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Cellular responses to SARS-CoV-2 vaccination after B-cell depletion: conflicting results from studies

I read with interest the Article by Matthias Moor and colleagues evaluating humoral and cellular immune responses to SARS-CoV-2 mRNA vaccination in patients with a history of B-cell depletion.[1] They described blunted humoral responses to vaccination in individuals with a variety of diseases treated with B-cell depletion, as reported now in numerous other studies. In addition, the authors reported blunted cellular responses in their study population and concluded that B-cell depletion impacts both the B-cell and T-cell response to SARS-CoV-2 vaccination.

The report of blunted cellular responses to SARS-CoV-2 vaccination contrasts with other studies that have described either preserved or more robust cellular responses in patients on B-cell-depleting therapies than in healthy volunteers or those on other therapies.[2–4]

The source of this discrepancy might relate to the methodology used—Moor and colleagues measured the amount of interferon γ (IFNγ) released following stimulation with SARS-CoV-2 peptides as opposed to measuring the number of cells producing IFNγ (via the enzyme-linked immune absorbent spot [ELISpot] assay[2,3]) or identifying the individual cells producing IFNγ or antigen-specific T cells (via flow-cytometry[4]), as has been done in other studies. Alternatively, the Methods section of their Article states that they stimulated with peptide or mitogen for 1 h before the IFNγ ELISA measurement.[1] This duration of stimulation is far shorter than the duration (16–24 h) recommended by the manufacturer (Qiagen, Hombrechtikon, Switzerland; category number 626715) and the time period in other assays.[2,3] It is important to clarify whether this was an intentional departure from the suggested protocol or a typographical error.

Besides the methodology, other explanations for the discrepancies could be that, in the study by Moor and colleagues, some patients were taking additional immunosuppressive medication, such as steroids, calcineurin inhibitors, and antimetabolites, as well as B-cell-depleting therapies, which probably also affect the cellular response to vaccination.[1] Indeed, studies on patients not receiving these additional agents show more robust cellular responses, suggesting that B-cell depletion alone[2,4] does not blunt this aspect of the immune response to SARS-CoV-2 vaccination. Perhaps the strongest evidence supporting this is the more robust cellular immune responses to SARS-CoV-2 vaccination in patients with X-linked agammaglobulinaemia than in healthy controls.[5]

Lastly, there are differences in demographic characteristics and treatments between the various conditions included in these studies,[1–4] making it difficult to establish whether alterations in vaccine response might be driven primarily by the underlying disease.

Thus, it is important to understand whether the discrepant results pertaining to cellular responses to SARS-CoV-2 vaccination are related to methodological issues and to highlight that the observed blunted cellular responses could potentially be attributed to factors besides B-cell depletion.

I declare no competing interests.

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1 Moor MB, Suter-Riniker F, Horn MP, et al. Humoral and cellular responses to mRNA vaccines against SARS-CoV-2 in patients with a history of CD20 B-cell-depleting therapy (RituxiVac): an investigator-initiated, single-centre, open-label study. *Lancet Rheumatol* 2021; **3:** e789–97.

2 Gadani SP, Reyes-Mantilla M, Jank L, et al. Discordant humoral and T-cell immune responses to SARS-CoV-2 vaccination in people with multiple sclerosis on anti-CD20 therapy. *EBioMedicine* 2021; **73:** 103636.

3 Prendecki M, Clarke C, Edwards H, et al. Humoral and T-cell responses to SARS-CoV-2 vaccination in patients receiving immunosuppression. *Ann Rheum Dis* 2021; **80:** 1322–29.

4 Madelon N, Lauper K, Breville G, et al. Patients treated with anti-CD20 therapy can mount robust T cell responses to mRNA-based COVID-19 vaccines. *medRxiv* 2021; published online July 23. https://doi.org/10.1101/2021.07.21.21260928.

5 Hagin D, Freund T, Navon M, et al. Immunogenicity of Pfizer–BioNTech COVID-19 vaccine in patients with inborn errors of immunity. *J Allergy Clin Immunol* 2021; **148:** 739–49.

Authors' reply

We thank Pavan Bhargava for pointing out an error in the Methods section of our Article. For the interferon γ (IFNγ) release assay, cells were indeed stimulated for 16 h, not 1 h. The original Article[1] has now been formally corrected. Bhargava then raises several points that we find difficult to agree with.

First, Bhargava appears to question the merit of studying a mixed population of patients that have different disease aetiologies and that are predominantly under co-treatment with additional immunosuppressives. We believe we have addressed this point, however. In the appendix of our Article, we present subanalyses for the different disease subpopulations and co-treatments.

Next, our analysis included only four patients with multiple sclerosis that were treated with ocrelizumab. The study by Gadani and colleagues[2]—one of the key studies Bhargava cited to support their argument—exclusively focused on patients with multiple sclerosis, the majority of whom were treated with ocrelizumab monotherapy, and hence the results of these two studies cannot be compared. As stated in the appendix of our Article (appendix p 7), ten (56%) of 18 participants on anti-CD20 monotherapy mounted a positive IFNγ release (table). Since our Article was published, we have

completed analyses in more patients; the full dataset shows a positive IFNγ release in 41 (44%) of 93 patients.

Furthermore, the results of different measures of cell-mediated immunity are not interchangeable and methodological differences might yield different results—as perhaps Bhargava was also alluding to. For example, previous literature on tuberculosis showed differences between the whole-blood-based quantitative IFNγ release assays and the more sensitive, but only semiquantitative, ELISpot analyses from peripheral blood mononuclear cells.[3] Most of the published studies on SARS-CoV-2-specific T-cell responses so far have used ELISpot assays. This highly sensitive, yet only semiquantitative, assay measures the number of T cells that recognise a given antigen. In our study, however, we measured the total amount of IFNγ released after stimulation. This approach integrates the number of reactive T cells with the amount of IFNγ produced per cell. Increasingly, data show that anti-B-cell monotherapy also affects T cells.[4] A recent study by Apostolidis and colleagues[5] reported a detectable, but altered, CD4+ T-cell response in patients on anti-CD20 therapies but also found a higher CD8+ T-cell response. Thus, it seems possible that, overall, B-cell depleting therapies impair the functional quality of T cells rather than their quantity. This finding is poorly understood for now, but points towards a key role of B cells in the promotion of cellular immunity.

Finally, as age is an important influencing factor regarding the strength of immune responses, it is worth considering that our anti-CD20-depleted patient population had a median age of 67 years, whereas the anti-CD20 treated population in the study from Gadani and colleagues was considerably younger (median age of 48 years). In addition, Gadani and colleagues' regression analysis was based on 30 patients and 12 controls and included four independent parameters. A model with such few participants and relatively many parameters may be at risk of overfitting, and thus might fail to correctly adjust for the age difference. Moreover, of the 38 patients, none of the 30 in whom cellular responses were analysed had been subjected to a serological assessment of previous SARS-CoV-2 exposure, as far as reported in Gadani and colleagues' paper.

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