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Durable T cell immunity to COVID-19 vaccines in MS patients on B cell depletion therapy

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Immune-mediated protection generated to COVID-19 mRNA vaccines is associated with anti-Spike (S) protein neutralizing antibodies. However, humoral immunity is compromised in B cell depleting (BCD) therapies, used to treat autoimmune diseases such as Multiple Sclerosis (MS). To study the effect of BCD on the durability and protective efficacy of vaccine-induced immunity, we evaluated S-reactive antibodies and T cell responses 1–70 weeks post-vaccination in MS cohorts treated with BCD compared to non-BCD therapies from four centers. BCD-treated participants had significantly reduced antibody levels and enhanced frequencies of S-reactive CD4$^+$ and CD8$^+$ memory T cells to COVID-19 vaccination compared to the non-BCD group, with some variations among different BCD formulations. T cell memory responses persisted up to 14 months post-vaccination in both BCD and non-BCD cohorts, who experienced similar clinical protection from COVID-19. Together, our results establish a critical role for T cell-mediated immunity in anti-viral protection independent of humoral immunity.

Mass immunization with COVID-19 mRNA vaccines has led to a significant reduction in morbidity and mortality following SARS-CoV-2 infection. Vaccine-induced neutralizing antibodies are the primary correlate of protection for most successful vaccines[1,2] including for the COVID-19 mRNA vaccines[3,4]. While antibody titers generated to many childhood vaccines can persist for decades, neutralizing antibodies generated to COVID-19 mRNA vaccines are less durable, exhibiting half-lives of several months[5–7]. However, these vaccines also induce a robust Spike protein (S)-reactive T cell response that is variably maintained over time post-vaccination[8,9]. The relative roles and synergistic effects of vaccine-induced antibodies and T cells in providing protective immunity is difficult to assess in humans.

Multiple sclerosis (MS) is an autoimmune neurological disease affecting nearly 1 million people in the United States[10,11], and the mainstay of management is long-term immunotherapy. One of these disease-modifying strategies involves monoclonal anti-CD20 antibodies, which specifically

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target B cells, resulting in significant B cell depletion in circulation and concomitant reduction in immunoglobulin levels[12–16]. These B cell depletion (BCD) therapies have emerged as a prevailing treatment for MS due to their efficacy in reducing the rate of clinical relapses[15,16]. However, BCD-treated individuals are at risk for opportunistic infections[17,18], inflammatory complications[19], and have diminished responses to vaccines compared to MS patients on other therapies[20]. Investigating the generation of immune memory following vaccination in this BCD-treated population offers insights into clinical management and an opportunity to dissect the role of lymphocytes in vivo in providing protective immunity in humans. The recent mass vaccination of the population with COVID-19 mRNA vaccines in the midst of a global pandemic provided an unprecedented opportunity to assess multiple aspects of immunity and protection in the context of BCD.

Studies have evaluated the anti-S response to COVID-19 mRNA vaccines in BCD participants at short intervals following vaccination, and they show greatly reduced but still measurable antibody responses[21–29]. BCD individuals also have an increased frequency of circulating COVID-19 vaccine-specific T cells compared to those on other therapies[21,23–28]. In healthy individuals, T cell responses are correlated with better outcomes[30–33], and vaccination in the BCD MS population reduced the odds of severe disease following infection[28] despite their diminished humoral response. These studies have been mostly limited to single cohorts and intravenously administered antibodies rituximab (RTX) and ocrelizumab (OCR)[15,34,35], and longitudinal data are limited.

Here, we present a multi-cohort longitudinal analysis integrating multiple aspects of the T and B cell immune response to COVID-19 mRNA vaccines in patients treated with three different BCD drugs, including the subcutaneously administered antibody, ofatumumab (OFA)[16]. We analyzed the generation of S-specific antibodies, CD4$^+$T cell, and CD8$^+$T cell responses and subsets in 101 participants with MS treated with BCD drugs ($n = 43$) compared to non-BCD ($n = 58$) therapies between 4 days and 70 weeks post-vaccination along with immune protection based on infection prevalence following vaccination. We further incorporated data from vaccinated cohorts from other institutions, totaling 216 participants (119 BCD and 97 non-BCD). In BCD participants, S-specific antibodies were negligible while S-reactive memory T cells were induced at significantly higher frequencies than in non-BCD participants and maintained over time post-vaccination, with some variations between BCD drugs. Despite these distinct immune profiles, both BCD and non-BCD vaccinated participants experienced a similar low frequency of mild SARS-CoV-2 infection. Our results show that durable T cell immunity to COVID-19 mRNA vaccines develops independently from circulating B cells and can provide protection from symptomatic infection even in the absence of circulating antibodies.

Results

Study cohort
To evaluate the longitudinal effects of various BCD therapies including OCR, RTX, and OFA on the induction and maintenance of COVID-19 vaccine induced immune memory, we recruited 101 participants with MS treated with various BCD and non-BCD disease modifying therapies who had received two doses of a COVID-19 mRNA vaccine (BNT162b2 or mRNA-1273) from two academic centers (Table 1). We collected 116 blood samples from participants taking BCD ($n = 43$) or non-BCD ($n = 58$) therapies between 4 and 483 days (mean 115 days) following the completion of their two dose vaccine series (Table 1, Fig. 1a). These donors were confirmed to have no clinical history of COVID-19 infection and their post-vaccine blood samples were negative for anti-Nucleocapsid antibodies (Table 1), allowing for investigation of a vaccine-induced response.

Diminished humoral response in MS participants treated with B-cell depleting therapies
BCD participants had significantly lower levels of circulating B cells compared to non-BCD participants (Fig. 1b, Supplementary Fig. 1), with undetectable or negligible antibody responses in the majority of BCD-treated individuals (Fig. 1c). When controlling for age, sex, vaccine type, and

the time interval since vaccination, BCD participants had significantly lower anti-S protein and anti-receptor binding domain (RBD) IgG compared to non-BCD participants (Fig. 1c). Within the BCD cohort, similar low antibody levels were observed in participants treated with various anti-CD20 agents (Fig. 1d). After vaccination, non-BCD participants exhibited a significant decline in both anti-S and anti-RBD IgG titers within 7 months post-vaccination (Fig. 1e) and in a linear model controlling for age, sex, and vaccine type (Supplementary Data 1), consistent with observations in the general population[5–7]. However, in BCD participants, there was no significant anti-S antibody response at either early or later time-points (Fig. 1e), nor was there a correlation between vaccine-induced antibody titers and time since last administration of anti-CD20 therapy (Supplementary Fig. 2). Together, these results show a greatly diminished humoral vaccine response in BCD participants from two geographically distinct centers that was observed up to one-year post-vaccination across all BCD drug types.

Enhanced cellular response in B-cell depleted participants
T cell responses are also generated following vaccine exposure and can serve important protective roles. We assessed the generation of S-reactive T cells to COVID-19 mRNA vaccination in our MS cohorts using the well validated Activation-Induced Marker (AIM) assay as described[36,37]. We stimulated peripheral blood mononuclear cells with S protein peptide megapools (MP) or DMSO as a control (see methods) and quantified S-reactive T cells based on co-expression of at least two surface markers on a given cell: among OX40, 4-1BB, and CD40L for CD4$^+$T cells and CD25, CD69, and 4-1BB for CD8$^+$T cells (see methods, Supplementary Fig. 1a). We detected AIM$^+$ (S-reactive) CD4$^+$ and CD8$^+$T cells in both non-BCD and BCD-treated participants, but not in the DMSO negative control (Fig. 2a, b). The total number of CD4$^+$ and CD8$^+$ T cells and memory (non-naïve) subsets in these cultures did not differ between the two treatment cohorts despite significant reduction in B cell numbers in the BCD compared to non-BCD group (Supplementary Fig. 1b). However, BCD participants had a significantly enhanced frequency of S-reactive CD4$^+$ and CD8$^+$T cells compared to non-BCD participants in a multivariate analysis controlling for time post-vaccination, age, sex, and vaccine brand (Fig. 2c, d). Within the BCD cohort, OFA-treated participants (who receive subcutaneous delivery of anti-CD20) exhibited significantly higher levels of S-reactive CD4$^+$ and CD8$^+$T cells compared to OCR-treated participants (who receive intravenous delivery), albeit with a low number of OFA-treated subjects (Fig. 2c, d). While the quantity of S-reactive CD8$^+$T cells was independent of the time since last administration of anti-CD20 therapy, the frequency of S-reactive CD4$^+$ T cells was higher when measured at longer intervals post-BCD treatment (Supplementary Fig. 2). The levels of S-reactive CD4$^+$ and CD8$^+$T cells did not decline over time post-vaccination and could be readily detected at 6 months post-vaccination and even over a year post-vaccination in select donors (Fig. 2e, f), contrasting the steep decline in S-reactive antibody titers at those timepoints (Fig. 1e, f). Together, these findings demonstrate that BCD participants develop long-lasting cellular immunity despite negligible humoral immunity.

To investigate whether differences in the magnitude of the T cell response were due to underlying differences in the composition of T cell memory subsets, we evaluated the S-reactive T cells for their memory phenotype: central memory (T$_{CM}$, CD45RA$^-$CCR7$^+$), terminally differentiated effector memory (T$_{EMRA}$, CD45RA$^+$, CCR7$^-$), or effector memory (T$_{EM}$, CD45RA$^-$, CCR7$^-$) in our multivariate model (Supplementary Data 1). S-reactive CD4$^+$T cells were predominately T$_{EM}$, and their phenotypes did not significantly differ between treatment cohorts in a multivariate analysis (Fig. 3a, b). By contrast, S-reactive CD8$^+$T cells were primarily T$_{EMRA}$ in both treatment cohorts, though BCD-treated participants had significantly fewer T$_{EM}$ compared to non-BCD participants (Fig. 3c, d).

T-follicular helper (T$_{FH}$) cells play an important role in promoting B cell affinity maturation and class switching in lymph nodes[38] and can circulate as memory T$_{FH}$[39]. We therefore evaluated the presence of circulating S-reactive memory T$_{FH}$ cells identified by PD-1 and CXCR5 expression[38].

Table 1 | Demographics and clinical data for MS participants

	Columbia, UPitt (Figs. 1–3)			All Centers (Fig. 4)		
	BCD	non-BCD	*p* value	BCD	non-BCD	*p* value
Donors, *n*	43	58		119	97	
Samples collected, *n*	49	67		161	120	
Age, mean years ± SD (range)[a]	44.4 ± 14.6 (24–73)	52.8 ± 11.6 (26–78)	0.0018	44.4 ± 11.7 (24–73)	52.3 ± 11.1 (26–78)	<0.0001
Time post-vaccination, mean days ± SD (range)[a]	92.3 ± 83.9 (4–383)	117.1 ± 95.2 (6–483)	0.2653	50.2 ± 65.3 (3–383)	83.7 ± 94.2 (6–483)	0.0001
Female Sex, *n* donors (%)	28 (65.1)	48 (82.8)	0.0614	82 (68.3)	81 (83.5)	0.0168
Ethnicity, *n* donors (%)[b]			0.1968			
Non-Hispanic/Latino	36 (83.7)	54 (93.1)		–	–	
Hispanic/Latino	7 (16.3)	4 (6.9)		–	–	
Unknown	0 (0.0)	0 (0.0)		–	–	
Race, *n* donors (%)[b]			0.8117			
Black/African American	6 (14.0)	9 (15.5)		–	–	
Asian	1 (2.3)	2 (3.5)		–	–	
Caucasian	33 (76.7)	46 (79.3)		–	–	
Native American	3 (7.0)	0 (0.0)		–	–	
Unknown	0 (0.0)	1 (1.7)		–	–	
Vaccine Type, *n* samples (%)[b]			0.4264			0.1185
BNT162b2	35 (71.4)	42 (62.7)		117 (72.7)	76 (63.4)	
mRNA-1273	14 (28.6)	25 (37.3)		44 (27.3)	43 (35.8)	
Unknown	0 (0)	0 (0)		0 (0)	1 (0.8)	
Dose Number, *n* samples[b]						0.354
2	49 (100)	67 (100)	1.000	128 (79.5)	101 (84.2)	
3	0 (0)	0 (0)		33 (20.5)	19 (15.8)	
aCD20 Drug, *n* donors (%)						
Rituximab	16 (37.2)	–		34 (28.6)	–	
Ocrelizumab	24 (55.8)	–		71 (59.7)	–	
Ofatumumab	3 (7.0)	–		14 (11.7)	–	
Other Therapy, *n* donors (%)						
Cladribine	–	2 (3.5)		–	2 (2.1)	
Dimethyl Fumarate	–	9 (15.5)		–	14 (14.4)	
Diroximel Fumarate	–	1 (1.7)		–	1 (1.0)	
Fingolimod	–	1 (1.7)		–	9 (9.3)	
Glatiramer Acetate	–	8 (13.8)		–	16 (16.5)	
Interferon beta-1a	–	4 (6.9)		–	5 (5.1)	
Interferon beta-1b	–	0 (0)		–	1 (1.0)	
Natalizumab	–	13 (22.4)		–	19 (19.6)	
Siponimod	–	0 (0)		–	2 (2.1)	
Teriflunomide	–	6 (10.4)		–	6 (6.2)	
None	–	14 (24.1)		–	22 (22.7)	
Anti-Nucleocapsid Antibody positivity, *n* donors (%)	0 (0)	0 (0)		–	–	

[a]Statistical comparison of demographics performed on continuous variables by Mann-Whitney *U*-test.
[b]Statistical comparison of categorical variables performed using Fisher's Exact test; comparison within race was performed between White and non-White subjects.

Participants taking BCD therapies had significantly lower levels of S-reactive T_{FH} compared to participants on non-BCD therapies (Fig. 3e). The frequency of S-reactive T_{FH} did not significantly decline following vaccination in either MS cohort (Fig. 3f). Similar to our findings of total S-reactive $CD4^+T$ cells, S-reactive T_{FH} were typically present at higher percentages in participants who were farther from their last administration of anti-CD20 therapy (Supplementary Fig. 2). Together, these findings demonstrate that B cell depletion does not overtly affect memory subset differentiation of vaccine-induced cells, but that optimal generation of vaccine-induced memory T_{FH} may require the presence of circulating B cells.

Effects of booster dose and various anti-CD20 drugs on COVID-19 vaccine response

To investigate the effects of a booster dose on the induction of immune memory following COVID-19 vaccination and to increase our sample size of participants taking various BCD agents, we performed a meta-analysis combining our data presented in Figs. 1, 2, and 3 with previously published data from two other centers[21,22]. The resultant dataset included 280 blood samples from 216 participants with MS (119 treated with BCD and 97 treated with non-BCD therapies) collected after administration of two or three doses of COVID-19 mRNA vaccines (Table 1). Across all
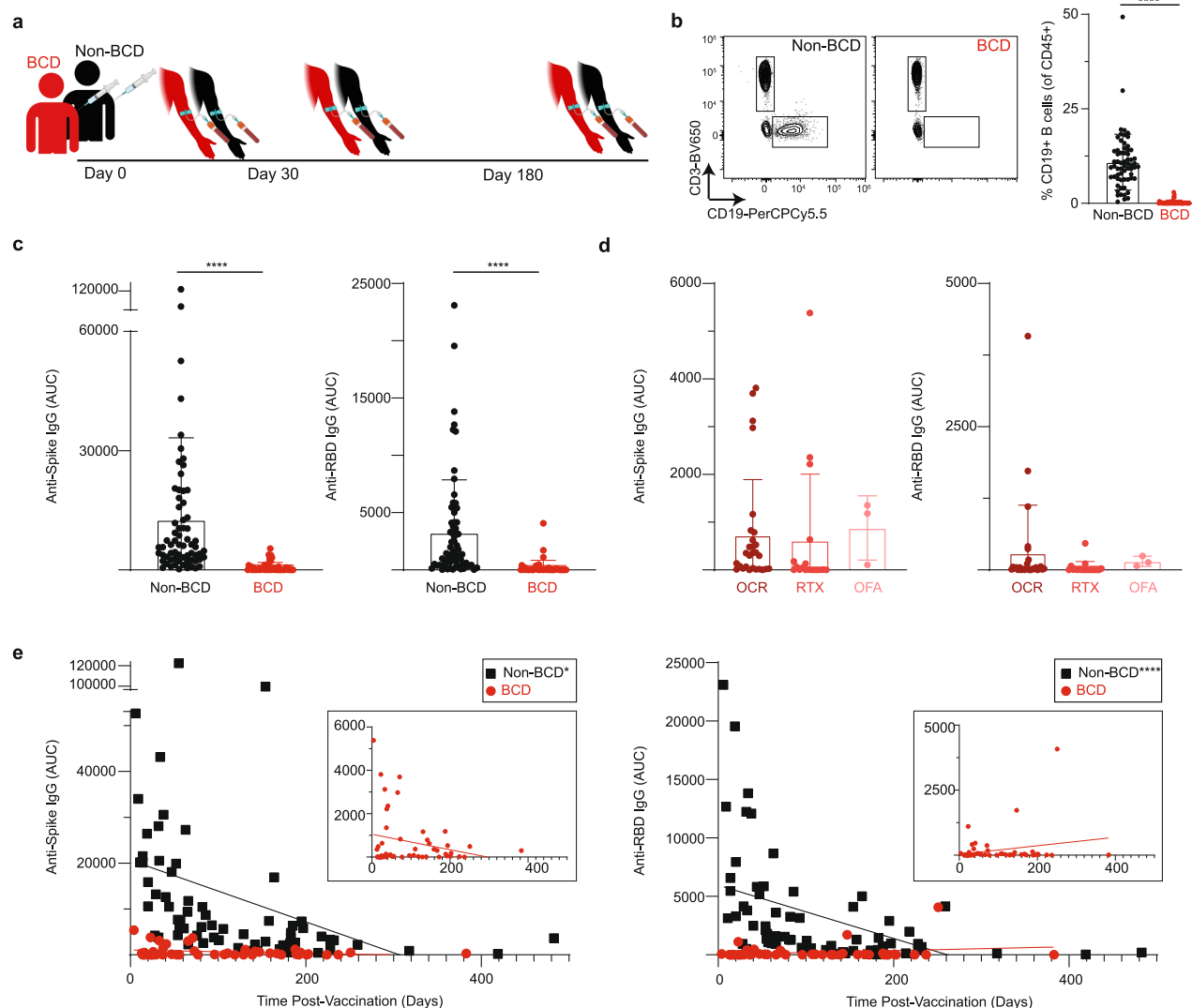
Fig. 1 | Long-term reduction of humoral responses to COVID-19 vaccines in participants with MS on BCD therapies. a Schematic of sample collection for participants with MS on non-BCD therapies (black) and BCD therapies (red) after initial two dose vaccine series. Samples were collected 4–483 days following completion of the vaccine series. b CD19+B cells in non-BCD (black) and BCD (red) participants shown in representative flow cytometry plots (left) and graphs of compiled data (right). c Area under the curve (AUC) of IgG specific for S (left) and RBD (right) in non-BCD (black) and BCD (red) participants. d Same as (c) for BCD participants taking various B cell depleting therapies. e Relationship of AUC of IgG specific for SARS-CoV-2 S (left) and RBD (right) with time post-vaccination in days for non-BCD (black) and BCD (red) participants. Inset graphs show results for only BCD participants. Statistics: (b–d) Bar plots represent data as mean ± standard deviation. b A t-test was used to compare the frequency of B cells in non-BCD and BCD participants. c, d A one-way analysis of covariance (ANCOVA) was used to evaluate the difference in antibody titers between cohorts after controlling for age, sex, vaccine type, and time-post vaccination. e Both Pearson's correlation and a linear model was used to investigate the relationship between antibody titers and time-post vaccination controlling for age, sex, and vaccine type. ****$p < 0.0001$, *$p < 0.05$ Abbreviations: BCD (B cell depleting/depleted), non-BCD (non-B cell depleting/depleted), S (Spike protein), RBD (receptor binding domain).

comparisons, there was no effect of sex, age, or vaccine type on the quantity of vaccine-induced immune memory between treatment cohorts (Supplementary Data 1).

To directly compare the response magnitude between vaccine doses in the compiled data, we needed to account for differences in the tests used to quantify vaccine induced antibody titers. We therefore normalized antibody titers to the percent maximum titer within each methodology (see methods). When controlling for age, sex, vaccine type, and time post-vaccination, we found that a third dose of an mRNA vaccine enhanced the humoral response in both BCD and non-BCD participants (Fig. 4a, b). However, the levels of both anti-S and anti-RBD antibodies remained lower in BCD participants compared to non-BCD participants regardless of dose number (Fig. 4a, b). In this larger cohort we identified a greater reduction of both anti-S and anti-RBD antibody levels in participants treated with RTX compared to the subcutaneously administered drug, OFA (Fig. 4a, b).

Together, these findings demonstrate the increased potency of RTX treatment in this participant population, and the clinical benefit of booster vaccines on the humoral response independent of age and sex, even in BCD participants.

All centers used the AIM assay to evaluate the quantity of S-reactive T cells (see methods). Here we found no increase in the quantity of S-reactive CD4+T cells following booster vaccine administration in either the BCD or non-BCD cohort (Fig. 4c). For S-reactive CD8+T cells, a booster vaccine enhanced their frequency in BCD, but not in non-BCD participants, and we confirmed the enhanced frequency of this subset in BCD participants compared to non-BCD participants after both two and three vaccine doses (Fig. 4d). When comparing the effects of various BCD therapies, we found that participants taking injectable OFA had significantly higher levels of CD4+T cells than participants taking intravenous OCR or RTX, but similar levels of CD8+T cells (Fig. 4c, d). These findings demonstrate that OFA may
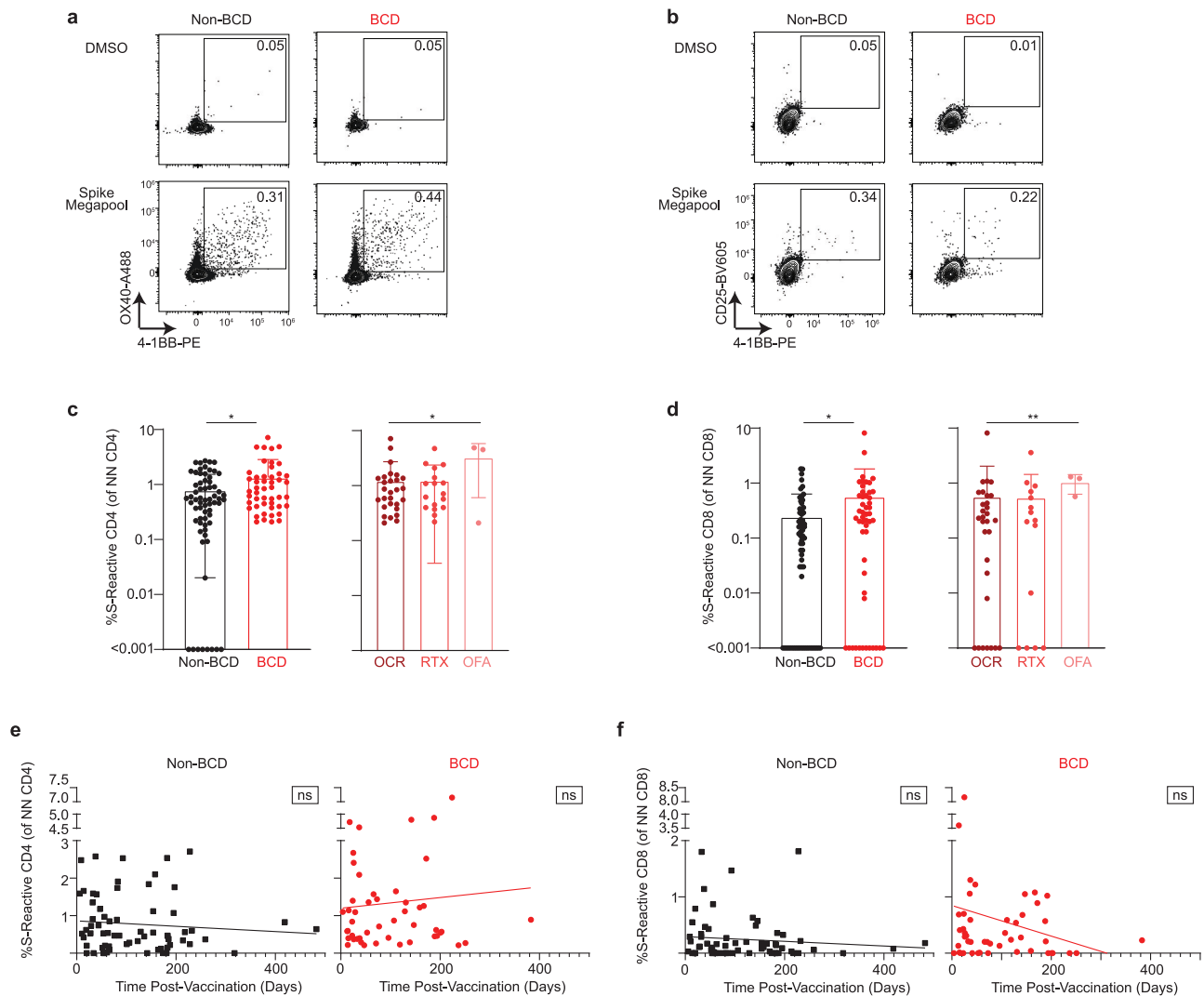
Fig. 2 | Enhancement of the T cell response to COVID-19 vaccines in BCD participants with MS. S-reactive T cells were identified following stimulation with S peptide pools using the activation-induced marker (AIM) assay (see methods). Representative flow cytometry plots of CD4+ (**a**) and CD8+ (**b**) T cells (see gating strategy Supplementary Fig. 1a) expressing activation markers after control DMSO stimulation (top) or S peptide stimulation (bottom) in non-BCD (left) or BCD (right) participants. S-reactive CD4+ (**c**) and CD8+ (**d**) T cells in non-BCD (black) vs BCD (red) participants (left) and BCD participants taking OCR (dark red), RTX (red), and OFA (pink) (right) shown as a percentage of non-naïve T cells in each site. **e** S-reactive CD4+ T cells over time post-vaccination for non-BCD (black, left) and BCD (red, right) participants. **f** Same as (**e**) for CD8+ T cells. Statistics: (**c**–**d**) Bar plots represent data as mean ± standard deviation and a one-way analysis of covariance (ANCOVA) was used to evaluate the difference in quantities of S-reactive T cells between cohorts after controlling for age, sex, vaccine type, and time-post vaccination. **e**–**f** A linear model was used to investigate the relationship between S-reactive T cell frequency and time post-vaccination controlling for age, sex, and vaccine-type. $^{**}p < 0.01$, $^{*}p < 0.05$, ns p $\geq$ 0.05 Abbreviations: BCD (B cell depleting/depleted), non-BCD (non B cell depleting/depleted), OCR (Ocrelizumab), RTX (Rituximab), OFA (Ofatumumab), S (Spike protein).

allow for a more favorable vaccine response compared to RTX or OCR and that a booster vaccine does not enhance the already substantial level of cellular immunity induced by a two-dose series.

Protective immunity in vaccinated MS participants

To investigate how the altered COVID-19 vaccine response of BCD participants affected clinical protection, we assessed the incidence of participant-reported COVID-19 infection within 280 days following completion of their initial two dose series. Reported infections were present in similar numbers of non-BCD (6 [6.2%]) and BCD participants (8 [8%]) ($p = 0.78$), and all reported infections were classified as mild, with only two requiring treatment (with monoclonal antibody therapy) and none requiring hospitalization. Many of these immunomodulated participants received booster vaccinations within the 9-month observation period and, on average, infection was reported 131 days (±81.5) after their last dose of vaccine. The low infection incidence in both cohorts indicates that the MS participants

with a negligible circulating SARS-CoV-2-specific antibody responses did not experience a greater infection rate or susceptibility compared to participants with MS with the full complement of vaccine-induced immunity. These results provide indirect evidence for the importance of T cell immunity in protection to SARS-CoV-2.

Discussion

We present here a multi-center longitudinal study of the induction and maintenance of humoral and cellular immunity following COVID-19 mRNA vaccines in blood from 217 individuals with MS, comparing responses in BCD-treated with non-BCD-treated participants. We reveal substantially impaired generation of S-specific antibodies following vaccination in BCD compared to non-BCD participants up to a year post-vaccination; by contrast, S-reactive memory CD4+ and CD8+ T cells were generated and maintained at a higher frequency in the BCD compared to the non-BCD cohort. Despite the absence of humoral immunity in BCD
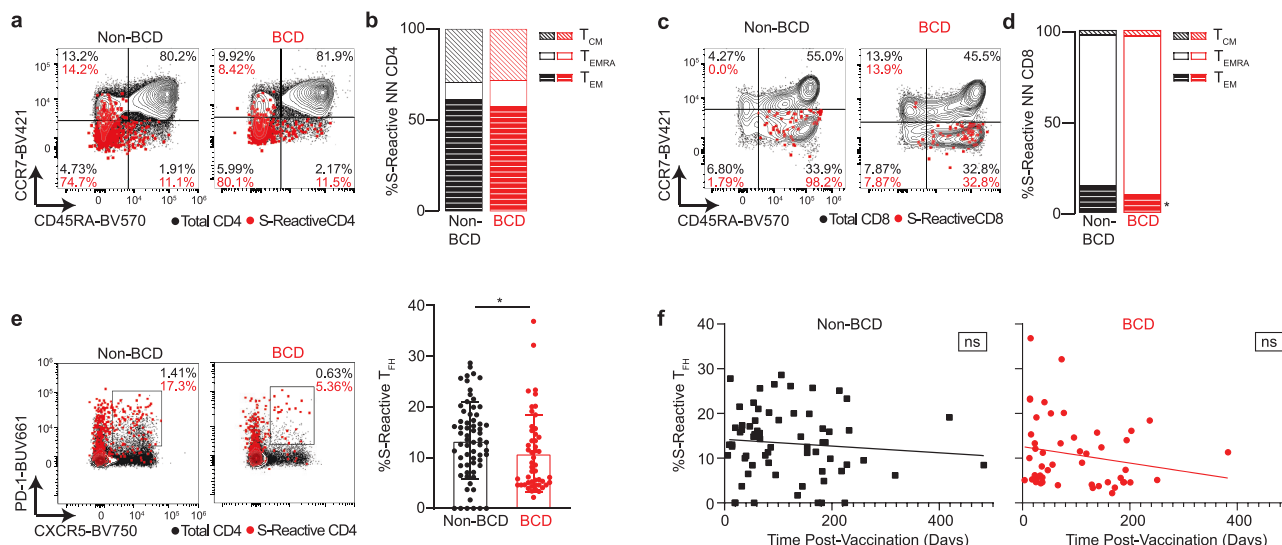
Fig. 3 | Variations in S-reactive T cell phenotypes in BCD participants with MS. **a** T cell subset phenotypes of S-reactive CD4+T cells shown in representative flow cytometry plots of total CD4+T cells (black contour) and S-reactive T cells (red dots) from non-BCD (left) or BCD (right) participants (**b**) or stacked bar plots of averaged data across all non-BCD (black) and BCD (red) participants. **c–d** Same as (A-B) for CD8+ cells. **e** Representative flow cytometry plots of total CD4+T cells (black contour) and S-reactive CD4+T cells (red dots) expressing T_{FH} markers PD-1 and CXCR5 in non-BCD (left) and BCD (right) participants (right) and compiled expression of S-specific T_{FH} shown as percentage of PD-1 and CXCR5 expressing S-reactive CD4+T cells. Bar plots represent data as mean ± standard deviation.

f Relationship of quantity of S-reactive T_{FH} with time post-vaccination in days for non-BCD (black, left) and BCD (red, right) participants. Statistics: (**b,d,e**) A one-way analysis of covariance (ANCOVA) was used to evaluate the difference in proportions of S-reactive T cell phenotypes between cohorts after controlling for age, sex, vaccine type, and time-post vaccination. **f** A linear model was used to investigate the relationship between S-reactive T_{FH} and time post-vaccination controlling for age, sex, and vaccine type. *$p < 0.05$, ns $p \geq 0.05$ Abbreviations: S (Spike protein), BCD (B cell depleting/depleted), non-BCD (non B cell depleting/depleted), T_{CM} (central memory T cells), T_{EMRA} (terminally differentiated effector memory T cells), T_{EM} (effector memory T cells), T_{FH} (T follicular helper cells).

participants, they experienced similar clinical protection from symptomatic infection as non-BCD participants. Together, these results reveal that cellular immunity comprises an important role in vaccine-induced immune protection and that vaccine-specific T cells in humans develop independently from vaccine-specific B cells.

It is well established that the level of anti-S antibodies induced by COVID-19 mRNA vaccines decline over time[5–7]. Here we also found that anti-S and anti-RBD antibodies waned over time in non-BCD-treated participants, while antibody levels were very low or undetectable in BCD participants over time post-vaccination. Nonetheless, antibody levels in both cohorts were slightly enhanced after booster vaccination, highlighting the utility of booster doses in the BCD population. Shorter time since last anti-CD20 treatment as well as lower cumulative exposure to BCD agents have been identified as predictors of a greater serologic response among BCD participants[24]. This small enhancement of antibody titers could thus be due to the timing of boosters between infusions, or BCD agents may not fully deplete B cells across lymphoid compartments, leaving tissue-localized B cells able to produce antibody even after multiple doses of vaccine. However, even with boosters, S-specific antibody titers in BCD participants remained very low overall.

Despite negligible B cell immunity, there was an enhanced frequency of vaccine-induced, S-reactive CD4+ and CD8+T cells in BCD compared to non-BCD participants—a finding also observed by others[21,23–28]. We show in our cohort that this T cell response is maintained for more than one year post-vaccination, which contrasts findings of reduced T cell immunity in blood post-vaccination in healthy (immune replete) individuals[8,40]. This enhanced memory T cell response in BCD subjects may be due to a BCD-induced increase in naïve T cells capable of responding to novel antigens[41]; however, we did not observe differences in numbers of naïve (or memory) T cells that could account for these distinct responses. Diminished B cell numbers can also affect antigen presentation for T cell priming. Early mouse studies showed efficient T cell priming in the absence of B cells[42]; however, reduced presentation of vaccine antigens by B cells may result in more T cell priming by other types of antigen presenting cells, such as dendritic cells

(DC) or by B cell subsets in lymph nodes not affected by BCD treatment. Vaccine-induced S-reactive T cells in BCD participants had lower frequencies of T_{FH}, also consistent with studies in mice that T_{FH} differentiation requires antigen presentation by B cells within follicles[43,44]. Our results therefore establish that robust generation and maintenance of memory T cells from COVID-19 mRNA vaccines can occur in the absence of B cells.

For the different BCD drugs examined here, we identified some variations in the vaccine response including reduced antibody production in RTX-treated participants and enhanced frequencies of S-reactive CD4+T cells in OFA-treated participants. These findings could result from the distinct formulation and dosing strategies for each drug, including the fraction of human immunoglobulin in OCR (humanized), OFA (fully human), and RTX (chimeric). Further, OFA binds to a distinct region of CD20[45], and is administered monthly with more rapid B cell repletion[15,46,47] while OCR and RTX are administered intravenously every six months. OFA is administered subcutaneously, which may allow the drug to better target the lymph nodes than intravenous infusions, which are less efficient on tissue B cells[48,49]. The unique timing of B cell repopulation and the extent of peripheral depletion by each drug should be considered when scheduling vaccinations in this population.

The humoral response is the protective correlate for many vaccines, including COVID-19 mRNA vaccines[3,4]. By contrast, the role of cellular immunity in vaccine-generated protection in humans is less understood for two main reasons: first, the contribution of T cells to protection cannot be fully assessed in the context of a robust antibody response and second, memory T cells often localize to tissues[50,51] and it can be difficult to evaluate their frequency from blood sampling. Here, we found that BCD participants lacking S-specific antibodies experienced similar low rates of infection with mild illness compared to their non-BCD counterparts having substantial antibody responses, consistent with the overall clinical observation that individuals on BCD therapies did not experience excess morbidity to SARS-CoV-2 infection[35]. Accordingly, in B cell-deficient mice, T cell immunity is sufficient to confer protection to SARS-CoV-2[52,53] and influenza virus infection[54,55]. Together, these findings provide compelling evidence for the
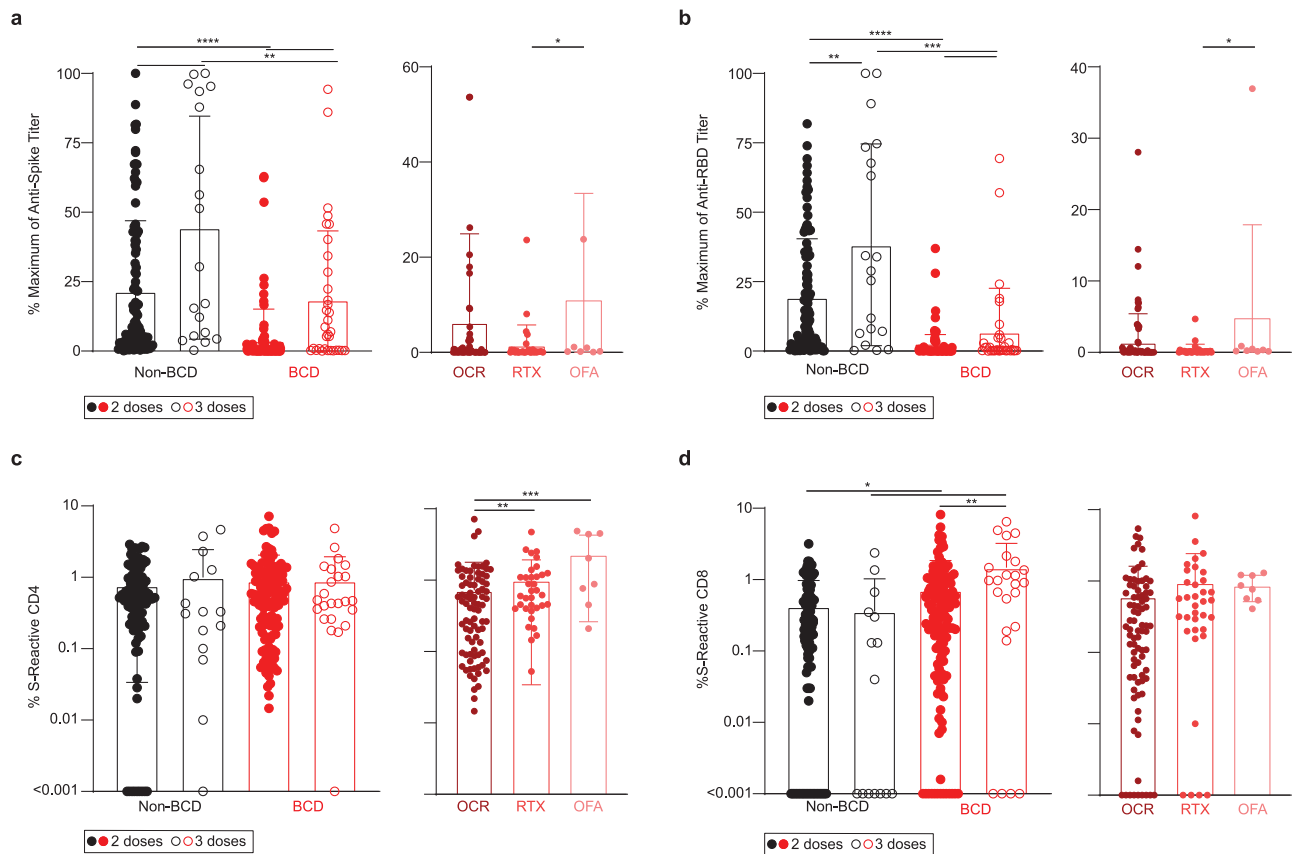
Fig. 4 | Role of dose and specific BCD drug in participants with MS from four academic centers. Quantification of the humoral and cellular response following two or three doses of COVID-19 vaccines in participants with MS compiled from four academic centers (see methods). **a–b** Quantity of anti-Spike and anti-RBD antibodies were normalized as a percent of the maximum of anti-Spike or anti-RBD antibody titer within each test method (see "methods"). **a** Normalized anti-Spike value is shown for non-BCD (black) and BCD (red) participants who have received two (closed circle) or three (open circle) doses of a COVID-19 vaccine (left) or BCD participants taking OCR, RTX, or OFA after two doses of the COVID-19 vaccine (right). **b** Same as (**a**) for anti-RBD antibodies. **c** Fraction of S-specific CD4$^+$T cells shown for non-BCD (black) and BCD (red) participants who have received two (closed circle) or three (open circle) doses of a COVID-19 vaccine (left) or BCD participants taking OCR, RTX, or OFA after two doses of the COVID-19 vaccine (right). **d** Same as (**c**) for CD8$^+$ T cells. Statistics: (**a–d**) Bar plots represent data as mean ± standard deviation and a one-way analysis of covariance (ANCOVA) was used to evaluate the difference in quantities of vaccine-induced immunity between cohorts after controlling for age, sex, vaccine type, and time-post vaccination. ****$p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, *$p < 0.05$ Abbreviations: BCD (B cell depleting/depleted), non-BCD (non B cell depleting/depleted), OCR (Ocrelizumab), RTX (Rituximab), OFA (Ofatumumab), S (Spike protein).

protective role of T cell immunity to SARS-CoV-2 in humans, that may also extend to other respiratory viruses.

The ability of T cells to protect in the absence of antibodies has implications for rapidly evolving strains such as SARS-CoV-2 and influenza which evade antibody neutralization. T cells recognize internal epitopes conserved between viral strains and have been shown in mouse influenza infection models to provide cross-strain protection[56,57]. In humans, SARS-CoV-2-specific T cells that can recognize multiple viral variants have been detected in the blood of vaccinated individuals[58], indicating their potential for robust protection against continuously evolving variant strains. It will be interesting to determine whether the increased T cell memory in BCD participants identified here is more cross-reactive and whether they will in fact, exhibit enhanced protection in the years to come.

Limitations of the study

This study examined the effects of BCD therapies on immune responses to vaccination in peripheral blood of individuals with MS. It was not possible to obtain lymphoid tissue (lymph nodes, spleen) where memory B and T cells are also maintained and data from peripheral blood may not be representative of tissues. The data on protection were based on clinical reporting and not on quantitative assessment of viral loads. The study was focused on MS patient cohorts and did not include other patient populations, such as individuals with certain lymphomas, that are also treated with BCD therapies.

Methods
Study design

Blood samples were collected from all participants following written, informed consent under Institutional Review Board approved protocols at Columbia University (#AAAT5674) and the University of Pittsburgh (STUDY19080007) and in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Participants with multiple sclerosis (MS) meeting 2017 MacDonald Criteria ages 18 to 70 were recruited at Columbia University MS Center and University of Pittsburgh prior to or after their initial two-dose COVID-19 mRNA vaccine series. Exclusion criteria included receipt of a SARS-CoV-2 vaccine other than an mRNA vaccine, pregnancy at time of enrollment or planning pregnancy during the 6-month period following vaccination, or additional immunotherapy for indication other than MS. Participants with a clinical history of COVID-19 infection prior to enrollment were excluded. Baseline demographic and clinical data such as age, gender, race, ethnicity, MS phenotype, disease modifying therapy, SARS-CoV-2 vaccine history (dates and vaccine type), and SARS-CoV-2 infection history were collected via REDCap. Clinical data were collected at the time of each sample collection. If participants reported a known clinical COVID-19 infection, subsequent samples were excluded from analysis. Incidence of confirmed COVID-19 infection within 9 months post-vaccination was recorded.

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Sample collection and processing

Sample collection was scheduled for 2–4 weeks and 6 months or more post mRNA vaccine series; all samples were collected between 1- 70 weeks post-vaccination. Blood was collected via venipuncture either at CUIMC or through home draws performed by ExamOne (up to 120 mL per visit) in heparinized tubes. Plasma supernatant was collected, and frozen and peripheral blood mononuclear cells were isolated using a Lymphoprep density gradient and frozen in Fetal Bovine Serum with 20% DMSO.

SARS-CoV-2 serology testing

Qualitative serology testing for the SARS-CoV-2 S and N protein was performed by the Center for Advanced Laboratory Medicine at Columbia University Irving Medical Center.

Enzyme-linked immunosorbent assays were used to determine the titers of SARS-CoV-2 S and RBD antibodies in participant plasma. Recombinant S protein (R&D Systems, cat # 11058-CV-100) was purchased and RBD protein from a SARS-CoV-2 Wuhan isolate (GenBank: MN908947.3) purified using the baculovirus expression system as described (Krammer et al., 2012; Margine et al., 2013) was generously provided by Dr. Florian Krammer (Mount Sinai School of Medicine). Ninety-six well plates were coated with these proteins, blocked with 3% milk (AmericanBio, cat # AB10109-01000) in DPBS containing 0.1% Tween 20 (Sigma-Aldrich, cat # P1379), incubated with heat-inactivated plasma in serial dilution, and then incubated with anti-human IgG horseradish peroxidase secondary antibodies as previously described[59]. Plates were developed and absorbance at 490 nm (OD_{490}) was measured using a BioTek 800 TS absorbance reader. Area under the curve was calculated for each sample using background normalized data.

Quantification of Spike-reactive T cells

Detection of S-reactive T cells was done by the AIM assay as described[37,40]. Briefly, single cell mononuclear suspensions isolated from blood samples prepared as above were plated in 96 well U-bottom plates (1×10^6 cells per well) in 200 μL of complete media (RPMI (Corning, cat # 10-040-CM), 10% inactivated human AB serum (Gemini, cat # 507533010) and penicillin-streptomycin-glutamate (Thermo Fisher Scientific, cat # 10378016)) and incubated overnight at 37 °C in 5% CO_2. Cells were then blocked for 15 min with anti-CD40 monoclonal antibody (0.5 μg/mL) (Miltenyi Biotec, cat # 130-094-133). Subsequently, a SARS-CoV-2 Spike (Wuhan strain) Megapool (S MP) (1 μg/mL), designed and synthesized as previously described[37], was added to the cell culture for 24 h. The S MP consists of 253 15-mer peptides overlapping by 10 residues and covering the entire S protein. As controls, additional cells were stimulated with an equimolar amount of DMSO or 25 μL/mL of Immunocult CD3/28 T Cell Activator. After 24 h, the cells were analyzed via flow cytometry.

Flow cytometry

For flow cytometry analysis of SARS-CoV-2 S-reactive T cells, stimulated cells were washed with FACS buffer (DPBS (Corning, cat # 20-030-CV) with 2% heat-inactivated FBS (GeminiBio, cat # 100–106) and 1 mM EDTA (Corning, cat # 46-034-CI)). Subsequently, fluorochrome-conjugated antibodies (see reporting summary) and dead stain eFluor780 (ThermoFisher Scientific, cat # 65-0865-18) in Brilliant Stain Buffer (BD Bioscience, cat # 566349) were added to cell suspensions for 20 min at RT protected from light, washed with FACS-buffer, centrifuged, and resuspended in fixation buffer (Tonbo, cat # TNB-0607-KIT) for 30 min at RT. S-specific $CD4^+$ T cells were identified based on expression of two or more of the following markers: CD40L, OX40, and 4-1BB using Boolean gating as described. S-specific CD8 T cells were identified by expression of CD25 and 4-1BB or CD25 and CD69 as done previously[59,60]. The quantity of S-specific T cells was calculated as the percent of non-naïve ($CCR7^+CD45RA^-$ or $CCR7^-CD45RA^-$ or $CCR7^-CD45RA^+$) $CD4^+$ or $CD8^+$ cells expressing the defined AIM markers following background subtraction of the percent of $CD4^+$ or $CD8^+$ T cells expressing these markers in the DMSO negative control well. S MP stimulated wells with fewer than 10 cells meeting these criteria were considered below the limit of detection. Flow cytometry data were collected using the five-laser Cytek Aurora flow cytometer and analyzed using FlowJo V10.7.1.

Meta-analysis of data from additional centers

Individualized data quantifying the humoral and cellular responses of Multiple Sclerosis participants from University of Pennsylvania[21] and University of California San Francisco[22] was generously provided by collaborators. Antibody titer data was normalized across centers by calculating the percent maximum titer within testing methodology (ELISA for University of Pennsylvania, Columbia University, and University of Pittsburg, and Luminex assay for University of California San Francisco).

Statistical analyses

Clinical and demographic variables reported in Table 1 were compared between treatment cohorts using the Mann-Whitney U test for continuous metrics and Fisher's exact test for categorical variables. Cell counts in Supplementary Fig. 1 were compared using the Mann-Whitney test. Pearson's correlation was used to investigate the relationship between the quantity of immune memory (anti-S antibodies, anti-RBD antibodies, S-reactive $CD4^+$ T cells, and S-reactive $CD8^+$ T cells) and time post-vaccination and time since last anti-CD20 treatment. These analyses were performed with GraphPad Prism software (v 8.4.3) To account for potentially confounding covariates including age, sex, and vaccine-type, a linear model was also used to investigate the association of these immune memory populations and time post-vaccination.

To compare the quantities of memory cell subsets as well as the phenotype of S-reactive T cells between treatment cohorts (BCD vs non-BCD), between specific drugs (ocrelizumab, rituximab, and ofatumumab), or before and after a booster vaccine, a one-way analysis of covariance (ANCOVA) was used. Age, sex, time post-vaccination, and vaccine type were included as covariates in the ANCOVA model. P-values from linear and ANCOVA models which are below 0.05 were considered to be significant. These analyzes were performed with R software (v 4.1.2.).

Sample-size estimation

The vast differences in antibody titers in BCD participants compared to non-BCD participants identified in previous studies preclude the need for a large sample size. Preliminary data from our center along with estimates of mean and variance from previous studies determined that a sample size of >64 allows us to detect $a \sim 1.6$ fold change in vaccine-responsive T cell frequencies between BCD and non-BCD participants with 80% power and a type-I error probability alpha = 0.05. We aimed to recruit as many vaccinated MS participants as possible in order to meet this sample size threshold while also being able to carefully exclude blood samples from participants with previous COVID-19 infection.

Blinding

For all data acquisition and flow cytometry data analysis, investigators were blind to treatment group.

Data availability

All new data generated in this study have been deposited at ImmPort (https://www.immport.org/home) with accession number SDY2869. All previously generated data can be accessed by contacting the authors of the published datasets[21,22]. No new code was generated in this study.

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Conceptualization: J.D.-P., C.T., C.A., Z.X., P.DJ., D.L.F., S.F.W., and R.S.F.; Formal Analysis: J.D.-P., C.T., and C.A.; Investigation: J.D.-P., C.A., and D.L.F.; Visualization: J.D.-P. and C.A.; Resources and Data Curation: Z.X., S.A., A.B.-O., R.B., D.E., S.F.B., DJ., M.K., K.O., A.R., J.S., E.W., EJ.W., L.Z., W.Z., P.DJ., S.F.W., R.S.F., and D.L.F.; Writing-Original Draft: J.D.-P, D.L.F., C.T., C.A., S.F.W., and R.S.F.; Writing-Review & Editing: All authors; Supervision: D.L.F., S.F.W., R.S.F.; Project Administration: D.L.F., S.F.W., and R.S.F.; Funding Acquisition: D.L.F., P.DJ., S.F.W., and R.S.F.

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Additional information
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