

Distinct Homologous and Variant-Specific Memory B-Cell and Antibody Response Over Time After Severe Acute Respiratory Syndrome Coronavirus 2 Messenger RNA Vaccination

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The durability of protective humoral immunity after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination and infection is largely dependent on the generation and persistence of antigen-specific isotype-switched memory B cells (MBCs) and long-lived plasma cells that reside in the bone marrow and secrete high-affinity neutralizing antibodies. The reactivity of vaccine-induced MBCs to emerging clinically significant SARS-CoV-2 variants of concern (VoCs) is largely unknown. In a longitudinal cohort study (up to 6 months following coronavirus disease 2019 messenger RNA vaccination), we measured MBCs in concert with other functional antibody measures. We found statistically significant differences between the frequencies of MBCs responding to homologous and VoC (Beta, Gamma, and Delta) receptor-binding domains after vaccination that persisted over time. In concert with a waning antibody response, the reduced MBC response to VoCs could translate to a weaker subsequent recall immune response and increased susceptibility to the emerging SARS-CoV-2 variant strains after vaccination.

Keywords. SARS-CoV-2; COVID-19; vaccine; antibodies, neutralizing; humoral immune responses; immunological memory; B cells.

A better understanding of the immune memory and functional humoral immunity after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination is essential for predicting and understanding the longevity of heterotypic protection from circulating variants of concern (VoCs). Longstanding functional humoral immunity is contingent on the generation, survival, and long-term maintenance of antigen-specific long-lived plasma cells, circulating and mucosal memory B cells (MBCs), and memory T cells (ie, memory T follicular helper cells) [1–6]. Antigen-specific MBCs, in particular, are one of the hallmarks of humoral immune memory and provide a robust secondary immune response upon antigen reencounter and/or reinfection. They are developed via both germinal center (GC)–dependent and GC-independent pathways and share common characteristics such as a long lifespan and the ability to quickly proliferate and differentiate into plasma cells, producing highly specific antibodies, when exposed to low doses of antigen [7]. The fine-tuned specificity of

MBCs can shape the B-cell functional immune responses upon encounter of identical and/or similar antigens, but is barely studied in the context of SARS-CoV-2 vaccination and clinically important VoCs [2, 8].

The goal of our study was the longitudinal characterization of functional humoral immunity following SARS-CoV-2 messenger RNA (mRNA) vaccination, and to assess and compare the reactivity of coronavirus disease 2019 (COVID-19) vaccine-induced MBCs (ie, the potential for recall immune response) to the emerging SARS-CoV-2 VoCs up to 6 months following vaccination series.

MATERIALS AND METHODS

For more details on the Materials and Methods, refer to the [Supplementary Data](#).

Human Subjects

The study sample consisted of 17 healthy subjects (15 naive and 2 recovered from COVID-19 infection) who provided a blood sample prior to vaccination (baseline) with BNT162b2 (Pfizer/BioNTech) or mRNA-1273 (Moderna) vaccine, at approximately 3 weeks after their first vaccine dose and before the second dose (first vaccine dose timepoint), at approximately 2 weeks after the receipt of their second vaccine dose (second vaccine dose timepoint), and at 6 months after the second vaccine dose. All study participants provided written informed

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consent, and all study procedures were approved by the Mayo Clinic Institutional Review Board.

SARS-CoV-2 Pseudovirus/Recombinant Vesicular Stomatitis Virus Microneutralization Assay

The neutralizing antibody response was assessed using a high-throughput, green fluorescent protein–based pseudovirus/vesicular stomatitis virus (VSV) microneutralization assay [9], further developed in our laboratory to allow rapid imaging/quantification of cell infection with the ImageXpress platform/software (Molecular Devices). Wuhan-Hu-1 spike-specific 50% end-point titer (neutralizing dose [ND₅₀]) for each sample was calculated using the Karber formula.

GenScript SARS-CoV-2 Surrogate Virus Neutralization Test

The capacity of sera to impair virus attachment/block the interaction between human angiotensin-converting enzyme 2 (hACE2) and different receptor-binding domains (RBDs) was determined using the SARS-CoV-2 surrogate virus neutralization test (sVNT) kit (RUO.3.0, GenScript USA) according to the manufacturer's specifications. The inverse dilution at which a sample reaches 50% inhibition is designated as the half-maximal inhibitory dose (ID₅₀).

Memory B-Cell Enzyme-Linked Immunosorbent Spot Assay

The frequencies of SARS-CoV-2–specific MBCs were quantified using the Mabtech (Cincinnati, Ohio) enzyme-linked immunosorbent spot assay (ELISPOT) for human immunoglobulin G (IgG) according to the manufacturer's specifications, and as previously described using SARS-CoV-2 recombinant antigens (Sino Biologicals, Beijing, China) [10, 11].

Statistical Analysis

The median of the replicates for each assay was calculated and used as the primary response. Two-sided Wilcoxon signed-rank tests were used to assess differences for all comparisons; the effect size (ES) is calculated taking the z-statistic divided by the square root of the sample size. Spearman correlation was used to assess the relationship between the ND₅₀ titers and the frequency of antigen-specific MBCs. All analyses were conducted using R version 4.0.3 [12].

RESULTS

Study Sample Characteristics

We enrolled and followed naive subjects and recovered COVID-19 subjects from Olmsted County, Minnesota, and surrounding areas following COVID-19 vaccination in January–June 2021. The study enrolled 17 generally healthy subjects; 59% were female and 94% were White/non-Hispanic or Hispanic with a median age at enrollment of 41 years (interquartile range [IQR], 24–54 years). The subjects received either the BNT162b2 (Pfizer/BioNTech) vaccine (n = 14) or mRNA-1273 (Moderna) vaccine (n = 3). Subjects provided a blood sample at baseline, at

approximately 3 weeks after their first vaccine dose (median, 21 days [IQR, 20–22 days]), at approximately 2 weeks after the receipt of their second vaccine dose (median, 14 days [IQR, 14–14 days]), and at approximately 6 months after the receipt of the second vaccine dose (median, 180 days [IQR, 175–181 days]).

Neutralizing Antibody Response

As expected, SARS-CoV-2 vaccination elicited robust anti-spike neutralizing antibody responses, quantified by a recombinant VSV pseudovirus neutralization assay based on the expressed Wuhan-Hu-1 spike [9], with significant interindividual variations in measured antibody titers. Neutralizing antibody response increased significantly after each vaccine dose ($P < .001$, $ES > 0.99$ for all comparisons up to ~2 weeks after the receipt of the second vaccine dose; [Figure 1A](#)). The median ND₅₀ approximately 2 weeks after the second SARS-CoV-2 mRNA vaccination was 3290 (IQR, 2572–4981). At 6 months following the vaccination series, the median ND₅₀ was 540 (IQR, 338–837), which was significantly lower (~6-fold lower) compared to the antibody response measured approximately 2 weeks after the second dose ($P = .0001$, $ES = 1.03$; [Figure 1A](#)).

Evaluation of Functional Antibody Activity to Different VoCs

We quantified the functional ability of sera to inhibit RBD-hACE2 binding using the GenScript sVNT with values expressed as ID₅₀. This assay allowed precise comparisons of serum reactivity to multiple RBD antigens and was performed on samples after the second vaccine dose (n = 15) and at 6 months (n = 14). The results from this assay demonstrated a reduction in functional antibody activity (compared to Wuhan-Hu-1 RBD) for all 4 tested VoC RBDs at both timepoints following mRNA vaccination ([Figure 1B](#) and [1C](#); [Supplementary Materials](#)). This reduction in antibody activity was more pronounced for the Beta (B.1.351) and Gamma (P.1) RBD variants ([Figure 1B](#) and [1C](#)). The calculated antibody median ID₅₀ values for the tested RBD antigens after the second vaccine dose were 1198 for the Wuhan-Hu-1 RBD, and 540, 333, 228, and 908 for the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2) RBD variants, respectively. The median ID₅₀ values at 6 months were 96 for the Wuhan-Hu-1 RBD, and 52, 40, 29, and 94 for the Alpha, Beta, Gamma, and Delta RBD variants, respectively. Statistical comparisons (Wilcoxon signed-rank test) at the second vaccine dose timepoint demonstrated a significant difference; that is, the mean antibody ID₅₀ value to Wuhan-Hu-1 RBD was significantly greater than the mean ID₅₀ values to the Alpha, Beta, and Gamma ($P < .0001$, $ES = 1.04$ for all comparisons) and Delta ($P < .001$, $ES = 0.86$) variant RBDs. At 6 months after the vaccination series, the mean antibody ID₅₀ value to Wuhan-Hu-1 RBD was also significantly greater than the mean ID₅₀ value to the Alpha, Beta, and Gamma ($P < .001$, $ES = 1.03$ for all comparisons) and Delta ($P = .025$, $ES = 0.60$) variant RBDs. The median ID₅₀ values for the Wuhan-Hu-1

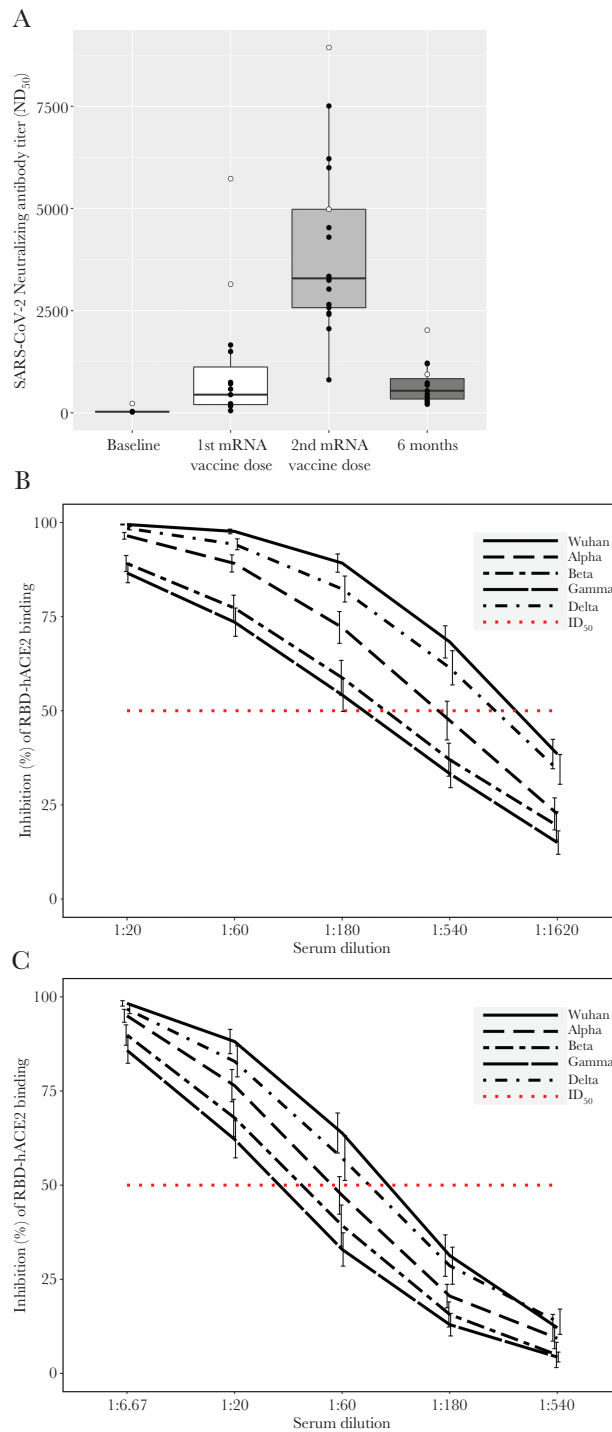


Figure 1. Functional antibody response after coronavirus disease 2019 (COVID-19) messenger RNA (mRNA) vaccination. *A*, The dynamics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralizing antibody response after vaccination was assessed using a vesicular stomatitis virus pseudovirus microneutralization assay. Neutralizing dose 50% (ND₅₀) titers were calculated as described in the Materials and Methods using dilution series from 1:20 up to 1:10240 with 4 replicates per dilution step. ND₅₀ values over timepoints relative to vaccination are displayed using boxplots for all subjects. The values are plotted on the log₂ scale, but the scales of the axis reflect the untransformed values for easier interpretation. Each box was plotted using the interquartile range (IQR) and the median is represented by the bold line in the box. The “whiskers” extend up to 1.5 times the IQR above or below the 75th or 25th percentiles, respectively. Black dots represent naive (at baseline) subjects, while white dots represent COVID-19-recovered (at baseline) subjects. *B* and *C*, Comparison of serum antibody-mediated inhibition of receptor-binding domain (RBD)–human angiotensin-converting enzyme 2 (hACE2) binding for different RBDs of variants of concern (Alpha [B.1.1.7], Beta [B.1.351], Gamma [P.1], and Delta [B.1.617.2]) and the Wuhan-Hu-1 RBD using the GenScript surrogate virus neutralization test kit after second vaccine dose (*B*) and at 6 months following the 2-dose vaccination series (*C*). Samples were serially diluted (3-fold) from 1:6.67 to 1:1620, and the different dilutions were assessed in duplicate for blockade/inhibition of RBD–hACE2 interaction. The inhibition curves represent mean inhibition (%) of RBD–hACE2 binding values across all tested subjects (second vaccine dose, *n* = 15; 6 months, *n* = 14), and error bars represent standard error. The dashed horizontal line represents a 50% reduction in RBD–hACE2 binding compared to negative controls (absence of blocking antibodies). Half-maximal inhibitory dose (ID₅₀) values to different RBDs were calculated as described in the Materials and Methods.

RBD and VoC RBDs significantly decreased ($P < .001$, ~7- to 12-fold lower) at the 6-month timepoint compared to the second vaccine timepoint.

Dynamics of Wuhan-Hu-1 MBC Response Over Time

We next sought to investigate the dynamics of MBC response after SARS-CoV-2 mRNA vaccination. We profiled the frequencies and specificities of isotype-switched IgG⁺ MBCs directed to different parts of the homologous Wuhan-Hu-1 spike protein over time, as an indication of mature, highly specific, and functional immune memory [1]. As expected, the frequency of antigen-specific MBCs changed/increased significantly over time during the mRNA vaccination series with large interindividual variations observed between subjects (Figure 2, $P < .004$, ES > 0.80 for all comparisons between the first 3 timepoints for S1 and RBD-specific MBC response). The primary vaccination elicited a significant SARS-CoV-2-specific MBC response directed mainly to the S1 portion of

the SARS-CoV-2 spike of Wuhan-Hu-1 and particularly to its RBD (Figure 2; median S1 MBC response of 13 spot-forming units [SFUs] per 2×10^5 cells [IQR, 9–16]; median RBD MBC response of 8 SFUs per 2×10^5 cells [IQR, 5–13]). This response was significantly boosted after the second vaccine dose to a median S1 MBC response of 45 SFUs per 2×10^5 cells (IQR, 25–57) and a median RBD response of 27 SFUs per 2×10^5 cells (IQR, 16–31). The measured MBC response at 6 months was variable between subjects with an increase observed for some individuals and a decrease observed for others (Figure 2). Of note, no detectable and/or increased SARS-CoV-2 N protein-specific antibody was identified for the subjects with a measured increase in MBC response (data not shown), suggesting that subclinical boosting from SARS-CoV-2 did not occur in our cohort. The overall S1 and RBD MBC response at 6 months (S1 MBC response, 21 SFUs per 2×10^5 cells [IQR, 11–73]; RBD MBC response, 11 SFUs per 2×10^5 cells [IQR, 9–42]) was not significantly different compared to the overall

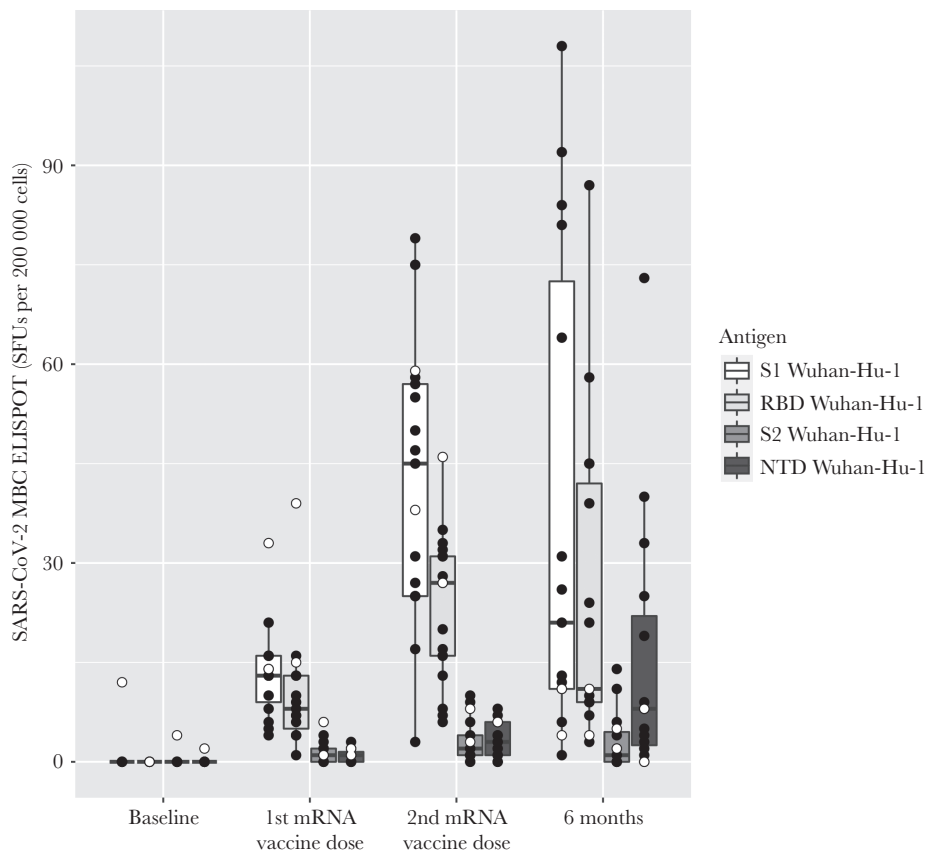


Figure 2. Dynamics of memory B-cell (MBC) response after coronavirus disease 2019 (COVID-19) messenger RNA (mRNA) vaccination for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The frequencies of immunoglobulin G (IgG)-positive MBCs were measured using the Mabtech ELISpot^{PLUS} kit (enzyme-linked immunosorbent spot assay [ELISPOT]) for human IgG, using the indicated antigens (dark/light gray and white boxes), after in vitro peripheral blood mononuclear cell 3-day stimulation with human recombinant interleukin 2 and R848. Detected responses are presented in spot-forming units (SFUs) per 2×10^5 cells, as subjects' antigen-specific medians (from 3 replicates with subtracted subject-specific no-antigen background measure). The values are plotted on the log₂ scale, but the scales of the axis reflect the untransformed values for easier interpretation. Each box was plotted using the interquartile range (IQR) and the median was represented by the bold line in the box. The "whiskers" extend up to 1.5 times the IQR above or below the 75th or 25th percentiles, respectively. Black circles represent naive subjects (at baseline), while white circles represent COVID-19-recovered subjects (at baseline). The MBC responses and changes over time of S1/receptor-binding domain (RBD)-specific MBC response and S2/N-terminal domain (NTD) MBC response are compared using the Wilcoxon signed-rank test and reported in the Results.

response measured after the first or after the second vaccine dose (Figure 2). Interestingly, IgG⁺ MBCs directed to the S2 portion of spike, as well as to the N-terminal domain (NTD), were detectable at a much lower frequency and present only in some of the subjects (Figure 2). The calculated median S2 MBC response after the second vaccine dose was 2 SFUs per 2×10^5 cells (IQR, 1–4), and the median NTD MBC response after the second vaccine dose was 3 SFUs per 2×10^5 cells (IQR, 1–6). Although the measured S2 and NTD MBC response was relatively low, we observed significant differences over time (eg, the response after the second vaccine dose was significantly higher than baseline ($P = .006$, ES = 0.80 and $P = .002$, ES = 0.87) for the S2 MBC response and NTD MBC response, respectively. Interestingly, the measured NTD-specific MBC response at 6 months (overall 8 SFUs per 2×10^5 cells [IQR, 3–22]) was markedly enhanced for some of the subjects and was significantly increased compared to baseline ($P = .02$, ES = 0.77) and first vaccine dose ($P = .009$, ES = 0.73), but not significantly different compared to the second vaccine dose (Figure 2).

Differential RBD-Specific MBC Reactivity Between Wuhan-Hu-1 and VoCs at Various Timepoints

While our data and other published results have demonstrated a reduction in antibody reactivity to SARS-CoV-2 variants after COVID-19 vaccination, it is not established if MBC responses against these variants are similarly impacted. Therefore, we probed the reactivity/ability of the IgG⁺ RBD-specific MBCs post-mRNA vaccination to target RBDs of all current, clinically significant VoC strains (Alpha, Beta, Gamma, and Delta RBDs). Although the interrogated VoC RBDs differed from the Wuhan-Hu-1 RBD by only one or a few amino acids, we noted differential and reduced reactivity of vaccine-induced MBCs to VoC RBDs as displayed in Figure 3 (displayed timepoints: ~3 weeks after the first mRNA vaccine dose; ~2 weeks after the second mRNA vaccine dose; and 6 months after the second vaccine dose). While RBD-specific MBCs were readily detectable to all variant RBDs at all timepoints following mRNA vaccination, we noted statistically significant reductions in the MBC frequencies to each of the variant RBDs after the first vaccine dose ($P < .029$, ES > 0.56 for all comparisons with the Wuhan-Hu-1 RBD, Figure 3A). Following the second vaccine dose, there remained a significant decrease in the number of reactive MBCs that recognized the Beta, Gamma, and Delta RBD variants compared to the Wuhan-Hu-1 RBD ($P < .005$, ES > 0.69 for the 3 VoC comparisons), while the difference in MBC response to the Alpha variant was nonsignificant (Figure 3B). Similarly, at 6 months following the vaccination series, the number of reactive MBCs to the Beta, Gamma, and Delta RBD variants remained significantly lower compared to the Wuhan-Hu-1 RBD MBC response ($P < .006$, ES > 0.71 for the 3 VoC comparisons; Figure 3C). The observed median RBD-specific MBC response

after the second vaccine dose (in SFUs per 2×10^5 cells) was 27 (IQR, 16–31) for the Wuhan-Hu-1 RBD; 22 (IQR, 16–33) for the Alpha variant RBD; 18 (IQR, 11–23) for the Beta variant RBD; 18 (IQR, 12–26) for the Gamma variant RBD; and 19 (IQR, 9–23) for the Delta variant RBD; that is, there was an approximate 30% decrease in MBC reactivity to the Beta, Gamma, and Delta VoC RBDs, and this trend was maintained over time (Figure 3).

DISCUSSION

The SARS-CoV-2 spike surface glycoprotein is expressed as a glycosylated trimer, which plays a key role in infection and viral entry via interaction with the hACE2 receptor. Its S1 subunit comprises 672 amino acids and is structured into 4 domains: the NTD, the RBD, and the 2 subdomains (SD1 and SD2). The S2 subunit is critical for viral fusion and comprises 588 amino acids containing the fusion peptide, the 2 heptad repeats (HR1 and HR2), the transmembrane domain, and the cytoplasmic tail [13]. In this study, we detected measurable MBC response to all major parts of the spike protein after mRNA vaccination (dominated by MBC response to S1 and its RBD domain) with significant boosting after administration of the second vaccine dose, but with no significant differences between the S1/RBD MBC response shortly after the second vaccine dose and the response 6 months later. Ciabattini et al also reported persistent spike-specific MBC response up to 6 months following 2 doses of mRNA vaccine (BNT162b2) [14]. This study reported a positive correlation between the spike-specific plasmablast response at day 7 after the second mRNA vaccine dose and the MBC response at 6 months following vaccination [14]. We noted moderate but consistent positive correlations between neutralizing antibody (ND₅₀) titers and the frequency of S1- and RBD-specific MBCs after the first vaccine dose ($r = 0.82$, $P = .00019$ and $r = 0.89$, $P = .00001$, respectively). The correlations were less robust and with marginal statistical significance after the second vaccine dose ($r = 0.49$, $P = .046$ and $r = 0.43$, $P = .09$, respectively) and were nonsignificant at the 6-month timepoint, likely due to the small sample size and interindividual variability in vaccine response.

Our results are similar to Goel et al, who investigated the antibody and spike/RBD-specific MBC response in 44 subjects following SARS-CoV-2 mRNA vaccination and found a robust neutralizing and MBC response upon completion of the vaccination series in naive individuals [2]. That study found a reduction in antibody response to the B.1.351 VoC but did not find statistically significant associations between MBC response (as quantified by flow cytometry using fluorescently labeled antigens as probes) and antibody titers [2]. However, the study demonstrated a strong association between the baseline MBC response in vaccinated COVID-19-recovered individuals and anti-spike/anti-RBD antibody titers following the first mRNA vaccine dose, which suggests that the quantity/

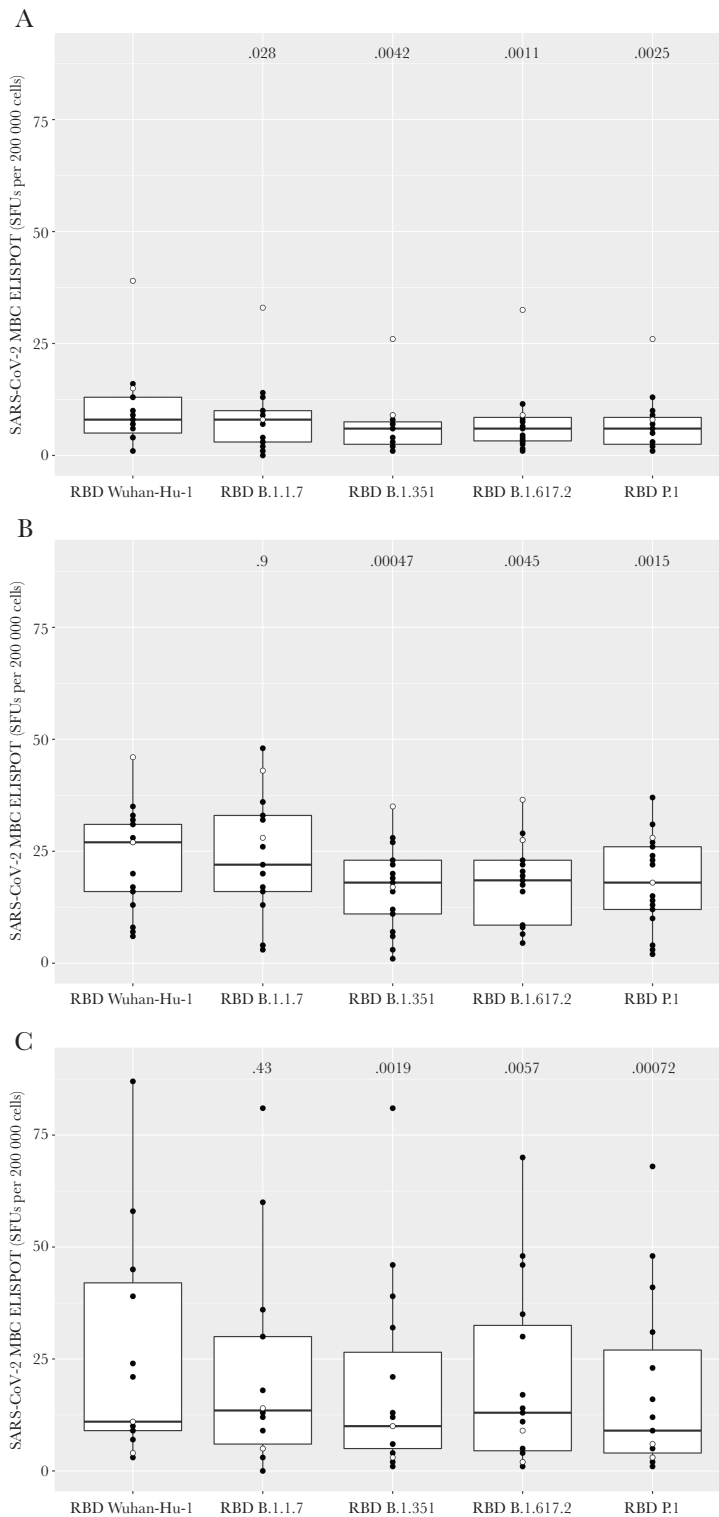


Figure 3. Reactivity of messenger RNA (mRNA) vaccine–induced memory B cells (MBCs) to different severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant of concern (VoC) receptor-binding domains (RBDs). The numbers of immunoglobulin G–positive MBCs reactive to the Wuhan-Hu-1 RBD and the RBDs of different VoC strains (Alpha [B.1.1.7], Beta [B.1.351], Gamma [P.1], and Delta [B.1.617.2]) were measured using enzyme-linked immunosorbent spot assay (ELISPOT) assay (spot-forming units [SFUs]) and assessed after the first mRNA vaccine dose (*A*), after the second mRNA vaccine dose (*B*), and at 6 months following the vaccination series (*C*). ELISPOT antigen-specific MBC response was quantified using 3 replicate measures. Each box was plotted using the interquartile range (IQR) and the median was represented by the bold line in the box. The “whiskers” extend up to 1.5 times the IQR above or below the 75th or 25th percentile, respectively. Black circles represent naive (at baseline) subjects, while white circles represent COVID-19–recovered (at baseline) subjects. The MBC response to each variant RBD was compared to the response directed to the Wuhan-Hu-1 RBD using the Wilcoxon signed-rank test. *A*, $P < .029$, $r > 0.56$ for all VoC comparisons with the Wuhan-Hu-1 RBD response after the first dose. *B*, $P < .005$, effect size > 0.69 for all comparisons after the second dose except for Alpha RBD, which was nonsignificant. *C*, $P < .006$, $r > 0.71$ for all comparisons at 6 months, except for Alpha RBD, which was nonsignificantly different compared to the Wuhan-Hu-1 RBD response.

quality of antigen-specific MBC response is indeed an indicator of recall protective humoral immunity [2]. Goel et al extended their findings to compare MBC response to variant RBDs (Alpha [B.1.1.7], Beta [B.1.351], and Delta [B.1.617.2]) with the Wuhan-Hu-1 RBD MBC response and found different magnitudes of cross-binding at 6 months postvaccination [8]. Our work extends the findings of this study by answering the critical question of what are the dynamics and reactivity of COVID-19 vaccine-induced MBCs to all emerging SARS-CoV-2 VoCs, and are these responses significantly different? We found comparable and significantly reduced RBD-specific MBC reactivity to the Beta, Gamma, and Delta RBD variants and no substantial loss of reactivity for the Alpha variant at either the second vaccine timepoint or 6 months following full vaccination. Interestingly, we observed a variable pattern of MBC response at 6 months compared to the second vaccine timepoint (ie, an increase for some individuals with no detectable anti-N protein antibody response, and a marked decrease for others). This finding is interesting with respect to the observed breakthrough infections in some individuals likely due to waning immunity, and is crucial for understanding the durability of heterotypic protection from circulating VoCs (since MBC recognition/cross-binding to VoC RBDs/antigens is lower, as demonstrated here).

We, and others, have demonstrated the dynamics of SARS-CoV-2-specific antibody response after mRNA vaccination characterized by a significant boost in functional humoral immunity after the second vaccine dose, followed by antibody waning [2, 6, 15–18]. Similarly, we also found a reduced functional antibody response to emerging VoCs [2, 6, 15–18]. We observed a robust neutralizing antibody response after COVID-19 mRNA vaccination, with a moderate reduction in the RBD-specific functional antibody response to the SARS-CoV-2 Alpha, Beta, Gamma, and Delta VoCs. Similarly, we observed this trend at 6 months. These findings corroborate the current understanding that COVID-19 vaccine-induced neutralizing antibody responses may be less effective against newly arising VoCs, including the highly prevalent Delta variant [16, 19–22]. A recent study of Planas et al [19] reported a 3-fold and 16-fold decrease in neutralizing antibody activity (as measured by whole SARS-CoV-2 VoC virus neutralization assays) against the Delta and the Beta variants when compared to the Alpha variant in vaccinees after 2 doses of the Pfizer vaccine. This difference is more pronounced than the moderate reductions in blocking antibody activity reported here, highlighting the fact that viral neutralization is a complex multifaceted process involving various viral epitopes and may occur through additional mechanisms beyond direct antibody occupancy of viral cell receptor-binding sites (eg, inhibition of postattachment events, inhibition of fusion with cell membranes and internalization, steric hindrance, inhibition/modulation of conformational changes).

The strengths of our study include a comprehensive evaluation of RBD-specific and VoC-specific MBC response as a key marker of immune memory and recall immune response following SARS-CoV-2 vaccination and infection over time [2, 3]. We evaluated the reactivity of COVID-19 vaccine-induced MBCs to important emerging SARS-CoV-2 VoC RBDs in a precise and systematic manner in a well-characterized cohort of generally healthy vaccinees (although at the time of the laboratory component of this study, the B.1.1.529 [Omicron] variant was not available for study). Of the described MBC isotypes, IgG is considered the dominant sustained isotype after COVID-19 vaccination and/or infection and was the focus of this study [1–3, 23]. Similar to recent findings, we found a robust S1/RBD-dominated MBC response after vaccination, which benefits from a booster dose and is positively correlated to a certain degree with antibody response [2]. A few studies have assessed the SARS-CoV-2-specific MBC response after disease and determined the longevity of antigen-specific MBC response beyond 6 months after infection, as well as the similar or slightly reduced MBC reactivity to VoCs [1, 8, 14, 24]. Our study builds on those findings by demonstrating differential specificity (statistically significant differences) of mRNA vaccine-induced MBC response to several emerging VoC RBDs, including the RBD of the Delta variant. While the MBC response to the VoC RBDs was consistently present after vaccination and boosted following the second vaccine dose, we noted a statistically significant reduction (~30% after the second mRNA vaccine dose) in the number of isotype-switched/IgG⁺ MBCs reactive to RBDs of Beta, Gamma, and Delta SARS-CoV-2 variants compared to Wuhan-Hu-1 RBD. Importantly, this difference was maintained 6 months following COVID-19 mRNA vaccination where we observed sustained vs waning MBC response in different individuals. A study following 54 convalescent subjects over time discovered sustained RBD-specific MBC response up to 15 months after infection [25]. Similar longitudinal studies are warranted in vaccinated individuals to untangle the longevity of SARS-CoV-2 protective immunity after vaccination. A recent study demonstrated lower MBC frequencies in subjects with breakthrough infection after vaccination than in vaccinated subjects who did not get infected, which emphasizes the importance of MBC response for protection [26]. The cross-reactivity of vaccine-induced switched MBC to the currently circulating B.1.1.529 VoC (Omicron) is largely unknown, but initial pilot studies indicate that the spike-specific MBCs cross-reacting with Omicron descend from “ancient,” rare preexisting MBCs [27]. Studies further investigating the durability and cross-protection of COVID-19 vaccine responses are urgently needed to curb the COVID-19 pandemic—particularly for newly emerging VoCs.

One limitation of our study is that whole spike-specific MBC responses, as well as different MBC isotypes, were not examined/compared for different VoCs. MBC response to the

Omicron VoC was not tested (due to the timing of our study), but such studies are now in progress. We also used an sVNT to capture the functional antibody reactivity to different VoCs based on hACE-2-RBD interaction inhibition. Although this assay is the only US Food and Drug Administration–approved SARS-CoV-2 surrogate neutralization test, it is focused on the RBD-hACE2 binding interaction and does not necessarily reflect the neutralization of SARS-CoV-2 VoC viruses by human sera in vitro, or protection in vivo.

Nevertheless, our study demonstrates distinctive homologous and variant-specific MBC responses after COVID-19 mRNA vaccination. Recently published reports point to the neutralizing antibody and anamnestic B-cell immune response as crucial and predictive of protection upon subsequent SARS-CoV-2 exposure [28, 29]. However, without a correlate of protection, the clinical significance of our findings remains uncertain at this time, but our data further point to the need for comprehensive testing and development of broadly protective vaccines inducing durable immunity against SARS-CoV-2 [30, 31]. In conclusion, our study suggests an attenuated recall humoral/MBC immune response to VoCs, which in concert with the lowered neutralizing antibody response to some of the variants and waning of antibody over time, could translate to an increased susceptibility to emerging SARS-CoV-2 variant strains in the face of waning immunity.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic conflict of interest policies. G. A. P. is the chair of a Safety Evaluation Committee for novel investigational vaccine trials being conducted by Merck Research Laboratories. G. A. P. offers consultative advice on vaccine development to Merck & Co, Avianax, Adjuvance, Valneva, Medicago, Sanofi Pasteur, GlaxoSmithKline, and Emergent Biosolutions. G. A. P. and I. G. O. hold 3 patents

related to measles and vaccinia peptide research. G. A. P. and R. B. K. hold a patent on vaccinia peptide research. R. B. K. has received funding from Merck Research Laboratories to study waning immunity to measles and mumps after immunization with the MMR-II vaccine. G. A. P., R. B. K., and I. G. O. have received grant funding from ICW Ventures for preclinical studies on a peptide-based COVID-19 vaccine. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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