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Correspondence

Impaired memory B-cell response to the Pfizer-BioNTech COVID-19 vaccine in patients with common variable immunodeficiency



To the Editor:

The recent article by Hagin et al¹ reports that most patients with inborn errors of immunity (IEI) generate humoral and cellular immune responses to the Pfizer-BioNTech COVID-19 vaccine. Neutralizing anti-receptor-binding domain (RBD) antibodies, RBD-specific B cells of the IgG⁺ and IgA⁺ isotype, and T cells producing IL-2 and IFN- γ were detected in most vaccinated patients.

Hagin et al¹ conclude that patients with IEI should be vaccinated because most of them are able to generate protective responses. Although we completely agree on the necessity of vaccinating patients with IEI, it is also indispensable to correctly evaluate the establishment and duration of protective immunity in this group of patients.

We conducted a similar study in a cohort of 33 patients with common variable immunodeficiency (CVID). We evaluated the level of SARS-CoV-2-specific serum antibodies and frequency of memory B cells (MBCs) following administration of the

Pfizer-BioNTech vaccine. Only 33% of our patients with CVID showed an antibody response, compared with 85.7% of the patients (12 of 14) reported by Hagin et al (see Fig 2, A in Hagin et al¹). Hagin et al¹ also measured RBD-specific MBCs, which play a fundamental role in long-term protection when serum antibody levels decline.² In Fig E2 of Hagin et al¹ (available in the Online Repository at www.jacionline.org), the gating strategy to identify RBD-specific IgG⁺ and IgA⁺ B cells and the percentage thereof are shown. Hagin et al¹ conclude that RBD-binding B cells are detected in healthy vaccinated donors and patients with CVID.

We have different results showing that healthy vaccinated donors generate RBD-specific MBCs after 2 vaccine doses, whereas patients with CVID are unable to do so (Fig 1).

Our different results might be explained by the difficulty of correctly quantifying cells present at low frequency in the sample analyzed. Flow cytometry can effectively and accurately manage extremely rare event analyses down to 10⁻⁵. In cases of rare event analysis, the nonspecific cell events can often outnumber the relevant cell frequency, making the count totally unreliable.³ Introduction of the concepts of limit of detection and limit of quantification in rare event analysis has been a remarkable advancement to ensure robust and reliable measurements of

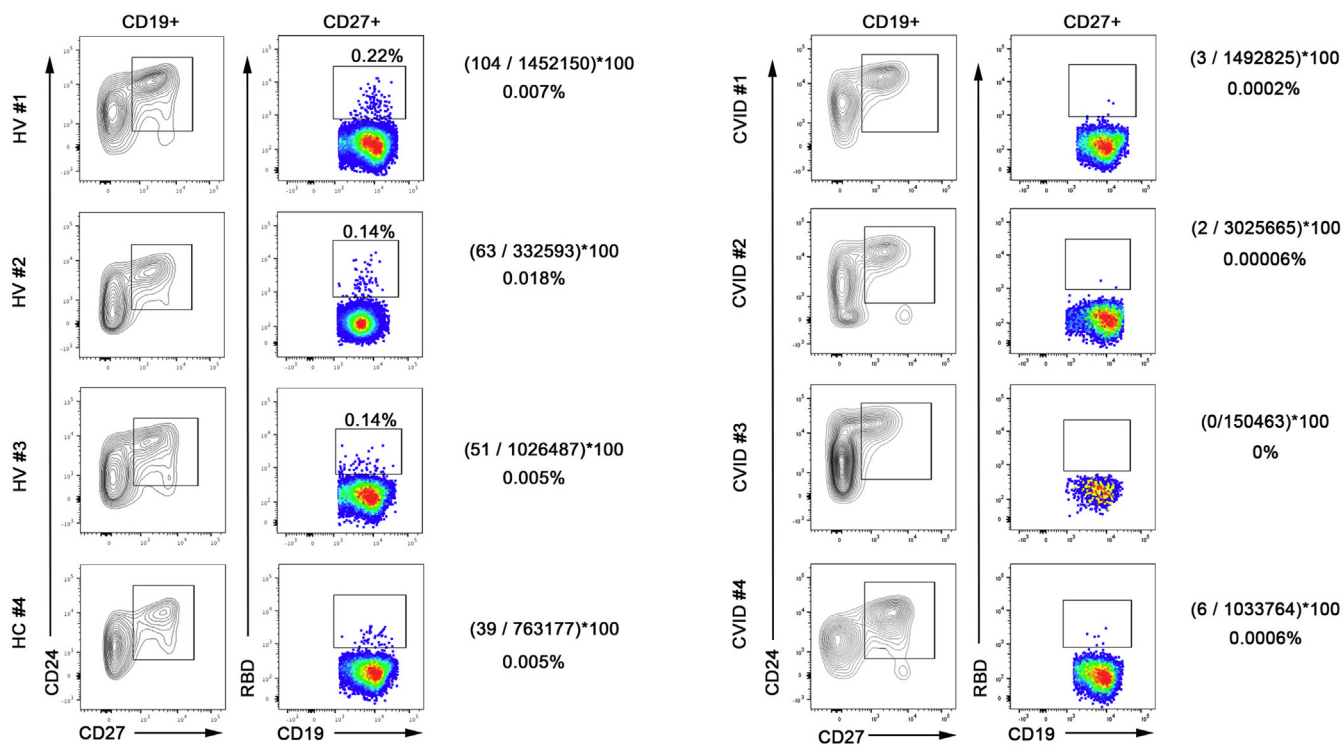


FIG 1. Detection of RBD-binding MBCs in healthy vaccinated donors (HVs) (*left plots*) and patients with CVID (*right plots*). MBCs, contained in the CD19 gate, were identified as CD24⁺CD27⁺. MBCs binding RBDs were detected by RBD-biotin labeled with streptavidin-fluorescein isothiocyanate. The numbers shown correspond to the RBD⁺ events divided by the total number of acquired live cells. HVs 1, 2, and 3 have the requisites to reach the limit of quantification (LOQ), and the percentages can be considered reliable. HV 4 is just under the limit of LOQ, but the number of events is sufficient for the limit of detection. None of the samples from patients with CVID had sufficient RBD⁺ events to reach either the limit of detection or the LOQ. Thus, frequencies are not reported.

rare events. Only when the limit of quantification is achieved can the frequency be considered reliable.⁴ The numbers of relevant events (RBD-specific B cells in this case) should be a defined percentage of the total acquired events.

RBD⁺ cells are a fraction of the MBCs generated by vaccination. B cells acquire increased specificity and affinity thanks to the mechanisms of somatic mutation and selection in the germinal centers. These mechanisms are severely impaired in patients with CVID.⁵

Beyond the technicalities, an inaccurate evaluation of the number of specific MBCs may lead to the conclusion that patients are protected and will be able to react to a SARS-CoV-2 encounter thanks to their MBCs. In contrast, when serum titers decline, patients with CVID will be unable to produce new specific antibodies because they lack the right MBCs. Administration of mAbs may prevent severe disease and emergence of new viral variants in these cases.

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REFERENCES

- Hagin D, Freund T, Navon M, Halperin T, Adir D, Marom R, et al. Immunogenicity of Pfizer-BioNTech COVID-19 vaccine in patients with inborn errors of immunity. *J Allergy Clin Immunol* 2021;148:739-49.
- Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. *Nat Rev Immunol* 2021;21:83-100.
- Hulspas R, O'Gorman MRG, Wood BL, Gratama JW, Sutherland DR. Considerations for the control of background fluorescence in clinical flow cytometry. *Cytometry B Clin Cytom* 2009;76:355-64.
- Illingworth A, Marinov I, Sutherland DR, Wagner-Ballon O, DelVecchio L. ICCS/ESCCA consensus guidelines to detect GPI-deficient cells in paroxysmal nocturnal hemoglobinuria (PNH) and related disorders part 3 - data analysis, reporting and case studies. *Cytometry B Clin Cytom* 2018;94:49-66.
- del Pino-Molina L, López-Granados E, Lecrevisse Q, Torres Canizales J, Pérez-Andrés M, Blanco E, et al. Dissection of the pre-germinal center B-cell maturation pathway in common variable immunodeficiency based on standardized flow cytometric euroflow tools. *Front Immunol* 2021;11:3889.

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Reply



To the Editor:

We would like to thank Salinas et al¹ for reading our article² and for their comments. However, they interpreted our conclusions completely incorrectly. Our study did not suggest that vaccinated

patients with inborn errors of immunity (IEI) are able to generate a *protective* immune response; rather, it described an early post-vaccine T-cell and B-cell immunogenicity in patients with IEI. This point is clearly emphasized in the Discussion section of our article.²

The recently reported “breakthrough” COVID-19 cases in vaccinated individuals, which are the result of both the emergence of new viral variants and waning immunity over time, are a challenge in healthy vaccinated individuals, let alone in vaccinated patients with IEI. That said, we are standing behind the presented data and believe that patients with IEI should be encouraged to get vaccinated.

As for the comments on our flow cytometry data that were expressed by Salinas et al,¹ we appreciate their concern regarding correct identification of rare events; however, it is absolutely unnecessary. As correctly noted by Salinas et al,¹ RBD-specific memory B cells are generated by vaccination. However, they should note that our analysis in Fig E2 of our article² identified RBD⁺, CD19⁺ IgG⁺/IgA⁺ B cells (ie, all IgG/IgA B cells that are specific to RBD) and not only memory B cells. Therefore, our analysis also includes activated B cells that may not be yet CD27⁺, as well as plasmablasts that are on their developmental route to becoming antibody-secreting plasma cells. This strategy is completely different, and it is by no means disputed by the results that Salinas et al¹ present in their Fig 1. The reasons why we chose this population are the early time point at which the samples were collected (2 weeks after vaccination) and the fact that the frequency of RBD-specific B cells, and not only RBD-specific memory B cells, is highly correlated with humoral activation and B-cell responses to SARS-CoV-2.

Regarding the suggestion by Salinas et al¹ that the number of RBD-specific B cells be defined in a way similar to the number of paroxysmal nocturnal hemoglobinuria cells, in their article on consensus guidelines to detect glycosylphosphatidylinositol-deficient cells, Illingworth et al³ specifically suggest that limit of detection and limit of quantification should be calculated out of the *gated* events acquired, and not the total number of events. Therefore, in our case, these events should be calculated out of the B-cell population. This approach would make sense, as patients with CVID can have B-cell lymphopenia, and calculating the number of RBD⁺ cells out of the total number of events could result in underestimation of the frequency of antigen-specific B cells within the B-cell population.

In addition, our Fig E2² shows representative plot figures of RBD⁺ B cells, and the percentage of RBD⁺ cells correlated well with the donor anti-S IgG levels. We used several methods to evaluate our patients' humoral response, including commercial anti-S antibody detection assay, in-house ELISA assay, and inhibition assay. All 3 methods showed similar results. It is therefore reasonable to assume that these antibodies were produced by RBD-specific B cells.

Salinas et al¹ detected humoral vaccine response in only 23.5% of their patients with CVID, and they explain this finding by impaired mechanisms of somatic hypermutation and selection in the germinal center. In that regard, the term *CVID* probably includes a group of mechanistically distinct pathologies, mostly affecting cell maturation and differentiation.⁴ Our study showed that patients with CVID exhibit a wide range of anti-SARS-CoV-2 antibody titers following vaccination, with a significantly better responses seen in younger patients (younger than 50 years), as opposed to older patients with CVID. In accordance with our