Neutralizing antibody responses gradually waned across several variants of concern (VOCs) after vaccination with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine messenger RNA-1273 (mRNA-1273). We evaluated the immune responses in nonhuman primates that received a primary vaccination series of mRNA-1273 and were boosted about 6 months later with either homologous mRNA-1273 or heterologous mRNA-1273.b, which encompasses the spike sequence of the B.1.351 Beta variant. After boost, animals had increased neutralizing antibody responses across all VOCs, which was sustained for at least 8 weeks after boost. Nine weeks after boost, animals were challenged with the SARS-CoV-2 Beta variant. Viral replication was low to undetectable in bronchoalveolar lavage and significantly reduced in nasal swabs in all boosted animals, suggesting that booster vaccinations may be required to sustain immunity and protection.

Systeamic D614G or B.1.1.7 Alpha (α) SARS-CoV-2 variant infection (1–5). Several SARS-CoV-2 variants of concern (VOCs) showed reduced sensitivity to neutralizing antibodies induced by vaccination or prior infection. The B.1.617.2 Delta (δ) variant is notable for its high transmissibility and an intermediate level of reduced neutralization by WA-1 immune sera (6, 7). The B.1.351 Beta (β) variant and the P.1 Gamma (γ) variant show the greatest reduction in neutralizability by the vaccine recipient or convalescent patient sera (8–13).

Since mRNA-1273 was granted Emergency Use Authorization (EUA) in December 2020 and deployed globally, many months have elapsed since the first individuals were vaccinated, and the durability of vaccine protection remains a concern. Additionally, the question of whether an additional booster vaccination is necessary, particularly as new VOCs become more prevalent, is clinically relevant. mRNA-1273 vaccination in humans reportedly induces antibody responses that persist beyond 6 months (14), but serum-neutralizing activity was significantly reduced against β and δ variants (7, 15, 16). Recent data show that boosting fully vaccinated individuals with mRNA-1273 several months after initial vaccine priming with mRNA-1273, mRNA-1273.β (S sequence based on the β variant), or a 1:1 combination of both vaccines (mRNA-1273.211) showed significantly increased neutralizing titers against the prototypic D614G virus and all variants, including β (17). Although these in vitro functional data are promising, there are no available efficacy data to define the level of antibodies that is sufficient to mediate protection against infection because the correlates of protection against VOCs are undetermined. Moreover, in nonhuman primates (NHPs), a higher threshold of neutralizing antibodies was reportedly needed for upper compared with lower airway protection (18), suggesting that a boost could be important to reach this threshold and contribute to reducing virus transmission.

NHPs have proven useful for vaccine development by facilitating the analysis of immunogenicity and protection against SARS-CoV-2 (19–24). Here, NHPs immunized with the clinically relevant mRNA-1273 vaccine regimen (100 µg × 2) were boosted ~6 months later with either mRNA-1273 (homologous) or mRNA-1273.β (heterologous) vaccines. Antibody, B cell, and T cell responses were assessed temporally after vaccination and infection to determine how boosting with mRNA-1273 or mRNA-1273.β influences the magnitude and quality of antibody responses against WA-1, β, and δ-specific responses and protection against SARS-CoV-2 β challenge. The data show that an additional, delayed boost with mRNA vaccines matched to either the original vaccine strain or to the heterologous challenge strain can have a marked and sustained effect on increasing the breadth of neutralization against all VOCs tested and protection in both the lower and upper airways against the β variant.

Results

Boosting increases mRNA-1273–induced serum antibody responses

The primary focus of this study was to assess the effect of homologous or heterologous boosting with the original WA-1 mRNA-1273 or mRNA-1273.β vaccines, respectively, on immunogenicity and protection against infection with the β variant. NHPs that received the clinically relevant primary regimen of mRNA-1273 (100 µg at weeks 0 and 4) were boosted with either mRNA-1273 or mRNA-1273.β vaccines ~6 months later (fig. S1). WA-1 and β S-specific or receptor-binding domain (RBD)–specific serum antibody responses were assessed at weeks 6, 29, 31, and 37. These time points were selected because they correspond to peak (19), memory before boost, 2 weeks after boost, and time of challenge (“prechallenge”) responses, respectively. WA-1 and β S-specific immunoglobulin G (IgG) geometric mean titers (GMTs) were 53,000 and 39,000 area under the curve (AUC), respectively, at the peak time point after a primary regimen of mRNA-1273. These responses dropped fourfold and sixfold,
respectively, after ~6 months (week 29). Boosting with mRNA-1273 or mRNA-1273.S induced fourfold and about sixfold increases in WA-1 and S-specific IgG titers such that, at 2 weeks after the memory boost, WA-1 and S-specific IgG GMTs were restored to peak levels (Fig. 1, A and B). By week 37, S-specific IgG GMTs remained >30,000 and >20,000 AUC, respectively, for WA-1 and S (Fig. 1, A and B), which, for WA-1, translated to ~1400 international units/ml (table S1). RBD-specific responses displayed similar kinetic trends (Fig. 1, C and D). Together, these data show that there is no difference in the ability of mRNA-1273 or mRNA-1273.S vaccines to boost primary mRNA-1273–elicited S-binding IgG responses.

Next, we assessed the kinetics of neutralizing antibody responses using a lentiviral-based neutralizing antibody assay for D614G, the benchmark strain, as well as several globally circulating variants [S, δ, P.1,ε, B.1.429-Epsilon (ε), and B.1.526-Iota (i)]. Consistent with previous data in NHPs and humans (7, 15), mRNA-1273 elicited a variant-dependent hierarchy of neutralizing antibody responses as determined by a lentiviral-based pseudovirus assay. At peak, the median inhibitory dilution (ID50) GMTs for D614G was 4700, followed by 5900, 1500, 1100, 830, and 770 for ε, γ, δ, and β, respectively, representing onefold to sixfold decreases compared with D614G. At the memory time point (week 24), neutralizing antibody titers decreased to 640 ID50 GMTs for D614G and 810, 280, 250, 740, and 350 ID50 GMTs for ε, γ, δ, and β, respectively. There was a greater fold reduction in neutralization titers from week 6 to week 24 for D614G compared with δ (P < 0.0001) (Fig. 1, E and F). Similar observations were made using D614G- and βVSV–based pseudovirus (fig. S2, A and B) or live virus neutralization assays (fig. S2, C and D). Last, WA-1 S-specific antibody avidity, one measure of affinity maturation, was significantly increased over the 6 months after the primary mRNA-1273 vaccination series in these NHPs (P < 0.0001) (Fig. 1, I and J). This indicates that continued affinity maturation occurs after primary vaccination, leading to an increase in antibody quality, and suggests that, for some variants, affinity maturation of mRNA-1273–elicited neutralizing antibody responses may occur over time, even in the absence of continued antigen exposure.

Six months after the primary vaccination series, either a homologous (mRNA-1273) or a heterologous (mRNA-1273.S) boost induced, on average, a 12-fold increase in pseudovirus-neutralizing titers for all the variants 2 weeks later, resulting in, e.g., 5000 and 3000 β-specific ID50 GMTs after mRNA-1273 or mRNA-1273.S boosts, respectively (Fig. 1, E and F). These responses to variants were significantly higher...
than at week 6 for both boost groups; e.g., week 31 S-specific neutralizing antibody responses were fivefold greater than week 6 responses ($P = 0.002$ and 0.003 for mRNA-1273 and mRNA-1273, S boosts, respectively). This postboost increase in lentiviral pseudovirus-neutralizing titers was confirmed using D614G- and VSV-based pseudovirus neutralization (fig. S2, A and B) and live virus neutralization assays (fig. S2, C and D). To assess the durability of neutralizing antibody responses after boost, we compared week 12, 8 weeks after the primary vaccination series, with week 37, 8 weeks after boost. D614G, S, and δ pseudovirus-neutralizing antibody responses were significantly higher at week 37 than at week 12 (Fig. 1, G and H). These data show that, despite significant waning of mRNA-1273–elicited neutralizing antibody responses against both $\beta$ and $\delta$ over the 6 months after the primary vaccine regimen, boosting with either homologous or heterologous mRNA-1273 restored and increased the potency and breadth of neutralizing antibody responses.

**Vaccine boosting increases antibody responses in the airway**

Vaccination-induced antibodies localized in the upper and lower airways may play a role in the initial control of SARS-CoV-2 replication (18, 25, 26). Therefore, we extended analysis beyond circulating antibody responses to assess WA-1, $\beta$, and $\delta$ S-specific IgG responses in bronchoalveolar lavage (BAL) and nasal swabs (NS) at weeks 6 and 36. At week 6, two doses of mRNA-1273 elicited WA-1, $\beta$, and $\delta$ S-specific GMTs of 13, 11, and 11 AUC, respectively, in the BAL (Fig. 2, A to C). Similar GMTs (11, 10, and 9 AUC, respectively) of S-specific IgGs were detected in NS samples (Fig. 2, D to F). After a boost with either mRNA-1273 or mRNA-1273, $\beta$, the levels of BAL and NS S-specific IgG were also 10 to 12 AUC GMTs (Fig. 2, A to F). WA-1 and $\beta$ S-specific IgA was also detected in BAL and NS (Fig. 2, G to J). To assess functional antibodies in these mucosal samples, we used an ACE2-binding inhibition assay. In the BAL of NHPs boosted with mRNA-1273, ACE2 binding to WA-1, $\beta$, and $\delta$ S proteins was reduced by a mean of 54, 37, and 58%, respectively (Fig. 2L). Similarly, in BAL and NS of NHPs that were boosted with mRNA-1273, $\beta$, ACE2 binding to WA-1 S was reduced by 42% and 55% and for $\beta$ S proteins by 30 and 46%, respectively (Fig. 2, K and L). These data confirm that either homologous or heterologous memory vaccination can induce upper and lower airway functional antibodies that are likely necessary to mitigate lower airway illness and transmission.

**Primary mRNA-1273, $\beta$ vaccination induces serum and mucosal antibody responses**

A secondary focus of this study was to evaluate the immunogenicity of mRNA-1273, $\beta$ as a primary vaccine regimen because this may have relevance for the design of future vaccines in naïve individuals; this group additionally served as a homologous vaccine control for the $\beta$ challenge (fig. S1). S-specific IgG GMTs for WA-1 and $\beta$ were 5100 and 8900, respectively, 2 weeks after the first vaccination of mRNA-1273, $\beta$ and increased fourfold to fivefold after a second vaccination (fig. S3A). Two doses of mRNA-1273, $\beta$ resulted in D614G and $\beta$ live virus ID$_{50}$ GMTs of 198 and 788, respectively (fig. S3B). Potent S-specific antibody responses against WA-1 or $\beta$ were also detected in BAL (fig. S3C) and NS (fig. S3D). Mucosal antibody responses were further exhibited by ACE2-binding inhibition, where mRNA-1273, $\beta$ induced BAL antibodies that reduced ACE2 binding to WA-1 and $\beta$ S by a median of 21 and 48%, respectively (fig. S3E). In NS, the median reduction of ACE2 binding to WA-1 and $\beta$ S binding was 30 and 56%, respectively.
(fig. S3F). Together, these data show mRNA-1273.β given as a primary regimen elicits higher β-specific responses compared with WA-1.

**Serum antibody repertoire elicited by homologous or heterologous prime boost**

To evaluate the impact of homologous (WA-1) or heterologous (β) boosting antigen on serum antibody epitope specificity, the absolute value [in response units (RUs)] and the relative proportion (percent competition) of serum antibodies against 16 distinct antigenic sites (table S2) on WA-1 SARS-CoV-2 S was measured using surface plasmon resonance (SPR). We evaluated serum antibody specificity at week 35 (6 weeks after boost), to allow sufficient time for B cell expansion after exposure to naïve β-S antigen in heterologous-boosted animals. The epitope specificity spanned the S2, N-terminal domain (NTD), S1, and RBD subdomains of the S protein, and the breadth was similar whether the boost was homologous or heterologous (Fig. 3A). The specificity of serum antibodies was qualitatively similar across all animals within each group for the NTD subdomain (average SD 32.1 to 36.9) but not for RBD subdomain (average SD 50.2 to 56.1) (Fig. 3A). Between vaccine groups, there was more NTD site A specificity in homologous- than in heterologous-boosted animals (Fig. 3A). Both absolute and relative serum reactivity to NTD site A [monoclonal antibody (mAb) 4-8; table S2] were higher in homologous- than heterologous-boosted animals at all time points after boost (Fig. 3B), suggesting that mutations found in the β S protein (A242-244, R246I) may render the heterologous boost unable to recall a primary mRNA-1273 vaccination memory B cell response to this antigenic site. By contrast, the RBD site H (mAb A23-97.1; table S2) showed differences in relative but not absolute serum reactivity, with heterologous boost inducing higher reactivity than homologous boosting at all postmemory boost time points (Fig. 3C). Because A23-97.1 binds equivalently to both WA-1 and β S (fig. S4), the higher relative (but not absolute) reactivity against this site for β is likely caused by the decreased contribution of NTD-A reactivity, as shown by the ratio of mRNA-1273.β to mRNA-1273 relative reactivity (fig. S5). Overall, mRNA-1273 to β ratios decreased over time, indicating a contraction of the naïve β-directed response and a return to a memory, long-lived epitope profile (fig. S5) and suggesting that heterologous boost does not alter absolute epitope reactivity over time. By contrast, after primary vaccination with mRNA-1273.β, we observed significantly increased absolute serum reactivity to RBD sites B, C, D, and F (fig. S6) compared with homologous boost, with sites C and D (A19-46.1 and A19-61.1, respectively; table S2) being associated with strong neutralization potency against β (27). These data highlight the differences between primary vaccination and heterologous boost with mRNA-1273.β and indicate that primary vaccination plays a key role in shaping the overall serum antibody epitope repertoire.

**Vaccine boosting expands mRNA-1273–induced memory B cell responses**

The durability of vaccine-induced antibody responses is an integral component of the pandemic response as efforts continue to mitigate the ongoing spread of SARS-CoV-2. Durable humoral immunity is driven by the ability to generate and sustain memory B cells and long-lived plasmablasts (28), and recent data in humans show the potential for such responses after vaccination with mRNA or primary infection (29, 30). To define how B cell specificity changes over time in mRNA-1273–immunized NHPs and to assess how mRNA-1273 priming
imprints homologous or heterologous boost, we performed temporal analysis of WA-1 and β S-specific memory B cells (fig. S7). After the primary vaccination series with mRNA-1273, at week 6, the frequency of memory B cells expressing antibody receptors dual reactive for both WA-1 and β S was 2 to 3%, with a much lower proportion of single WA-1- or β-specific B cells (Fig. 4). Six months later, at week 29, there was an ~10-fold reduction in the frequency of double-positive WA-1 and β memory B cells (Fig. 4C). This finding demonstrates a rapid recall response of primary vaccination-induced B cells and coincides with an increase in neutralizing antibody responses (Fig. 1, E to J). The NHPs that underwent primary mRNA-1273β vaccination had a memory B cell response that also consisted of WA-1 and β S-specific double-positive specificity but a higher proportion of single-positive β S-specific cells (Fig. 4), consistent with β-specific skewing of antibody responses after mRNA-1273β primary vaccination (fig. S3). These data confirm the observation from serum antibody epitope mapping that both homologous and heterologous boosting can efficiently expand memory B cell responses that are maintained after primary vaccination.

Vaccine boosting restimulates mRNA-1273–induced $T_\text{H}1$ and $T_\text{FH}$ T cell responses
mRNA-1273 has been shown to elicit CD4+ responses composed of $T_\text{H}1$ helper (T$_\text{H}1$) and $T_\text{FH}$ follicular helper (T$_\text{FH}$) cells and a lower frequency of CD8 T cells in both humans and NHPs (18, 19, 31). However, the definition of longitudinal T cell development and the potential for boosting contracted memory T cells have not yet been reported in mRNA-1273–vaccinated NHPs. Here, consistent with prior studies, S-specific T$_\text{H}1$ and T$_\text{FH}$ responses, but...
not T_{H}2 responses, were induced at week 6, 2 weeks after mRNA-1273 vaccination (Fig. 5). In these NHPs, T_{H}1 and T_{H}1 responses were contracted by week 29 but were both boosted by either mRNA-1273 or mRNA-1273b (Fig. 5, A, C, and D). Week 36 responses revealed that mRNA-1273b vaccination also induced S-specific T_{H}1 and T_{H}1 responses, but not T_{H}2 responses (Fig. 5). These data suggest that homologous and heterologous mRNA vaccine boosts are equally capable of restimulating T_{H}1 and T_{H}1 cell responses.

**NHPs are protected in the upper and lower airway against SARS-CoV-2 b challenge**

NHPs were challenged at week 38, which was ~9 weeks after vaccine boost in animals receiving three immunizations, and ~5 weeks after the primary mRNA-1273b vaccination series (i.e., two vaccinations), with a total dose of 2 × 10^5 plaque-forming units (PFUs) of SARS-CoV-2 b via intratracheal and intranasal routes (Fig. S1 and Fig. 6A). Two days after challenge, control NHPs that received mock mRNA had a median of ~6 log_{10} SARS-CoV-2 envelope (E) subgenomic RNA (sgRNA_E) copies/ml in BAL. By contrast, all eight NHPs that received primary mRNA-1273b vaccination had undetectable BAL sgRNA_E. Day 2 after challenge, four of eight NHPs that received primary mRNA-1273b vaccination with homologous mRNA-1273 boost had undetectable BAL sgRNA_E, and seven of eight NHPs boosted with heterologous mRNA-1273b had undetectable BAL sgRNA_E. By day 4, all boosted NHPs, with the exception of one mRNA-1273b-boostered animal, had undetectable BAL sgRNA_E (Fig. 6B). In NS, at days 2 and 4 after challenge, both groups of boosted NHPs showed significantly less (~3 to 4 log_{10} reduction) sgRNA_E compared with controls (P < 0.0014 and 0.0004 for mRNA-1273 and mRNA-1273b, respectively). By day 7, the majority (13 of 16) of boosted NHPs had undetectable sgRNA_E compared with control NHPs, for which NS sgRNA_E persisted at a median of 4 log_{10} NS sgRNA_E (Fig. 6C).

Although sgRNA is a sensitive measurement of viral replication that can be used diagnostically, we also determined the ability of live virus to be propagated from postchallenge BAL and NS samples, an alternative measure of viral load that is relevant for indicating lung disease or transmission from the upper airway. On day 2 after challenge, all boosted animals had low to undetectable [i.e., <4 log_{10} median tissue culture infectious dose (TCID_{50})/ml] of culturable SARS-CoV-2 in BAL (Fig. 6D). In NS, SARS-CoV-2 was unculturable for three of eight or five of eight NHPs that were boosted with mRNA-1273 or mRNA-1273b, respectively, which was similar to the mRNA-1273b primary vaccination group (Fig. 6E). sgRNA and viral titers were highly correlated (Fig. 6, F and G); in fact, there was no culturable SARS-CoV-2 from BAL or NS that had sgRNA_E levels <1.0 × 10^3 RNA copies/ml (Fig. 6F) or <4.7 × 10^3 RNA copies/swab (Fig. 6G), respectively. Nucleoplasid (N)-specific sgRNA measurements followed the same trend, albeit with higher detection sensitivity (fig. S9).

We also evaluated lung samples for pathology and detection of viral antigen 7 to 9 days after SARS-CoV-2 b challenge. Influenza was minimal and was similar across lung samples from vaccinated NHPs, with rare cases showing a moderate to severe response (fig. S10 and table S3). The inflammatory changes in the lung were characterized by a mixture of macrophages and polymorphonuclear cells present within some alveolar spaces and mild to moderate expansion of alveolar capillaries with mild type II pneumocyte hyperplasia at day 7 after challenge to changes more consistent with lymphocytes, histiocytes, and fewer polymorphonuclear cells associated with more prominent and expanded alveolar capillaries, occasional areas of perivascular and peribronchiolar inflammation, and type II pneumocyte hyperplasia at days 8 to 9 after challenge. SARS-CoV-2 antigen was detected in six of six control NHPs evaluated at days 7, 8, and 9 after infectious challenge, with three of six showing antigen present in multiple lobes. In vaccinated NHPs, antigen was detected in only one of 24 animals and in a single lobe (mRNA-1273x3; table S3). These results, together with sgRNA data, confirm that boosting mRNA-1273–vaccinated NHPs limits β viral replication in the lower and upper airway.

**Concluding remarks**

The SARS-CoV-2 mRNA-1273 vaccine shows between 90 and 100% protection against WA-1 (2), α, or β variants (32) when administered as two doses 4 weeks apart and assessed within a 2-month window. Kinetic analyses of antibody responses after vaccination with mRNA-1273 (7) or BNT162b2 (30) show reduction of neutralizing activity from the peak humoral response after the second immunization through day 209 (7, 17). Consistently, the β variant is the most neutralization resistant of all VOCs to date. However, when we initiated these studies, it was not yet established how this resistance would influence the durability of protective efficacy in humans against β. Of additional concern are recent clinical reports from Israel showing that cohorts immunized with the BioNTech/Pfizer COVID-19 vaccine, BNT162b2, >6 months previously may present...
Fig. 6. Efficacy of mRNA-1273 against upper and lower respiratory B.1.351 viral replication. (A) Abbreviated schema: Rhesus macaques were immunized and challenged. (B to E) BAL [(B) and (D)] and NS [(C) and (E)] samples were collected on day 2 (circles), day 4 (squares), and day 7 (triangles) after challenge, where applicable. Viral replication was assessed by detection of SARS-CoV-2_E sgRNA [(B) and (C)], and viral titers were assessed by TCID$_{50}$ assay [(D) and (E)]. For (B) to (E), boxes and horizontal bars denote the IQRs and medians, respectively; whisker end points are equal to the maximum and minimum values. For (F) and (G), plots show correlations between viral titers and sgRNA_E in BAL (F) and NS (G) 2 days after challenge. Black and gray lines indicate linear regression and 95% confidence interval, respectively. $r$ and $P$ represent Spearman’s correlation coefficients and corresponding $P$ values, respectively. Symbols represent individual NHPs and may overlap for equal values. Dotted lines indicate assay limits of detection.
with symptomatic disease or increased frequency of severe disease when infected with the δ variant (33). Like β, δ also includes mutations in S that reduce neutralization sensitivity by vaccinee sera (34). Thus, the combination of lack of vaccination, waning immunity, reduced neutralization capacity against VOCs, and increased transmissibility of δ are key factors responsible for the current status of the ongoing COVID-19 pandemic. It may be important to boost antibody responses, especially against VOCs, to sustain and increase protection against severe disease, particularly in at-risk cohorts, and to reduce the potential for mild infection and transmission.

Current assessments of COVID-19 vaccine efficacy in both preclinical and clinical studies are concerned with whether an additional boost after the primary vaccine regimen should be matched to the most epidemiologically relevant VOCs or if homologous boosting with the original vaccine will be sufficient to generate broadly neutralizing antibody responses against VOCs. Here, we show that boosting NHPs 6–8 months after their primary vaccination series with either mRNA-1273 or mRNA-1273.β significantly increased serum-neutralizing activity against all VOCs before the boost and mediated high-level protection in the upper and lower airseways against β challenge. Neutralizing titers against all VOCs tested were significantly higher at both 2 and 8 weeks after the boost compared with the same time points after the primary vaccination series (17). These data suggest that boosting could lead to a higher antibody setpoint, potentially altering the need and timing for additional boosting. Last, we could not discern a significant difference in neutralizing antibody titers or reduction in viral replication between the homologous and variant mRNA boost. These data suggest that for the currently circulating strains, the ancestral WA-1 S encoded by mRNA vaccines will significantly enhance immunity and confer high-level protection. It remains possible that in the future, viral evolution of SARS-CoV-2 may require a different boost regimen, and this will be an area of continued investigation.

Longitudinal assessment of neutralizing antibody responses showed a relatively slower decay in serum-neutralizing activity against VOCs compared with the benchmark D614G strain. These data suggest that although humoral antibody responses are an established correlate for short-term protection in NHPs and humans (18, 35), deeper analysis may be needed to define correlates of protection with contracted memory responses. These data also propose the possibility that despite a quantitative decrease in the magnitude of antibody responses over time after vaccination, the loss of functional activity may be partially mitigated by affinity maturation and improved quality of the response. It was recently reported that there may be discordance in antibody evolution between convalescent individuals and those who receive an mRNA vaccine. Specifically, the report indicated less affinity maturation and a limited increase in breadth to the RBD subdomain in isolated mAbs between 2 and 5 months after mRNA vaccination (30). Our assessment of polyclonal serum antibody affinities (avidity) using the intact WA-1 S protein showed that vaccination with mRNA-1273 led to an increase in avidity over time, confirming a qualitative improvement in the antibody response in NHPs (29, 36, 37).

The increase in antibody responses after boost suggests that there is significant B cell memory induced by primary mRNA vaccination that can be rapidly recalled after the boost (29). Here, after primary vaccination with mRNA-1273, the peak response yielded a total frequency of WA-1 and β-specific memory B cells of ~3%, and the majority (~80%) of these responses recognized both S proteins, with a small proportion specific for WA-1 or β only. After 6 months, the magnitude of the responses contracted but were rapidly restored to a frequency of 3% after boosting. The relative frequency of B cells specific for WA-1, β, or both did not change after boost with either the homologous or heterologous mRNA, suggesting that priming with mRNA-1273 imprinted the B cell repertoire. Additionally, a substantial proportion of the S-specific B cells 7 weeks after boost were resting memory B cells, which suggests that boosting rapidly restores the resting memory B cell population that is seen after primary mRNA-1273 vaccination and is consistent with the sustained antibody responses after boost.

Naïve animals primed with only mRNA-1273.β showed between 25 and 40% of the total B cell response specific for β only. Likewise, serum antibody epitope profiling demonstrated a repertoire that was qualitatively similar after either homologous or heterologous memory boost after mRNA-1273 primary vaccination, whereas a primary vaccination series with mRNA-1273.β yielded a unique repertoire (both qualitative and quantitative) with marked increases in reactivity to epitopes associated with broad and potent neutralization of VOCs. These data have implications for how future mRNA vaccines can be designed to imprint B cell repertoires in naïve individuals for increased potency and breadth of neutralizing activity (38).

Although the data presented here focus on the role of boosting vaccine responses with mRNA, as a proof of principle, we also show that priming with mRNA-1273.β in naïve NHPs induced potent neutralizing antibody responses against β and provided high-level protection in the upper and lower airseways after challenge. Parallel studies in preclinical rodent models have shown that priming with a variant mRNA or a bivalent vaccine containing the WA-1 and the β variant induces higher neutralizing responses to D614G and a number of variants tested relative to either mRNA-1273 or mRNA-1273.β alone (38). These data highlight how vaccines in unprimed individuals may be changed depending on the evolution of the virus in the future.

The primary aim of this study was to assess whether homologous or heterologous mRNA-1273 boost in naïve animals that underwent a primary vaccine regimen elicits comparable protective immune responses to inform clinical development, and the experimental design was powered to specifically assess this aim given our limited number of available animals. Recently, broad cross-clade neutralizing antibodies were isolated from SARS-CoV-1 survivors who then received the BNT162b2 vaccine (39), so it is notable that the close relatedness of the homologous and heterologous mRNA-1273 boosters used here may limit our ability to fully gauge postboost cross-reactivity. Another limitation of this study is that we could not directly establish whether boosting increased protection compared with vaccinated animals that did not receive a boost. As to whether the boost increased protection, in our recent NHP study (40), animals received a primary vaccination series of 100 μg of mRNA-1273 and were challenged at 8 weeks after vaccination with the same β isolate used here (JHU B.1351 P2). There was a 1-log10 reduction in sgRNA in the upper airway, and the β-specific neutralizing antibody ID50 GMT was 300 at the time of challenge. Here, we show that 8 weeks after boost, β-specific neutralizing GMTs were ~3000, and there was a 5- to 4-log reduction in sgRNA in the upper airway. These data strongly suggest that the increased neutralizing responses induced by the additional boost leads to better protection in the upper airway.

In conclusion, the data reported here show that a homologous mRNA-1273 boost can significantly improve both the breadth and potency of neutralizing antibody responses and confer protection against upper and lower airway infection to a heterologous challenge virus, which is relatively resistant to in vitro neutralization. The potential clinical utility of a boost would be to sustain high-level protection against severe disease and possibly limit the duration and extent of mild infection in the setting of waning immunity, especially in the elderly and others with preexisting health conditions or poor response to vaccination. We have previously shown that there is a higher threshold for antibody-mediated protection in the upper airway (18), which would be more important for limiting mild infection and transmission than for protecting the lower airways to limit severe disease. Thus, boosting will potentially provide more
durable and broader immunity and could reduce morbidity and mortality until greater population immunity is achieved.

**Materials and Methods**

**Preclinical mRNA and lipid nanoparticle production**

A sequence-optimized mRNA encoding prefusion-stabilized SARS-CoV-2 S protein containing 2 proline stabilization mutations (S-2P) (41, 42) for WA-1 and β was synthesized in vitro and formulated as previously reported (17, 43). Control mRNA "UNFIX-01 (Untranslated Factor 9)" was synthesized on the basis of the sequence in table S4 and similarly formulated into lipid nanoparticles.

**Rhesus macaque model**

Animal experiments were performed in compliance with all pertinent US National Institutes of Health regulations and approval from the Animal Care and Use Committees of the Vaccine Research Center and BIOQUAL Inc. (Rockville, MD). Studies were conducted at BIOQUAL Inc. The experimental details of VRC-20-857.3b (fig. S1) are similar to prior studies (18, 19, 26, 40). Briefly, 3- to 15-year-old rhesus macaques of Indian origin were stratified into groups based on sex, age, and weight. Animals were immunized with mRNA-1273 at weeks 0 and 4 with a dose of 100 μg intramuscularly in 1 ml of formulated in phosphate-buffered saline (PBS) into the right hindleg. Placebo-control animals were administered control mRNA. At week 29 (~25 weeks after the second immunization), a group of animals was boosted with 50 μg of mRNA-1273 or 50 μg of mRNA-1273.β. An additional group of animals was immunized at weeks 29 and 33 with 50 μg of mRNA-1273.β. At week 38 (9 weeks after the homologous or heterologous mRNA boost or 5 weeks after the mRNA-1273.β prime and boost) all animals were challenged with a total dose of 2 × 10⁷ PFUs of SARS-CoV-2 β (JHU P2) as previously described (40). The viral inoculum was administered as 1.5 × 10⁶ PFUs in 3 ml intratracheally and 0.5 × 10⁵ PFU in 1 ml intranasally in a volume of 0.5 ml into each nostril. Pre- and postchallenge sample collection is detailed in fig. S1.

**Quantification of SARS-CoV-2 sgRNA**

BAL and NS subgenomic SARS-CoV-2 E mRNA was quantified by reverse transcription polymerase chain reaction (RT-PCR) as previously described (26). Subgenomic SARS-CoV-2 N mRNA was quantified similarly, as described in (40). The lower limit of quantification was 50 copies.

**TCID₅₀ quantification of SARS-CoV-2 from BAL**

Viral load (TCID₅₀) from BAL samples was calculated using previously described methods (40). (JHU P2) as previously described (41, 42) for WA-1 and β was synthesized in vitro and formulated as previously reported (17, 43). Control mRNA "UNFIX-01 (Untranslated Factor 9)" was synthesized on the basis of the sequence in table S4 and similarly formulated into lipid nanoparticles.

**Histopathology and immunohistochemistry**

After challenge, on days 7 to 9, animals were euthanized, and lung tissue was processed and stained with hematoxylin and eosin for routine histopathology and analyzed for detection of SARS-CoV-2 virus antigen as previously described (40). Inflammation was often characterized by a mixture of inflammatory cells including polymorphonuclear cells, macrophages, and lymphocytes; cellular infiltrates were often observed in association with perivascular areas, alveolar interstitium, and surrounding small- and medium-sized airways. Antigen-positive foci were often associated with alveolar-lining cells (pneumocytes), immune cells, and infrequently with the airway epithelium of small- and medium-sized airways. All samples were blinded and evaluated by a board-certified veterinary pathologist.

**Multiplex MSD ELISA for serum antibody responses**

For 10-plex meso scale discovery (MSD) enzyme-linked immunosorbent assay (ELISA), 96-well plates were precoated with SARS-CoV-2 S-2P (44) and RBD proteins from multiple variants, SARS-CoV-2 N protein, and bovine serum albumin (BSA) and supplied by the manufacturer (Meso Scale Diagnostics). Determination of serum antibody binding was performed as previously described (7), and reagent details are provided in table S5. All calculations were performed within Excel and GraphPad Prism software version 7.0. Readouts are provided as AUC. For 4-plex ELISA, 96-well plates were precoated with SARS-CoV-2 S-2P, RBB, N, and a BSA control in each well. Determination of serum antibody binding was performed as previously described (7, 18, 26). Calculated electrochemiluminescence immunoassay (ECLIA) parameters to measure binding antibody activities included interpolated concentrations or assigned arbitrary units (per milliliter) read from the standard curve. International units (IU) were established for each antigen on the basis of parallelism between the MSD reference standard and the WHO International Standard. The S-specific IgG lower limit of detection was 0.3076 IU/ml, and the RBD-specific IgG lower limit of detection was 1.5936 IU/ml.

**MSD ELISA for mucosal antibody responses**

Total S-specific IgG in BAL and NS was determined by MSD ELISA as previously described (26), with the following minor change: MSD panel 13 was used.

**Serum antibody avidity assay**

Avidity was assessed using a sodium thiocyanate (NaSCN)-based avidity ELISA against SARS-CoV-2 S-2P as previously described (18, 26). The avidity index was calculated using the ratio of IgG binding to S-2P in the absence or presence of NaSCN and is reported as the avidity of two independent experiments, each containing duplicate samples.

**Serum antibody epitope definition**

Serum epitope mapping competition assays were performed using a Biacore 8K+ (Cytiva) SPR spectrometer. Following the manufacturer’s protocol, anti-histidine IgG1 antibody was immobilized on Series S Sensor Chip CM5 (Cytiva) through primary amine coupling using a His capture kit (Cytiva). His-tagged SARS-CoV-2 WA.1 S-2P was captured on active sensor surface.

Competitor mAbs or negative control antibodies at set concentrations were injected over both active and reference surfaces to saturation. The competitor human IgG mAbs used included S2-specific mAb S652-112; NTD-specific mAbs 4-8, S652-118, and N3C; S1-specific mAb A20-361; and RBD-specific mAbs B1-182, C6, A20-29, A19-461, LY-COV555, A19-611, S309, A23-971, A19-301, A23-801, and CR3022. NHP sera were flowed over both active and reference sensor surfaces for 40 min. Then, 1× PBS+ (Cytiva) was used as the running buffer and diluent for all samples. Active and reference sensor surfaces were regenerated between each analysis cycle using 10 mM glycine, pH 1.5 (Cytiva).

Before analysis, sensorgrams were aligned to Y (RUs = 0), beginning at the serum association phase using Biacore 8K Insights Evaluation Software (Cytiva). Reference-subtracted relative “analyte binding late” report points (RUs) were collected and used to calculate absolute and relative competition. Relative percent competition (% C) was calculated using the following formula: % C = {1 - (100 * (RUs in presence of competitor mAb)/RUs in presence of negative control mAb))}. Absolute competition (ARUs) was calculated with the following formula: ARUs = [(RUs in presence of negative control mAb) − (RUs in presence of competitor mAb)]. Results are reported as absolute serum epitope reactivity and percent competition, and statistical analysis was performed using unpaired, two-tailed t test (GraphPad Prism software version 8). All assays were performed in duplicate, with average data points for each animal represented on the corresponding graphs.

**SPR-binding assay**

Series CM5 Sensor Chips (Cytiva) were activated by immobilizing anti-histidine IgG1 antibody on surface using a His capture kit (Cytiva) according to the manufacturer’s protocol. His-tagged SARS-CoV-2 WA.1 or β S protein containing S-2P mutations was captured on an active sensor surface at a set concentration for 10 min. mAbs were injected over both active and reference surfaces and allowed to

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bind to saturation. Graphs represent reference-subtracted relative “analyte binding late” report points (RUs), determined by aligning sensorgrams to Y (RUs) = 0, beginning at the mAb association stage using Biacore 8K Insights Evaluation Software (Cytiva). All assays were performed using a Biacore 8K+ (Cytiva) SPR spectrometer.

**ACE2-binding inhibition assay**

ACE2-binding inhibition was completed, as previously described (7), using 1:5 diluted BAL and NS samples.

**Lentiviral pseudovirus neutralization assay**

Pseudotyped lentiviral reporter viruses were produced by the cotransfection of plasmids encoding SARS-CoV-2 proteins from multiple variants, a luciferase reporter, lentivirus backbone, and human transmembrane protease serine 2 (TMPRSS2) genes as previously described (17, 44). Reagent details are shown in table S5. Sera were tested, in duplicate, for neutralizing activity against the pseudovirus present in the 90% ID50. Sera were tested, in duplicate, for neutralizing activity against the pseudovirus present in the 90% ID50.

**VSV pseudovirus neutralization assay**

SARS-CoV-2 pseudotyped recombinant VSV-ΔG-firefly luciferase viruses were made by cotransfection of plasmid expressing full-length S, and subsequent infection with VSVΔG-firefly-luciferase and neutralization assays were completed on sera samples as previously described (15, 45); reagent details are described in table S5. The lower limit of quantification was 1:40 ID50.

**Focus reduction neutralization test**

Focus reduction neutralization tests were performed on sera samples, in duplicate, as previously described (40). Reagent details are provided in table S5. For samples that did not neutralize 50% of virus at the limit of detection, “)” was plotted and used for geometric mean calculations.

**B cell probe-binding assay**

Cryopreserved peripheral blood mononuclear cells (PBMCs) were thawed and rested overnight in a 37°C, 5% CO2 incubator. The next morning, cells were stimulated with SARS-CoV-2 S protein peptide pools (S1 and S2) that were matched to the vaccine insert [composed of 158 and 157 individual peptides, respectively, as 15mers overlapping by 11 amino acids (11.5)]. The following mAbs were used: CD3 APC-Cy7 (clone SP34.2, BD Biosciences), CD4 PE-Cy5 (clone 5S3.5, Invitrogen), CD8 BV570 (clone RPA-T8, BioLegend), CD45RA PE-Cy5 (clone 5H9, BD Biosciences), CCR7 BV650 (clone G043H7, BioLegend), CXCR5 PE (clone M5U5EBEE, Thermo Fisher Scientific), cells were washed twice in wash buffer, and residual red blood cells were lysed using BD FACs Lysing Solution (BD Biosciences) for 10 min at room temperature. After two additional washes, cells were fixed in 0.5% formaldehyde (Thousimis Research) and subsequent infection with VSV for 10 min at room temperature. After two additional washes, cells were fixed in 0.5% formaldehyde (Thousimis Research) and subsequently analyzed using FlowJo software version 10.7.2 (BD Biosciences).

**Intracellular cytokine staining**

Cryopreserved PBMCs were thawed and rested overnight in a 37°C, 5% CO2 incubator. The next morning, cells were stimulated with SARS-CoV-2 S protein peptide pools (S1 and S2) that were matched to the vaccine insert (composed of 158 and 157 individual peptides, respectively, as 15mers overlapping by 11 amino acids (11.5)). The following mAbs were used: CD3 APC-Cy7 (clone SP34.2, BD Biosciences), CD4 PE-Cy5 (clone 5S3.5, Invitrogen), CD8 BV570 (clone RPA-T8, BioLegend), CD45RA PE-Cy5 (clone 5H9, BD Biosciences), CCR7 BV650 (clone G043H7, BioLegend), CXCR5 PE (clone M5U5EBEE, Thermo Fisher Scientific), cells were washed twice in wash buffer, and residual red blood cells were lysed using BD FACs Lysing Solution (BD Biosciences) for 10 min at room temperature. After two additional washes, cells were fixed in 0.5% formaldehyde (Thousimis Research) and subsequently analyzed using FlowJo software version 10.7.2 (BD Biosciences).

**Statistical analysis**

Graphs show data from individual animals, with dotted lines indicating assay limits of detection. Groups were compared for viral load and immune responses using Welch’s t tests; data were analyzed on the log10 scale for viral loads and appropriate immune assays. Changes over time were summarized using the change on the log10 scale, and statistical significance was determined using paired t tests. When more than two variants were compared, as in Fig. 1, A to F, a Holm’s adjustment for multiple comparisons was used on the set of P values to determine significance. There were no adjustments for multiple comparisons across different analyses in this descriptive study. Comparisons drawn between vaccination groups for serum antibody epitope analysis were performed using unpaired t tests, within each time point evaluated. Analyses were performed in R software version 4.0.2 and GraphPad Prism software versions 8.2 and 9.0.2.

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**Competing interests:** K.S.C. and B.S.G. are inventors on US Patent Application 62/972,886 entitled “2019-nCoV Vaccine.” J.M., L.W., C.A.S., J.R.M., D.D., N.J.S., A.R.H., W.S., Y.Z., and M.R. are inventors on US Patent Application 63/147,419 entitled “Antibodies Targeting the Spike Protein of Coronaviruses.” K.S.C., B.S.G., L.W., W.S., and Y.Z. are inventors on multiple US Patent applications entitled “Anti-Coronavirus Antibodies and Methods of Use.” A.C., M.K., A.C., and D.K.E. are employees of Moderna. M.S.S. serves on the scientific board of advisors for Moderna. D.V., A.V.R., Z.F., A.C., L.P., H.A., and M.G.L. are employees of BIOQUAL Inc. Data and materials availability: All data are available in the main text or the supplementary materials. This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. To view a copy of this license, visit https://creativecommons.org/licenses/by/4.0/.

**SUPPLEMENTARY MATERIALS**

science.org/doi/10.1126/science.abl8912
Figs. S1 to S10
Tables S1 to S5
MDAR Reproducibility Checklist

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