LETTER

The second COVID-19 mRNA vaccine dose enhances the capacity of Spike-specific memory B cells to bind Omicron BA.2

To the editor,

Following the outbreak of the SARS-CoV-2 pandemic, the scientific community has responded with unprecedented pace to develop new vaccines with high efficacy in eliciting neutralizing antibodies targeting the spike receptor binding domain (RBD) of up to 95% for the BNT162b2 (Pfizer-BioNTech) mRNA vaccine.[1–3] Despite the rapid vaccine roll-out in 2021, multiple new variants of concern (VoC) have emerged that increase transmission, disease severity and/or undermine public health measures such as vaccination.[4] While previous VoC Beta (P.1), Gamma (B.1.351), and Delta (B.1.617.2) with two to three mutations in the RBD partially evaded the antibody responses,[2,5] the currently circulating Omicron (B.1.1.529) subvariants BA.2 and BA.5 with over 15 mutations readily evade antibody responses and neutralization.[4] Booster vaccinations enhance the
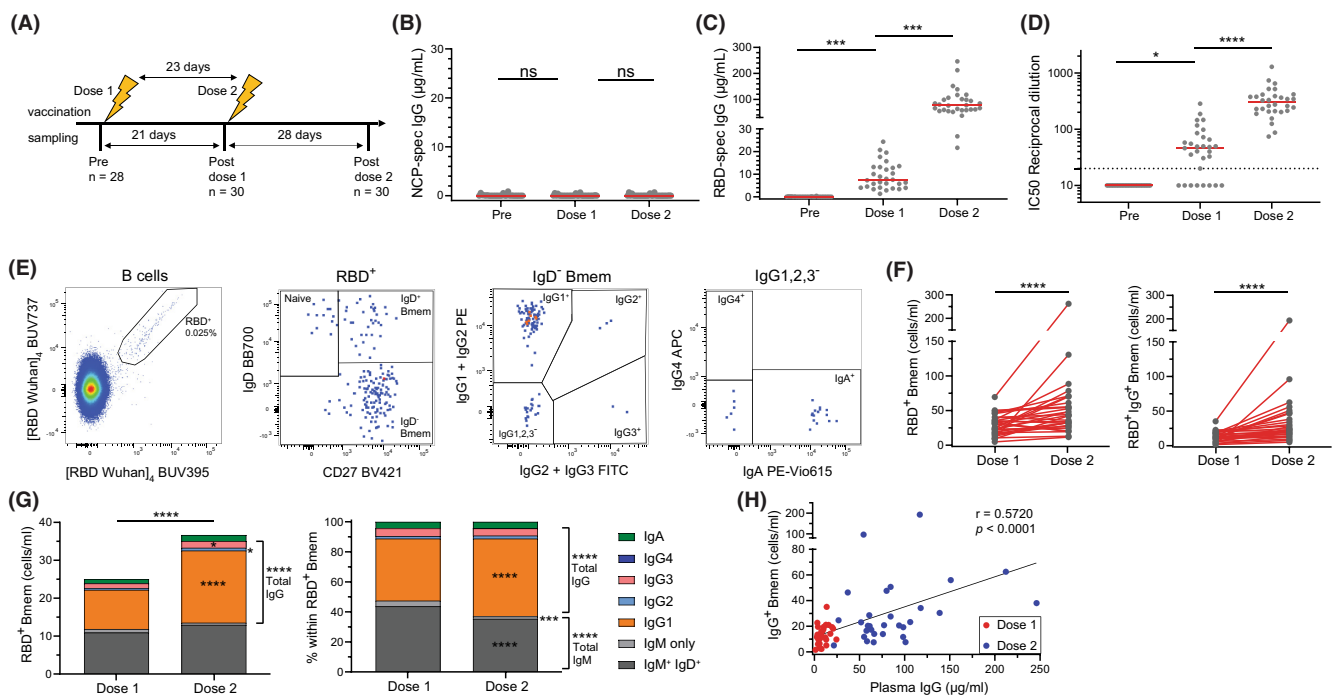

FIGURE 1 Serological and RBD-specific Bmem response to BNT162b2 vaccination. (A) Participants were sampled pre-BNT162b2 vaccination, 3 weeks post-dose 1, and 4 weeks post-dose 2 with a median of 23 days (range: 21–39 days) between doses. (B) NCP-specific and (C) RBD-specific plasma IgG postvaccination. (D) Neutralizing antibodies to Wuhan Spike RBD postvaccination. Dotted line in D depicts IC50 = 20, the cut-off for neutralization.[3] Prevaccination (n = 28), post-dose 1 (n = 30), post-dose 2 (n = 30). Horizontal lines represent median values. (E) Gating strategy and immunophenotype of RBD-specific memory B cells (Bmem) using RBD Wuhan tetramers. (F) Absolute number of RBD-specific Bmem and IgG+ Bmem in vaccinated individuals. (G) Absolute number and proportion of Ig switched RBD-specific Bmem. Significant population changes post-dose 2 vs dose 1 are depicted. Friedman test with Dunn's multiple comparisons test for B-D. Wilcoxon matched-pairs signed-rank test for F and G, $*p < 0.05$, $***p < 0.001$, $****p < 0.0001$. (H) Correlation between plasma IgG and IgG+ RBD-specific Bmem post-dose 1 and 2. Trend line depicts linear correlations; nonparametric Spearman's rank correlation

protection against VoC; however, it is unclear whether this is due to higher levels of antibodies and memory B cells (Bmem), or whether these have an enhanced VoC binding capacity.

We collected blood from 30 healthy COVID-19 naive individuals before and after first and second dose BNT162b2 (Figure 1A, Table S1). Whilst Australia did not escape infections in 2020 and 2021, cases were minimal, and testing and surveillance were high. All participants reported the absence of infection throughout the study and were negative for nucleocapsid (NCP)-specific IgG (Figure 1B).[3] All participants generated anti-RBD IgG post-dose 1, which increased significantly post-dose 2 (Figure 1C). Most individuals (22/30) generated neutralizing antibodies after dose 1, and all reached neutralizing levels above an IC50 of 70 after dose 2 (Figure 1D). However, it is unclear whether plasma antibodies are representative of those in the upper airways, the entry site for SARS-CoV-2, and therefore may not accurately reflect vaccine protection and efficacy. Furthermore, as levels of neutralizing antibodies decline beyond 1-month post-vaccination,[2] these are unlikely to represent the durable protection from severe disease that lasts 3–6 months.

In contrast to plasma Ig, SARS-CoV-2-specific Bmem are maintained in stable numbers after infection and vaccination.[2,3] Using two fluorescent tetramers of Wuhan RBD for double discrimination,[3] RBD-specific Bmem were extensively immunophenotyped (Figure 1E). RBD-specific Bmem were detected in all donors after first and second doses, with significantly higher numbers (1.7-fold) post-dose 2 (Figure 1F, Figure S1A–D). The RBD-specific Bmem

compartment consisted mostly of IgM[+] or IgG1^{+} cells, the latter increasing in number and frequency post-dose 2 (Figure 1F,G, Figure S1A–D). In contrast, the total Bmem population contained fewer IgG1^{+} cells and more IgG2^{+} cells than RBD-specific Bmem and did not change post-dose 2 (Figure S2A,B). The total numbers of RBD-specific IgG[+] Bmem were positively correlated with RBD-specific plasma IgG after both vaccine doses (Figure 1H, Figure S3).

RBD-specific IgG[+] Bmem expressed high levels of CD27 indicating increased levels of antigen-driven replication (Figure S1E,F).[3] RBD-specific Bmem had a resting memory phenotype with low frequencies of CD21^{lo} cells and CD71^{+} cells (Figure S1G,H, Figure S2C), which are phenotypes of recent activation.[3,6] Thus, we have established that 4 weeks after BNT162b2 vaccination, RBD-specific Bmem display a resting, mature Bmem immunophenotype.

We next examined the capacity of the BNT162b2 vaccine to protect against VoC with antibody evasion mutations: Beta, Gamma, Delta, and Omicron subvariants BA.2 and BA.5 (Figure 2A).[5] Reactivity to all variants except Delta increased after dose 2, but recognition of BA.2 and BA.5 by plasma IgG was significantly lower than the other variants (Figure 2B,C). Using RBD Gamma, Delta, and Omicron BA.2 and BA.5 tetramers, RBD-specific Bmem were evaluated for their binding of VoC (Figure 2D). The Gamma and Delta RBDs were each recognized by approximately 60–70% of RBD-specific Bmem. However, only 20%–30% of RBD-specific Bmem recognized Omicron BA.2 and BA.5 RBDs (Figure 2E). After dose 2, the proportions of RBD-specific Bmem that recognized Omicron
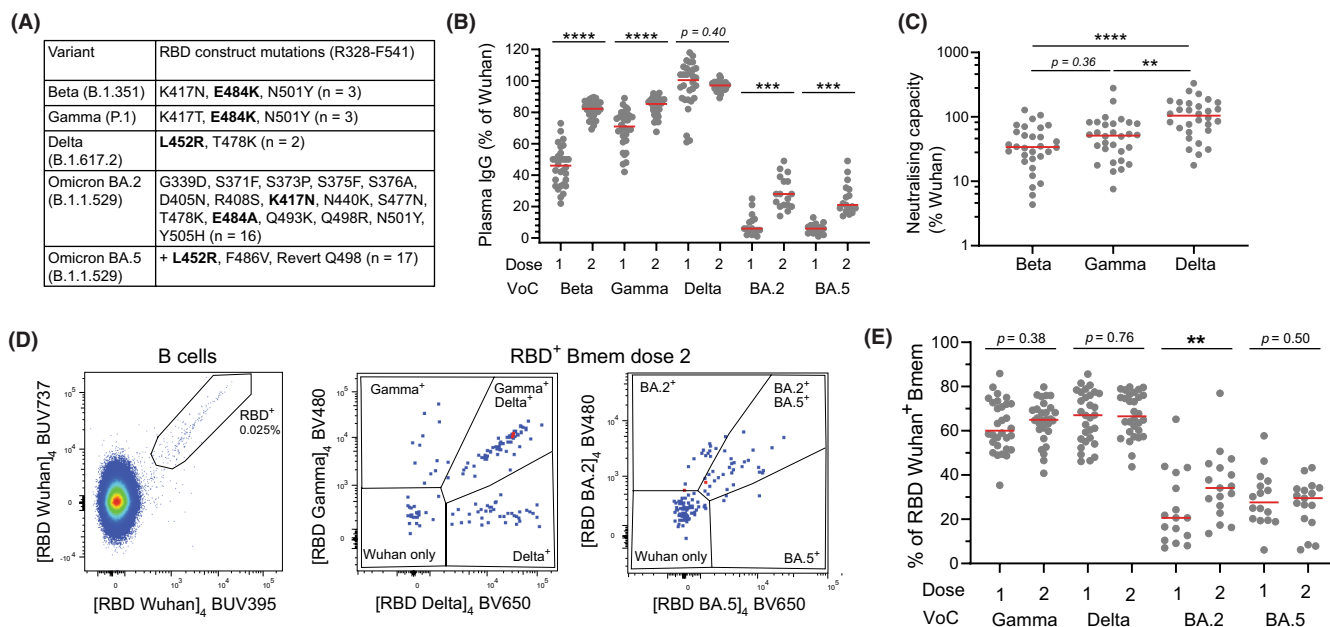

FIGURE 2 Second BNT162b2 dose increases recognition of variants of concern by RBD-specific antibodies and Bmem. (A) List of mutations in the RBD of Beta, Gamma, Delta, and Omicron BA.2 and BA.5 variants. Mutations in bold cause a reduction in antibody recognition.[2,5] (B) Proportion of RBD-specific IgG that also bind VoC Beta, Gamma, Delta, and Omicron BA.2 and BA.5 post-dose 1 and 2. Beta, Gamma, and Delta (n = 30); Omicron BA.2 and BA.5 (n = 16 post-dose 1 and n = 18 post-dose 2). (C) Neutralizing capacity of Wuhan spike-specific antibodies against Beta, Gamma, and Delta VoC post-dose 2. (D) Gating strategy to identify RBD-specific Bmem that also bind RBD Gamma and/or Delta and Omicron BA.2 and/or BA.5. (E) Proportion of RBD-specific Bmem that also bind Gamma, Delta, BA.2 or BA.5. Wilcoxon matched-pairs signed-rank test for B and E with Bonferroni correction for multiple comparisons, Friedman test with Dunn's multiple comparisons test for C, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

BA.2 significantly increased to nearly 40% (Figure 2E). The increased proportion of RBD-specific Bmem that recognized variants were due mainly to an increase in IgG1^{+} Bmem (Figure S4).

In summary, we show that BNT162b2 elicits robust antibody and Bmem responses. The second vaccine dose increases the capacity of serum IgG to bind Beta, Gamma, and Omicron BA.2 and BA.5; the numbers of IgG1 switched Bmem and the capacity of Bmem to bind Omicron BA.2. The combined boosting of Bmem numbers and their capacity to bind variant RBDs may contribute to the protection against severe disease provided by third and fourth dose boosters.

CONFLICT OF INTEREST

MCvZ, REO'H, and PMH are inventors on a patent application related to this work. SJB is an employee of and owns stock in BD Biosciences. All the other authors declare no conflict of interest.

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