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Immunogenicity of a third COVID-19 messenger RNA vaccine dose in primary immunodeficiency disorder patients with functional B-cell defects

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Clinical Implications

Antibody responses associated with protection from SARS-CoV-2 infection significantly improved after a third COVID-19 messenger RNA vaccine dose in this cohort of primary immunodeficiency disorder patients with functional B-cell defects.

We recently reported the humoral and T-cell immune responses to SARS-CoV-2 vaccination in primary immunodeficiency disorder (PID) patients with functional B-cell defects.[1] We evaluated the spike protein-specific antibody response after two doses of either the Pfizer-BioNTech or Moderna vaccines. This evaluation included using a receptor blocking activity competition assay that evaluates the ability of the antibody in serum or plasma to bind to the spike protein receptor-binding domain (RBD) and prevent its interaction with the ACE2 cellular receptor, which correlates with the antibody neutralization of viral infection (see Supplemental Methods in this article's Online Repository at www.jaci-inpractice.org).[2,3] We originally found that only two of 35 patients with humoral defects (one of 15 common variable immunodeficiency [CVID] patients) had an ACE2 blocking level of 50% or greater, and that such activity was undetectable in most of these patients. These findings raised the possibility that two injections with messenger RNA (mRNA) vaccines may leave patients with humoral defects with minimal protection from SARS-CoV-2 infection. Recently, the Centers for Disease Control and Prevention recommended a third mRNA dose in patients with moderate and severe immunodeficiencies.[4] The quantity of spike protein-specific antibody has been reported to be significantly increased after a third dose of SARS-CoV-2 mRNA vaccine both in adults aged 60 years and greater and in patients with kidney transplants.[5,6] Notably, the ability of these antibodies to block the interaction of the spike protein RBD with ACE2 was not evaluated.

Here, we report the humoral responses of PID patients after a third COVID-19 vaccine (Table I). We focused on evaluating the efficacy of mRNA vaccination for PID patients with humoral defects, including patients with moderately low to normal levels of B cells and impaired or absent specific antibody responses. We excluded patients with absent B cells and globally reduced antibody production (eg, those diagnosed with X-linked agammaglobulinemia). We did not include patients with hemophagocytic lymphohistiocytosis or autoinflammatory conditions. Median age was 60.9 years and 92% of the cohort was female. All except one of the 14 patients were receiving immunoglobulin replacement therapy (Table I; see Table E1 in this article's Online Repository at www.jaci-inpractice.org). All patients had received three doses of either mRNA-1273 (Moderna, Cambridge, MA, USA) or BNT162b2 (Pfizer-BioNTech, Mainz, Germany) SARS-CoV-2 mRNA vaccines. Half of the patients received the BNT162b2 (Pfizer-BioNTech) vaccine; the remainder received the mRNA-1273 (Moderna) vaccine. There was no vaccine mixing. Median time between the second and third doses was 21.3 weeks. Median timing of laboratory values checked after the third dose and bloodwork was 4.8 weeks. No patients had a known history of SAR-CoV-2 infection before vaccination and none developed a SARS-CoV-2 infection during the study period. No significant adverse events were reported.

To evaluate the immunogenicity of the vaccine, we measured the spike protein-specific antibody response using a SARS-CoV-2 IgG antibody enzyme-linked immunosorbent assay coating with S1 RBD antigen, with reflex to SARS-CoV-2 ACE2 receptor blocking activity. These assays were performed at the Stanford Health Care Clinical Virology Laboratory, a Clinical Laboratory Improvement Amendments of 1988–certified laboratory (see Supplemental Methods).

All 10 patients with CVID mounted an RBD IgG-specific antibody response, and half (n = 5) had functional antibodies that had 50% or greater ACE2 blocking activity. All four specific antibody deficiency (SAD) or hypogammaglobulinemia patients mounted a positive RBD IgG-specific antibody response and had 50% or greater ACE2 blocking activity. Overall, median ACE2 blocking activity increased from 2% to 79% in CVID patients ($P = .007$; n = 10; Wilcoxon matched-pairs test) and from 0% to 94.5% in patients with SAD or hypogammaglobulinemia ($P < .0002$; n = 4; Wilcoxon matched-pairs test) (Figure 1). Only two patients, both with CVID, had decreased ACE2 blocking activity after the third SARS-CoV-2 mRNA vaccine compared with activity after the second dose. These two results were repeated and/or reevaluated. No parameter (e.g. age, gender, IgG at baseline, CD4 and CD8 T cell count, or absolute B-cell count) was significantly associated with higher ACE2 blocking activity. T-cell function in this patient population was normal; all had normal interferon-gamma production after mitogen stimulation. All except one had normal interferon production when stimulated with antigen (SARS-CoV-2) (Table E1).

To the best of our knowledge, this study of PID patients with functional B-cell defects was the first to evaluate ACE2 receptor blocking activity after three doses of the SARS-CoV-2 mRNA vaccine in patients with PID. Based on results showing low ACE2 blocking activity after two SARS-CoV-2 mRNA vaccine doses, immunity after two doses appears to be poor or transient. In the current cohort, most patients had an increase in ACE2 blocking activity, with the most significant change seen in patients with SAD or hypogammaglobulinemia. Median ACE2 blocking activity for this subgroup increased from 2% after two COVID-19 vaccine doses to 94.5% after three doses. In the CVID group, in which B-cell dysfunction was more severe, median ACE2 blocking activity after three COVID-19 doses was 79%. These results show improvement and emphasize the recommendation for three doses of SARS-CoV-2 mRNA

TABLE I. Subjects' characteristics

Subject	Age, y	Sex	Primary immunodeficiency disorder diagnosis	Immunoglobulin replacement therapy	SARS-CoV-2 mRNA vaccine	Weeks between first and second vaccines	Weeks between second and third vaccines	Weeks between second vaccine dose and serology	Weeks between third vaccine dose and serology	SARS-CoV-2 spike protein IgG after two vaccine doses	SARS-CoV-2 ACE2 blocking activity after two vaccine doses	SARS-CoV-2 spike protein IgG after three vaccine doses	SARS-CoV-2 ACE2 blocking activity after three vaccine doses
1	33	F	CVID	Yes	Moderna	4	24.4	8.7	6	+	49%	+	83%
2	41	M	CVID	Yes	Moderna	4	15.7	5.6	3.2	+	31%	+	19%
3	63	F	CVID	Yes	Moderna	4	19.3	7.0	5.4	+	4%	+	50%
4	58	F	CVID	Yes	Pfizer-BioNTech	3	20.6	4.9	3.2	+	2%	+	48%
5	60	F	CVID	Yes	Pfizer-BioNTech	3	18.7	9.6	4.5	+	37%	+	96%
6	63	F	CVID	Yes	Moderna	4	17.1	9.9	5.7	+	37%	+	11%
7	71	F	CVID	Yes	Moderna	4	22.3	10.7	6.4	+	26%	+	87%
8	73	F	CVID	No	Pfizer-BioNTech	3	25	24.7	4	+	0%	+	24%
9	79	F	CVID	Yes	Pfizer-BioNTech	5	20.1	11.3	4	+	0%	−	42%
10	41	F	CVID	Yes	Pfizer-BioNTech	3	23.1	9.1	5	−	7%	+	79%
11	67	F	Hypogammaglobulinemia	Yes	Pfizer-BioNTech	5	23.3	9.4	4.3	+	6%	+	92%
12	74	F	SAD	Yes	Moderna	3	16.4	14.4	4	+	15%	+	95%
13	64	F	SAD	Yes	Moderna	4	22.3	18.0	6.2	+	0%	+	97%
14	66	F	SAD	Yes	Pfizer-BioNTech	3	30.1	19.0	6.2	+	0%	+	94%

CVID, Common variable deficiency; *SAD*, specific antibody deficiency.

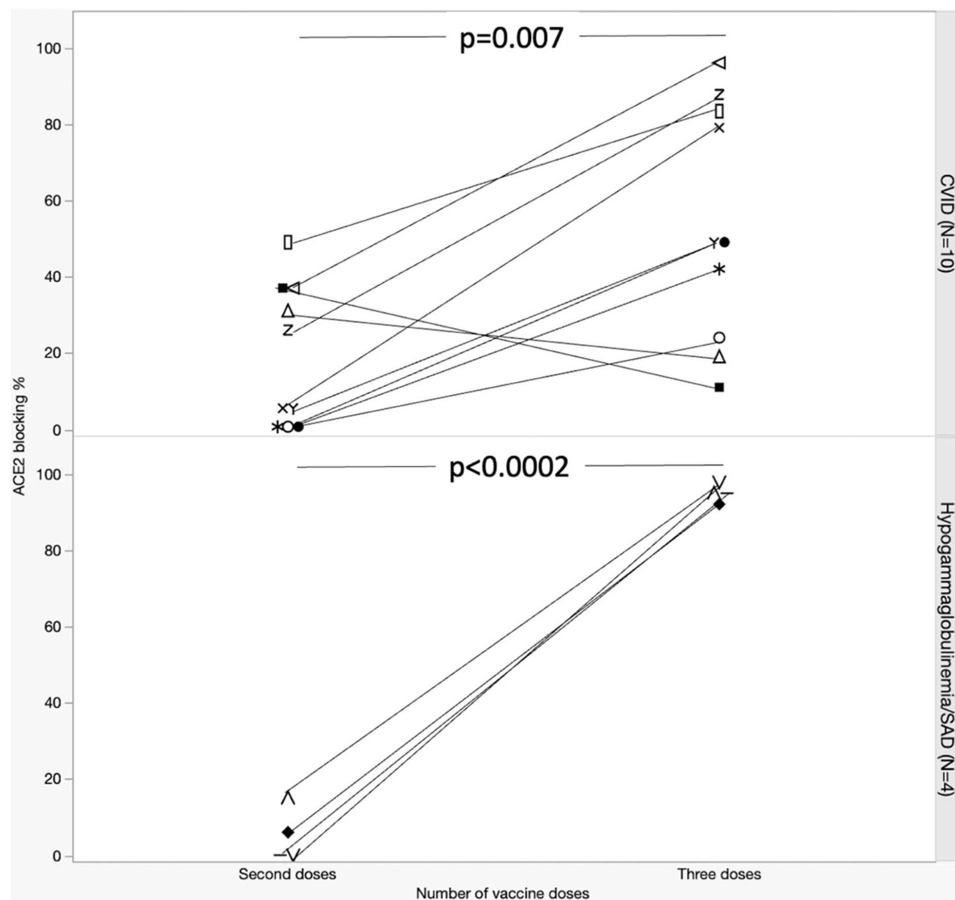

FIGURE 1. SARS-CoV-2 ACE2 blocking activity level in primary immunodeficiency disorder patients with functional B-cell defects (n = 14). Patients were subdivided according to the diagnosis of common variable immunodeficiency (CVID) (n = 10) or specific antibody deficiency (SAD)/hypogammaglobulinemia (n = 4). The data analysis was performed using the Wilcoxon matched-pairs test.

vaccines in patients with primary immunodeficiencies. Although improvement was seen in patients with functional B-cell defects, it is unclear whether such improvement is enough to protect against infection from COVID-19 and how long this ACE2 receptor blocking activity will be sustained. Many patients remain unprotected, and other therapies should also be considered. Emerging treatments such as SARS-CoV-2 monoclonal antibody, which has recently received US Food and Drug Administration emergency use authorization for preexposure prevention of COVID-19, may be another avenue to protect this high-risk group.

This study had several limitations, including the relatively small size of the cohort and the lack of healthy controls. The study population was also predominantly female and older (median age, 60.9 years). We chose a threshold of ACE2 receptor blocking activity of 50% and greater for a positive result for this report, but further studies are needed to establish reference ranges that denote protection. Testing was also performed a median of 4.8 weeks after the third dose. We plan to monitor SARS-CoV-2 ACE2 receptor blocking activity longitudinally to assess the durability of this response. Nevertheless, this small study suggests that a third dose of SARS-CoV-2 mRNA vaccines seems to be beneficial in PID patients with CVID, SAD, or hypogammaglobulinemia, and emphasizes the importance of testing the function of these antibodies in this patient population.

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ONLINE REPOSITORY

Supplemental methods

Enzyme-linked immunosorbent assay to detect anti–SARS-CoV-2 and anti-SARS-CoV antibodies in plasma samples and competition enzyme-linked immunosorbent assay to detect antibodies blocking the binding of ACE2 to receptor-binding domain. This assay, along with its references, was performed at the Stanford Health Care Clinical Virology Laboratory, a Clinical Laboratory Improvement Amendments of 1988–certified laboratory. In brief, 96-well Corning Costar high binding plates (Catalog No. 9018, Thermo Fisher, Waltham, MA, USA) were coated with SARS-CoV-2 spike receptor-binding domain protein in phosphate-buffered saline (PBS) at a concentration of 0.1 μg/well overnight at 4°C. Each sample was run once. All competition Enzyme-linked immunosorbent assay steps were carried out on the next day at room temperature. Wells were washed three times with PBS-T and blocked with PBS-T containing 3% nonfat milk powder for 1 hour. Wells were then incubated with plasma samples from the cohort of patients at a dilution of 1:10 in PBS-T containing 1% nonfat milk for 1 hour. Two quality controls (Access SARS-CoV-2 IgG QC, QC1-QC2, two levels; Catalog No. C58964, Beckman Coulter, Miami, FL, USA) and two blank wells incubated with PBS-T containing 1% nonfat milk were included for each plate. We added ACE2-mFc diluted to 0.5 μg/mL in 1% nonfat milk powder without washing steps and incubated it for an additional 45 minutes. After that was washed three times with PBS-T, horseradish peroxidase-conjugated goat anti-mouse IgG (Fc-specific; Catalog No. 31439, Invitrogen, Waltham, MA, USA; 1:10,000 dilution) in PBS-T containing 1% nonfat milk was added and incubated for 45 minutes. Wells were washed three times with PBS-T and dried by vigorously tapping plates on paper towels. TMB substrate solution was added, and the reaction was stopped after 12 minutes by the addition of 0.16 M sulfuric acid. The optical density (OD) at 450 nm was measured with an EMax Plus microplate reader (Molecular Devices, San Jose, Calif). The OD values were converted to the percentage of blocking using the formula: $100 \times (1 - [\text{sample OD} - 0.2] / [\text{QC1 OD} - 0.2])$, considering the background noise of the assay of 0.2 as determined after testing pre-pandemic control plasma samples.

Analysis and data visualization. Software used to analyze and visualize data was from JMP (version 15, SAS Institute Inc, Cary, NC, 1989-2021) and Microsoft Corporation (Microsoft Excel, Redmond, WA, 2018).

TABLE E1. Subjects' characteristics

Subject	Age, y	Primary immunodeficiency disorder diagnosis	Absolute lymphocyte count, K/μL	B cells, μL	T cells (CD3), μL	T cells (CD4), μL	T cells (CD8), μL	T-cell function (interferon/ mitogen), IU/mL	T-cell function (interferon/ antigen), IU/mL	Baseline IgG, mg/dL	Immunosuppressant therapy
1	33	CVID	1,630	212	1,320	799	1,456	4.19	>10.00	–	None
2	41	CVID	1,344	40	981	524	417	>10.00	>10.00	542	None
3	63	CVID	1,113	71	796	504	292	2.07	>10.00	333	None
4	58	CVID	1,870	449	1,253	804	411	4.07	>10.00	361	None
5	60	CVID	1,887	226	1,189	774	396	>10.00	>10.00	399	None
6	63	CVID	1,822	109	1,330	875	474	>10.00	>10.00	413	Budesonide
7	71	CVID	2,982	209	2,117	1,700	388	>10.00	>10.00	473	None
8	73	CVID	1,495	194	912	628	254	>10.00	3.51	377	Hydroxychloroquine
9	79	CVID	1,066	11	831	725	117	0.79	0.15	374	None
10	41	CVID	1,566	266	1,237	626	407	>10.00	7.88	–	None
11	67	Hypogammaglobulinemia	1,359	82	1,182	761	408	>10.00	2.84	611	None
12	74	SAD	1,600	48	1,312	1,136	176	>10.00	1.85	1,130	None
13	64	SAD	1,274	153	981	739	319	4.82	>10.00	664	None
14	66	SAD	1,920	173	1,440	1,267	192	0.77	0.74	923	None

CVID, Common variable immunodeficiency.

An IFN-gamma response of more than 0.35 IU/mL is considered positive for both antigen and mitogen.