

Correspondence on 'SARS-CoV-2 vaccination in rituximab-treated patients: evidence for impaired humoral but inducible cellular immune response'

We read with a great interest the article published by Bonelli *et al* suggesting an inducible cellular immune response in rituximab (Rtx) treated patients.¹ The CD20-antibody Rtx is one of the most widespread biologicals worldwide with a broad spectrum of oncological and rheumatological indications. Due to its depleting effect on circulating B cells, the generation of antibodies against novel pathogens is impaired in Rtx-treated patients.^{2,3} Accordingly, the last EULAR recommendations on vaccination advised that 'vaccination should be provided at least 6 months after the last administration and 4 weeks before the next course of B cell-depleting therapy'.⁴ To ensure appropriate SARS-CoV-2 vaccination, the last EULAR advise was to refer to a rheumatologist.⁵ The American College of Rheumatology (ACR) has recommended to vaccinate Rtx-treated patients not earlier than 5 months after the last administration with the next cycle given not earlier than 2–4 weeks thereafter.⁶ In any case, the combination of B cell-depleting therapy with vaccination has been quite a challenge for patients and physicians—especially since it became clear that Rtx therapy may be associated with unfavourable outcomes in B cell-depleted patients.⁷ Fortunately, very recent data by Bonelli *et al* have now suggested that a cellular response is mounted after SARS-CoV-2 vaccination in Rtx-treated patients despite a failed humoral immune response.¹ The authors demonstrated that peripheral blood cells of vaccinated patients do produce Interferon γ (IFN γ) after stimulation with SARS-CoV-2 spike (S) protein-derived overlapping peptides.¹ These results increase the scientific interest into a more detailed characterisation of vaccine-reactive T-cell immunity, which has recently been in the focus of our group as well due to a frequent Rtx application in our settings.

Applying multiparameter flow cytometry, we explored the efficacy of SARS-CoV-2 vaccination as defined by quantification and in-depth characterisation of T cell immunity in nine Rtx-treated patients diagnosed with autoantibodies against myeloperoxidase (MPO), proteinase 3 (PR3), MPO/PR overlap and immunoglobulin A (IgA) vasculitis or membranous glomerulopathy (table 1). Their mean age was 65 years, 33.3% were women, and the mean time after the last application of Rtx was 4.5 months (2–7 months). The vaccination by two doses of BNT162b2 was performed within 3 weeks. SARS-CoV-2-reactive immunity was

analysed before the first dose, and 3 weeks after the first and the second dose, respectively. Vaccinated healthcare workers (n=14) served as controls.

All but two Rtx-treated patients had neglectable levels of CD19⁺ B cells (figure 1D). Consequently, the development of antibodies to the SARS-CoV-2 S-protein was substantially impaired in Rtx group excluding two of the patients with detectable CD19⁺ B cells, who showed seroconversion. In contrast, virus specific IgG antibodies were detected in all healthy controls.

Importantly, vaccine-reactive T cells were found in the majority of Rtx-treated patients. Due to pre-existing SARS-CoV-2-cross-reactive T cells known to be detectable in unexposed patients as demonstrated by other and our groups,^{8,9} vaccine-directed T cell response was defined by a >twofold increase of SARS-CoV-2 S-protein-reactive T cell frequencies compared with the prevaccination (TP0) state. Accordingly, CD4⁺ T cell vaccination response was observed in 78% of Rtx-treated patients 3 weeks after the first vaccination and 86% after the second vaccination (figure 1A), while CD8⁺ T cell responses were only found in 22% and 43% of the patients after the first and second vaccinations, respectively. Of note, there were no statistically significant differences in the frequencies of vaccine-reactive CD4⁺ and CD8⁺ T cells between patients and controls (figure 1B). The substantial number of activated T cells produced GranzymeB, interleukin (IL)-2, interferon γ (IFN γ) or tumour necrosis factor α (TNF α) as monofunctional or polyfunctional T cells suggesting their protective function¹⁰ (figure 1C). Again, there were no statistically significant differences between patients and controls.

T cell reactivity against SARS-CoV-2 variants of concern (VOC), including B.1.1.7 and B.1.351 strains, after vaccination with the Wuhan wild type S-protein are of special interest. Importantly, 75% of Rtx-treated patients had T cells directed against the S-protein derived from both mutant strains after the second vaccination, respectively (figure 1B). These T cells were able to produce several cytokines simultaneously suggesting antiviral potential of these polyfunctional T cells¹⁰ (figure 1C). Again, the magnitude and functionality of B.1.1.7 and B.1.351 S-reactive T cells were not significantly different between patients and controls (figure 1B), with a tendency towards lower frequencies in the former.

In conclusion, despite the lack of seroconversion in most patients, Rtx-treated patients are able to raise T cells reactive not only to SARS-CoV-2 wild type strain but also to B.1.1.7 and B.1.351 VOC. Although not a confirmation of antiviral protection of vaccine-reactive T cells, their polyfunctional properties suggest an antiviral potential.

Table 1 Patient characteristics

	Age	Sex	Body mass index (kg/m ²)	Months since rituximab	Overall rituximab treatment duration	Aetiology	GFR (CKD-EPI; mL/min/1.73 ²)
1	73	F	21.5	2	8	MPO	31.7
2	65	M	32.2	2	26	PR3	38.6
3	60	M	35.4	7	26	IgAV	17.3
4	57	M	23.3	6	6	MPO	47.1
5	74	M	32.1	7	7	MGN	45.2
6	79	M	25.1	5	27	MGN	43.7
7	52	F	25.7	4	17	MPO/PR3	43.2
8	64	M	27.5	4	21	PR3	27.5
9	64	F	34.6	4	10	PR3	47.9

GFR, glomerular filtration rate

; IgAV, IgA-associated vasculitis; MGN, membranous glomerulonephritis; MPO, myeloperoxidase; PR3, proteinase 3.

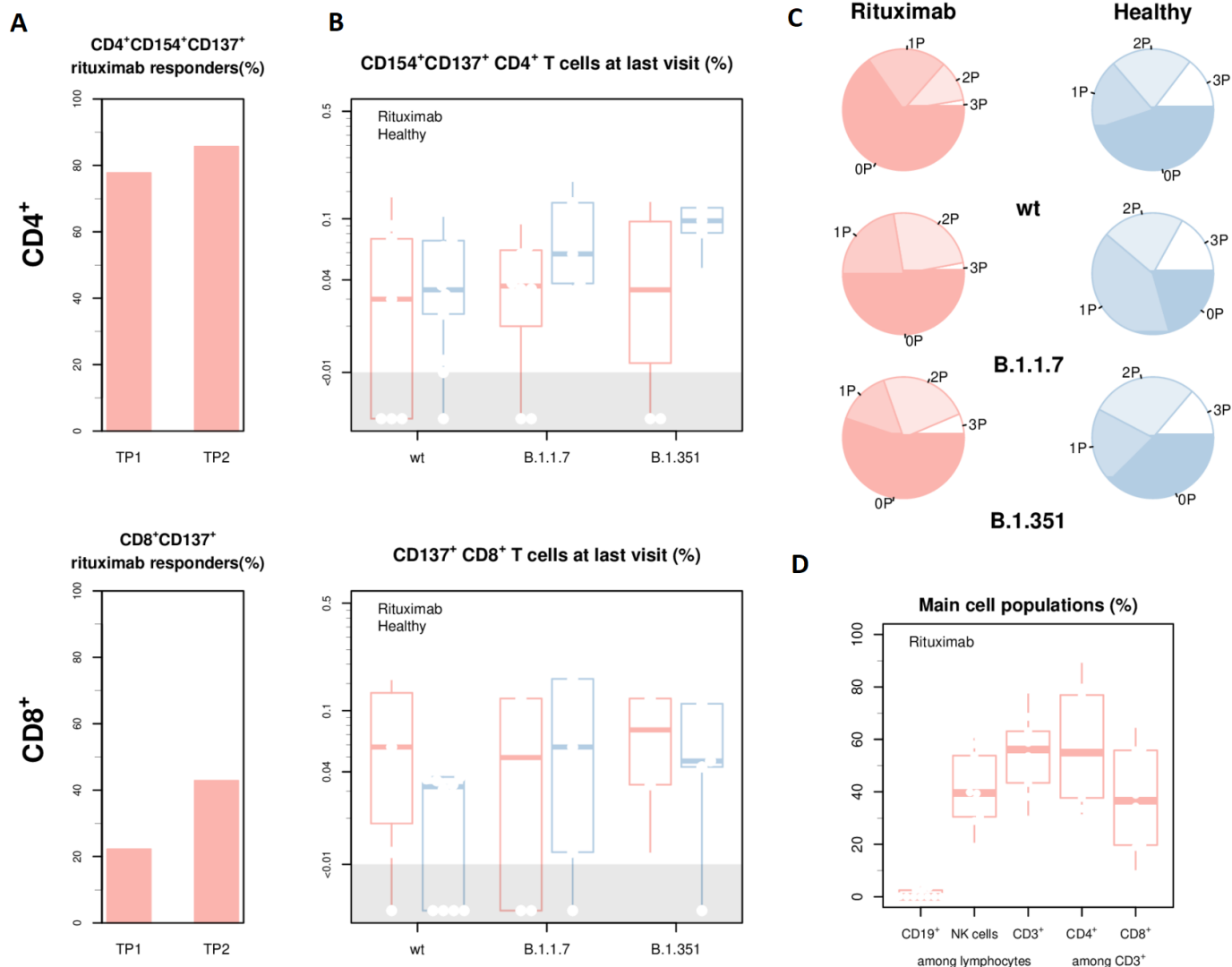


Figure 1 Robust polyfunctional T cell response directed against spike (S) wild type (wt) and variants of concern (VOC) strains can be detected in rituximab (Rtx) treated patients following SARS-CoV-2 vaccination. (A) Incidence of Rtx-treated patients responded to the vaccination 3 weeks after the first dose (TP1) and 3 weeks after the second dose (TP2). Vaccine-directed T cell response was defined as >twofold increase of S-reactive T cell frequencies as compared with the prevaccination (TP0) T cell response (CD4⁺ T cells top; CD8⁺ T cells bottom). (B) The relative frequency of SARS-CoV-2 reactive CD4⁺ and CD8⁺ T cells directed against S-protein of wt, B.1.1.7 and B.1.351 VOC strains in Rtx-treated patients and healthy donors. For CD4⁺ T cells, activation is defined by CD154⁺ and CD137⁺ double expression (top) and for CD8⁺ T cells by CD137⁺ (bottom). Presented are data obtained from the last visit (3–5 weeks) after the second vaccination dose. For wt strain, 9 Rtx-treated patients and 14 controls were available. For VOC strains, eight Rtx-treated patients and five controls were available. PBCMs were isolated 3–5 weeks after the second BNT162b2 dose and stimulated overnight with SARS-CoV-2 S-protein of wt, B.1.1.7 and B.1.351 strains, respectively. T cell reactivity was determined by flow cytometry as CD154⁺, CD137⁺ and CD137⁺ for CD4⁺ and CD8⁺, respectively, together with antibodies for Granzyme B, IFN γ , TNF α and IL-2. (C) Total frequency of monofunctional (1P), bifunctional (2P) or trifunctional (3P) T cells concurrently producing one, two or three cytokines or no cytokines (0P), respectively, in response to S-protein from wt, B.1.1.7 or B.1.351. PBCMs were isolated 3–5 weeks after the second BNT162b2 dose and stimulated overnight with SARS-CoV-2 S-protein from wt, B.1.1.7 and B.1.351. T cell reactivity was determined by flow cytometry as CD154⁺ and CD137⁺ together with antibodies for Granzyme B, IFN γ , TNF α and IL-2. (D) Reduced CD19⁺ but normal frequencies of other lymphocytes in Rtx-treated patients. The frequencies of CD19⁺, NK, CD3⁺, CD4⁺/CD3⁺ and CD8⁺/CD3⁺ were evaluated in fresh whole blood by flow cytometry. Each point signifies a patient. IFN γ , Interferon γ ; IL, interleukin, NK, natural killer, TNF α , tumour necrosis factor α .

The role of SARS-CoV-2-specific antibodies in COVID-19 is not well established: reports indicate an increased risk of severe COVID-19 infections in Rtx-treated patients,⁷ whereas other data have suggested that B cells are dispensable for resolving such infections.¹¹ The here presented data indicate that polyfunctional antiviral T cell responses are raised after SARS-CoV-2 vaccination in this high-risk population suggesting protection in the absence of virus-specific antibodies.

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