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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2023.02.010.

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See related commentary on pg 1340

SARS-CoV-2 Vaccination Effectiveness in Rituximab-Treated Patients Affected by Pemphigus Vulgaris

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TO THE EDITOR

Rituximab (RTX), a chimeric monoclonal antibody targeting CD20 on B lymphocytes, is an effective disease-modifying agent in the treatment of pemphigus vulgaris (PV), a rare autoimmune blistering disease (Witte et al.,

Abbreviations: CTL, CD8+ cytotoxic T lymphocyte; EM, effector memory; HC, healthy control; PV, pemphigus vulgaris; RTX, rituximab

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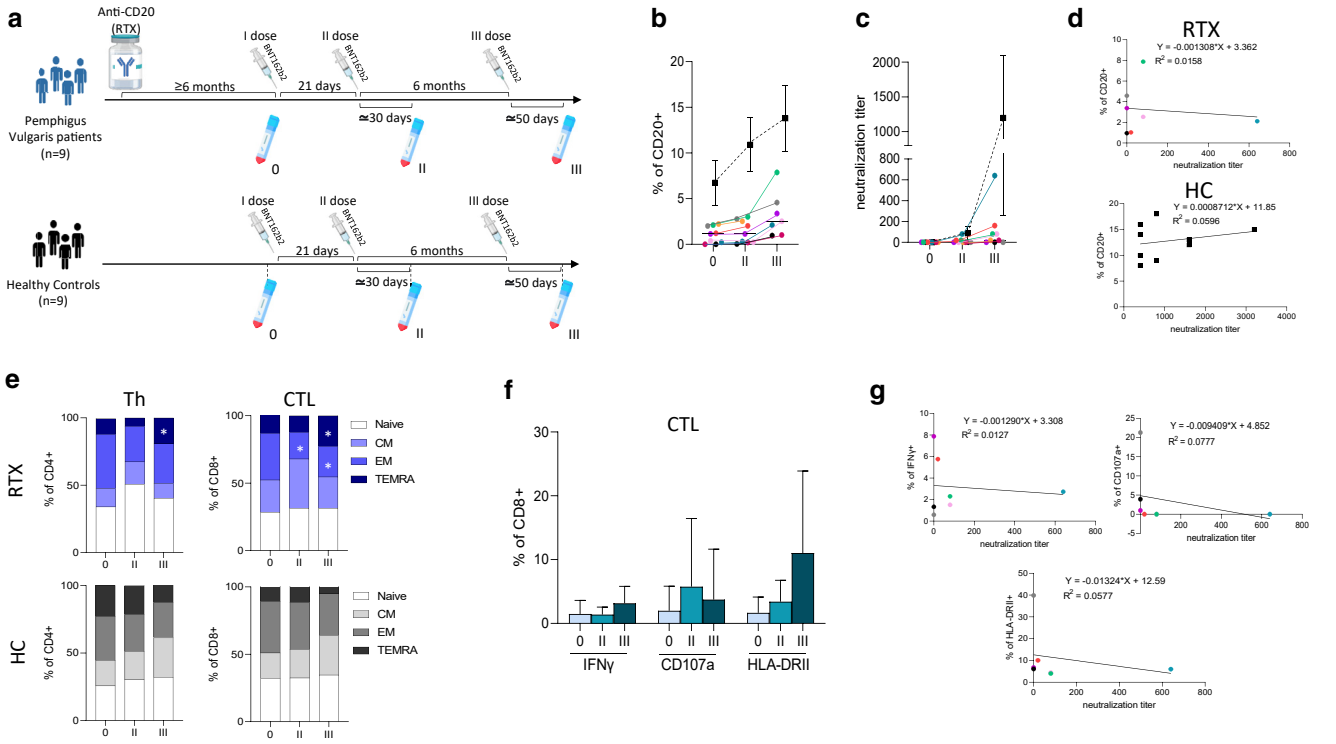

Figure 1. Humoral and cell-mediated immunological response against SARS-CoV-2 vaccine in RTX-treated subjects with PV. (**a**) Synoptic representation of the study design. (**b**–**d**) Specific anti–SARS-CoV-2 humoral response. (**b**) Flow cytometric analyses of the percentage of CD19+ B lymphocytes in HCs (depicted as average with SD by black squares and dotted lines) and RTX+ subjects (individually depicted by colored circles and solid lines; average is shown by black bars) on SARS-CoV-2–specific in vitro stimulation before vaccination (0) and after the second (II) and the third (III) dose (REML $P < 0.0001$). (**c**) Anti–SARS-CoV-2 plasmatic neutralization titer in HCs (depicted as average with SD by black squares and dotted lines) and RTX+ subjects (individually depicted by colored circles and solid lines; average is shown by black bars) at time points 0, II, and III (REML $P < 0.0006$). (**d**) Correlation between the percentage of CD19+ B lymphocytes and the neutralization titer calculated at time point III in RTX+ (left) and HC (right) subjects. (**e**–**g**) Specific anti–SARS-CoV-2 cell-mediated response. (**e**) Flow cytometric analyses of T-lymphocyte memory subsets at 0, II, and III time points on SARS-CoV-2–specific in vitro stimulation. Percentages of naïve, CM, EM, and TEMRA Th CD4+ (left) and T cytotoxic CD8+ (CTL; right) are depicted for RTX+ (top) and HC subjects (bottom) (*$P \leq 0.05$). (**f**) Flow cytometric analyses of CTL T lymphocytes expressing IFN-γ, CD107a, or HLA-DRII activation markers at pre, II, and III time points, on SARS-CoV-2–specific in vitro stimulation. (**g**) Correlation between the percentage of IFN-γ-, CD107a,- or HLA-DRII-expressing CTL and the neutralization titer calculated at time point III in RTX+ subjects. CM, central memory; CTL, CD8+ cytotoxic T lymphocyte; EM, effector memory; HC, healthy control; PV, pemphigus vulgaris; REML, restricted maximun likehood; RTX, rituximab; TEMRA, terminally differentiated EM re-expressing CD45RA; Th, T-helper.

2018; Yuan et al., 2022). PV is caused by serum IgG autoantibodies targeting adhesion molecules of the cadherin family, particularly desmoglein 3 and 1, two major components of desmosomes (Spindler and Waschke, 2018). PV usually arises with oral erosions first and then spreads to the skin with a chronic-relapsing course (Feliciani et al., 2018).

Some recent reports have already highlighted the putative risk of SARS-CoV-2 infection in patients treated with RTX (Beyzaee et al., 2021). Besides the risk of a more severe disease course during B-cell–depleting therapy, a major concern relates to the risk of reduced immunogenicity of vaccination (Apostolidis et al., 2021; Avouac et al., 2022; Baker et al., 2020; Felten et al., 2022). Therefore, the question arises whether patients should withhold or interrupt RTX therapy around COVID-19 vaccination or delay vaccination. However, to date, no scientific evidence has been produced on the efficacy of SARS-CoV-2 vaccines on RTX-treated patients with PV.

To address this question, we prospectively enrolled 9 subjects (5 men and 4 women) affected by PV with a median age of 46 (interquartile range: 41.5–50.5) years, undergoing primary Pfizer-BNT162b2 COVID-19 vaccine cycle and booster dose (the protocol number 464_2020 was approved by the Institutional Review Board of the Ethics Committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy). Written, informed consent was provided by all the enrolled subjects. All patients with PV were treated with 2× 1,000 mg RTX for 6 to 12 months before the first vaccine dose. Nine healthy controls (HCs) following the same vaccine schedule were included as controls. We collected peripheral blood 2 weeks before the first dose of vaccine (time point 0), 7 weeks after the second dose (time point II), and 12 weeks after the third booster dose (time point III), as summarized in Figure 1a.

Clinical and demographical features of the patients enrolled in this study are summarized in Table 1. In all cases, the clinical diagnosis of PV has been confirmed by skin biopsy, direct and indirect immunofluorescence, and ELISA. Almost all patients (n = 8) presented with mucosal involvement; the other most frequently involved sites were the trunk (n = 4), scalp (n = 3), back (n = 2), and upper limbs (n = 1). Five patients had a partial response, whereas four achieved complete clinical remission.

Table 1. Demographics and Clinical Features of Reported Cases

No.	Sex/Age (Y)	Age at Diagnosis (Y)	Involved Sites	Comorbidities	Treatment	Follow-Up
1	F/56	51	Mucosal involvement	Hypothyroidism	Topical and systemic CSs, rituximab	PR
2	M/65	60	Scalp, back, trunk	Thromboangiitis obliterans; hypertension	Topical and systemic CSs, immunosuppressants, rituximab	CR
3	M/49	46	Mucosal involvement, upper limbs	Obesity, hypertension	Systemic CSs, immunosuppressants, rituximab	CR
4	M/56	50	Mucosal involvement, scalp, back	Hypercholesterolemia, type 2 diabetes mellitus, osteoporosis, surgically treated bladder cancer	Topical and systemic CSs, rituximab	PR
5	F/42	40	Mucosal involvement, trunk	None	Systemic CSs, rituximab	CR
6	F/22	20	Mucosal involvement, trunk	Autoimmune thyroiditis	Systemic CSs, rituximab	PR
7	M/51	43	Mucosal involvement	None	Systemic CSs, rituximab	PR
8	M/50	48	Mucosal involvement, trunk	Osteoporosis	Topical and systemic CSs, immunosuppressants, rituximab	PR
9	F/46	44	Mucosal involvement, scalp, trunk	None	Topical and systemic CSs, rituximab	CR

Abbreviations: CR, complete remission; CS, corticosteroid; F, female; M, male; PR, partial response.

The specific anti−SARS-CoV-2 humoral response was evaluated for all the enrolled subjects at the considered time points, both as B-cell immunophenotypic analyses and as a neutralization titer. On 24-hour stimulation with SARS-CoV-2 spike antigens (180 μg/ml), we detected an increase of specific B cells both in the RTX and HC groups (restricted maximun likehood (REML) $P < 0.0001$) (Figure 1b). However, in the patients treated with RTX, the percentage of SARS-CoV-2−specific B cells was significantly lower than in the HCs (REML $P < 0.0001$). Neutralization capability was titrated in plasma specimens collected at time points II and III (Figure 1c) by the gold standard neutralization titer assay. Our results showed that the SARS-CoV-2−specific plasmatic neutralization titer increased in both groups overtime (REML $P = 0.0004$), but it was significantly lower in the RTX group (REML $P = 0.0006$). The direct correlation between B-cell percentage and neutralization titer assay was not significant at time point II (not shown) or time point III (Figure 1d) in either of the groups. Although neutralizing antibodies are considered the key effector of the vaccine-induced immune response and routinely tested to measure vaccine effectiveness, evidence points out a pivotal role for the T-cell−mediated immune response (Bange et al., 2021; Dan et al., 2021; Fenizia et al., 2022; Sekine et al., 2020). Therefore, we assessed naïve and memory T-cell subsets by flow cytometry on SARS-CoV-2 in vitro stimulation of CD4+ T helper (Th) and CD8+ cytotoxic T lymphocytes (CTLs) (Figure 1e). Throughout the time points, naïve cell percentages marginally varied in a nonsignificant manner both for Th and CTL cells as well as the central memory subset. Although no significant variations were observed in Th effector memory (EM), significant differences were observed over time in CTL EM both at time point II ($P \leq 0.05$) and time point III ($P \leq 0.05$). In both Th and CTL, the terminally differentiated EM re-expressing CD45RA subset was significantly expanded after the third vaccine dose ($P \leq 0.05$). Terminally differentiated EM re-expressing CD45RA subsets in both Th and CTL cells were significantly expanded compared with the HC group (Figure 1e, lower panels). This is a sign of a functional and responsive adaptive immune response against the SARS-CoV-2 infection (Fenizia et al., 2022; Sallusto et al., 2004). EM typically resides in the peripheral bloodstream, where they ensure a strong enhanced recall response. Furthermore, terminally differentiated EM lymphocytes re-expressing CD45RA are considered the terminal stage of differentiation of EM lymphocytes, expressing senescence and exhaustion markers; indeed, an increased level of terminally differentiated EM re-expressing CD45RA is a characteristic trait of patients infected with SARS-CoV-2, as we previously showed, which is even more pronounced in patients treated with RTX (De Biasi et al., 2020; Fenizia et al., 2022). On the CTLs, the proinflammatory cytokine IFN-γ, the degranulation marker CD107a, and the activation marker HLA-DRII were evaluated (Figure 1f). While observing an increasing trend for all three considered activation markers, no significant differences were reached over time, nor compared with the HC group (data not shown). The direct correlation between the neutralization titer assay and the percentage of cells expressing the activation markers (i.e., IFN-γ, CD107a, or HLA-DRII) was not significant in the RTX group (Figure 1g) or in the HC group (not shown).

Our results showed poor B-lymphocyte reconstitution on RTX treatment, together with a strongly decreased neutralization ability than to RTX-untreated HCs. However, both Th and CTL lymphocytes developed a specific anti−SARS-CoV-2 response in patients treated with RTX. Similarly, a robust T-lymphocyte−driven response is evident in convalescent and protected individuals, even despite a poor antibody response, such as in patients with multiple sclerosis treated with disease-modifying therapies, such as RTX (Apostolidis et al., 2021).

Overall, our results provide previously unreported evidence that the SARS-CoV-2 vaccine–elicited specific T-cell–mediated immunologic memory is largely intact in RTX-treated patients affected by PV, despite the extremely low humoral antibody response, which has been considered the most representative of vaccine efficacy by far. Finally, we provide relevant insight on vaccine efficacy in immunocompromised subjects that will possibly be of paramount importance for clinical management of this cohort and for defining appropriate public health guidelines for vulnerable populations.

Data availability statement

No large datasets were generated or analyzed during this study. Data related to this article will be made available by the authors, without undue reservation.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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Response to Gattinger et al

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Abbreviations: AD, atopic dermatitis; ALEX[2], allergy xplorer; CCD, cross-reactive carbohydrate determinants; Der p, Dermatophagoides pteronyssinus*; HDM, house dust mite; ISAC, immuno solid-phase allergen chip; MeDALL, mechanisms of the development of allergy*

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TO THE EDITOR

With great interest, we read the controversy between González-Pérez et al. (2022), challenging the supposedly elevated prevalence of IgE sensitization to Der p 11 from the house dust mite