than naive individuals in response to vaccination, most likely due to the boosting of the pre-existing antibody repertoire developed during infection.

To further assess differences in repertoire breadth between naive and recovered individuals, we depleted RBD-specific antibodies from the plasma of naive and recovered vaccinees and determined neutralizing capacity. We previously published that neutralizing activity in acute SARS-CoV-2 patients is highly dependent on RBD-specific antibodies.[4] Similarly, the recovered group largely lost neutralizing activity when RBD-specific antibodies were depleted, suggesting that vaccination in recovered individuals is likely recalling B cell responses established during previous infection. However, naive individuals vaccinated with Moderna were able to retain more plasma

neutralizing capacity in the absence of RBD-binding antibodies, suggesting that they may produce an antibody response with greater breadth. In the recovered group, responses to vaccination highly correlated with pre-existing immunity, both post-infection and immediately preceding vaccination. This correlation, the recovered group's dependence on RBD-specific antibodies for neutralization, and our previous finding that acute SARS-CoV-2 patients also exhibit similar RBD-specific antibody dependence support that mRNA vaccination is skewing the antibody repertoire toward RBD specificity through the recruitment of pre-existing clones as opposed to *de novo* activation of naive B cells. Naive Pfizer vaccinees also exhibited significantly more RBD-specific antibody-dependent neutralization compared with their Moderna-vaccinated counterparts. This difference may be due to the different formulations or dosages of the vaccines. The exact implication of these differences is as yet unclear given that they failed to correlate with binding or neutralizing titers to either WT or variant SARS-CoV-2. Further experiments to determine the antibody specificities of these non-RBD neutralizers will be needed to ascertain their significance to the overall immune response.

A continuous debate throughout the development of SARS-CoV-2 vaccines is whether one vaccine brand elicits a broader or more durable immune response than the other. In our study, we were able to compare the two mRNA vaccines available, Moderna's mRNA-1276 and Pfizer's BNT16b2, in both recovered and naive individuals. We observed no difference in the generation of a robust RBD-specific MBC population in response to vaccination, nor did we observe significant differences in ability of individuals to neutralize WT or variant SARS-CoV-2 1 month after vaccination, suggesting that both mRNA vaccines elicit a strong immune response against SARS-CoV-2. However, we did find that the S-specific IgG response in recovered individuals receiving Moderna peaked significantly faster than in individuals receiving Pfizer. This difference could likely be explained by the higher dosage (100 μg) of immunogen given in mRNA-1276, and the impact of this faster and its stronger peak is unclear, as S-specific titers between two vaccinee groups normalize quickly. We also found that naive Moderna vaccinees retained greater neutralizing titers in the absence of RBD-binding antibodies than both naive and recovered Pfizer vaccinees. This finding points to a potential difference in the breadth of the antibody repertoire generated between Moderna and Pfizer. While we were unable to identify the epitope specificities of this non-RBD fraction, it is interesting to note this difference in the face of recent reports that Moderna is more efficacious in preventing hospitalization when compared with Pfizer, especially many months out from vaccination.[11] Further investigation into the specific differences in the repertoire generated by these two vaccines is necessary.

SARS-CoV-2 continues to be a critical worldwide public-health threat. Vaccination, especially with highly efficacious mRNA vaccines, remains the best possible strategy for combatting the continuing pandemic. The comparison of antibody-binding, B cell memory, and neutralizing activity in recovered and naive individuals provides a possible prediction of how individuals may respond to repeated exposure, either through vaccination or infection. Our study also underlines that vaccination is equal or better at inducing immunity compared with infection alone, examines specificity of neutralizing activity in both Pfizer and Moderna immunization, and highlights the durability of the humoral immune response to vaccination.

Limitations of the study

Limitations of this study include a bias toward Pfizer vaccination in both naive and recovered groups and a small sample size. In addition, while our study evaluates vaccine responses in both SARS-CoV-2 recovered and naive groups, it reports only up to 6 months following vaccination and does not assess booster responses. Because this study continues to collect participant samples, including following booster administration, we will be able to further assess and evaluate our predictions on this important immune response.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2022.100603.

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AUTHOR CONTRIBUTIONS

G.M. and L.E.N contributed to the acquisition, analysis, and interpretation of data and writing the manuscript. V.-V.E., K.E.M., and M.E. performed *in vitro* neutralization experiments. M.W.F. and C.N. contributed to data acquisition and editing the manuscript. V.I.Z. performed half-life calculations and approval of the final manuscript. C.R.C., L.H., and K.S. provided clinical support for the study and contributed to sample collection. E.J.A., R.A., M.S.S., and J.W. contributed to the conception and design of the work and the writing and approval of the final manuscript.

DECLARATION OF INTERESTS

E.J.A. has consulted for Pfizer, Sanofi Pasteur, Janssen, and Medscape, and his institution receives funds to conduct clinical research unrelated to this manuscript from MedImmune, Regeneron, PaxVax, Pfizer, GSK, Merck, Sanofi-Pasteur, Janssen, and Micron. He also serves on a safety monitoring board for Kentucky BioProcessing, Inc., and Sanofi Pasteur. His institution has also received funding from NIH to conduct clinical trials of Moderna and Janssen COVID-19 vaccines. M.S.S. serves on the advisory board for Moderna.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse Anti-Human CD71/PerCP-Cy5.5	Biolegend	334114; RRID:AB_2563175
Mouse Anti-Human CD14/AF700	eBiosciences	56-0149-42; RRID:AB_2574497
Mouse Anti-Human CD16/AF700	eBiosciences	56-0168-42; RRID:AB_2574499
Mouse Anti-Human CD38/V450	BD Biosciences	646852; RRID:AB_1937284
Mouse Anti-Human CD4/PE-Cy5	BD Biosciences	555348; RRID:AB_395753
Mouse Anti-Human CD8/BV605	BD Biosciences	564115; RRID:AB_2744466
Mouse Anti-Human IgM/BV650	Biolegend	314525; RRID:AB_2563382
Mouse Anti-Human CD27/BV711	Biolegend	302834; RRID:AB_2563809
Mouse Anti-Human CD27/BV711	BD Biosciences	564893; RRID:AB_2739003
Mouse Anti-Human CD3/BV786	BD Biosciences	563800; RRID:AB_2744384
Mouse Anti-Human IgD/BUV395	BD Biosciences	563813; RRID:AB_2738435
Mouse Anti-Human CD19/BUV737	BD Biosciences	612756; RRID:AB_2870087
Mouse Anti-Human CD20/BUV805	BD Biosciences	612905; RRID:AB_2870192
Mouse Anti-Human IgA/PE	Miltenyi Biotech	130-113-476; RRID:AB_2733861
Mouse Anti-Human IgG/PE-Cy7	BD Biosciences	561298; RRID:AB_10611712
LIVE/DEAD Fixable Yellow	Thermo Fisher Scientific	L34968
Bacterial and virus strains		
nCoV/USA_WA1/2020	Dr. Vineet D. Menachery (UTMB, Galveston, TX)	N/A
B.1.351	Dr. Andy Pekosz (John Hopkins University, Baltimore, MD)	hCoV-19/South Africa/KRISP-K005325/2020
B.1.617.2	Mid-turbinate nasal swab	hCoV-19/USA/PHC658/2021
Biological samples		
Human PBMC/Plasma/Serum Samples	Emory University	N/A
Chemicals, peptides, and recombinant proteins		
Recombinant Protein (RBD-S1)	Suthar, et al	Custom
ACK Lysis Buffer	Quality Biological	118-156-101
Paraformaldehyde (16%)	Thermo Fisher Scientific	28906
DMEM	VWR	45000-304
Methylcellulose	Sigma-Aldrich	M0512-250G
Critical commercial assays		
AF488 Protein Labeling Kit	Thermo Fisher Scientific	A10235
V-PLEX COVID-19 Coronavirus Panel 1 (IgG) Kit	Mesoscale Discovery	K15362U
V-PLEX COVID-19 Coronavirus Panel 1 (IgA) Kit	Mesoscale Discovery	K15364U
V-PLEX SARS-CoV-2 Panel 7 (IgM) Kit	Mesoscale Discovery	K15438U
V-PLEX SARS-CoV-2 Panel 11 (IgG) Kit	Mesoscale Discovery	K15455U
V-PLEX SARS-CoV-2 Panel 13 (IgG) Kit	Mesoscale Discovery	K15463U
Dynabeads Antibody Coupling Kit	Thermo Fisher Scientific	14311D
Experimental models: Cell lines		
VeroE6 C1008 cells	ATCC	Cat# CRL-1586, RRID:CVCL_0574
Software and algorithms		
FlowJo	BD Biosciences	10.8.0
Discovery WorkBench 4.0	Mesoscale Discovery	N/A
GraphPad Prism (8,9)		N/A
Viridot	Katzelnick et al	https://github.com/leahkatzelnick/Viridot

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact Author Jens Wrammert (jwramme@emory.edu).

Materials availability

No unique reagents were generated in this study.

Data and code availability

This paper does not report new data sets of a standardized datatype and does not report custom code. Any additional information required to reanalyze the data reported in this work paper is available from the Lead Contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study population

A longitudinal cohort of recovered and naïve vaccinated individuals were recruited at Emory University with approval from the institutional review board (IRB00022371). Informed consent was obtained from all participants before conduct of study procedures. Further demographic details of this cohort can be found in Table S1.

Cell lines

VeroE6-TMPRSS2 cells were generated and cultured as previously described.[37] Briefly, VeroE6-TMPRSS2 cells were generated by transfecting VERO E6 cells (ATCC CRL-1586) with pCAGGS plasmid in which chicken actin gene promoter drives the expression of an open reading frame comprising Puromycin N-acetyl transferase, GSG linker, 2A self-cleaving peptide of thosea asigna virus (T2A), human transmembrane serine protease 2 (TMPRSS2). Two days post-transfection, cells were trypsinzed and transferred to a 100 mm dish containing complete DMEM medium (1x DMEM, Thermo Fisher, # 11965118, 10% FBS, 1x penicillin/streptomycin) supplemented with puromycin (Thermo Fisher, #A1113803) at a final concentration of 10 μg/mL. Approximately ten days later, individual colonies of cells were isolated using cloning cylinders (Sigma) and expanded in medium containing puromycin. Clonal cell lines were screened for expression of TMPRSS2 by flow cytometry. VeroE6-TMPRSS2 cells were cultured in complete DMEM in the presence of Gibco Puromycin 10 mg/mL (# A11138-03). VeroE6-TMPRSS2 cells were used to propagate all virus stocks.

Viruses

nCoV/USA_WA1/2020 (WA/1), closely resembling the original Wuhan strain and resembles the spike used in the mRNA-1273 and Pfizer BioNTech vaccine, was propagated from an infectious SARS-CoV-2 clone as previously described.[38] icSARS-CoV-2 was passaged once to generate a working stock. The B.1.351 variant isolate, kindly provided by Dr. Andy Pekosz (John Hopkins University, Baltimore, MD), was propagated once to generate a working stock. hCoV-19/USA/PHC658/2021 (herein referred to as the B.1.617.2 variant) was derived from nasal swab collected in May 2021. Using VeroE6-TMPRSS cells, the B.1.617.2 variant was plaque purified directly from the nasal swab, propagated once in a 12-well plate, and expanded in a confluent T175 flask to generate a working stock. All viruses used in this study were deep sequenced and confirmed as previously described.[37]

METHOD DETAILS

Sample preparation

Briefly, plasma and PBMC were isolated from peripheral blood collected in CPT or heparin tubes from these participants following infection or vaccination. CPT and heparin tubes were processed according to the manufacturer's protocol, and separated plasma and PBMCs were collected separately. PBMCs were treated with ACK lysis buffer (catalog no. 118-156-101, Quality Biological) for 5 min and washed three times with PBS with 2% FBS before counting and analysis by flow cytometry. PBMC and plasma were frozen at −80 °C prior to long-term storage at −80 °C (plasma) or in liquid nitrogen (PBMC).

Flow cytometry

Freshly isolated or thawed PBMCs were stained first for viability with LIVE/DEAD Fixable Yellow (Thermo Fisher Scientific) and then for markers with the following mAbs: IgA (IS11-8E10; Miltenyi Biotec), IgD (IA6-2; BD Biosciences), IgG (G18-145; BD Biosciences), IgM (MHM-88; BioLegend), CD3 (SK7, BD Biosciences), CD4 (RPA-T4, BD Biosciences), CD8 (SK1; BD Biosciences), CD14 (61D3; eBioscience), CD16 (CB16; eBioscience), CD19 (SJ25C1; BD Biosciences), CD20 (2H7; BD Biosciences), CD27 (O323; BioLegend or MT271; BD Biosciences), CD38 (HB7; BD Biosciences), and CD71 (CY1G4; BioLegend). Ag-specific B cells were detected by staining with RBD-conjugated to Alexa Fluor 488 (Alexa Fluor 488 Protein Labeling Kit; Thermo Fisher Scientific). RBD was conjugated as previously described.[4] After staining, PBMCs were washed and then fixed for 15 min using 2% paraformaldehyde (PFA; Thermo Fisher Scientific). Data were acquired on a BD FACSymphony A5 and analyzed using FlowJo 10.8.0 (BD Biosciences).

Antibody binding assay

Binding analyses were performed on plasma and serum samples using one or more of the following multiplexed antigen panels: V-PLEX COVID-19 Coronavirus Panel 1 (K15362/64U), V-PLEX SARS-CoV-2 Panel 7 (K15438U), V-PLEX SARS-CoV-2 Panel 11 (K15455U), and/or V-PLEX SARS-CoV-2 Panel 13 (K15463U). Briefly, plates were blocked with 150 μL/well of PBS +5% BSA for 30 minutes shaking at 700 rpm. After washing 3x with PBS+0.05% Tween 20, 50 μL/well of sample diluted at 1:20,000 was added to the plate in duplicate and incubated for 2 hours shaking at 700 rpm. After washing, 50 μL/well of SULFO-TAG secondary (Anti-Human IgM, IgG, or IgA as appropriate) was incubated for 1 hour shaking at 700 rpm. After a final wash, 150 μL/well of MSD GOLD Read Buffer was added, and plates were read immediately on the MESO QuickPlex SQ 120. Antibody titers were calculated with Discovery Workbench 4.0 using a standard curve and are reported in arbitrary units per mL (AU/mL).

Focus reduction neutralization test

FRNT assays were performed as previously described.[39] Briefly, samples were diluted at 3-fold in 8 serial dilutions using DMEM (VWR, #45000-304) in duplicates with an initial dilution of 1:10 in a total volume of 60 μL. Serially diluted samples were incubated with an equal volume of WA1/2020 or B.1.351 or B.1.617.2 (100–200 foci per well based on the target cell) at 37°C for 1 hour in a round-bottomed 96-well culture plate. The antibody-virus mixture was then added to VeroE6-TMPRSS2 cells and incubated at 37°C for 1 hour. Post-incubation, the antibody-virus mixture was removed and 100 μL of pre-warmed 0.85% methylcellulose (Sigma-Aldrich, #M0512-250G) overlay was added to each well. Plates were incubated at 37°C for 16 hours. After 16 hours, methylcellulose overlay was removed, and cells were washed three times with PBS. Cells were then fixed with 2% paraformaldehyde in PBS for 30 minutes. Following fixation, plates were washed twice with PBS and 100 μL of permeabilization buffer, was added to the fixed cells for 20 minutes. Cells were incubated with an anti-SARS-CoV spike primary antibody directly conjugated with alexaflour-647 (CR3022-AF647) for up to 4 hours at room temperature. Cells were washed three times in PBS and foci were visualized and imaged on an ELISPOT reader (CTL).

RBD depletion assay

Depletion of RBD-specific antibodies from plasma was conducted as previously described.[4] Briefly, plasma samples were diluted 1:10 with superparamagnetic beads coupled to RBD according to the manufacturer's protocol. Samples were incubated with rotation at RT for 1 hour after which the diluted plasma was separated from beads and transferred to tubes containing the same amount of RBD-coupled beads separated from storage buffer. Samples were incubated again rotating at RT for 1 hour, and the diluted plasma was separated from beads and transferred to fresh tubes for analysis. Removal of RBD-binding antibodies was confirmed through binding analysis (as described previously), and neutralization assays were performed as described previously using an initial dilution of 1:50 in 100 μL.

QUANTIFICATION AND STATISTICAL ANALYSIS

FRNT quantification

Antibody neutralization was quantified by counting the number of foci for each sample using the Viridot program.[40] The neutralization titers were calculated as follows: 1 - (ratio of the mean number of foci in the presence of sera and foci at the highest dilution of respective sera sample). Each specimen was tested in duplicate. The FRNT-50 titers were interpolated using a 4-parameter nonlinear regression in GraphPad Prism 9.2.0. Samples that do not neutralize at the limit of detection at 50% are plotted at the initial plasma dilution.

Statistics

Data were analyzed using GraphPad Prism 8.4.3. Statistics were calculated using mixed-effects model with Geisser-Greenhouse correction for all comparisons of cell populations and antibody titers across timepoints. Brown-Forsythe ANOVA and Dunnet's T3 multiple comparison test or mixed-effects model with Geisser-Greenhouse correction was used to calculate statistics between groups. Pearson correlation coefficients and linear regressions were applied as appropriate. Mixed-effects models implemented in MonolixSuite 2020R1 (Lixoft) were used to estimate the corresponding half-lives of antigen-specific antibodies. The equation $dAb/dt = -k*Ab$ was fitted to the longitudinal data starting from day 21 after the second vaccine dose (data for each individual are shown in Figure 7 as circles connected with thin lines), where Ab is the antibody level and k is the exponential decay rate. The corresponding half-lives were calculated as $t1/2 = \ln(2)/k$. The individual-level parameters were lognormally distributed for the initial Ab level (at day 21) and normally distributed for the decay rate k with an assumption of no correlations between the random effects. We assumed multiplicative independent lognormal observation error. The estimation of the population parameters was performed using the Stochastic Approximation Expectation Maximization (SAEM) algorithm, and corresponding fits are shown with thick lines in Figure 7.