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Robust T-Cell Responses in Anti-CD20-Treated Patients Following COVID-19 Vaccination: A Prospective Cohort Study

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Background. Patients treated with anti-CD20 therapy are particularly at risk of developing severe coronavirus disease 2019 (COVID-19); however, little is known regarding COVID-19 vaccine effectiveness in this population.

Methods. This prospective observational cohort study assesses humoral and T-cell responses after vaccination with 2 doses of mRNA-based COVID-19 vaccines in patients treated with rituximab for rheumatic diseases or ocrelizumab for multiple sclerosis (n = 37), compared to immunocompetent individuals (n = 22).

Results. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific antibodies were detectable in only 69.4% of patients and at levels that were significantly lower compared to controls who all seroconverted. In contrast to antibodies, Spike (S)-specific CD4 T cells were equally detected in immunocompetent and anti-CD20 treated patients (85–90%) and mostly of a Th1 phenotype. Response rates of S-specific CD8 T cells were higher in ocrelizumab (96.2%) and rituximab-treated patients (81.8%) as compared to controls (66.7%). S-specific CD4 and CD8 T cells were polyfunctional but expressed more effector molecules in patients than in controls. During follow-up, 3 MS patients without SARS-CoV-2-specific antibody response had a mild breakthrough infection. One of them had no detectable S-specific T cells after vaccination.

Conclusions. Our study suggests that patients on anti-CD20 treatment are able to mount potent T-cell responses to mRNA COVID-19 vaccines, despite impaired humoral responses. This could play an important role in the reduction of complications of severe COVID-19.

Keywords. anti-CD20; COVID-19 vaccination; multiple sclerosis; rheumatoid arthritis; T-cell response.

In patients with immune-mediated rheumatic diseases (RD) and multiple sclerosis (MS), immunosuppressive drugs and in particular anti-CD20 therapy are associated with an increased risk of severe coronavirus disease 2019 (COVID-19) [1–3]. Although generally identified as priority groups for vaccination, these patients were not included in pivotal studies evaluating the efficacy of COVID-19 vaccines and their effectiveness in this population is still unknown. Anti-CD20 treatment depletes B cells and impairs antibody responses to classical vaccines [4–6]. Several studies have now confirmed reduced antibody levels and seroconversion rates in anti-CD20 treated patients following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [7] and COVID-19 vaccination, irrespective of the underlying disease [8–11]. Although antibodies are likely to play a critical role in preventing infection, recovery from COVID-19 in patients with X-linked agammaglobulinemia suggests that antibodies are not mandatory to overcome disease [12]. T cells may also be involved in protection against COVID-19 [12–14] and memory T cells are readily detectable several months after infection [15]. As B cells could play a role as antigen-presenting cells to naive T cells, the question remains as whether B-cell depleted patients could still mount functional T-cell responses to COVID-19 vaccines, which may provide some level of protection against severe disease.

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The aim of our study was thus to characterize and compare T-cell responses to mRNA-based COVID-19 vaccines between patients with rheumatic diseases and multiple sclerosis treated with anti-CD20 therapy and immunocompetent controls.

METHODS

Study Design and Approval

We included individuals ≥ 18 years of age scheduled to receive the COVID-19 vaccine or having received ≤ 2 COVID-19 vaccine doses in the last 5 weeks. Subjects with SARS-CoV-2 documented infection <3 months prior to inclusion or ongoing signs of febrile or nonfebrile infection were excluded. Further study details are found in Supplementary Methods.

Study Approval

This prospective observational study was conducted at the Geneva University Hospitals (HUG), Switzerland, according to the principles of good clinical practice and was approved by the Geneva Cantonal Ethics Commission (2021-00430). Informed consent was obtained from all participants.

Immunological Read-Outs

Antibodies were measured in sera using the Elecsys Anti-SARS-CoV-2 nucleoprotein (anti-N total antibodies) and Anti-SARS-CoV-2 Spike (anti-receptor binding domain [RBD] total antibodies) on the Cobas e801 analyzer (Roche Diagnostics, Switzerland). Seroconversion was defined as > 0.8 IU/mL for the anti-RBD antibodies and > 1 cutoff index (COI) for anti-N antibodies as previously reported [16]. For the AIM assay, PBMC were stimulated with 1 µg/mL of SARS-CoV-2 megapool peptides (15-mer peptides overlapping by 10 amino acids spanning the entire S-antigen, n = 253) or in dimethyl sulfoxide (DMSO) (negative controls). For intracellular cytokine production, Brefeldin A (Golgiplug, BD) was added to the culture overnight. Cells were stained with specific antibodies and analyzed by flow cytometry (see Supplementary Methods for further details). Percentage of S-specific T cells, AIM^+, cytokine $^+$ or granzyme B $^+$ cells were calculated by subtracting the value of the corresponding DMSO stimulation control samples.

Statistics

Statistical analysis was performed in GraphPad Prism software (version 8.0.2). Kruskal-Wallis test with Dunn multiple comparisons was used (unless otherwise stated in the figure legends) and categorical variables were compared using Fisher exact test (3 × 2). Correlation analyses were performed using Spearman test. *P* values from correlations were corrected for multiple comparisons using the false discovery rate method, and $P < .05$ was considered statistically significant.

RESULTS

Clinical Characteristics of Participants

In order to assess the effect of B-cell depletion on vaccine-induced T-cell responses, we studied a total of 37 patients treated with either ocrelizumab (n = 26) for multiple sclerosis (MS) or rituximab (n = 11) for rheumatic diseases (RD) compared to 22 age-matched immunocompetent controls (for details see Table 1). Most patients with RD were treated for rheumatoid arthritis (n = 7) and had more comorbidities compared to MS patients or controls. Mean age was balanced between groups. Regarding concomitant medication, 5/11 RD patients received another immunosuppressor (mainly methotrexate or corticosteroids), whereas all MS patients were treated with ocrelizumab only. Hence, this cohort is likely to reflect the effect of short- to long-term treatment with anti-CD20 on vaccine response in the absence of other major confounding factors, in particular age and concomitant immunosuppressive drugs. The interval between last anti-CD20 treatment and first vaccine dose was shorter in patients under ocrelizumab (median 24.9 weeks) than in patients treated with rituximab (median 42 weeks), which explained the absence or low (<2%) percentage of CD19-positive B cells among total PBMC at time of vaccination, especially in ocrelizumab-treated patients (Supplementary Figure 1). Most patients (34/37, 91.9%) and a majority of controls (15/22, 68.2%) had no history of SARS-COV-2 reverse transcription polymerase chain reaction (RT-PCR)-confirmed infection (review of medical record) prior to vaccination (Table 1) nor a positive anti-nucleoprotein serology (measured 30 days after vaccination, Figure 1A).

Reduced Humoral Responses in Anti-CD20 Treated Patients Following COVID-19 Vaccination

Participants were vaccinated either with 2 doses of BNT162b2 (Pfizer/BioNTech; n = 13) or mRNA-1273 (Moderna, n = 46) COVID-19 mRNA vaccine at 28-day intervals (median 28 days, interquartile range [IQR] = 0). Immune responses were measured 30 days after the second dose and in a subset of participants (n = 20) at time of first vaccination (Supplementary Figure 2). The level of antibodies specific to the anti-receptor binding domain (RBD) of the SARS-CoV-2 spike (S) protein was significantly lower in both anti-CD20 treated patient populations as compared to controls, irrespective of the vaccine used (geometric mean: 5371 U/mL in controls, 69.3 U/mL in rituximab- and 8.3 U/mL in ocrelizumab-treated patient, Figure 1A). Although all controls had seroconverted 30 days after vaccination and regardless of their history of COVID-19, significantly fewer anti-CD20 treated patients had detectable anti-RBD antibodies ($P = .001$), with a higher seropositivity rate in patients treated with rituximab (8/11; 72.7%) compared to ocrelizumab (16/26; 61.5%). RD patients with undetectable antibodies had all received concomitant treatment with methotrexate or corticosteroids. As expected, antibody levels

Table 1. Demographic and Clinical Patient Characteristics

	Healthy Controls	MS Patients	RD Patients
n	22	26	11
Female, n (%)	15 (68.2)	14 (53.8)	7 (63.6)
Age, median [IQR]	54.5 [43.5, 58.8]	45.6 [39.8, 52.7]	58.0 [46.4, 64.6]
Comorbitities,[a] n (%)	3 (13.6)	6 (23.1)	7 (63.6)
History COVID-19 (RT-PCR), n (%)	3 (13.6)	0	2 (18.2)
Positive anti-N serology, n (%)	4 (18.2)	1 (3.8)	0 (0)
Vaccine, n (%)			
BNT162b2	5 (22.7)	3 (11.5)	5 (45.5)
mRNA-1273	17 (77.3)	23 (88.5)	6 (54.5)
Neurologic disease, n (%)	…		…
PPMS		3 (11.5)	
RRMS	…	21 (80.8)	…
SPMS	…	2 (7.7)	…
Rheumatologic disease, n (%)	…	…	
Rheumatoid arthritis	…	…	7 (63.6)
CTD	…	…	3 (27.3)
Vasculitis	…	…	1 (9.1)
Anti-CD20 therapy, n (%)[b]			
Ocrelizumab (600 mg)	…	26 (100)	…
Rituximab	…	…	11 (100)
500 mg	…	…	1 (9.1)
1000 mg	…	…	5 (45.5)
1500 mg	…	…	1 (9.1)
2000 mg	…	…	4 (36.4)
Time between last treatment and 1st vaccine dose, weeks [IQR]	…	24.9 [17.3, 26.4]	42.0 [30.6, 58.7]
Other treatment, n (%)	…		
Corticosteroids		0	2 (18.2)
Methotrexate	…	0	4 (36.4)
Leflunomide	…	0	1 (9.1)

Abbreviations: COVID-19, coronavirus disease 2019; CTD, connective tissue disease; IS, immunosuppressive treatment IQR, interquartile range; PPMS, primary progressive multiple sclerosis; RD, rheumatic disease; RRMS, relapsing remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.

[a]Comorbidities: chronic lung disease, diabetes, hypertension, obesity, depression, cardiovascular disease.

[b]The dose mentioned is the total dose that the individual received in around 2 weeks.

in patients correlated with level of circulating CD19-positive B cells (measured at day 30 after vaccination, Figure 1B), which were lower in patients on ocrelizumab due to a more recent treatment. There was no correlation between age and antibody response. The three patients with a known history of COVID-19 or with detectable anti-N antibodies did not have higher antibody responses as compared to those unexposed (Figure 1A, Supplementary Figure 2*A*).

Robust T-Cell Responses in Anti-CD20 Treated Patients Following COVID-19 Vaccination

T-cell immunity against SARS-CoV-2 is thought to play a role in protection against severe disease [14]. To assess if mRNA vaccines could elicit T-cell responses in our patient cohort as reported for healthy individuals [17, 18], we stimulated PBMC collected 30 days after the second vaccine dose with a pool of peptides covering the S-protein [19] and identified S-specific T cells using the activation-induced marker (AIM) assay. S-specific $OX40^+$ $41\text{-}BB^+$ CD4 T cells were equally induced in immunocompetent and anti-CD20 treated patients (Figure 1C), with a high frequency of responders (85–91%). S-specific $CD69^+$ $41BB^+$ CD8 T cells were detectable at similar levels in all groups (Figure 1E); however, there was a statistically significant higher response rate found in ocrelizumab- (96.2%; 25/26) and rituximab-treated patients (81.8%; 9/11) compared to controls (66.7%; 14/21, $P = .02$). Previous history of COVID-19, the type of mRNA vaccine, and time since last anti-CD20 treatment had no impact on the level of S-specific T cells, and results were similar when participants with an history of COVID-19 were excluded from the analysis (data not shown). Interestingly, there was a weak inverse correlation between the magnitude of S-specific CD8 T cells and anti-RBD antibody responses considering all participants (R = -0.32, $P = .049$, Figure 1F, not significant for CD4 T cells, Figure 1D). Finally, the higher frequency of patients with AIM-positive CD8 T cells compared to controls was probably not due to higher levels of pre-existing cross-reactive T cells: in a subset of previously uninfected patients (n = 13), S-specific AIM-positive + CD4 and CD8 T cells were undetectable at time of first vaccination (Supplementary Figure 2*B*, 2*C*).

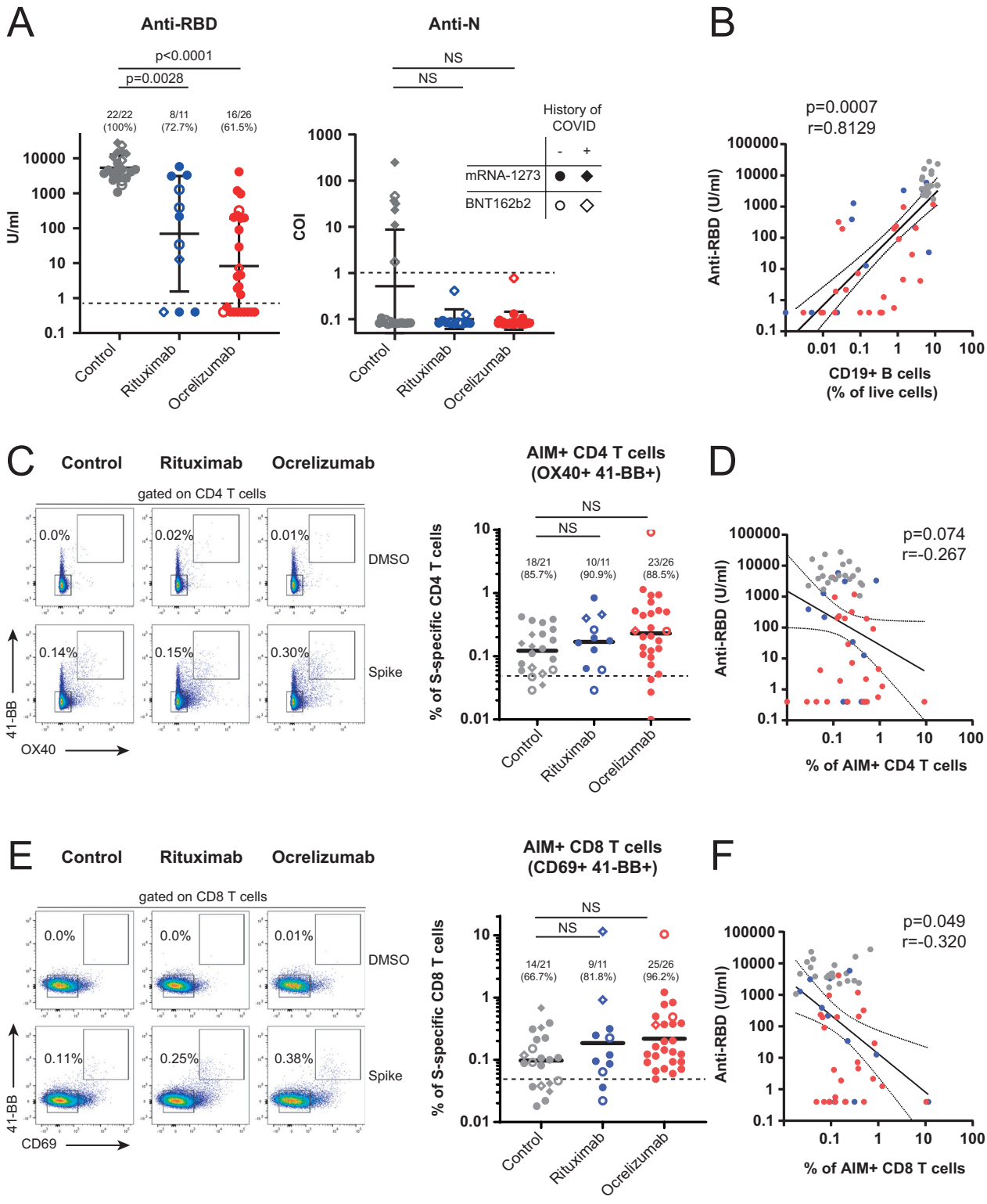

Figure 1. SARS-CoV-2 mRNA vaccination induces antigen-specific CD4 and CD8 T cells in Rituximab and Ocrelizumab-treated patients. *A*, Levels of anti-SARS-CoV-2 N and RBD total Ig measured in sera of healthy controls (n = 22), rituximab (n = 11) and ocrelizumab-treated patients (n = 26) 30 days after the second dose of BNT162b2 (open symbol) or mRNA-1273 (closed symbol) COVID-19 mRNA vaccines. Dotted line indicates cut-off for seropositivity: anti-RBD; 0.8 U/mL; anti-N: 1 COI. *B*, Spearman correlations of anti-RBD antibodies and frequency of CD19-positive B-cells in all patients (n = 58) (*C*, *E*) Representative flow cytometry plots of CD4 (C) and CD8 (E) T cells after PBMC stimulation with DMSO (negative control) and S-peptide pool 30 days after the second vaccination. S-specific AIM-positive T cells are gated as OX40$^+$ 41-BB$^+$ for CD4 T cells (*C*) or CD69$^+$ 41-BB$^+$ for CD8 T cells (*E*). Individual data are represented on the right-hand panel with geometric mean. The dotted line represents the limit of detection. Percentages of responders (those with level above limit of detection) are indicated (Fisher's test 3 × 2 $P = .02$). (*D*, *F*) Correlation of anti-RBD total antibodies and AIM-positive CD4 (D) and CD8 (F) T cells in all patients (n = 58). Abbreviations: COI, cut-off index; COVID-19, coronavirus disease 2019; DMSO, dimethyl sulfoxide; Ig, immunoglobulin; NS, not significant; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

S-Specific T-Cell Responses Are Polyfunctional

We then assessed the functionality of antigen-specific T cells to understand if the quality of T-cell responses is altered in the absence of B cells. The mRNA COVID-19 vaccines are known to predominantly induced Th1 CD4 T cells expressing interleukin (IL)-2, interferon (IFN)-γ, and the transcription factor Tbet rather than Th2 (IL-13$^+$, GATA3$^+$) or Th17 (IL-17$^+$, ROR γ T$^+$) cells [20]. First, we observed that a majority of S-specific AIM-positive CD4 T cells expressed Tbet in all patients and controls and found that some ocrelizumab-treated patients had a higher percentage of GATA-3-positive T cells, however not reaching statistical significance at the group level (Figure 2A). Next, we used intracellular cytokine staining to evaluate if S-specific T cells express several cytokines, given that polyfunctional T cells are often associated with improved vaccine-induced protection to viral infection. We found that anti-CD20 treated patients had similar level of S-specific CD4 T cells expressing at least 2 of the markers IL-2, tumor necrosis factor (TNF)-α, IFN-γ, or granzyme B as compared to controls, suggesting a similar polyfunctionality (Figure 2B). The frequency of S-specific CD4 T cells producing IL-2 and IL-2$^+$ TNF- α$^+$ in both ocrelizumab and rituximab-treated patients was higher, however, as compared to immunocompetent controls, although the percentage of CD4 T cells expressing IFN-γ alone or in combination with other cytokines was similar (Figure 2C and Supplementary Figure 3*A*). There were no detectable IL-13 or IL-17-expressing CD4 T cells (Supplementary Figure 3*A*).

We next assessed whether S-specific CD8 T cells were also functional in our patient cohorts. S-specific CD8 T cells expressing either IL-2 or IFN-γ were detected in more patients treated with anti-CD20 than in controls (Figure 3A). The percentage of IL-2-expressing cells was significantly higher ($P = .013$) in ocrelizumab-treated patient as compared to controls, although only a trend was observed for IFN-γ ($P = .07$) and for rituximab-treated patients for both cytokines. Similar to CD4 T cells, patients on anti-CD20 treatment had polyfunctional S-specific CD8 T cells, with a trend for more cells expressing at least 3 markers and significantly more single IL2$^+$ cells than controls (Figure 3B, 3C). In general, a higher frequency of S-specific CD8 T cells co-expressing granzyme and cytokines were found in patients as compared to controls.

Finally, we assessed the memory phenotype of S-specific AIM-positive CD4 and CD8 T cells and did not find any difference between groups (Supplementary Figures 3 and 4). CD8 T cells had predominantly an effector memory phenotype (CD45RA$^-$ CCR7$^-$), whereas CD4 T cell phenotypes were equally effector (CD45RA$^-$ CCR7$^-$) and central memory (CD45RA$^-$ CCR7$^+$).

Altogether, our data suggest that S-specific T cells induced by mRNA vaccines have a similar functional and memory profile but express more effector molecules in anti-CD20 treated patients as compared to controls.

Observed Breakthrough Cases in MS Patients Treated With Ocrelizumab

Up to end of October 2021, 3 COVID-19 breakthrough cases with the Delta Variant were self-reported in the entire cohort of Ocrelizumab-treated MS patients. Two of the patients were already included in this interim analysis (2 females, aged between 40 and 50 years). None of the 3 patients had humoral immune responses at 1 month after the second vaccine dose (Supplementary Figure 5*A*) or at the time of COVID-19 infection. The 3 patients had low CD19-positive B-cell counts (at d60 post-vaccination) but not particularly in the lower range of B cell levels measured in the entire patient cohort. Both female patients had detectable SARS-CoV-2 specific CD4 or CD8 T-cell responses at day 60, with a polyfunctionality similar to the other participants in the same group (Supplementary Figure 5 *B*–5*E*, red and blue stars). Upon diagnosis of the 3rd patient (male, 33 years old), we performed T-cell analyses on the bio-banked sample collected one month after the second dose. No SARS-CoV-2-specific T-cell responses were detected (Supplementary Figure 5*B* and 5*C*, square), and the patient is further referred to as "nonresponder." The interval between last vaccine dose and infection varied between 10 weeks (nonresponder) and 20 weeks, respectively 24 weeks for the other 2 patients. Clinical symptoms were mild in all 3 patients, and both responders received monoclonal anti-SARS-CoV-2 antibodies (REGN-COV2) in the days following diagnosis. The nonresponder was treated only after 2 weeks with REGN-COV2, when he presented with persistent symptoms to outpatient clinics and was still PCR-positive and SARS-CoV-2-seronegative. All patients recovered quickly.

DISCUSSION

Immunosuppressed patients are at higher risk of developing a severe COVID-19 [1–3] but were not included in pivotal studies evaluating the efficacy of COVID-19 mRNA vaccines. Therefore, there is an urgent need to decipher humoral and cellular immune responses induced by mRNA vaccines in these populations. In this study, we assessed the impact of B-cell depletion on S-specific T-cell responses induced by the BNT162b2 (Pfizer/BioNTech) or mRNA-1273 (Moderna) COVID-19 mRNA vaccines in rituximab- and ocrelizumab-treated patients.

Patients under anti-CD20 therapy had lower levels of RBD-specific antibodies as compared to controls, in line with what was reported by others and as expected from experience with other vaccines [10, 11, 21]. In general, more patients treated with Rituximab had higher CD19-positive B-cell counts than patients treated with ocrelizumab, likely due to a longer interval between last treatment and vaccination in the RD group. However, some patients developed RBD-specific antibodies and these responses correlated with levels of circulating CD19-positive B cells measured at day 30 after vaccination but not

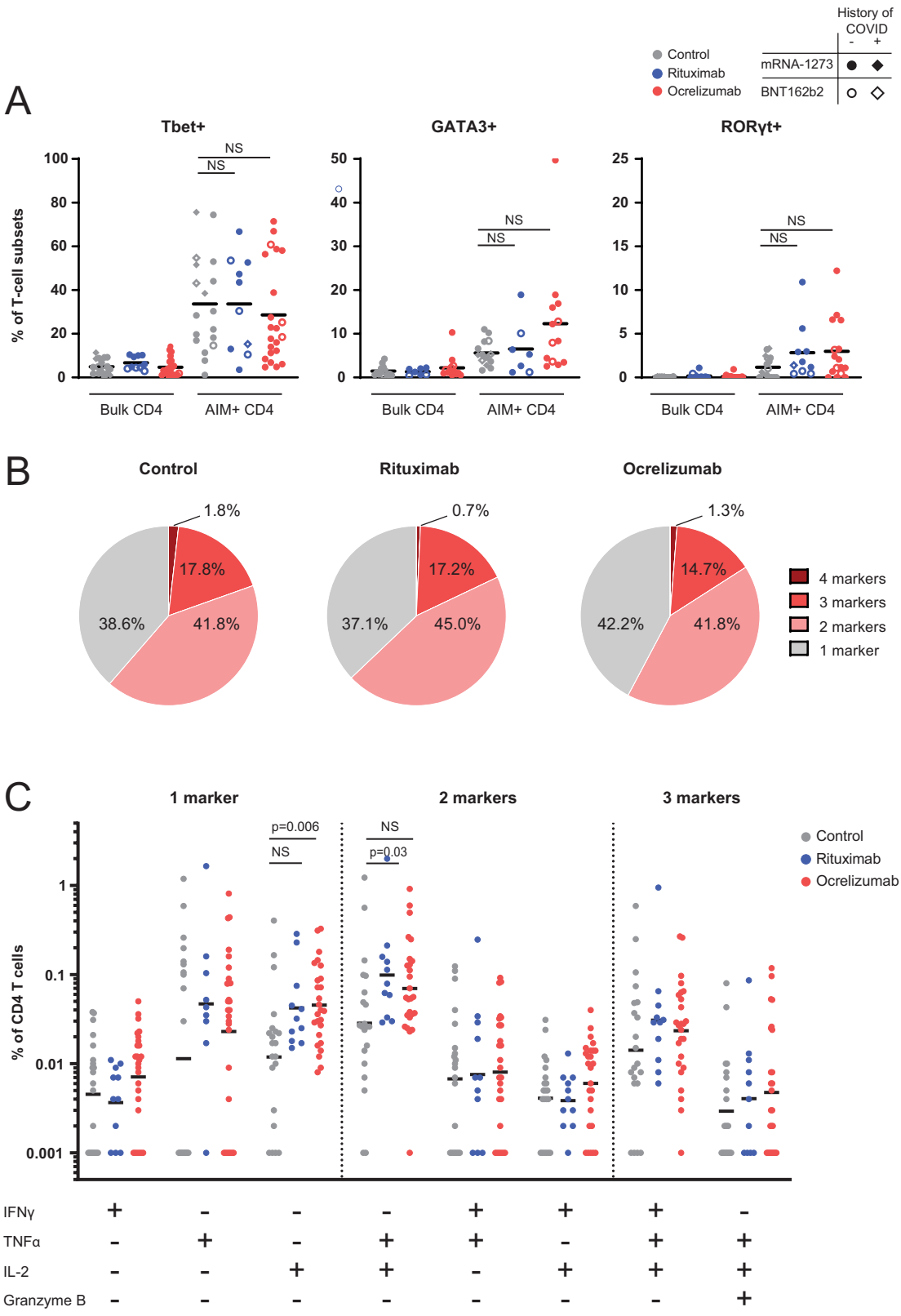

Figure 2. S-specific CD4 T-cell vaccine responses are polyfunctional in patients treated with anti-CD20. *A*, Expression of Tbet, GATA3, and RORγt in nonspecific CD4 T cells ("bulk") and AIM-positive S-specific CD4 T cells of healthy controls (n = 14–18), rituximab-treated patients (n = 7-10), and ocrelizumab-treated patients (n = 13–22) after stimulation with peptide pool. Analyses were restricted to individuals with detectable AIM-positive + CD4 T cells. *B*, Pie chart showing polyfunctionality of S-specific CD4 T cells of healthy controls (n = 21), rituximab-treated patients (n = 11) and ocrelizumab-treated patients (n = 26). Proportions of CD4 T cells expressing 1, 2, 3, or 4 of the activation markers IL-2, TNF-α, IFN-γ, or Granzyme B after peptide pool stimulation are shown. *C*, Individual data of S-specific CD4 T cells expressing different combination of markers (in % of total CD4 T cells, background subtracted). Abbreviations: COVID, coronavirus disease; IFN, interferon; IL, interleukin; NS, not significant; TNF, tumor necrosis factor.

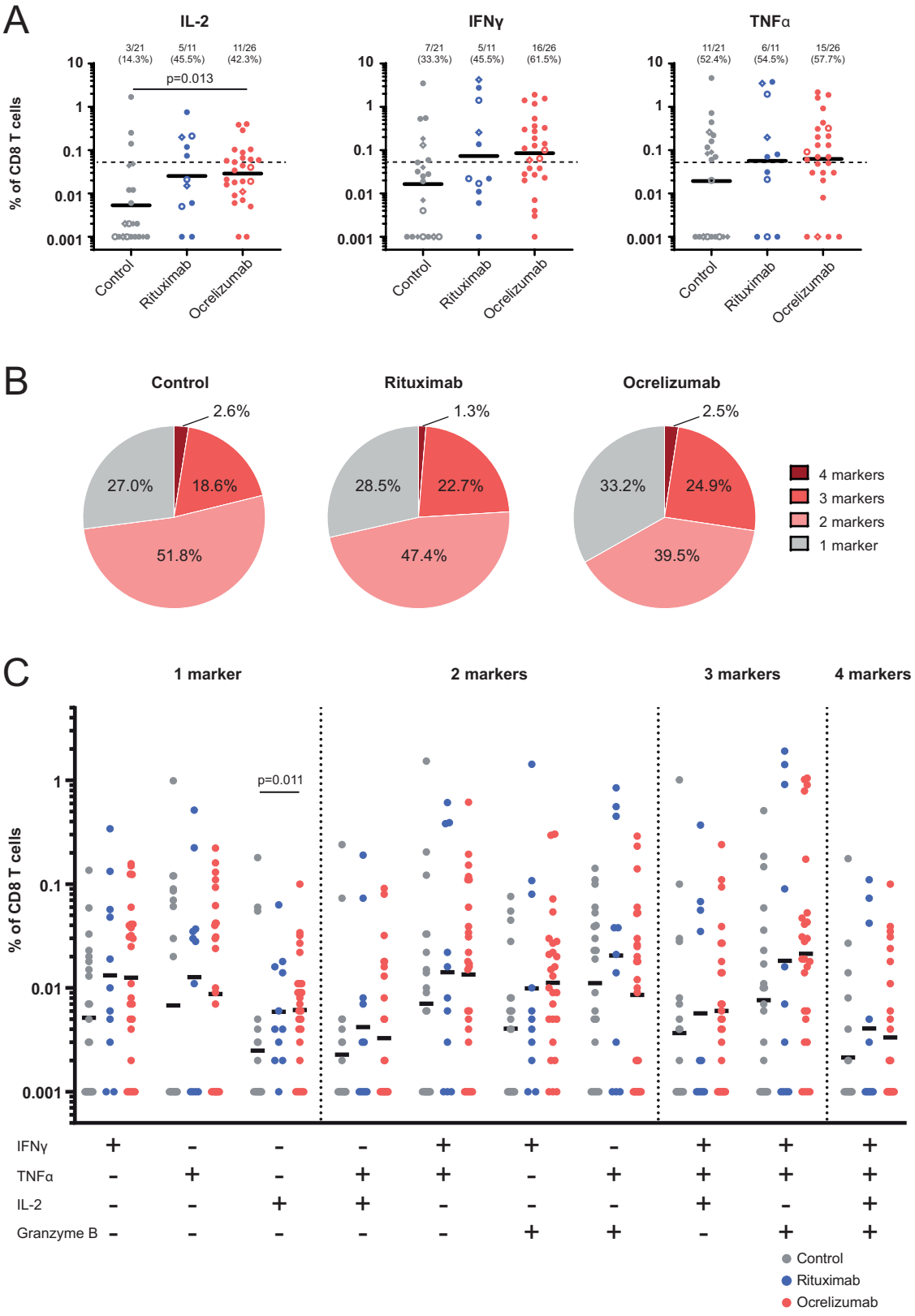

Figure 3. S-specific CD8 T cells express more effector molecules in patients treated with anti-CD20 as compared to controls. *A*, Expression of S-specific CD8 T cells expressing IFN-γ, IL-2, or Granzyme B in healthy controls (n = 21), rituximab-treated patients (n = 11) and ocrelizumab-treated patients (n = 26) upon stimulation with peptide pool (background subtracted). *B*, Pie chart showing polyfunctionality of S-specific CD8 T cells shown as proportions of CD8 T cells expressing 1, 2, 3, or 4 of the effector molecules IL-2, TNF- α, IFN- γ, or Granzyme B after peptide pool stimulation. *C*, Individual data of S-specific CD8 T cells expressing different combination of markers (in % of total CD8 T cells, background-subtracted). Abbreviations: IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

with time since last treatment (data not shown). It is unclear at this stage whether the level and neutralizing capacities of the antibodies in those patients who seroconverted will be sufficient to prevent infection or severe COVID-19.

We found that patients under anti-CD20 therapy with a known history of COVID-19 or with detectable anti-N antibodies did not have higher antibody responses as compared to those unexposed. This suggests that on anti-CD20 treatment, previous exposure to SARS-CoV-2 does not provide an advantage in terms of humoral vaccine response, in contrast to what we (Figure 1A) and others [22] observed in immunocompetent individuals.

Although it is not yet demonstrated that T-cell immunity against SARS-CoV-2 plays a direct role in protection against severe disease in humans (but in animals [14]), it may provide some level of protection to vaccinated patients under anti-CD20 treatment despite their limited antibody response. The number and functionality of T cells is generally maintained after treatment with B-cell targeting drugs, although depletion of some $CD20^+$ T cells, an increase in memory and loss of terminally differentiated CD4 T cells have been reported [23, 24].

Despite lower antibody responses, anti-CD20 treated patients mounted robust S-specific CD4 and CD8 - (T-cell responses) following COVID-19 mRNA vaccination, a finding similar to a recent study measuring pan-T-cell responses by IFN-γ-ELISpot in rituximab-treated patients [25]. In line with our results, SARS-CoV-2-specific CD4 and CD8 T cells are also detectable in exposed family members and in convalescent patients with asymptomatic/mild COVID-19 who remained seronegative [26].

Surprisingly, patients on anti-CD20 therapy developed strong S-specific CD8 T-cell responses and presented higher response rates compared to controls. The level of S-specific CD8 T cells was inversely associated with anti-RBD antibody responses. One hypothesis to explain higher T-cell activation in those patients could be the presence of more activated APCs (eg, monocytes) at time of vaccination as a result of B-cell depletion [27]. Interestingly, in hematologic cancer patients treated with anti-CD20, a greater number of CD8 T cells is associated with improved COVID-19 survival, despite impairment in humoral immunity, and 77% of patients had detectable SARS-CoV-2-specific T-cell responses [28]. Additionally, it has been recently shown that robust and functional CD8 T-cell responses are elicited already one week after the BNT162b2 prime vaccination when neutralizing antibodies are only weakly detected [29] and at a time when protective effect of COVID-19 mRNA vaccines can be observed [30, 31]. The question remains whether the strong CD8 T-cell responses elicited in anti-CD20 treated patients would be sufficient to prevent severe COVID-19. All 3 seronegative patients who presented with a breakthrough COVID-19 infection had mild symptoms only and were rapidly and successfully treated with monoclonal anti-SARS-CoV-2 antibodies (REGN-COV2) as part of our institutional protocol. The patient without detectable T cells had persistent symptoms and viral load, although earlier treatment with antibodies of the two other patients does not allow a fair comparison between these breakthrough cases. Data in larger breakthrough cohorts are needed to establish a potential role of T cells in preventing severe or prolonged diseases in vaccinated anti-CD20 treated patients.

Patients on anti-CD20 had polyfunctional S-specific CD4 and CD8 T cells, with more T cells producing at least 2 or 3 cytokines. In addition, the frequency of S-specific CD4 and CD8 T cells producing IL-2 in patients under anti-CD20 therapy was higher as compared to controls. Finally, rituximab- and ocrelizumab-treated patients developed antigen-specific memory T-cell responses similar to immunocompetent controls. CD8 T cells had predominantly an effector memory phenotype, whereas CD4 T-cell phenotypes were equally effector and central memory, as described by others following infection [15]. This suggest that in addition to generating polyfunctional T cells, it is likely that mRNA vaccines can generate long-lasting memory T cells in patients treated with anti-CD20 as shown for healthy individuals [32]. This will be confirmed in a follow-up of the current study by looking at the persistence of the cellular response in these patients at 6 and 12 months postvaccination.

Limitations of our study include the small sample size and the short follow-up after vaccination. We also were not able to correlate T-cell findings with clinical protection as the study was not designed to measure efficacy, which is pivotal in the identification of vaccine-responders and the indication for a potential third vaccine dose. Strengths of our study is the prospective design and the ability to evaluate 2 patient populations under anti-CD20 therapy who have different underlying conditions that do both not greatly affect other axes of immune responses, such as in poly-immunosuppressed patients, or those suffering from lymphoma or leukemia.

In summary, our study suggests that patients with anti-CD20 treatment are able to mount potent T-cell responses to mRNA COVID-19 vaccines similar to immunocompetent controls. Although patients treated with anti-CD20 treatment have decreased humoral responses to mRNA COVID-19 vaccines, elicited T-cell memory response could reduce complications of SARS-CoV-2 infection in this vulnerable population.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. A. S. is a consultant for Gritstone, Flow Pharma, CellCarta, Arcturus, Oxfordimmunotech, and Avalia. L. J. I. has filed for patent protection for various aspects of vaccine design and identification of specific epitopes. A. F. reports support from Pfizer for an unrelated project concerning safety analyses of JAK-inhibitors and serving on Advisory Board/DSMB for Pfizer for JAK-inhibitor therapy (tofacitinib). A. D. reports honorarium for lecture paid to the University from Roche; serving on Advisory Board for Speranza and a consultant for Lubrizol; and serving as Chairman for WHO Technical Advisory Group (TAG) on Emergency Use Listing of COVID-19 vaccines. C.-A. S. reports receiving research grant on novel adjuvants from Sanofi Pasteur; research grant on COVID-19 infection immunity from Swiss National Research Foundation; ADVAC course from Bill and Melinda Gates Foundation; reports honoraria for educational vaccinology event from Merck; has served on advisory board on Pertussis vaccines for Bionet Asia. K. L. reports consulting fees from Pfizer to their institution; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events to their institution from Pfizer, Viatris, and Celltrion. All other authors report no potential conflicts.

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