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Rituximab Impairs B Cell Response But Not T Cell Response to COVID-19 Vaccine in Autoimmune Diseases

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Objective. Antibody response to the messenger RNA (mRNA) COVID-19 vaccine has been shown to be diminished in rituximab (RTX)–treated patients. We undertook this study to compare humoral and T cell responses between healthy controls, patients with autoimmune diseases treated with RTX, and those treated with other immunosuppressants, all of whom had been vaccinated with 2 doses of the mRNA COVID-19 vaccine.

Methods. We performed anti-spike IgG and neutralization assays just before and 28 days after the second BNT162b2 (Pfizer-BioNTech) vaccine dose. The specific T cell response was assessed in activated CD4 and CD8 T cells using intracellular flow cytometry staining of cytokines (interferon-γ, tumor necrosis factor, and interleukin-2) after stimulation with SARS–CoV-2 spike peptide pools.

Results. A lower proportion of responders with neutralizing antibodies to the vaccine was observed in the RTX group (29%; n = 24) compared to the other immunosuppressants group (80%; n = 35) ($P = 0.0001$) and the healthy control group (92%; n = 26) ($P < 0.0001$). No patients treated with RTX in the last 6 months showed a response. Time since last infusion was the main factor influencing humoral response in RTX-treated patients. The functional CD4 and CD8 cellular responses to SARS–CoV-2 peptides for each single cytokine or polyfunctionality were not different in the RTX group compared to the other immunosuppressants group or the control group. In RTX-treated patients, the T cell response was not different between patients with and those without a humoral response.

Conclusion. RTX induced a diminished antibody response to the mRNA COVID-19 vaccine, but the functional T cell response was not altered compared to healthy controls and autoimmune disease patients treated with other immunosuppressants. Further work is needed to assess the clinical protection granted by a functionally active T cell response in the absence of an anti-spike antibody response.

INTRODUCTION

Immunosuppressed patients have experienced an increased mortality rate during the COVID-19 pandemic. Particularly, patients with autoimmune diseases treated with rituximab (RTX) have an odds ratio of death of 4.04 (95% confidence interval 2.32–7.03) (1). The global COVID-19 pandemic is starting to be controlled by countries having benefited from mass vaccination. Unfortunately, RTX treatment that increases the risk of death in patients with autoimmune diseases also diminishes the immune response to the COVID-19 vaccine, with only ~40% of patients showing detectable humoral response (2–4). Other treatments such as mycophenolate mofetil (MMF) and steroids have also been shown to diminish antibody response to COVID-19 vaccine (5). There is still controversy regarding the T cell response, with preliminary studies showing preserved T cell response among patients treated with RTX (3,4,6,7),

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while others have shown that the T cell response was impaired upon RTX treatment (8). Notably, those studies used either interferon-γ (IFNγ) release assay bulk measurement (6–8) or IFNγ enzyme-linked immunospot (ELISpot) assays (4,5). These techniques can detect an overall T cell response to IFNγ only but do not have high sensitivity and lack the fine-tuning provided by the characterization of the production of multiple cytokines by specific T cells.

The objective of this study was to assess humoral and cellular responses (using cytokine production of CD4 and CD8 T cells) to the messenger RNA (mRNA) COVID-19 vaccine in a population of patients with autoimmune diseases treated with RTX and to compare them to healthy controls and patients treated with other immunosuppressive or immunomodulatory agents.

PATIENTS AND METHODS

Study patients. Consecutive patients with autoimmune diseases treated in a single tertiary rheumatology center, the National Reference Center for Rare Systemic Autoimmune Diseases, were included between January and March 2021. Patients were divided into 2 groups: 1) patients who had received an RTX infusion in the previous year, and 2) patients who had been treated with other immunosuppressive or immunomodulatory agents. Healthy controls were age- and sex-matched to the included patients.

All patients and controls provided informed consent. The study was approved by the Ethics Committee "CPP Sud Méditérrannée" (no. 2020-A00509-30). All patients received a BNT162b2 (Pfizer-BioNTech) vaccine injection on days 0 and 28, according to local guidelines at the time. Participants with a detectable response against the SARS–CoV-2 nucleocapsid at any time were excluded, as it is a hallmark of previous SARS–CoV-2 infection. Humoral response was assessed on day 28 and 1 month after the second dose, on day 56. The cellular response was assessed on day 56.

Patient and public involvement. Due to the urgency and time constraints of such a study, patients were not involved in the design, conduct, or reporting of data. They will be involved in the research dissemination plan, where results will be provided to patient advocacy groups and findings applied during therapeutic education sessions.

SARS–CoV-2 serology. Elecsys anti–SARS–CoV-2 and Elecsys anti–SARS–CoV-2 S immunoassays (Roche Diagnostics) were used for the qualitative detection of total antibodies to nucleocapsid protein and the quantitative determination of antibodies to the spike protein receptor-binding domain (RBD), respectively. In this assay, both neutralizing and non-neutralizing anti-spike antibodies were detected. Anti-nucleocapsid antibodies were considered detected when the assay index result was >1 unit; the quantification range of anti-spike antibodies was 0.4–250 units. Results <0.4 units were considered nonreactive, and anti-spike titers higher than the quantification range were expressed as 250 units.

Surrogate virus neutralization assay. The iFlash-2019-nCoV neutralizing antibody assay (Shenzhen YHLO Biotech) allows for the quantitative determination of total anti-spike antibodies able to block the interaction between soluble angiotensin-converting enzyme 2 and RBD peptides (neutralizing antibodies) coated on microparticles, in an automated immunoassay format. Surrogate neutralizing antibody titers are expressed in IU/ml. According to the manufacturer, results ≥24 IU/ml are considered reactive.

T cell response. We analyzed the T cell response in samples collected 1 month after the second vaccine dose. The percentages of cytokine-secreting cells among activated T cells (CD4+CD154+ and CD8+CD137+) were assessed using frozen human peripheral blood mononuclear cells (PBMCs) from immunized patients. Cytokine production of T cells was assessed after stimulation with peptide pools spanning the wild-type sequence of SARS–COV-2 spike. Briefly, PBMCs were thawed and rested for 1 hour in complete medium (RPMI 1640 GlutaMax [Gibco] supplemented with 10% fetal calf serum, 1% penicillin/streptomycin, and 2% HEPES 1 m*M* [Ozyme]) at 37°C in a CO_2 incubator. Live PBMCs were then counted and stimulated at 1×10^6 per ml with the PepMix SARS–COV-2 spike glycoprotein (JPT Peptide Technologies). Two pools of 15-mer peptides overlapping by 11 amino acids were used for the stimulation of PBMCs (spike 1 domain [S1] and spike 2 domain [S2]) at 2 μg/ml for 18 hours at 37°C in a 5% CO_2 incubator. Brefeldin A (Sigma) was added to the PBMCs at 5 μg/ml 2 hours after the beginning of the incubation. Control cells were treated with phorbol 12-myristate 13-acetate (62 ng/ml; Sigma) and ionomycin (720 ng/ml; Sigma) (positive control), or with complete medium only (unstimulated). After 2 washing steps with phosphate buffered saline (PBS) 1× (Lonza), PBMCs were stained with a viability marker (Live/Dead Near-IR; ThermoFisher) for 20 minutes at 4°C.

Next, PBMCs were fixed and permeabilized for 20 minutes using a Cytofix/Cytoperm kit according to the guidelines of the manufacturer (BD Biosciences). PBMCs were then stained with a panel of antibodies targeting surface markers and cytokines: CD3 BV605, CD8 BV771, CD4 BV421, CD154 PercPCy5.5, CD137 PEDazzle594, tumor necrosis factor (TNF) PECy7, IFNγ allophycocyanin, perforin fluorescein isothiocyanate (all from BioLegend), and granzyme B Alexa Fluor 700 (BD Bioscience), in Perm/Wash buffer supplemented with 10 μl of Fc blocking reagent (Miltenyi) and 10 μl of Brilliant Buffer Plus (BD Biosciences) for 30 minutes at 4°C. Finally, PBMCs were washed in Perm/Wash buffer and resuspended in PBS 1% paraformaldehyde (Sigma) until sample acquisition on a Fortessa Flow Cytometer (BD Biosciences). Data were analyzed using FlowJo software, version 10. Percentages of activated cytokine-secreting CD4 and CD8 T cells were obtained for each of the S1 and S2 pools. For comparison to the nonstimulated condition, values of the S1 and S2 peptide pools were summed. For comparison between groups, the percentage of the

Table 1. Demographic and clinical characteristics of the subjects*

	Controls (n = 26)	RTX-treated patients (n = 24)	Patients treated with other immunosuppressants (n = 35)
Female	20 (78)	22 (92)	23 (66)
Age, median (range) years	60 (26–97)	62 (42–91)	64 (28–94)
Diagnosis			
Rheumatoid arthritis	–	12 (50)	14 (40)
Sjögren's syndrome	–	9 (38)	6 (17)
Myositis	–	1 (4)	1 (3)
SLE	–	1 (4)	3 (8)
Vasculitis	–	0 (0)	2 (6)
GCA	–	0 (0)	3 (8)
PMR	–	0 (0)	1 (3)
Systemic sclerosis	–	1 (4)	2 (6)
Psoriatic arthritis	–	0 (0)	1 (3)
Ankylosing spondylitis	–	0 (0)	1 (3)
Renal transplant recipient	–	0 (0)	1 (3)
Treatment			
Glucocorticoids	–	5 (21)	15 (43)
Mean dose (mg/day)	–	2.9	3.1
RTX			
Time between last infusion of RTX and first vaccine injection, median (range) days	–	162 (0–295)	–
DMARDs			
MTX	–	10 (42)	12 (34)
bDMARDs			
TNFi	–	0 (0)	4 (11)
Tocilizumab	–	0 (0)	3 (9)
Tofacitinib	–	0 (0)	1 (3)
Belimumab	–	0 (0)	1 (3)
Other immunosuppressants			
MMF	–	1 (4)	7 (20)
Bendamustine	–	1 (4)	0 (0)
Gammaglobulinemia (<8 gm/liter)	–	3 (12.5)	–

* Except where indicated otherwise, values are the number (%) of subjects. RTX = rituximab; SLE = systemic lupus erythematosus; GCA = giant cell arteritis; PMR = polymyalgia rheumatica; DMARDs = disease-modifying antirheumatic drugs; MTX = methotrexate; bDMARDs = biologic DMARDs; TNFi = tumor necrosis factor inhibitors; MMF = mycophenolate mofetil.

nonstimulated condition was subtracted from each stimulated peptide pool. The plotted percentages were the sum of percentages of the S1 and S2 pools.

Statistical analysis. Continuous variables are expressed as the mean ± SD. Categorical variables were compared using Fisher's exact test, correlations were assessed using Spearman's test, continuous variables were compared using the Mann–Whitney U test, and multiple comparisons of continuous variables were performed using the Kruskal-Wallis test. Analyses were performed using GraphPad Prism, version 9.

RESULTS

Patient characteristics. Two patients in the control group, 2 in the RTX group, and 2 in the other immunosuppressant group were excluded because of positive anti-nucleocapsid antibodies, which indicate previous SARS–CoV-2 infection. Thus, we included 26 controls and 59 patients with autoimmune diseases (24 in the RTX group and 35 in the other immunosuppressant group) (Table 1).

Delayed and diminished antibody and neutralizing response in RTX-treated patients. On day 28, there was a significantly diminished anti-spike antibody response in both the RTX group (mean ± SD 16.64 ± 52 units/ml) and the other immunosuppressant group (26.75 ± 58 units/ml) compared to the control group (83.79 ± 92 units/ml) (Figure 1A). This highlights a significantly delayed response among most autoimmune disease patients. On day 56, one month after the second vaccine injection, only the RTX group (mean ± SD 69 ± 110 units/ml) had lower levels of anti-spike antibody response compared to healthy controls (235 ± 58 units/ml) and those in the other immunosuppressant group (180 ± 100 units/ml) (Figure 1B). The neutralization assay on day 56 showed that the RTX group (mean ± SD 480 ± 1,064 IU/ml) had much lower levels of neutralizing

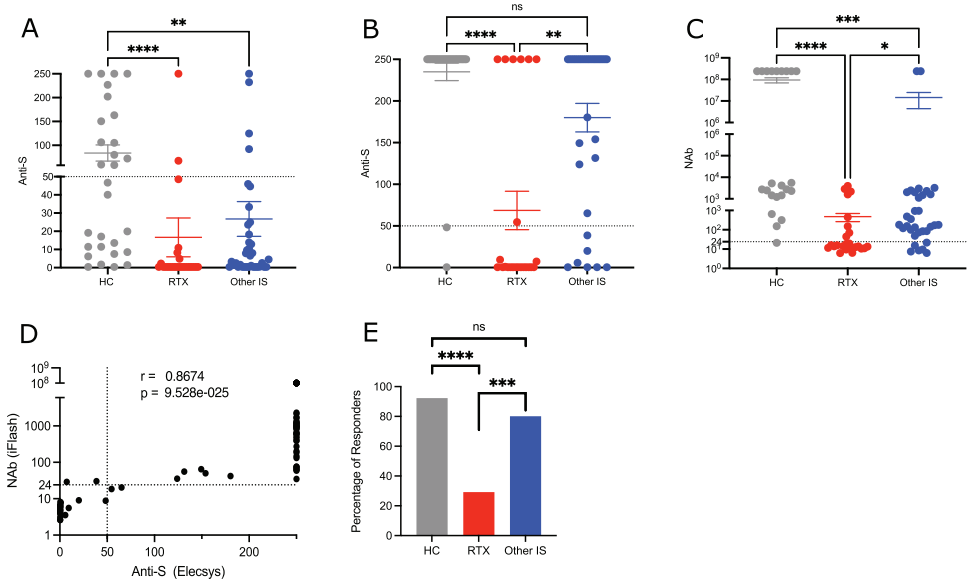

Figure 1. Humoral response after mRNA vaccination against COVID-19. **A** and **B**, Anti-spike (anti-S) antibody response on day 28 (n = 90) (**A**) and day 56 (n = 87) (**B**) after the first injection with the BNT162b2 (Pfizer-BioNTech) vaccine, in healthy controls (HC), rituximab (RTX)–treated patients, and patients treated with other immunosuppressants (IS). **C**, Neutralizing antibody (Nab) response on day 56 after the first injection with the BNT162b2 vaccine (n = 80). **D**, Correlation between anti-spike antibody and neutralizing antibody titers that were used to set the threshold of response. **E**, Percentages of responders, defined as subjects with an anti-spike antibody level of ≥50 units/ml (n = 78). In **A–C**, symbols represent individual subjects; bars show the mean ± SD. In **A–D**, the dotted line indicates the cutoff value. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$. NS = not significant. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.42058/abstract.

antibodies compared to healthy controls ($9.4 \times 10^8 \pm 1.2 \times 10^9$ IU/ml; $P < 0.0001$) and the other immunosuppressant group ($1.5 \times 10^8 \pm 5.8 \times 10^8$ IU/ml; $P = 0.042$) (Figure 1C). The other immunosuppressant group also had significantly reduced levels of neutralizing antibodies compared to healthy controls ($P = 0.001$) (Figure 1C).

There was a strong correlation between neutralizing antibody titers and anti-spike antibody titers (r = 0.87, $P < 0.0001$) (Figure 1D). Ninety-seven percent of patients with detectable neutralizing antibodies (>24 IU/ml) had an anti-spike concentration >50 units/ml. Antibody responders were defined as such if they met this anti-spike concentration threshold, indicating that they were very likely to have detectable neutralizing antibodies. On day 56, 29.2% of patients in the RTX group were responders, compared to 80% in the other immunosuppressant group ($P = 0.0001$) and 92.3% in the healthy control group ($P < 0.0001$) (Figure 1E).

Impact of B cell depletion on anti-spike response among RTX-treated patients. Demographic factors and cumulative dosing of RTX did not influence antibody responses, as shown in Supplementary Table 1 (available on the *Arthritis & Rheumatology* website at https://onlinelibrary.wiley.com/doi/10.1002/art.42058). However, we identified that in RTX-treated patients, significantly more time had passed since the last infusion for responders (mean ± SD 233 ± 48 days) compared to nonresponders (106 ± 93 days) (Figure 2A). No patient who received an infusion in the last 6 months showed a response. There was also a strong correlation between the number of B cells and the anti-spike antibody response (Figure 2B).

Preservation of functional specific T cell response in RTX-treated patients compared to healthy controls and patients treated without RTX. We analyzed the T cell response using percentages of cytokine-secreting cells among

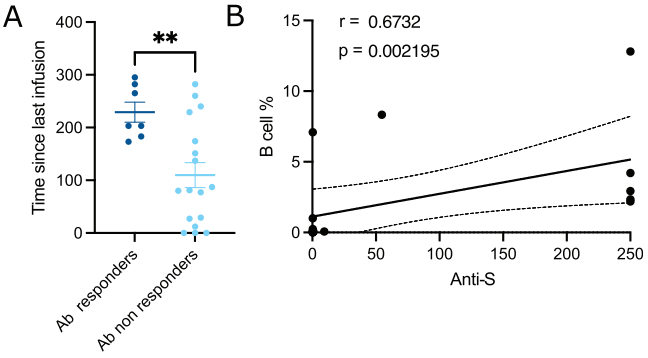

Figure 2. Factors influencing the humoral response in the RTX-treated group. **A**, Time between the last infusion of RTX and the first vaccination in responders and nonresponders. Symbols represent individual subjects; bars show the mean ± SD. **B**, Correlation between percentage of B cells and anti-spike antibody (Ab) response (n = 24). ** = $P < 0.01$. See Figure 1 for other definitions. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.42058/abstract.

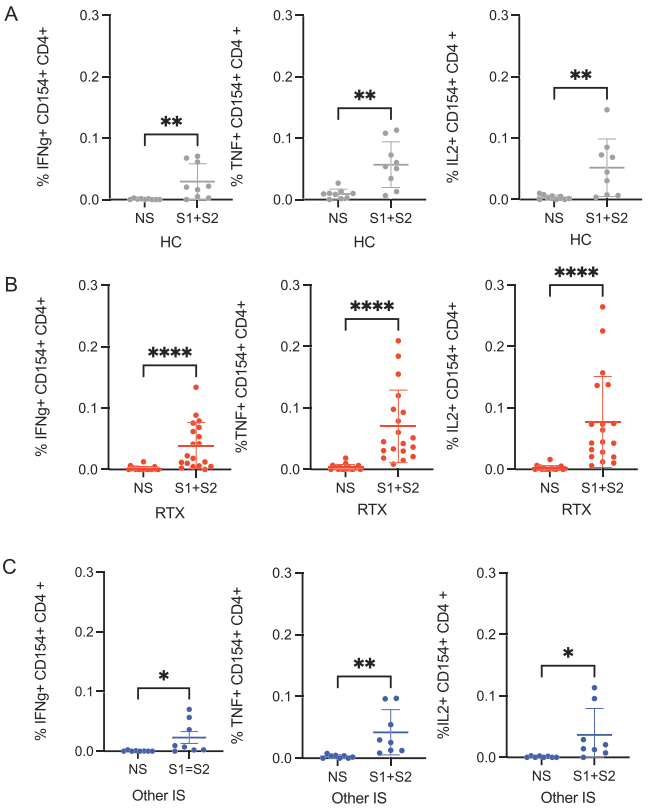

Figure 3. Comparison of unstimulated (NS) and spike peptide–stimulated cytokine secretion in CD4 T cells. In peripheral blood mononuclear cells from healthy controls (HC) (n = 9) (**A**), rituximab (RTX)–treated patients (n = 19) (**B**), and patients treated with other immunosuppressants (IS) (n = 8) (**C**), the T cell response was measured as the percentage of activated CD154+ T cells secreting cytokines (interferon-γ [IFNγ], tumor necrosis factor [TNF], and interleukin-2 [IL-2]) following stimulation with spike peptides (S1 + S2) or in unstimulated conditions. Values in the stimulated samples are the sum of the percentages of cells stimulated by the S1 and S2 pools. Symbols represent individual subjects; bars show the mean ± SD. * = $P < 0.05$; ** = $P < 0.01$; **** = $P < 0.0001$.

activated T cells (CD4+CD154+ and CD8+CD137+) in 9 healthy controls, 19 RTX-treated patients, and 8 patients treated with other immunosuppressants. In all groups, the percentage of CD4+CD154+ T cells producing IFNγ, TNF, and interleukin-2 (IL-2) were significantly higher in response to anti-spike peptide pools (S1 + S2) compared to the unstimulated condition (Figure 3). This validated the specificity of the T cell response detected by our assay. The specific anti-spike CD8 T cell response was significantly different compared to the unstimulated condition for all 3 cytokines in the RTX group, 2 cytokines (IFNγ and TNF) in the healthy control group, and only TNF in the other immunosuppressant group (Supplementary Figure 1, available on the *Arthritis & Rheumatology* website at https://onlinelibrary.wiley.com/doi/10.1002/art.42058). T cell response was not influenced by the age of the participants (data not shown).

Between the 3 groups, there was no difference in the CD4 T cell response in cells secreting IFNγ, TNF, or IL-2 (Figure 4A). Similarly, cytokine-secreting activated spike-specific CD8 T cells were not different between the healthy controls (n = 9), RTX-treated patients (n = 19), and patients treated with other immunosuppressants (n = 8) (Figure 4B). We did not observe any specific granzyme B or perforin response.

Finally, we studied the ability of the CD4 and CD8 T cells to secrete multiple cytokines upon stimulation with spike peptides.

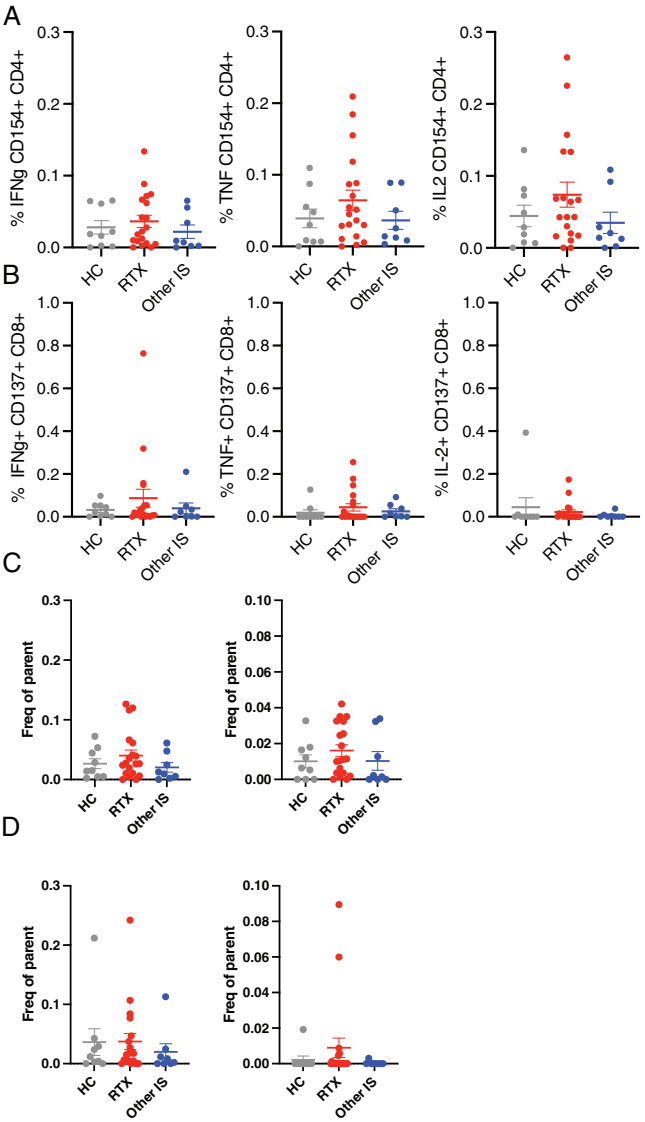

Figure 4. Comparison of specific CD4 and CD8 T cell responses between groups. **A,** Percentage of activated CD154+ cytokine-secreting cells among CD4 T cells. **B**, Percentage of activated CD137+ cytokine-secreting cells among CD8 T cells, minus the percentage among CD8 T cells in the unstimulated condition. Percentages in the stimulated samples are the sum of the percentages of cells stimulated by the S1 and S2 pools. **C** and **D**, Percentages of activated CD154+ cells among CD4 T cells (**C**) and activated CD137+ cells among CD8 T cells (**D**) that secreted 2 cytokines (left) or 3 cytokines (right) at the same time (polyfunctionality). Symbols represent individual subjects; bars show the mean ± SD. No *P* values obtained by Kruskal-Wallis test were significant. See Figure 3 for definitions.

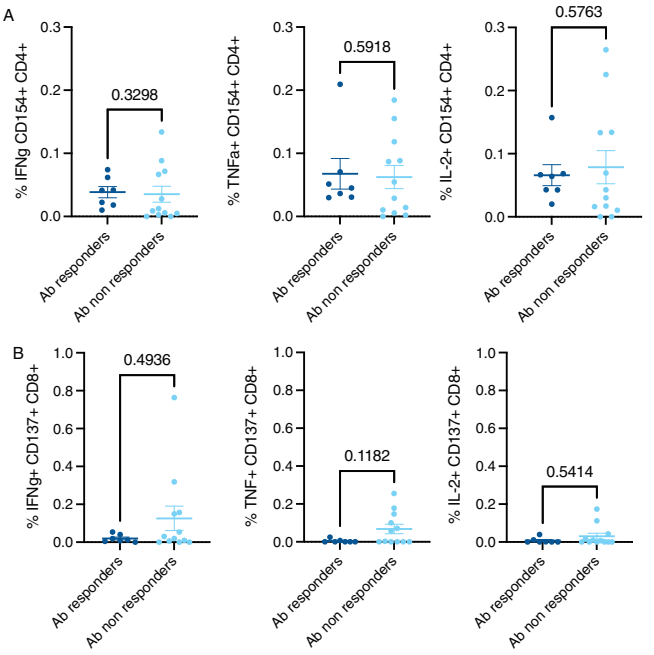

Figure 5. Relationship between cellular and humoral response in RTX-treated patients. Comparisons of percentages of activated CD154+ cytokine-secreting CD4 T cells (**A**) and activated CD137+ cytokine-secreting CD8 T cells (**B**) between antibody (Ab) responders and nonresponders in the RTX-treated group (n = 19) are shown. Symbols represent individual subjects; bars show the mean ± SD. *P* values show comparisons between indicated groups. See Figure 3 for other definitions. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.42058/abstract.

Once again, we did not find any significant difference in the percentages of polyfunctional CD4 T cells (Figure 4C) or CD8 T cells (Figure 4D) between healthy controls, RTX-treated patients, and patients treated with other immunosuppressants.

Similar T cell response in antibody responders and nonresponders among RTX-treated patients. To assess whether the T cell response was different in patients with versus those without an antibody response to the vaccine, we compared the percentages of cytokine-secreting cells stimulated by spike peptides. No difference in the CD4 or CD8 T cell response was observed when comparing antibody responders and nonresponders in the RTX group for IFNγ, TNF, or IL-2 (Figure 5). Notably, there was a trend toward a better CD8 T cell TNF response against spike peptides in patients without a humoral response ($P = 0.11$) (Figure 5B).

DISCUSSION

This single-center prospective study confirmed the diminished anti-spike antibody and neutralizing antibody response to mRNA COVID-19 vaccines in patients with autoimmune diseases who are being treated with RTX, compared to patients treated with other immunosuppressants and healthy controls. Detailed analysis of the CD4 and CD8 T cell responses using intracellular cytokine staining (ICS) revealed a preserved response in RTX-treated patients compared to healthy controls and patients treated with other immunosuppressants, even in those lacking a humoral response.

To provide a clinically relevant level of neutralizing response, we identified a cutoff of 50 units/ml for the anti-spike antibody assay, which guarantees optimal specificity in detecting neutralizing antibodies. Using this threshold, less than one-third of patients treated with RTX had an effective humoral response to the vaccine, which is consistent with findings from previous studies. We and others (3,4,8) have identified that the time since last infusion and not the cumulative dose of RTX is an important risk factor for nonresponse. This could help to guide clinicians, since we showed, like Spiera et al (3), that no patient had a humoral response if the last infusion of RTX had been conducted in the previous 6 months.

A key finding of our study relies on the maintenance of the T cell response against the vaccine, regardless of the humoral response. Data on the cellular response against the COVID-19 vaccine in patients treated with RTX remain controversial. Our results are consistent with other studies (3,4,6,7) that also provide evidence of a similar T cell response in controls as in patients treated with RTX, but they are in opposition to a recent publication that identified a diminished T cell response in RTX-treated patients, using an IFN release assay (8). In the latter study, patients in the RTX group were co-treated with other immunosuppressants such as steroids (75%), MMF or azathioprine (42%), and calcineurin inhibitors (33%), versus only 4% in our study who were co-treated with MMF.

The main difference between our study and previous studies is the technique used for assessing T cell response. ICS is the reference technique used by Pfizer-BioNTech to address the question of cellular response in the general population (9). Most published studies used the IFNγ release assay, which measures secretion from all cells in the tube (6–8), or IFNγ ELISpot (4,5). These techniques can detect an overall T cell response to IFNγ only but lack the fine-tuning provided by ICS that allows for assessment of several cytokines secreted specifically by activated CD4 and CD8 T cells. ICS also allows for detection of polyfunctional T cells, which can secrete 2 or 3 cytokines at the same time. Again, there was no difference in the percentage of polyfunctional T cells between healthy controls, RTX-treated patients, and patients treated with other immunosuppressants.

Studies have shown a correlation between polyfunctional T cells and control of HIV infection (10). Restricting our analysis of T cell response to activated and cytokine-secreting T cells greatly enhanced the specificity. This provides a more stringent method of assessing the cellular response. We confirmed the ability of autoimmune disease patients to build a specific CD4 T cell response to BNT162b2. Autoimmune disease patients showed similar percentages of cytokine-specific CD4 T cells compared to a study performed in healthy controls by Pfizer-BioNTech using the same ICS technique (9). We also confirmed, both in healthy

controls and patients, a lower response for IL-2 in the CD8 T cells, as previously described by Sahin et al (9). Spike-specific CD8 T cells are known to be less frequently detected compared to CD4 T cells (11) in convalescent patients.

Studies in rhesus macaques showed that depletion of CD8 T cells in animals with low titers of neutralizing antibodies leads to higher viral replication in rechallenge experiments (12). We did not find diminished cytokine secretion of spike-specific CD8 T cells in the RTX group compared to the other groups, and conversely, observed a trend toward a better CD8 T cell TNF response in RTX-treated patients without a humoral response. Interestingly, a recent study in multiple sclerosis patients treated with anti-CD20 demonstrated an increase in spike-specific CD8 T cells compared to controls (13).

This study has a number of limitations. Fewer patients were analyzed in the T cell experiments compared to those using antibody assays. This is due to the time and cost constraints of the ICS technique that requires nonautomated processing, staining, acquisition, and analysis of the samples. Notably, the number of samples analyzed is similar to the reference article using the same technique (9). Therefore, our study may be underpowered to demonstrate a higher T cell response in antibody nonresponders. However, we are confident that there is no difference in T cell response between antibody responders and nonresponders. Finally, our study did not assess other cytokines such as IL-21, which can be secreted by CD4 Tfh, and Th17, which can play a role in antiviral defense.

Further human studies are needed to assess whether this preserved T cell response, despite the impaired humoral response to the COVID-19 vaccine, will be sufficient to protect these RTX-treated patients from severe forms of COVID-19 and to decrease the excess mortality observed in these patients when infected. Taken together, our findings highlight the probable usefulness of vaccination in RTX-treated patients, even if they do not develop a humoral response.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Bitoun had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Bitoun, Henry, Seror, Roque Afonso, Le Grand, Mariette.

Acquisition of data. Bitoun, Henry, Desjardins, Vauloup-Fellous, Dib, Belkhir, Mouna, Joly, Bitu, Ly, Pascaud, Mariette.

Analysis and interpretation of data. Bitoun, Henry, Desjardins, Vauloup-Fellous, Dib, Belkhir, Mouna, Joly, Seror, Roque Afonso, Le Grand, Mariette.

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