LETTER TO THE EDITORS

Anti-SARS-CoV-2 humoral and cellular responses in multiple sclerosis patients treated with anti-CD20 monoclonal antibodies

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Dear Sirs,

Multiple sclerosis (MS) patients treated with anti-CD20 monoclonal antibodies are at higher risk of severe COVID-19 [1, 2]. Vaccination against SARS-CoV-2 has been shown to be highly efficient in the general population, but has not been fully evaluated in anti-CD20 treated MS patients. These patients present an impaired anti-SARS-CoV-2 antibody response, but recent data suggest that anti-SARS-CoV-2 T cell response could be conserved as measured by T cell activation markers and interferon production [3, 4]. However, T cell proliferation, a key feature of specific T cell response, has never been measured in this population. In this study, we compared anti-SARS-CoV-2 humoral and cellular immune responses in anti-CD20 treated MS patients and healthy volunteers and further investigated clinical and biological factors that could have influenced those immune responses in anti-CD20 patients.

We performed a prospective observational single-center study in the neurological hospital of Lyon, France, from November 2021 to February 2022. We included anti-CD20-treated MS patients with at least two anti-SARS-CoV-2 vaccine doses. This study is in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Patients were included in the French MS registry (OFSEP—Observatoire Français de la Sclérose en Plaques) and provided written informed consent. Ten healthy volunteers (HV) were included as controls after informed consent as part of the REA-IMMUNO-COVID (RICO) clinical study (N°IRB/IORG #: IORG0009918; Agreement Number 2020-A01079-30; NCT04392401). This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines. Clinical and demographic data were collected for each patient. Blood samples were collected during clinical routine care, 6 months after previous anti-CD20. S1-RBD-specific IgG were measured using the Atellica IM SARS-CoV-2 IgG (sCOVG) (Siemens) diagnosis kits according to the manufacturer's recommendations. Cellular response was assessed by CD3 + T cell proliferation assay measured under antigenic peptide stimulation covering SARS-CoV-2 Spike as previously described [5, 6]. Statistical analyses were conducted using Prism (version 5.03, Graphpad Software). Categorical variables were analyzed with a Fisher's exact or Chi^2 test. Continuous variables were analyzed with a Kruskal–Wallis test for multiple group comparisons and a Mann–Whitney test for the comparison of two independent groups.

We tested 61 anti-CD20-treated MS patients (anti-CD20 patients) and 10 healthy volunteers. The clinical and demographic characteristics of anti-CD20 patients are presented in Table 1.

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Table 1 Clinical and biological characteristics of anti-CD20 patients

	Non-responders	Partial responders	Full responders	Total anti-CD20 patients	p value*
n	15	38	8	61	ns
Female/male (sex ratio)	11/4 (2.75)	23/15 (1.5)	6/2(3)	40/21 (1.9)	ns
Age, year	47 (39–48)	44 (32–55)	42 (32–45)	44 (34–53)	ns
Disease					ns
RR MS, n (%)	11 (73.3)	22 (57.9)	7 (87.5)	40 (65.6)	ns
SP MS, n (%)	3 (20)	12 (31.6)	1 (12.5)	16 (26.2)	
PP MS, n (%)	1 (6.7)	4 (10.5)	0 (0)	5 (8.2)	
EDSS, median (IQR)	3 (0–6)	2.5 (1.4–5)	1.5 (0–5.6)	2.5 (0.5–5)	ns
Disease duration, month	220 (107–255)	134 (73–219)	71 (49–140)	148 (70–238)	ns
Ocrelizumab (%)	11 (73.3)	21 (55.3)	7 (87.5)	39 (63.9)	ns
Rituximab (%)	4 (26.7)	17 (44.7)	1 (12.5)	22 (36.1)	
Number of anti-CD20 perfusion	5 (3–9)	6 (4.8–8)	3 (2.3–4.8)	5 (4–8)	ns
Previous immunosuppressant (%)	11 (73.3)	22 (57.9)	6 (75)	39 (63.9)	ns
COVID status					ns
Infected, n (%)	2 (13.3)	7 (18.4)	1 (12.5)	10 (16.4)	ns
2 Shots of anti-COVID vaccine, n (%)	0 (0)	5 (13.1)	0 (0)	5 (8.2)	ns
3 Shots of anti-COVID vaccine, n (%)	8 (53.3)	23 (60.6)	4 (50)	35 (57.4)	
4 Shots of anti-COVID vaccine, n (%)	7 (46.7)	10 (26.3)	4 (50)	21 (34.4)	
BNT162b2 vaccine, n (%)	14 (93.3)	34 (89.4)	7 (87.5)	55 (90.2)	ns
mRNA-1273 vaccine, n (%)	1 (6.7)	2 (5.3)	0	3 (4.9)	
Heterologous vaccination, n (%)	0	2 (5.3)	1 (12.5)	3 (4.9)	
Delay from last boost, days	152 (49–216)	73.5 (41.3–190.5)	45 (32.8–59)	73 (42.5–195.5)	0.03
Mean delay anti-CD20—Vaccine, days	96 (51–116)	109 (71–147)	110 (70–139)	108 (70–135)	ns
Mean delay between anti-CD20 infusions, days	182 (180–189)	182 (177–187)	183 (177–188)	182 (179–187)	ns
Immunological status					ns
T cell at baseline (cell/mm^3)	1282 (786–1957)	990 (720–1496)	840 (703–1820)	1037 (727–1575)	ns
CD4 + T cell at baseline (cell/mm^3)	704 (531–1201)	662 (473–902)	571 (444–1332)	674 (475–994)	ns
CD8 + T cell at baseline (cell/mm^3)	356 (133–816)	302 (187–472)	306 (113–433)	315 (186–498)	ns
CD20 + cell at baseline (cell/mm^3)	119 (74–237)	178 (146–303)	140 (87–275)	164 (110–279)	ns
T cell at sampling (cell/mm^3)	1050 (827–1480)	1233 (824–1507)	1060 (831–1695)	1198 (827–1509)	ns
CD4 + T cell at sampling (cell/mm^3)	675 (606–768)	776 (552–982)	656 (588–1142)	721 (585–947)	ns
CD8 + T cell at sampling (cell/mm^3)	375 (189–612)	344 (236–525)	349 (219–642)	346 (212–563)	ns
CD20 + cell at sampling (cell/mm^3)	0 (0–0)	0 (0–3)	8 (1.5–27.3)	0 (0–3)	< 0.0001
Number of patients with residual B cells (%)	0 (0)	10 (26.3)	7 (87.5)	17 (27.9)	< 0.0001
IgG level (g/L)	7.6 (6.7–9.8)	8.9 (7.7–10.2)	11.2 (10.2–13.1)	9 (7.5–10.6)	0.0037
IgM level (g/L)	0.6 (0.3–1.3)	0.6 (0.3–0.7)	0.8 (0.5–1)	0.6 (0.3–0.9)	ns
IgA level (g/L)	1.4 (1.2–2.1)	1.5 (1.1–1.9)	2.3 (1.3–2.8)	1.5 (1.2–2.2)	ns

Reference values from the clinical laboratory were: T cells: 595–1861 per mm^3; CD4 + T cells: 314–1270 per mm^3; CD8 + T cells: 147–836 per mm^3; CD20 + B cells: 109–890 per mm^3; IgG level: 7–16 g/L; IgM level: 0.4–2.80 g/L; IgA level: 0.78–4.11 g/L

RR MS, relapsing–remitting multiple sclerosis; *SP MS* secondary progressive multiple sclerosis; *PP MS* primary progressive multiple sclerosis; *EDSS* Expanded Disability Status Scale; *ns* not significant

[a]*p* values represented here correspond to Kruskal–Wallis test for multiple group comparisons

The median [IQR] anti-SARS-CoV-2 IgG levels were significantly lower in anti-CD20 patients (1.9 BAU/mL [0–12.1]) compared to HV (3077 BAU/mL [506–3270]) ($p < 0.0001$) (Fig. 1a). The anti-SARS-CoV-2 serology was positive in 18% (11/61) of anti-CD20-treated patients compared to 100% (10/10) of HV ($p < 0.0001$) (Fig. 1b).

In contrast, detectable anti-SARS-CoV-2 specific T cell response was found in 70% (43/61) of anti-CD20 patients compared to 100% (10/10) of HV ($p = 0.056$) (Fig. 1d). However, the percentage of SARS-CoV-2-specific T cells was lower in anti-CD20 patients (1.3% [0–2.6]) compared to HV (2.5% [1.2–5]) ($p = 0.048$) (Fig. 1c).

In total, 100% (10/10) of HV developed both humoral and cellular immune response following SARS-CoV-2 vaccination. In contrast, 13% (8/61) of anti-CD20 patients developed both humoral and cellular immune response, 57% (35/61) developed only a cellular response, 5% (3/61) developed only a humoral response, and 25% (15/61) developed neither a humoral nor a cellular immune response (Fig. 1e).

We then compared the clinical and biological characteristics of MS patients treated with anti-CD20 depending on their response to vaccination: full responders (both humoral and cellular specific responses), partial responders (either humoral or cellular specific responses), and non-responders (neither humoral nor cellular response) (Table 1). We showed that full responders have a shorter delay from last vaccine boost (45 days [32.8–59]) compared to non-responders (152 days [49–216]) ($p = 0.0089$) (Fig. 1f). Patients whose last vaccine boost was performed beyond 5 months were at higher risk of being non-responders compared to patients whose last vaccine boost was performed within 5 months ($p = 0.033$) (Fig. 1g).

B cells count 6 months after last anti-CD20 infusion was higher in full responders (8 CD20 + B cell/µL [1.5–27.3]) compared to partial responders (0 [0–3], $p = 0.0012$) and compared to non-responders (0 [0–0], $p < 0.0001$) (Fig. 1h). The repartition of immune response status was significantly different in patients with or without B-cell repletion (B-cell repletion being defined as ≥ 1 CD20 + B cell/mm^3 6 months after previous anti-CD20 infusion) ($p < 0.0001$) (Fig. 1i). 41% (7/17) of patients with B-cell repletion were full responders and 59% (10/17) were partial responders. In contrast, only 2% (1/44) of patients with no B-cell repletion were full responders, 64% (28/44) were partial responders, and 34% (15/44) were non-responders. Accordingly, IgG level was higher in full responders (11.5 g/L [10.2–13]) compared to partial responders (8.9 [7.7–10.2], $p = 0.0056$)) and non-responders (7.7 g/L [6.2–10.3], $p = 0.0078$).

The other clinical or biological parameters that were evaluated did not differ between non-responders, partial responders, and full responders (Table 1).

As observed in other cohorts [3, 4] through measurement of T cell IFNγ production or expression of activation-induced markers on T cells, we confirmed that most anti-CD20-treated MS patients develop SARS-CoV-2 specific T cell response despite a poor humoral response as measured by T cell proliferation after 1 week of SARS-CoV-2 antigen stimulation.

Large studies evaluating the clinical impact of anti-SARS-CoV-2 vaccine in anti-CD20 patients are still missing. Nevertheless, we can hypothesize that patients able to develop both humoral and cellular responses might be better protected against severe COVID-19 than patients developing only humoral or only cellular response or no response. Hence, we believe that cellular response should be routinely measured, in addition to serology, to better evaluate individual patient's risk of infection.

We showed that patients able to develop both humoral and cellular response had a shorter delay from previous vaccine injection compared to non-responders, suggesting that vaccine boost should be repeated for an optimal immune response.

In addition, patients presenting B-cell repletion were more likely to develop both humoral and cellular immune responses following vaccination. Early B-cell repletion following anti-CD20 treatment is not associated with MS relapses [7], contrary to neuromyelitis optica spectrum disorders [8]. Early B-cell repletion period might therefore be the most appropriate window for vaccination.

This study has some limitations including the sample size and the lack of comparison with other disease-modifying therapies such as S1P agonists. As mentioned above, additional studies evaluating COVID-19 protection in patients developing humoral and/or cellular immunity should be performed.

We showed that most anti-CD20-treated MS patients presented with impaired humoral response following anti-SARS-CoV-2 vaccination, but developed specific cellular response although in a lower extend than in healthy volunteers. Clinical impact of such incomplete immune response to vaccination should now be evaluated. A shorter delay from previous vaccine boost and B-cell repletion were associated with a better overall immune response. We might postulate that, to optimize the development of an anti-SARS-CoV-2-specific immune response, vaccine boosts should be repeated in anti-CD20-treated MS patients in an individualized time frame based on B-cell repletion.
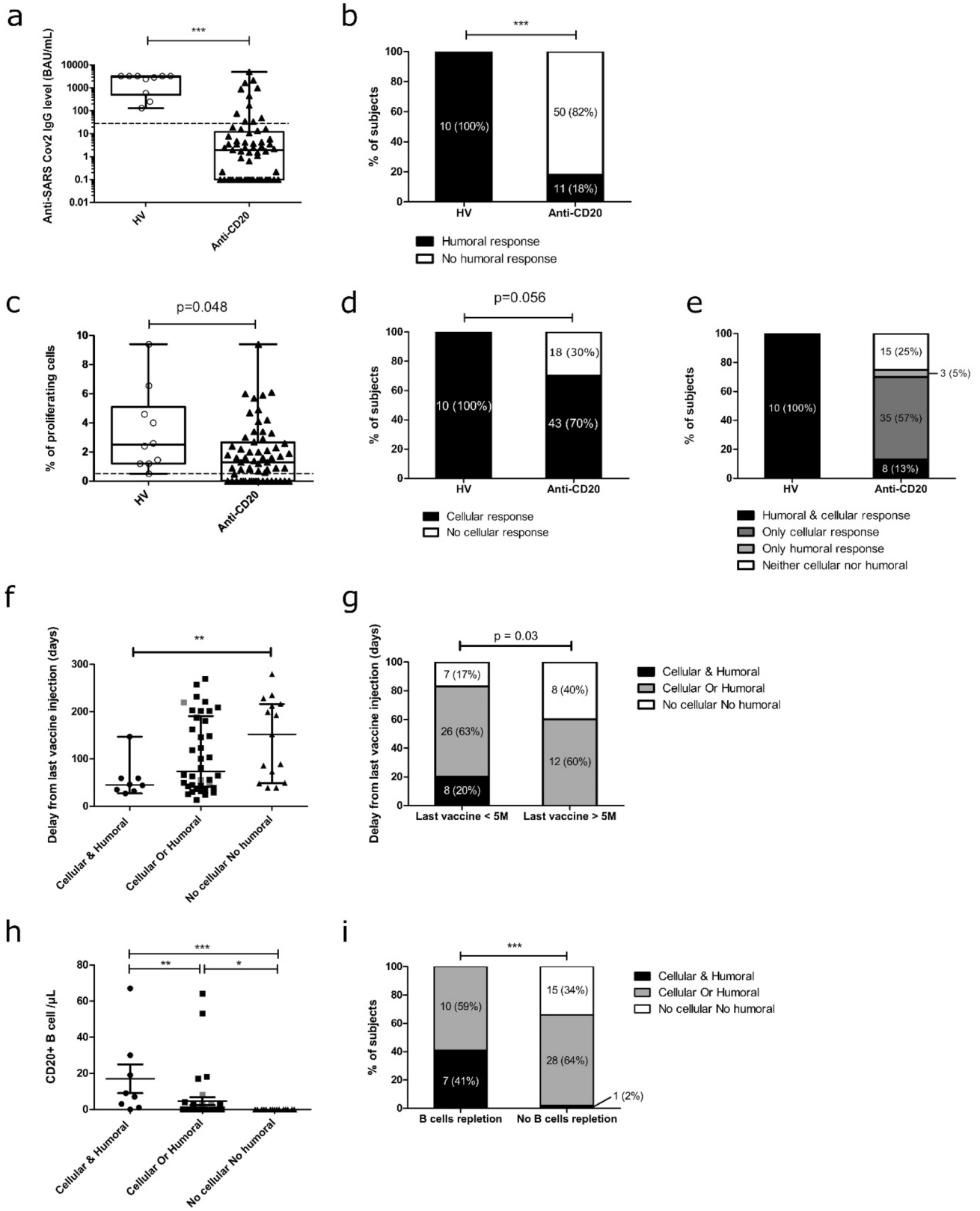
◄Fig. 1 Characteristics of anti-SARS-CoV-2 humoral and cellular responses in anti-CD20 patients and healthy volunteers. **a** Anti-SARS-CoV-2 IgG level (BAU/mL) in Healthy Volunteers (HV, white dots) and anti-CD20 patients (black triangles) in log(10) scale. The 0 value is represented as 0.1BAU/mL. The dashed line represents the positive serology threshold (21.8 BAU/mL) **b** Repartition of subjects with negative (white) or positive (black) anti-SARS-CoV-2 serology in HV and anti-CD20 patients. **c** Percentage of proliferating CD3 + T cells following SARS-CoV-2-specific antigen stimulation in HV and anti-CD20 patients. **d** Repartition of subjects without (< 0.5%, white), or with (> 0.5%, black) a specific T cell proliferation following SARS-CoV-2-specific antigen stimulation in HV and anti-CD20 patients. **e** Repartition of subjects with neither humoral nor cellular SARS-CoV-2-specific response (white), only humoral SARS-CoV-2-specific response (light grey), only cellular SARS-CoV-2-specific response (dark grey), or both humoral and cellular SARS-CoV-2-specific responses (black). **f–i** Characteristics of anti-CD20 patients with different SARS-CoV-2-specific immune response status. **f** Delay (days) between last vaccine booster and sampling in non-responders (no cellular no humoral response), partial responders (cellular or humoral response), and full responders (cellular and humoral responses). In **f** and **h**, the 3 grey squares represent the patients developing only a humoral response and the 35 black squares represent the patients developing only a cellular response. **g** Repartition of the SARS-CoV-2-specific immune response status (no humoral no cellular response, humoral or cellular response, and cellular and humoral responses) in patients with a last vaccine booster within or beyond 5 months. **h** CD20 + B-cell count at sampling, before the next anti-CD20 infusion, in non-responders (no cellular or no humoral response), partial responders (cellular or humoral response) and full responders (cellular and humoral responses). **i** Repartition of the SARS-CoV-2 specific immune response status in patients with or without B-cell repletion

Declarations

Conflicts of interest S. Vukusic has received lecturing fees, travel grants, and research support from Biogen, BMS-Celgene, Janssen, Merck, Novartis, Roche, Sanofi-Genzyme, and Teva. P. Nicolas, H. Marion-Moffet, M. Gossez, G. Monneret, R. Marignier, and F. Venet report no disclosure relevant to the subject of this manuscript.

Ethical approval and consents This study is in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Patients were included in the French MS registry (OFSEP—Observatoire Français de la Sclérose en Plaques) and provided written informed consent, and healthy volunteers were included after informed consent as part of the REA-IMMUNO-COVID (RICO) clinical study (N°IRB/IORG #: IORG0009918; Agreement Number 2020-A01079-30; NCT04392401).

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