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Convergent CDR3 homology amongst Spike-specific antibody responses in convalescent COVID-19 subjects receiving the BNT162b2 vaccine

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ABSTRACT

Convalescent coronavirus disease 2019 (COVID-19) subjects who receive BNT162b2 develop robust antibody responses against SARS-CoV-2. However, our understanding of the clonal B cell response pre- and post-vaccination in such individuals is limited. Here we characterized B cell phenotypes and the BCR repertoire after BNT162b2 immunization in two convalescent COVID-19 subjects. BNT162b2 stimulated many B cell clones that were under-represented during SARS-CoV-2 infection. In addition, the vaccine generated B cell clusters with >65% similarity in CDR3 V_H and V_L region consensus sequences both within and between subjects. This result suggests that the CDR3 region plays a dominant role adjacent to heavy and light chain V/J pairing in the recognition of the SARS-CoV-2 spike protein. Antigen-specific B cell populations with homology to published SARS-CoV-2 antibody sequences from the CoV-AbDab database were observed in both subjects. These results point towards the development of convergent antibody responses against the virus in different individuals.

1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the beta-coronavirus responsible for coronavirus disease-2019 (COVID-19), has been a pressing public health concern ever since its global pandemic spread in early 2020. Rapid successes in vaccine development and distribution in the past year, including the emergency use authorization and deployment of mRNA vaccine BNT162b2 (Pfizer/BioNTech), have helped to impede the spread of the virus. BNT162b2 is generally delivered under a two-dose prime-boost regimen, exhibiting ~95% vaccine efficacy when doses are administered 21 days apart [1,2]. Vaccine mRNA, delivered intracellularly via a lipid nanoparticle shell as a vector, encodes for the membrane-anchored Spike protein found on the surface of SARS-CoV-2 viral particles [3,4]. Production of this "foreign" Spike protein elicits a robust adaptive immune response,

promoting the development of T cell help/cytotoxicity and B cell antibody responses against the virus that are equal to or stronger than a natural infection [2].

For individuals infected with SARS-CoV-2, a robust immune response dominated by activated B cells, plasmablasts, and short-lived plasma cells is generated upon acute infection. During convalescence, long-lasting plasma cell and memory B cell subsets that remain active for up to 8 months post-symptom onset are generated [5,6]. The long-term immune response elicited following mRNA vaccination is similar to that of SARS-CoV-2 infection, with studies reporting strong circulating memory B cell responses after two consecutive doses [7]. For convalescent COVID-19 subjects, strong Spike-specific and neutralizing antibody responses appear rapidly following the initial mRNA vaccine dose, with several studies suggesting that additional booster doses may have limited immunological benefit in this subject population [8–15]. This

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expediated protective immunity likely stems from the pre-existent SARS-CoV-2-specific B cell memory responses present in these individuals that may be driven by germinal centre formation, which produces high affinity, long-lasting memory B cells able to secrete virus-neutralizing antibodies [4,16].

Many studies have separately assessed the effects of both natural SARS-CoV-2 infection [17–20] and prophylactic mRNA vaccination [17,20–23] on B cell populations by single-cell RNA sequencing. These next-generation sequencing technologies have facilitated the elucidation of clonality amongst specific B cell subsets [17,20], as well as the detection of sequence homologies amongst published SARS-CoV-2 clonotypes [21] within and between subject cohorts. However, there has been limited research thus far utilizing these techniques to analyze B cell responses in convalescent COVID-19 vaccinees. With the phenomenon of convalescent individuals requiring only a single dose to generate optimal protective immunity, further investigation into the transcriptomic and antibody phenotypic changes elicited by vaccination in this population is warranted.

In this study, we compared longitudinal changes in B cell populations, B cell transcriptomes, and BCR repertoires following BNT162b2 vaccination in two convalescent COVID-19 subjects. Higher levels of SARS-CoV-2 neutralization and Spike-reactive B cells were noted for both subjects after vaccination. Single-cell RNA sequencing of Spike-reactive B cells revealed that many B cell clones were activated following SARS-CoV-2 infection and BNT162b2 vaccination. Strikingly, many clones between subjects shared similar CDR3-loop amino acid sequences in the V_H and V_L regions post-vaccination, suggesting that clones with similar CDR3 regions are expanded by the vaccine and dominate SARS-CoV-2 Spike protein recognition. Finally, we observed that BNT162b2 elicited expansion of B cell subsets in both subjects bearing sequence homology to published SARS-CoV-2 antibodies in the CoV-AbDab database. These observations provide a description of the impact of BNT162b2 in our subject cohort, while suggesting possible implications regarding appropriate vaccine dosage regimens for convalescent COVID-19 subjects.

2. Materials and methods

2.1. Study cohort

Subjects were chosen and consented based on prior SARS-CoV-2 infection and BNT162b2 vaccination through an REB approved study protocol at St. Michael's Hospital, Unity Health (REB20-044). Peripheral blood mononuclear cells (PBMCs) were isolated from blood drawn from each subject over several timepoints post symptom onset and after vaccination. Cells were viably frozen until use. PBMCs from a healthy subject were also isolated and used as a control for flow cytometry.

2.2. Flow cytometry and tetramer generation

Frozen PBMCs were thawed and washed once in R-10 with DNase (Sigma) and once with Dulbecco's phosphate-buffered saline (D-PBS). Cells were resuspended in D-PBS and stained with Fixable Live-Dead Violet (Thermofisher). Cells were washed once in D-PBS, resuspended in FACS staining buffer (DPBS+2% FBS + 0.1 Na_3N), and stained with the following antibodies and reagents: PE SARS-CoV-2 Spike protein tetramers, APC SARS-CoV-2 Spike tetramers, FITC anti-human IgA (Miltenyi), PE-Cy 7 anti-human CD19 (BD), APC-H7 anti-human IgG (BD), AF700 anti-human Ig D (BD), BV-605 anti-human CD38, BV510 anti-human IgM (BD), BV786 anti-human CD27 (BD), PE-CF594 anti-human CD21, BV421 anti-human CD3/14/16 (BD). Cells were washed and resuspended in FACS staining buffer before acquisition. Flow analysis was performed on FlowJo (BD). Spike-specific tetramers consisting of biotinylated SARS-CoV-2 Spike protein linked to streptavidin SA-APC or SA-PE were generated as described previously [24,25]. Briefly, biotinylated proteins (RBD, Spike trimer) were mixed with SA-fluorophores (PE, APC) at a ~4:1 M ratio by slowly titrating in the latter with the former in 1/10 amounts every 10′ on ice until the full amounts were mixed. Tetramers were stored in the dark at 4 °C until use.

2.3. Memory B cell ELISPOT

The IgG or IgA Basic ELISPOT kit's protocol (Mabtech) was followed with modification. Briefly, frozen PBMCs were thawed and washed once in R-10 with DNase (Sigma) and once with R-10. Cells were cultured in R-10 with IL-2 and R848 (Mabtech) for 6 days and plated in Millipore MAIP plates (Sigma) pre-coated with anti-human IgG or IgA. Plates were incubated at 37 °C for 16 h; cells were washed away with D-PBS after incubation. Biotinylated SARS-CoV-2 Spike protein was added to the wells to detect Spike-specific B cells, and biotinylated anti-human IgG or IgA was added for detection of total IgG or IgA B cells. Streptavidin-HRP and TMB substrate were used for spot development. Spots were counted with ImmunoSpot Analyzer (CTL).

2.4. Neutralizing antibody titre assay

Subject sera were heat inactivated at 56 °C for 30 min. Sera were diluted in serum-free media by a factor of 20, then serially diluted 6 steps at 2-fold intervals and incubated with 100 TCID50/mL of SARS-CoV-2 virus (Wuhan variant) for 1 h at 37 °C, 5% CO2. After incubation, VeroE6 cells were inoculated with the blood-virus mixture for 1 h at 37 °C, 5% CO_2 in quadruplicate. After incubation, the inoculum was removed and fresh DMEM with 2% FBS was added to the VeroE6 cells and returned to the incubator. The VeroE6 cells were incubated for 5 days and Cytopathic Events (CPE) was microscopically assessed. CPE was recorded in GraphPad Prism (GraphPad Software) and analyzed using a 4-parameter nonlinear regression to calculate the IC50. IC50 is defined as the serum concentration at which 50% of the VeroE6 cells are protected from infection.

2.5. Cell sorting and single cell RNA sequencing

PBMCs were thawed and treated with 100 μg/ml DNase1 for 15 min before staining. Tetramers were stained alongside fixable viability dye-eFluor 780 (Thermofisher), CD3-Pacific Blue, CD19-FITC, CD27-PerCP Cy5.5, and IgD-BV605 antibodies (Biolegend or BD) for cell sorting stains. Spike-reactive B cells were sorted based on double positive tetramer staining and submitted to the Princess Margaret Genomics Centre (Toronto, ON) for single cell RNA sequencing. Single-cell library preparation and sequencing was performed using a 10× Genomics Chromium controller to prepare libraries for gene expression (GEX) (Single Cell 5′ R2-only Chemistry) and B cell receptor (BCR) (Single Cell V(D)J R2-only). Libraries for samples were sequenced on an Illumina-2500 for single-cell gene expressions (5000 cells, 50 K reads per cell) and the single-cell BCR sequences (5000 cells, 5 K reads per cell). Our data consisted of single-cell GEX and BCR sequencing data for 3 S-reactive enriched samples and computational antibody discovery was based on the single-cell RNAseq gene expression and BCR sequencing data. Due to the low numbers of purified S-protein reactive cells, purified T cells were added as carriers to facilitate processing.

2.6. Pre-processing and removal of carrier T cell signals

For GEX data, we used 10× genomics CellRanger (ver.3.1.0) with default settings [26] to demultiplex UMI GEM barcodes and embedded STAR [27] to align GEX data to the human genome version GRCh38–3.0.0 and used gene count and filtered barcodes as the default output of the pipeline for further analysis. We used the Seurat R package [28] as the main framework for single-cell transcriptome analysis. Cells with coverage on less than 200 genes and more than 6000 genes and cells with a mitochondrial RNA count of more than 20% were removed. We normalized cell counts using Seurat's *NormalizeData* with

"LogNormalize" parameters and a scale factor of 10,000 and used 5000 most variant genes to perform PCA. The first 20 principal components were used to identify clusters in the UMAP space and further visualization. We used standard cell markers to identify carrier T cells (CD3E, CD4, CD8A, CD8B) and removed them from further transcriptome analysis. Specifically, we used *FindIntegrationAnchors* (30 dimensions, default parameters) to identify pairwise anchors between 4 samples and used *IntegrateData* with (default parameters) to integrate our 4 data sets. We then scaled the data using *ScaleData*, computed the PCA using *RunPCA,* found clusters in 20 lower dimensions using *FindNeighbors* and *FindClusters* (resolution = 1), and utilized *RunUMAP* for the visualization. To address whether S-protein enrichment altered the samples' B cell composition, we computed the ratio of the fraction of cells in each cluster normalized by the total number of cells in each sample after clustering the combined tonsillar GEX data and clustering in UMAP space.

2.7. Single-cell BCR analysis, pre-processing

We used 10× genomics CellRanger (ver.4.0) with default settings to demultiplex UMI GEM barcodes and used the pipeline to align the contigs to IMGT BCR loci germline reference [29]. Only BCR data of cells with productive heavy and light chain sequences we included for further analysis; BCR records that did not have a corresponding gene-expression records in our GEX dataset were removed.

2.8. BCR repertoire analysis

After pre-processing the single-cell BCR sequencing data, our datasets consisted of (440, 439, 31, 690) paired antibody sequences for pre-vaccination and post-vaccination of P1 and P2, respectively. We performed clonal analysis of BCR repertoires by clustering antibody sequences of all 4 samples together. We defined each pair of antibodies in the same clone if they matched for V and J genes in both chains, had equal-length CDR3 loops in both chains, and had at least an average of 90% amino-acid similarity (*hamming distance)* in their CDR3 regions of both the heavy and light chains.

2.9. Sequence homology with publicly available antibody sequences in CoV-AbDab

Our publicly available reference antibody dataset for sequence homology search consisted of 2700 SARS-CoV-2 reactive antibodies in CoV-AbDab [30]. We used *MAFFT* to compute pairwise alignments between our BCR data to the reference dataset antibodies and selected any sequences for further analysis if they passed the sequence homology criterion of matched VH, JH, and VL genes and at least an average of 70% amino-acid similarity (*hamming distance*) in their CDR3 loops.

3. Results

3.1. B cell memory responses are elicited after BNT162b2 in convalescent SARS-CoV-2 subjects

To determine the transcriptomic and phenotypic impact of BNT162b2 vaccination on convalescent COVID-19 subjects, we studied two previously infected COVID-19 subjects who received either one or two doses of BNT162b2. Both subjects were between 40 and 64 in age and both were symptomatic after contracting the virus; Subject 2 (P2) required hospitalization after infection (Table 1). Serum and PBMC samples were collected at several timepoints during the course of infection leading up to and following vaccination (Fig. 1A). Subject 1 (P1) was followed for 10 months with samples collected only up to one week after the first dose of BNT162b2; P2 was followed for 2 months, with samples collected up to 5 days after the second immunization.

The ability of SARS-CoV-2 infection and BNT162b2 immunization to

Table 1
Study Cohort demographics and disease history.

Patient ID	Sex	Age	Disease severity	Hospital admission (Y/N)	Vaccine doses at sampling
OM8080 (P1)	F	41	Mild	N	1
OM8126 (P2)	M	53	Moderate	Y	2

stimulate protective immunity was first assessed via serological assays. An increase in viral neutralizing capability was noted based upon a sharp increase in the IC50 sera titres in both subjects following vaccination measured using a SARS-CoV-2 live virus neutralization assay (Fig. 1B). Additionally, the frequency of different circulating B cell subsets was measured before and after vaccination by flow cytometry (Supplementary Fig. 1A). The proportions of Spike-specific B cells (Fig. 1C) and memory B cell Spike-specific immunoglobin frequency (Fig. 1D) were increased after vaccination in both subjects, as measured by flow cytometry and ELISpot, respectively, compared to both pre-vaccination and a healthy control sample (flow cytometry only). This is consistent with prior studies identifying sharp increases in neutralizing antibody titres and Spike-specific IgG in double-dosed individuals, highlighting an induction of the memory response against the virus [2,3]. Notably, P1 – who received one dose of BNT162b2 at the time of sampling – displayed equal or greater antibody-specific responses compared to double-dosed P2, underscoring the potent existing immunological memory in convalescent individuals that has been repeatedly observed in similar studies [8–15].

Expansion of different memory B cell and plasmablast or plasma cell populations was also noted in Spike-specific B cells in both subjects after receiving BNT162b2 (Fig. 1E). Both subjects displayed an increase in plasmablast/plasma (P1: 0% to 5.93%; P2: 0% to 7.84%) and activated memory (P1: 0% to 54.24%; P2: 10% to 25.49%) Spike-specific B cell populations after vaccination, emphasizing the strong recall response elicited by mRNA vaccination in convalescent COVID-19 subjects. Additionally, an increase in IgG-switched Spike-specific B cells was observed following immunization in P2 (Fig. 1F), but this was not seen in P1 (Fig. 1F), possibly because this subject was sampled shortly after their initial dose of the vaccine. IgA-switched Spike-specific B cells were also increased post-vaccination (Fig. 1F), which can be linked to the simultaneous expansion of plasmablasts observed in these subjects, as these B cell subsets are known to be primarily IgA$^+$ when in steady-state circulation [19,31].

3.2. Expansion of Spike-reactive plasmablast and IgA-positive B cells following BNT162b2 vaccination

To determine whether vaccination played a role in activating pre-existing SARS-CoV-2-reactive B cell clones following infection, we performed single-cell RNA sequencing on SARS-CoV-2 Spike-specific B cells (10× Genomics) (Supplementary Fig. 1B). We analyzed transcriptomes and B cell receptor (BCR) sequences of 451 pre-vaccine and 512 post-vaccine B cells for P1, and 30 pre-vaccine and 754 post-vaccine cells for P2 (Fig. 2A, Supplementary Fig. 2A-B). We integrated these 4 datasets for further analysis (Supplementary material 1). Seven distinct clusters of B cells were observed from the enriched populations based upon expression of typical surface markers, forming three memory (c0, c1, c3), one naïve (c5), one plasma (c6), and two plasmablast (c2, c4) cell subsets when all samples were pooled together (Fig. 2A). Memory subset clusters c0, c1, and c3 were defined by high *CD19, CD69,* and *MS4A1* expression combined with low *IGHD* expression (Fig. 2B); memory cluster c0 notably had high *CD27* levels. Plasma cluster c6, plasmablast c2, and plasmablast c4 were defined by low *CD19, MS4A1,* and *CD69*, while displaying high *CD27, CD38, XBP1*, and *IRF4* (Fig. 2B, Supplementary Fig. 2C); plasmablast clusters c2 and c4 showed some
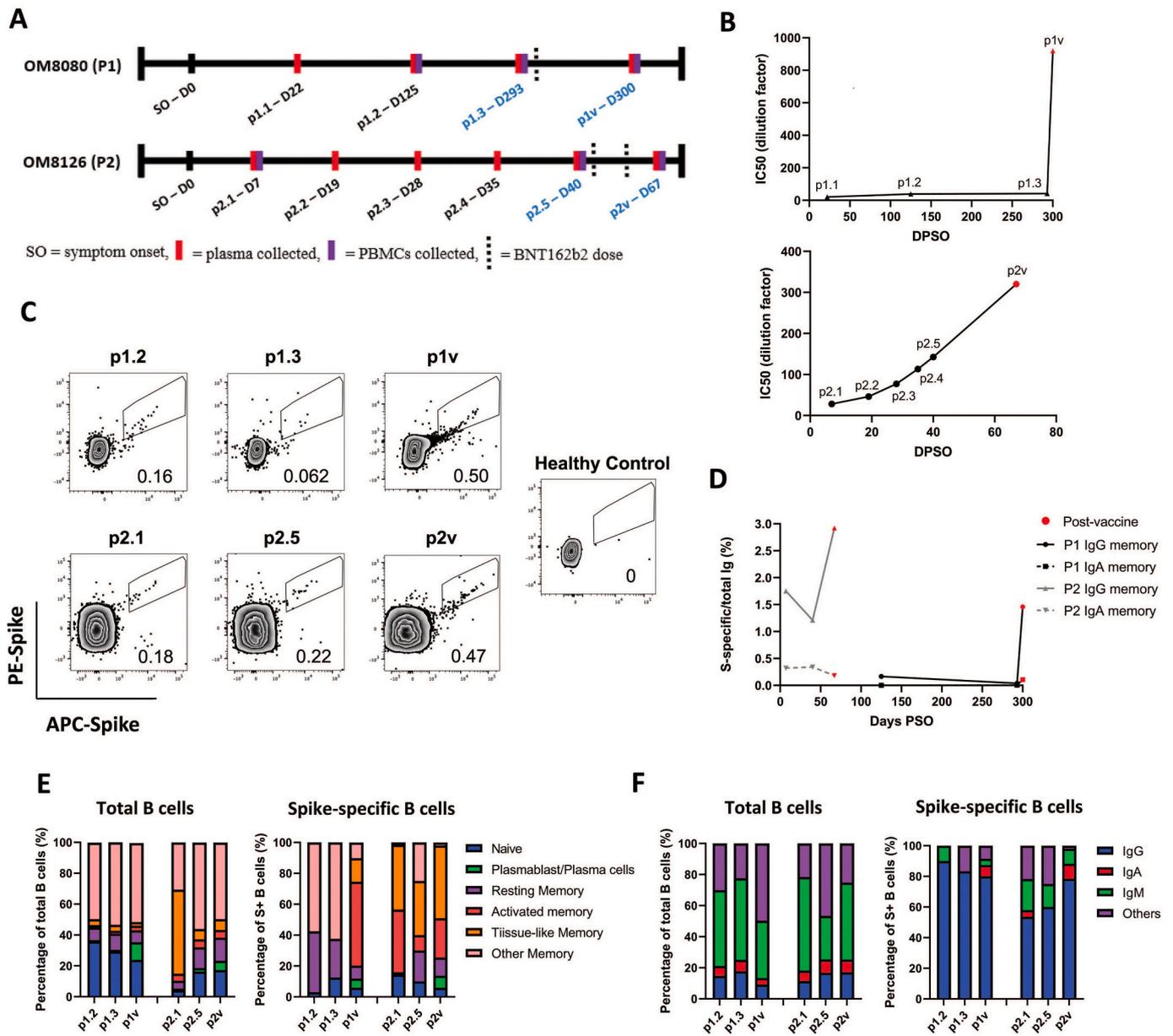
Fig. 1. Spike-specific B cell frequency and subsets are altered following vaccination in recovered SARS-CoV-2 subjects. (A) Disease and vaccination timeline for subjects in study cohort. Timepoints highlighted in blue were submitted for single cell RNA sequencing. (B) IC50 of serum antibody titres from all follow-up visits post-diagnosis. IC50 was determined by 4 parameter non-linear regression of cytopathic effect (CPE) recorded after inoculation of VeroE6 cells with SARS-CoV-2 previously incubated with serial dilutions of subject sera. Each sample was performed in quadruplicate. Post-vaccination timepoints are highlighted in red. (C) Representative flow cytometry graphs showing frequency of SARS-CoV-2 Spike-specific B cells from thawed peripheral blood mononuclear cells (PBMCs) stained with PE-/APC-labelled SARS-CoV-2 Spike tetramers and gated on $CD3^-$, $CD14^-$, $CD16^-$ live $CD19^+$ cells. p1v and p2v represent samples taken following 1 or 2 doses of BNT162b2, respectively. PBMCs from a healthy donor were used as staining control. (D) Frequency of SARS-CoV-2 Spike-specific immunoglobins (Ig) out of total Ig from memory B cells by enzyme-linked immune absorbent spot (ELISPOT). PBMCs from SARS-CoV-2 convalescent subjects at different time points were stimulated and detected on an ELISPOT plate coated with anti-human IgG or anti-human IgA antibodies. Shown is the percentage of Spike-specific IgG or IgA spots in the total IgG or total IgA spots. Red markers indicate post-vaccination timepoints. (E) Distribution of B cell subsets based on surface marker expression. B cells were defined as $CD19^+$. Plasmablasts were defined as $CD27^{Hi}$, $CD38^{Hi}$, while IgD^+ were naïve B cells. Non-naïve B cells were defined as IgD^-: $CD21^+$, $CD27^+$ were resting memory; $CD21^-$, $CD27^+$ were activated memory; $CD21^-$, $CD27^-$ were tissue-like memory; and $CD21^+$, $CD27^-$ were other memory B cells. (F) Distribution of B cell subsets based on immunoglobin class. "Other" indicates B cells that were either IgD^+ or IgG^-, IgA^-, IgM^-, IgD^-. Data are shown as percentages of the B cell subsets in total or Spike-specific B cells. PSO – post symptom onset, DPSO – days post symptom onset. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PRDM1 expression while minimal expression was observed in the plasma c6 subset. Naïve cluster c5 B cells were classified primarily based on high *IGHD* expression (Fig. 2B).

While vaccination resulted in expansion of plasmablast subsets in both subjects, increases in memory B cells were predominantly observed in P2 (Fig. 2C). Differences in B cell subset expansion can be attributed

to the number of doses received at the time of sampling as well as the stage of recovery – P1 was immunized only once 10 months post-symptom onset, while P2 received their second booster dose 2 months after diagnosis. Interestingly, an increase in the proportion of IgA+ B cells was also noted for both subjects following immunization (Fig. 2D), a phenotype that was also apparent by flow cytometry, albeit at a lower
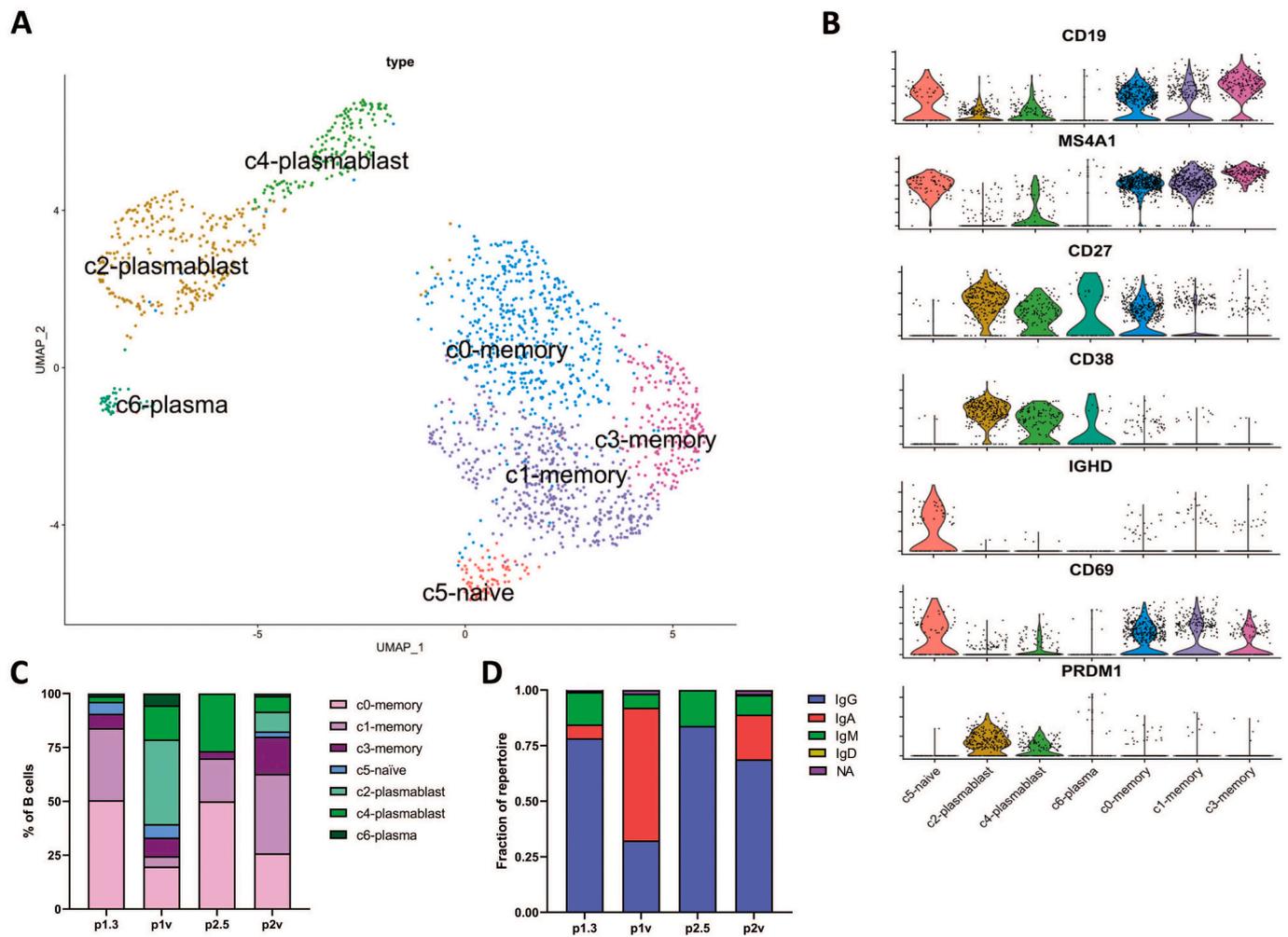
Fig. 2. RNA sequencing of Spike-reactive B cells reveals shifted B cell subsets and increased IgA-positive B cells following vaccination. Thawed PBMCs from timepoints prior to and after vaccination from both donors were enriched for antigen-specific B cells (CD19$^+$, CD3$^-$) by FACS sorting using SARS-CoV-2 Spike tetramers and submitted for single cell RNA sequencing. Transcriptomic data was processed as described in the methods and the first 20 principal components were used to identify cell clusters within the (A) UMAP space. Components consisted of (B) descriptive markers for CD19$^+$, CD3$^-$ B cell subsets. (C) B cell subset cluster frequency and (D) antibody isotype fraction were subsequently calculated based on this transcriptomic data. NA – cells with data not available.

frequency (Fig. 1F).

3.3. Spike-reactive B cells harbor many V$_H$-J$_H$ pairs in convalescent vaccinees suggesting that many B cell clones are involved in the SARS-CoV-2 response

To examine the B cell receptor (BCR) repertoire in SARS-CoV-2 Spike-reactive B cells pre- and post-vaccination, we assessed V$_H$-J$_H$ usage in the sorted B cell population. In both subjects, we observed many BCR heavy chain gene pairs based on V$_H$ and J$_H$ usage (Fig. 3A; light green, 0). However, following BNT162b2 vaccination, we observed selective expansion of specific V$_H$-J$_H$ heavy-chain gene pairs in both subjects (Fig. 3A; darker green, 1–4), underscoring a shift in gene segment usage following mRNA vaccination of convalescent subjects. We also observed several novel V$_H$-J$_H$ heavy-chain gene pairs that were not observed pre-vaccination (dark green, 5) (Fig. 3A). Additionally, there was a significant increase in non-synonymous mutations present in the BCR V-regions in several P1 subsets post-vaccination, especially within the memory and c4 plasmablast clusters (Fig. 3B). This result corroborates prior studies that posit mRNA vaccination as a primer for somatic hypermutation in B cells via germinal centre formation [16,17]. P1 also displayed higher non-synonymous mutation rates in almost every subset compared to P2 (Fig. 3B), which may suggest that extended

time between infection and vaccine boost (P1's 10 months vs P2's 2 months) could lead to higher affinity antibody-producing B cells after vaccination due to increased germinal centre-mediated development of long-lived memory B cells. Collectively, these data show extensive usage of different V$_H$-J$_H$ gene pairs in Spike-reactive B cells pre- and post-BNT162b2 vaccination, which suggests 1) that reactivity to Spike protein can be accomplished with many V$_H$ and J$_H$ elements, and 2) that a polyclonal, higher affinity B cell memory response is stimulated post-vaccination in convalescent COVID-19 subjects.

3.4. Spike-specific antibodies induced by vaccination possess high CDR3 sequence similarity and are homologous to published SARS-CoV-2 antibodies

Vaccination of healthy (no prior COVID-19 history) individuals has been shown to induce the production of antibodies that undergo clonal expansion and that are homologous to known SARS-CoV-2 targeting antibodies elicited during natural viral infection [21,32]. To determine whether these phenomena would similarly be observed in convalescent COVID-19 subjects, we compared the clonal structure of BCR repertoires of SARS-CoV-2 Spike-specific B cells in both individuals before and after vaccination. Antibodies were classified as clonal if they were matched for the V and J genes of both the heavy and light chains and had CDR3
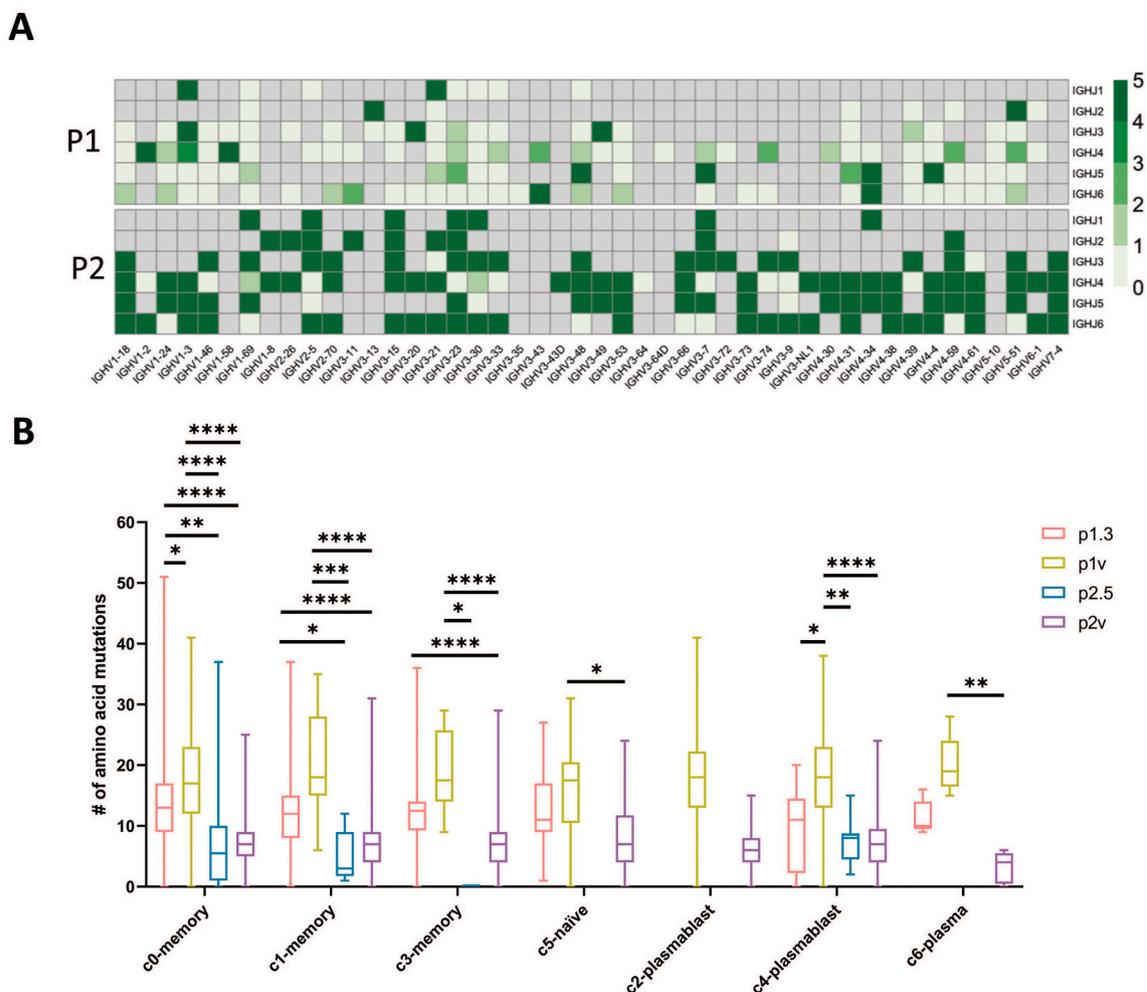
A

B

Fig. 3. Extensive V_H and J_H usage in Spike-reactive B cell clones pre- and post-vaccination. (A) Repertoire heavy chain gene-usage post-vaccination compared to pre-vaccination for P1 (top heatmap) and P2 (bottom heatmap): the fraction of VH-JH gene pairs (heavy-chain only) of BCR repertoire was computed for each time point, and the ratio between the fractions pre- and post-vaccination was computed. Each square corresponds to a gene pair and the values represent the ratio of the gene-usage fraction of the post-vaccination sample with respect to the pre-vaccination sample for the same subject. The values are capped at 5 for clarity of lower values. When a gene pair is not observed pre-vaccine, the value is represented by 5. Grey boxes indicate absence of the gene pair in either timepoint. (B) BCR V-region non-synonymous amino acid mutations between subject timepoints. *Kruskal-Wallis test followed by Dunn's multiple comparisons test.

regions of the same length in both chains with at least 90% amino acid sequence similarity (see Methods). We found that the response was polyclonal pre-vaccination (all singletons) in both subjects (Fig. 4A-B), supporting the notion mentioned above that many different B cell clones participated in the SARS-CoV-2 infection response. Interestingly, vaccination did not lead to expansion of specific B cell clones in either subject, but instead appeared to result in the activation of many different B cell clones that appeared to vary from those elicited pre-vaccination. These data suggest that the clonal response to BNT162b2 immunization after SARS-CoV-2 infection is diverse and not dominated by specific clones. As vaccination of previously infected individuals should lead to specific amplification of pre-existing B cell memory cells, the apparent lack of a link between clones before and after vaccination is likely due to the high numbers of B cell clones involved in the response pre- and post-vaccination.

Interestingly, when B cells were clustered solely based on heavy and light chain CDR3 sequence similarity (>90% average amino acid similarity), several clusters were observed containing both pre- and post-vaccination clones from each subject (Fig. 4C). Within these clusters, CDR3 heavy and light chain consensus sequences displayed high similarity (Supplementary Fig. 3, Supplementary material 1). In most cases, clustered B cells also had matching V and J pairs in both the heavy and light chain (Supplementary material 1), indicating that differences in B

cell clonal clusters in Fig. 4A were driven by differences in the length of the CDR3 loop (average lengths shown in Supplementary Fig. 4) and that each cluster consisted of many different clones. However, some clusters (such as clusters 2 and 3 for P2; Supplementary Fig. 3B) had clones with similar CDR3 sequences but not matching V/J regions (Supplementary Data 1C). This was also the case for the light chain CDR3 in P2 which were similar between cluster 1 and 2 (Supplementary Fig. 3B), but these two clusters use different V_L and J_L elements (Supplementary Data 1C). This suggests that V/J pairing was not solely responsible for CDR3 sequence similarity. CDR3 similarity was also observed between subjects, with some inter-subject B cell clusters appearing when CDR3 homology was assessed at 65% amino acid similarity (Fig. 4D). These CDR3 clusters were dominated by post-vaccine B cells in both subjects, indicating that this common CDR3 usage developed after vaccination. This result suggests that despite a lack of clonal dominance to SARS-CoV-2 Spike protein in this cohort pre- and post-vaccination, antigen specificity was maintained with similar paratopes encoded primarily by the CDR3 loops that were generated as a result of vaccination.

To assess possible similarities with antibodies from other subject cohorts and to investigate the potential functions of B cell clones from our subjects, we performed a virtual screening and homology assessment (Fig. 5A) against the publicly available CoV-AbDab database [30], which contains published SARS-CoV-2-specific antibodies generated
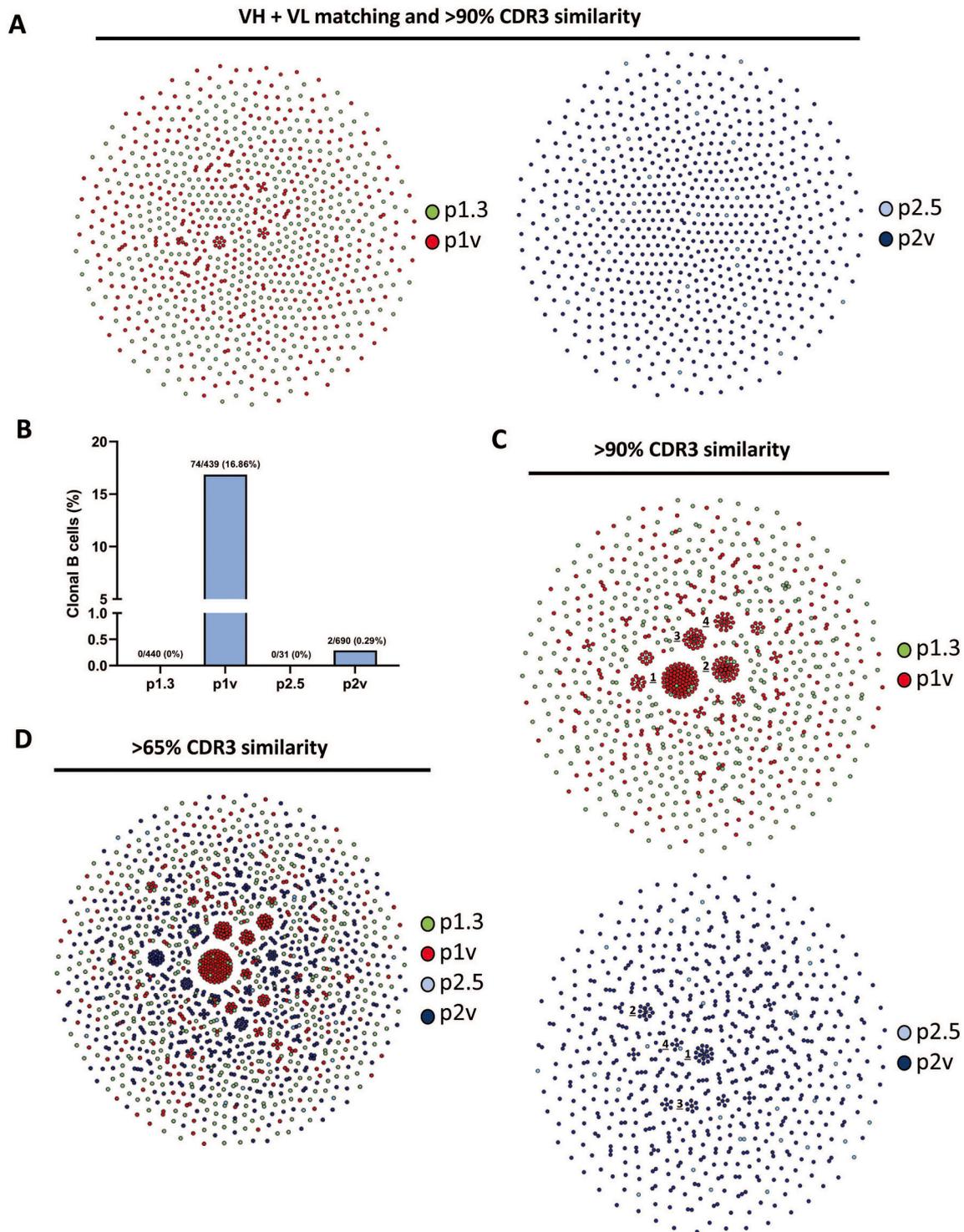
Fig. 4. Clonal similarity observed pre- and post-BNT162b2 vaccination between subjects based on CDR3 homology. (A) Cluster plots displaying Spike-reactive B cell clones based on matching heavy and light chain V/J regions along with similarity in CDR3 loop sequences (>90% amino acid similarity and matching length) in both subjects pre- and post-vaccination (B) Percent of B cells that are clones as defined in (A) amongst sorted B cell populations. (C) Cluster plots displaying B cell clusters based solely on similarity in CDR3 loop sequences >90% between timepoints, or (D) similarity in CDR3 sequences >65% between subjects. Each dot represents an individual B cell, clusters represent clonally expanded subsets. Four of the largest cell clusters labelled (to the left in Fig. 4C) and assessed for consensus sequences (Supplementary Fig. 3, Supplementary Data 1B–C) for each subject.

after natural infection. BCRs were considered homologous to a published antibody if their V_H, J_H, and V_L genes were matched and possessed at least 70% sequence similarity within their CDR3 loops (both heavy and light chains). A substantial number of BCRs were identified to be homologous to published antibodies, with the majority found within the

plasmablast and memory B cell subsets in P1 and P2, respectively (Fig. 5B). Both subjects harbored 27 or more antibody groups containing >4 BCRs (both clonal and non-clonal) homologous with different published antibodies after vaccination, many of which were known to be SARS-CoV-2 neutralizing, while P1 additionally had 20 antibody groups
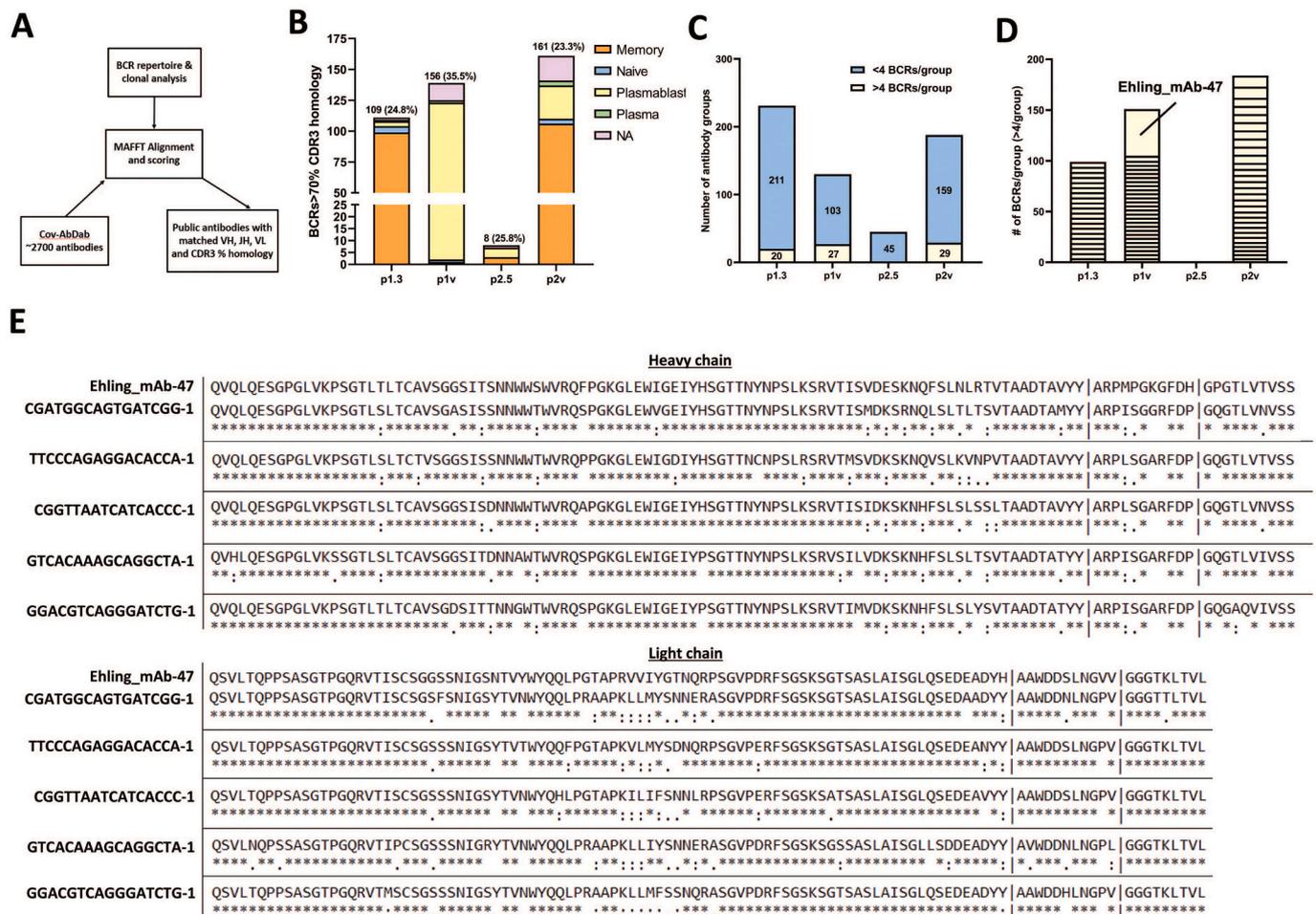
Fig. 5. BNT162b2 vaccination induces the generation of B cell clones with high CDR3 homology with published SARS-CoV-2 antibodies in convalescent subjects. (A) Pipeline of clonal and homology analysis based on MAFFT alignment and comparison with the CoV-AbDab database. Number of (B) BCRs amongst B cell subsets with matching heavy/light chains and >70% CDR3 homology to antibody sequences found in CoV-AbDab as count and percent frequency of total B cells. (C) Antibody groups with greater or less than 4 BCRs harboring homology to published antibody clonotypes. Each group can contain both clonal and non-clonal B cells. (D) Number of BCRs present in antibody groups with greater than 4 BCRs homologous to published antibodies between subject timepoints. Each group has a minimum of 4 BCRs and arrow indicates group with the most number of BCRs with homology to published SARS-CoV-2 antibody Ehling_mAb-47. (E) Similarity of representative p1v clonal CDR1, CDR2, and junction (CDR3 and flanking residues) heavy and light chain sequences to Ehling_mAb-47. Barcodes for five representative clones are listed on the left. Alignment score is presented under sequences. "*" – match, ":" – strongly conserved, "." – weakly conserved, "|" – separation between region sequences, NA – cell identity not available.

with this level of similarity even before their initial immunization (Fig. 5C). Hundreds of other B cells in our subjects also displayed similar published clonotype homology, albeit with fewer BCRs (<4) per group. Most homologous subject antibody groups contained 4–11 members (Fig. 5D), but one large group in P1 contained 46 BCRs that were homologous to a published monoclonal SARS-CoV-2 antibody, Ehling_mAb-47 [33]. Strikingly, seven of the largest clones (mostly IGHV4-4/IGHJ5, defined by >90% CDR3 similarity) in P1 displayed high amino acid sequence similarity to Ehling_mAb-47 in both their heavy and light chain CDR regions (five shown in Fig. 5E, four consensus sequences shown in Supplementary Fig. 3). The discovery of similarities in clonal antigen-specific antibody sequences between our two subjects and with those of published SARS-CoV-2 antibodies from CoV-AbDab suggests that vaccination induces the development of antibody clonotypes with antigen specificity and variable region sequences that are similar to those found during natural infection. Moreover, it supports the case for convergent antibody maturation that has also been observed in uninfected mRNA vaccinees [21,22,32].

Overall, these findings highlight a shift in B cell phenotype, function, and clonality following the vaccination that may favour the development of memory B cell responses that harbor similar variable region

sequences across convalescent COVID-19 vaccinees.

4. Discussion

Matching B cell heavy chain V and J genes and assessing sequence similarity in the heavy chain CDR3 (CDRH3) region are criteria typically used to identify similar B cell clonotypes in different subject cohorts [19,21,22,32,34–38]. Clonotypes from both naïve BNT162b2 vaccinees [21,22,32] and convalescent unvaccinated individuals [19,34–38] have been found to be homologous with published antibodies and/or amongst different individuals based on the above criteria, showing convergent development of conserved variable region genes and sequences that work to target SARS-CoV-2. Many of these clonotypes have been identified to specifically target RBD, with subsets of these antibody clones shown to be neutralizing [32,34,36]. Although clonality and homology of the SARS-CoV-2-specific BCR repertoire have been commonly reported in convalescent COVID-19 subjects and individuals immunized with mRNA vaccines, these assessments have not been thoroughly performed in convalescent BNT162b2 vaccinees. Here, we report that many B cell clones participate in the response to SARS-CoV-2 Spike protein as a result of SARS-CoV-2 infection and following BNT162b2 vaccination

in our subject cohort. Furthermore, we noted CDR3 sequence-specific similarity between different convalescent BNT162b2 recipients (Fig. 4D). Many of the similar CDR3 sequences in our subjects were associated with matching V/J pairs but different CDR3 loop lengths, indicating that different B cell clones expanded post-vaccination harbored similar Spike-specific variable regions. This observation suggests that the CDR3 loop in V/J-matched clones forms the dominant antigen recognition domain of the antibody to the SARS-CoV-2 Spike protein.

Antibody homology based on variable region gene matching and CDR3 sequence similarity was also noted with several published SARS-CoV-2 antibodies, many of which were known to have virus-neutralizing capability. In our cohort, sequence similarity with published antibodies was observed in both CDRH3 and the light chain CDR3 (CDRL3) variable regions. Many of the clones that were homologous to Ehling_mAb-47 showed high amino acid sequence similarity especially in CDRL3, several of which were identical in these light chain sequences to the published antibody (Fig. 5E). This contrasts with some reports that have noted CDRL3 as less conserved compared to its heavy chain counterpart, showing heterogeneity in the light chain amino acid sequences of un-vaccinated convalescent subjects and naïve vaccinees [21,35], although this disparity may be due to differences in cohorts assessed. Overall, this remarkable similarity in CDR3 sequences strengthens the case for convergent antibody responses, which refers to the independent development of similar antibody variable region sequences targeting conserved SARS-CoV-2 antigens (e.g. RBD) in convalescent BNT162b2 vaccinees and during SARS CoV-2 natural infection amongst different individuals. Additionally, these results underscore the importance of CDR3 diversification in the SARS-CoV-2 booster response within convalescent vaccinees, suggesting that modification of this region could have implications for future vaccine or therapeutic design.

For B cell subsets induced following vaccination, disparity in expanded populations was evident between subjects likely due to differences in doses received and stages of recovery. We observed robust plasmablast expansion in P1, who received one dose at time of sampling, which contrasted with the maintenance of memory subsets identified in P2, who received two doses (Fig. 2C). These results suggest that plasmablasts are primarily expanded 1 week after initial vaccination in convalescent COVID-19 subjects, but shift towards a memory B cell phenotype as early as 1 week after the booster dose; this is synchronous with observations seen in naïve double-dosed SARS-CoV-2 mRNA vaccinees [4,17,22]. Importantly, viral immunity was still elevated in P1 after their initial dose, as noted by high viral neutralizing capability and Spike-specific B cell frequency, indicating enhanced anti-SARS-CoV-2 immunity regardless of subsets expanded. Interestingly, one of the B cell subsets expanded post-vaccination was IgA$^+$ B cells, a population thought to primarily line mucosal sites. As mRNA vaccines are not mucosal vaccines, an increase in IgA$^+$ B cells was unexpected, but may also be linked to a simultaneous increase in plasmablast populations, which are known to be IgA+ in circulation [19,31]. Further research into this expanded subset of cells could reveal informative details on the breadth of immunological stimulation by BNT162b2.

Overall, this study uncovered a polyclonal B cell response and high CDR3 sequence similarity in B cells following BNT162b2 vaccination in two convalescent COVID-19 subjects. With the ongoing implementation of additional booster vaccine doses to combat waning immunity, further studies assessing the diversification of convergent antibody clonotypes and the long-term kinetics of virus-specific immune memory after subsequent doses will be crucial in the continuing control of SARS-CoV-2 and its variants.

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Author contributions

MW, AZ, MO, AM designed the experiments, MW, AZ, JL, PB, JC, EY performed the research and analyzed the data; MO, AM supervised; MW, AZ, JL, PB, AM wrote the manuscript.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgements

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