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Immunogenicity and tolerability of COVID-19 messenger RNA vaccines in primary immunodeficiency patients with functional B-cell defects

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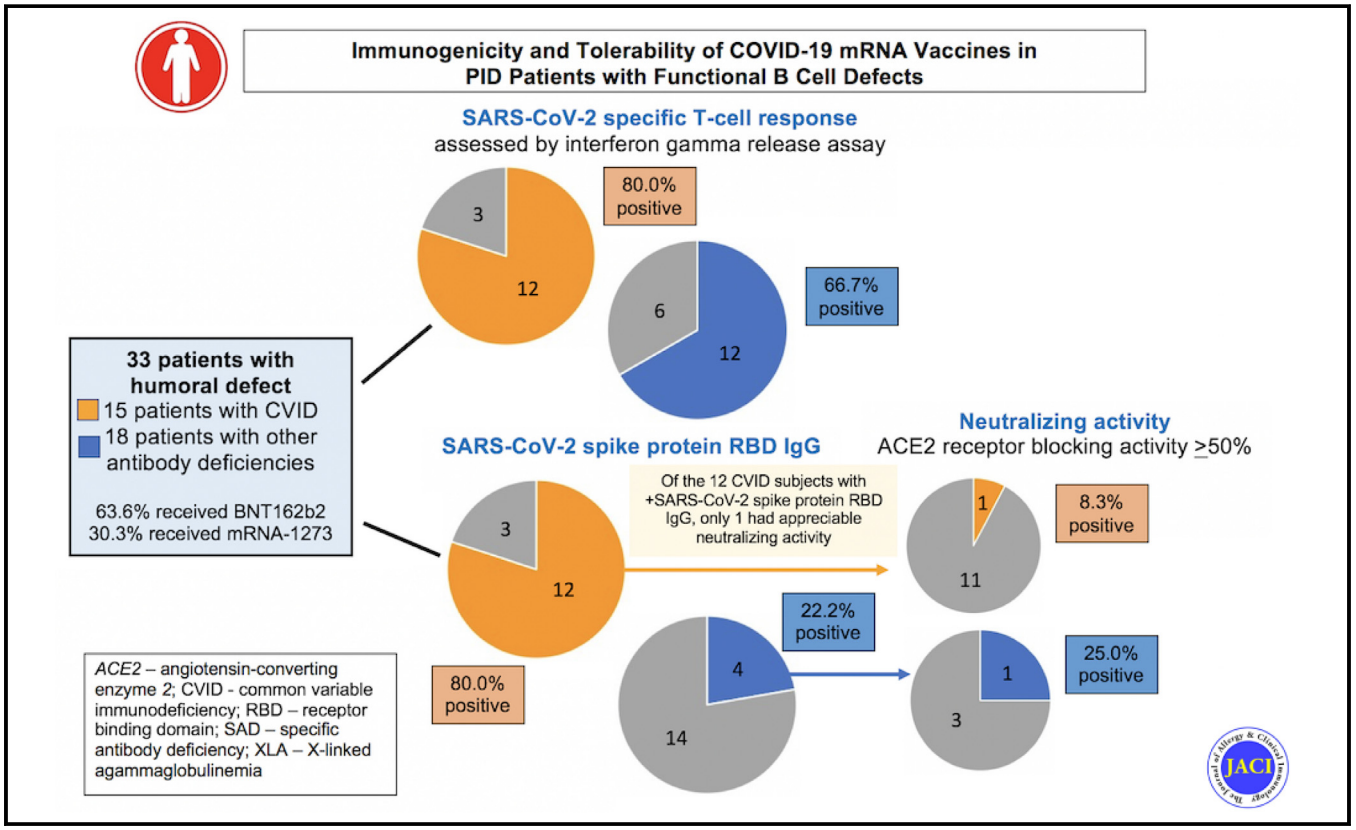
GRAPHICAL ABSTRACT



Background: Data on the safety and efficacy of coronavirus disease 2019 (COVID-19) vaccination in people with a range of primary immunodeficiencies (PIDs) are lacking because these patients were excluded from COVID-19 vaccine trials. This information may help in clinical management of this vulnerable patient group.

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Objective: We assessed humoral and T-cell immune responses after 2 doses of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) messenger RNA (mRNA) vaccines in patients with PID and functional B-cell defects.

Methods: A double-center retrospective review was performed of patients with PID who completed COVID-19 mRNA vaccination and who had humoral responses assessed through SARS-CoV-2 spike protein receptor binding domain (RBD) IgG antibody levels with reflex assessment of the antibody to block RBD binding to angiotensin-converting enzyme 2 (ACE2; hereafter referred to as ACE2 receptor blocking activity, as a surrogate test for neutralization) and T-cell response evaluated by an IFN-γ release assay. Immunization reactogenicity was also reviewed.

Results: A total of 33 patients with humoral defect were evaluated; 69.6% received BNT162b2 vaccine (Pfizer-BioNTech) and 30.3% received mRNA-1273 (Moderna). The mRNA vaccines were generally well tolerated without severe reactions. The IFN-γ release assay result was positive in 24 (77.4%) of 31 patients. Sixteen of 33 subjects had detectable RBD-specific IgG responses, but only 2 of these 16 subjects had an ACE2 receptor blocking activity level of ≥50%.

Conclusion: Vaccination of this cohort of patients with PID with COVID-19 mRNA vaccines was safe, and cellular immunity was stimulated in most subjects. However, antibody responses to the spike protein RBD were less consistent, and, when detected, were not effective at ACE2 blocking. (J Allergy Clin Immunol 2022;149:907-11.)

Key words: *SARS-CoV-2, SARS-CoV-2 vaccination, primary immunodeficiency, ACE2 blocking antibody, SARS-CoV-2 spike protein antibody, antibody deficiency, common variable immunodeficiency, Good syndrome, mAb, SARS-CoV-2 IFN-γ release assay*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains a serious threat to global health and a significant cause of morbidity and mortality, especially in patients with primary immunodeficiencies (PIDs).[1] Two safe and effective messenger RNA (mRNA) vaccines targeting the spike protein of SARS-CoV-2 have been granted emergency use authorization (EUA).[2] SARS-CoV-2-specific humoral and T-cell responses both contribute to protection against coronavirus disease 2019 (COVID-19) infection.[3-5]

Although about 10 million people in the United States are immunocompromised, patients with immunodeficiencies including PIDs were excluded from the SARS-CoV-2 vaccine trials leading up to the EUAs. Thus, data on safety and immune responses to COVID-19 vaccination in recipients with immunodeficiencies and dysregulation syndromes are limited. Recent publications have suggested good tolerance and immunogenicity in patients with PID, but more and larger studies are needed[6-8] that include evaluation of antibody responses that predict protection from infection.

Thirty-three patients with diverse PIDs ranging in age between 19 and 79 years (mean [SD], 50.2 ± 18.35 years) followed at the allergy and immunology clinics at Stanford University and the University of California, San Francisco, were studied. All had received 2 doses of either mRNA-1273 (Moderna) or BNT162b2 (Pfizer-BioNTech) SARS-CoV-2 mRNA vaccines (Table I and see Table E1 in this article's Online Repository at www.jacionline.org). We focused on the evaluation of safety and 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 as well as those with low or absent B cells and globally reduced antibody production. 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 (ELISA) coating with S1 receptor binding domain (RBD) antigen, with reflex to SARS-CoV-2 angiotensin-converting enzyme 2 (ACE2) receptor blocking activity,[9] which correlates well with antibody virus neutralization.[10] Spike protein–specific T-cell responses were evaluated using a SARS-CoV-2 IFN-γ release assay (IGRA).[11] These assays were performed at Stanford Health Care Clinical Virology Laboratory, a Clinical Laboratory Improvement Amendments–certified laboratory.

Abbreviations used

ACE2:	Angiotensin-converting enzyme 2
COVID-19:	Coronavirus disease 2019
CTLA-4:	Cytotoxic T lymphocyte–associated protein 4
CVID:	Common variable immunodeficiency
ELISA:	Enzyme-linked immunosorbent assay
EUA:	Emergency use authorization
IGRA:	IFN-γ release assay
IVIG:	Intravenous immunoglobulin
mRNA:	Messenger RNA
PID:	primary immunodeficiency
SARS-CoV-2:	Severe acute respiratory syndrome coronavirus 2

RESULTS AND DISCUSSION

Testing was performed a mean of 10.9 ± 5.3 weeks after the second vaccine dose, and most subjects had positive immune results to some degree (Fig 1). Twenty-four (77.4%) of 31 patients had positive IGRA results. About half of our subjects (16 of 33) had detectable RBD-specific IgG responses, but only 2 had an ACE2 receptor blocking activity level of ≥50%. Our subjects had impaired antibody responses as their predominant clinical immunodeficiency, such as common variable immunodeficiency (CVID) (n = 15), hypogammaglobulinemia (n = 4), selective antibody deficiency (n = 2), Good syndrome with absent B cells (n = 4), agammaglobulinemia (n = 2), hyper IgM syndrome (n = 2), *PIK3R1* deficiency (n = 1), cytotoxic T lymphocyte–associated protein 4 (CTLA-4) haploinsufficiency (n = 1), and combined immunodeficiency (ataxia telangiectasia, n = 1; *ATP6AP1* gene/immunodeficiency 47, n = 1) (Table I). Thirty-two subjects (96.9%) were receiving immunoglobulin replacement therapy. Sixty-nine percent of the patients received the BNT162b2 (Pfizer-BioNTech) vaccine; the remainder received the mRNA-1273 (Moderna) vaccine. Five had a SARS-CoV-2 spike protein IgG level checked before COVID-19 vaccination, which was undetectable in all cases. None of our patients had a known history of SARS-CoV-2 infection before vaccination, and none developed a SARS-CoV-2 infection during the study period. Clinical data for up to 9 months after vaccination are reported. Tolerability/reactogenicity information was gathered through chart review and revealed that the vaccines were well tolerated (Table II). There were no severe adverse reactions.

TABLE I. Subject characteristics and test results

Subject no.	Age (years)	Sex	PID diagnosis	Antibody deficiency	Ig therapy	SARS-CoV-2 mRNA vaccine	Time between second vaccine dose and serology (weeks)	SARS-CoV-2 spike protein IgG after vaccine	SARS-CoV-2 ACE2 blocking activity	SARS-CoV-2 IGRA
1	21	M	Agammaglobulinemia	Yes	Yes	Pfizer-BioNTech	4.43	Negative	—	Positive
2	30	M	XLA	Yes	Yes	Moderna	4.00	Negative	—	Positive
3	30	F	CVID	Yes	Yes	Pfizer-BioNTech	5.86	Positive	50-60%	Positive
4	32	F	CVID	Yes	Yes	Pfizer-BioNTech	8.71	Negative	—	Positive
5	38	F	CVID	Yes	Yes	Pfizer-BioNTech	4.14	Positive	40-50%	Positive
6	40	M	CVID	Yes	Yes	Moderna	5.57	Positive	40-50%	Positive
7	41	F	CVID	Yes	Yes	Pfizer-BioNTech	9.14	Positive	<10%	Positive
8	53	M	CVID	Yes	Yes	Moderna	9.43	Negative	—	Positive
9	56	M	CVID	Yes	Yes	Pfizer-BioNTech	15.00	Positive	<10%	Negative
10	58	F	CVID	Yes	Yes	Pfizer-BioNTech	4.86	Positive	<10%	Positive
11	59	M	CVID	Yes	Yes	Pfizer-BioNTech	7.00	Negative	—	Negative
12	60	F	CVID	Yes	Yes	Pfizer-BioNTech	9.57	Positive	30-40%	Positive
13	63	F	CVID	Yes	Yes	Moderna	9.86	Positive	30-40%	Positive
14	71	F	CVID	Yes	Yes	Moderna	10.71	Positive	20-30%	Positive
15	72	M	CVID	Yes	Yes	Moderna	17.57	Positive	NA	Positive
16	73	F	CVID	Yes	No	Pfizer-BioNTech	24.71	Positive	<10%	Positive
17	79	F	CVID	Yes	Yes	Pfizer-BioNTech	11.29	Positive	<10%	Negative
18	39	F	HGG	Yes	Yes	Moderna	9.57	Positive	60-70%	Positive
19	55	F	HGG	Yes	Yes	Pfizer-BioNTech	6.85	Negative	—	Positive
20	67	F	HGG	Yes	Yes	Pfizer-BioNTech	9.43	Positive	<10%	Positive
21	75	M	HGG	Yes	Yes	Moderna	16.77	Negative	—	Negative
22	53	F	SAD	Yes	Yes	Pfizer-BioNTech	6.57	Positive	40-50%	Positive
23	74	F	SAD	Yes	Yes	Moderna	14.43	Positive	10-20%	Positive
24	43	M	GS with HGG	Yes	Yes	Pfizer-BioNTech	9.86	Negative	—	Negative
25	65	F	GS with HGG	Yes	Yes	Pfizer-BioNTech	5.86	Negative	—	Positive
26	68	F	GS with HGG	Yes	Yes	Moderna	19.00	Negative	—	Negative
27	70	F	GS with HGG	Yes	Yes	Pfizer-BioNTech	19.14	Negative	—	Negative
28	39	M	Hyper IgM syndrome	Yes	Yes	Pfizer-BioNTech	15.71	Negative	—	Positive
29	40	M	Hyper IgM syndrome	Yes	Yes	Pfizer-BioNTech	13.14	Negative	—	Positive
30	19	M	CTLA-4 deficiency	Yes	Yes	Pfizer-BioNTech	6.43	Negative	—	Positive
31	29	M	*PIK3R1*	Yes	Yes	Pfizer-BioNTech	18.25	Negative	—	—
32	26	F	Ataxia telangiectasia	Yes	Yes	Pfizer-BioNTech	5.71	Negative	—	—
33	20	M	*ATP6AP1* gene/ immunodeficiency 47	Yes	Yes	Pfizer-BioNTech	4.43	Negative	—	Positive

GS, Good syndrome; *HGG,* hypogammaglobulinemia; *NA,* not applicable; *SAD,* specific antibody deficiency; *XLA,* X-linked agammaglobulinemia.

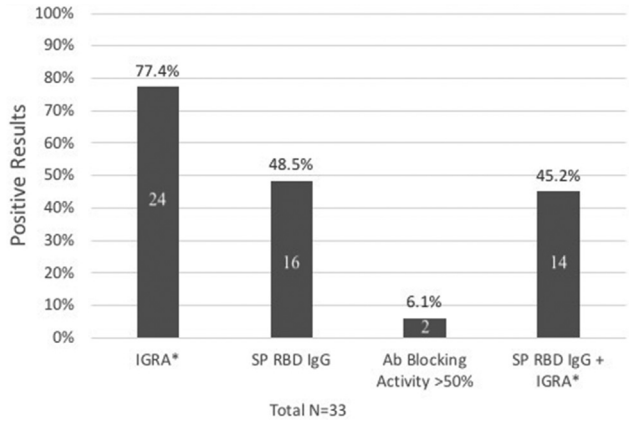

FIG 1. Immunogenicity of the SARS-CoV-2 vaccines in PID patients with functional B-cell defects. SP RBD IgG antibody to the SARS-CoV-2 RBD domain of the spike protein (SP). Antibody blocking activity was ≥50%; ACE2 blocking antibody activity was also ≥50%. *Numbers in bars* signify number of subjects. Unless otherwise noted, sample size is 33. *Denominator is 31.

All our patients had an antibody deficiency, which is the most common general category of PID. As expected, our 4 patients harboring inborn errors that markedly impair IgG antibody production (2 with agammaglobulinemia and 2 with hyper IgM syndrome resulting from CD40 ligand deficiency) had negative SARS-CoV-2 IgG antibody results (Table I, Fig 2). IGRA results were positive for all 4 of these patients, consistent with the relative selectivity of these immunodefects and their largely sparing T-cell immunity. In our subjects with humoral defects as part of CVID (n = 15), 80% had a positive SARS-CoV-2 spike protein RBD–specific IgG result, and 80% had a positive IGRA result. CVID patients with a positive RBD-specific IgG level had a statistically significant higher average of circulating CD3 T-cell level than those who had a negative result (1317 ± 431.9/μL vs 531 ± 157.0/μL, respectively) (*t* test $P = .029$). The mean baseline IgG levels were also higher in CVID patients who had a positive RBD-specific IgG responses than those who did not (364.7 ± 102.0 mg/dL vs 91.0 ± 58.0 mg/dL, respectively (*t* test $P = .004$). A striking finding was that only 1 of the 15 CVID patients with a positive RBD IgG-specific antibody

TABLE II. Adverse effects after SARS-CoV-2 vaccination

Adverse effect	No. (%)
Sore arm	6 (18.2)
Fatigue	4 (12.1)
Headache	5 (15.1)
Local reaction/rash	2 (6)
Fever/chills	1 (3)
Myalgias	1 (3)
Neck stiffness	1 (3)
Vertigo/paresthesia	1 (3)
Nausea/vomiting	1 (3)
Flare of enteropathy*	1 (3)
Flare of chronic urticaria	1 (3)
Total subjects with symptoms	14/33 (42)

*Flare occurred 1 week after vaccination.

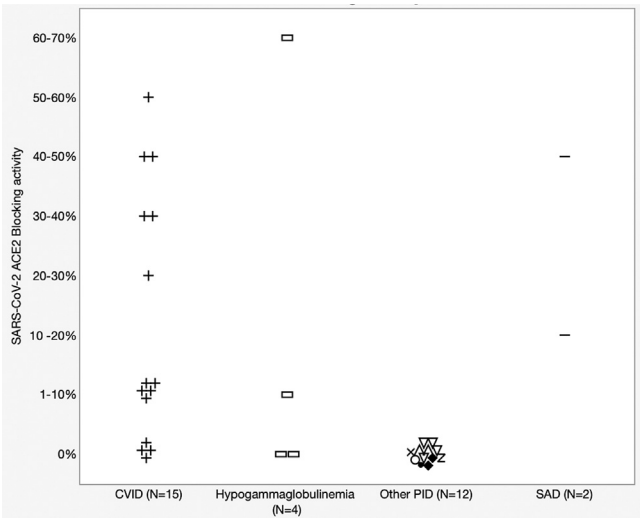

FIG 2. SARS-CoV-2 antibody ACE2 blocking activity in 33 PID patients with B-cell functional defect. Patients were subdivided according to different disease categories. "Other PID" includes X-linked agammaglobulinemia (XLA) patients (n = 2), Good syndrome (n = 4), CTLA-4 haploinsufficiency (n = 1), *PIK3R1* (n = 1), AT (n = 1), and *ATP6AP1* (n = 1). *AT,* Ataxia telangiectasia.

response also had functional antibodies that blocked the interaction of the RBD with ACE2, as assessed in the ACE2 blocking antibody assay.[9] Because this blocking activity correlates well with antibody that effectively neutralizes SARS-CoV-2 for entry into host cells,[10] this indicated that the bodies of most CVID patients did not mount antibody responses that would be protective against SARS-CoV-2 infection.

The responsiveness the disease of patients with PID to COVID-19 vaccination might be potentially difficult to assess if the patient is receiving immunoglobulin replacement therapy with a product that contains SARS-CoV-2 antibody derived from donors who have had SARS-CoV-2 infection, who have received COVID-19 vaccines, or both. We anticipated that this was unlikely to account for the presence of 46.9% of our 32 patients who were receiving immunoglobulin replacement therapy having any spike protein–specific antibody, given the usual lag between seroprevalence in the blood donor population and the specific antibody in manufactured immunoglobulin products.[12-16] To evaluate the potential impact of immunoglobulin therapy on SARS-CoV-2 spike protein RBD–specific humoral responses, we evaluated 2 patients (patients 24 and 32; Table I) for SARS-CoV-2 ACE2 receptor blocking antibody levels before and after intravenous immunoglobulin (IVIG) therapy. For patient 24, both before and after IVIG therapy, ACE2 receptor blocking activity was <10%, and for patient 32, the post-IVIG ACE2 receptor blocking activity minimally changed from <10% before infusion to 14% after infusion. Thus, in these 2 subjects, the IVIG products they received in September 2021 (over 1.5 years since the start of the global COVID-19 pandemic) did not appreciably alter their levels of protective neutralizing antibody.

To our knowledge, this study of PID patients with functional B-cell defects is the first to evaluate the ACE2 receptor blocking activity after 2 doses of the SARS-CoV-2 mRNA vaccines. The receptor blocking activity competition assay evaluates the ability of the antibody in serum or plasma to bind to the spike protein RBD and prevent its interaction with ACE2.[9] The level of receptor blocking activity may correlate with antibody-mediated neutralization assays that use viruses pseudotyped with the spike protein.[10] Thus, our finding that only 1 of 15 CVID patients had an ACE2 blocking level of ≥50% and that such activity was undetectable in most of these patients raises the possibility that mRNA vaccination may provide minimal protection from SARS-CoV-2 infection for CVID patients. It is also important to consider that the ACE2 receptor blocking assay used the RBD similar to that encoded by the current EUA-approved mRNA vaccines, and protection might be even further reduced with SARS-CoV-2 variants that have amino acid changes in the RBD domain.

Similar to Hagin et al,[7] 80% of our patients with CVID had a spike protein RBD–specific IgG response. Additionally, 80% of our CVID patients had spike protein–specific T-cell immune response. In the antibody-deficient patients in our cohort, and in contrast to the study of Hagin et al, there was no difference between age or IgG at baseline and a positive SARS-CoV-2 spike protein result, but those with a positive IGRA result were younger, with a mean age of 48.2 ± 17.7 versus 64.3 ± 12.4 years (*t* test $P = .032$).

This study has several limitations, including the relatively small size of our cohort and the relatively short period of the vaccine observations. We plan to measure and report on additional data including our patients' responses to a third vaccine dose, given new recommendations by the US Centers for Disease Control and Prevention for a third mRNA dose in patients with moderate and severe immunodeficiencies.[17] We also did not include any patients with hemophagocytic lymphohistiocytosis or autoinflammatory conditions. Additionally, the ability to interpret the clinical significance of individual patient ACE2 receptor blocking activity for providing protection will require additional clinical studies to establish validated cutoff values. The threshold of ACE2 receptor blocking activity of ≥50% for a positive result was chosen for this report, but further studies are needed to more precisely establish protective ranges.

Currently, SARS-CoV-2 mAb therapies are granted EUA for use in older and high-risk individuals, such as some PID patients, for postexposure prophylaxis or infection with SARS-CoV-2. In patients with humoral defects where functional antibody protection is not achieved, either through vaccination or immunoglobulin replacement therapy, it would be reasonable to expand mAb therapy to serve as a prophylactic in this high-risk patient population. In fact, an EUA has recently been requested for a mAb cocktail (AstraZeneca) to serve as preexposure prophylaxis in vulnerable populations, such as the immunocompromised. Studying vaccinated PID patients

and their neutralizing antibody may help determine those who can benefit from such prophylactic therapy.

In our cohort of PID patients with functional B-cell defects, mRNA vaccines were well tolerated, and although antibody responses to the spike protein that are associated with protection were not reliably induced in most of our subjects, T-cell responses were elicited in most of our patients. These T-cell immune responses are anticipated to be helpful in limiting virus replication in cases of established infection.[5] Further long-term studies will aid in determining effective therapies and recommendations in patients with PID during this SARS-CoV-2 pandemic.

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Clinical implications: mRNA vaccination may be less effective at preventing acquisition of SARS-CoV-2 in our cohort of PID patients with functional B-cell defects. Induction of SARS-CoV-2 spike protein–specific T-cell immunity by vaccination might help reduce disease severity in these patients.

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METHODS

X-linked agammaglobulinemia is a humoral immunodeficiency caused by mutations in Bruton tyrosine kinase, a key signal transduction molecule required for B-cell development. Patients have low to absent B cells (most patients have a small number of B cells), and reduced levels of all immunoglobulin classes. CVID is an antibody deficiency syndrome characterized by decreased serum IgG (IgM and IgA are often decreased) with impaired specific antibody responses. CTLA-4 haploinsufficiency is an immunodysregulatory disorder that can present a CVID-like phenotype when hypogammaglobulinemia is present. Immunodeficiency 47 is a complex immunodeficiency syndrome characterized by hypogammaglobulinemia, recurrent bacterial infections, defective glycosylation of serum proteins, and liver disease with neonatal jaundice and hepatosplenomegaly. Good syndrome with immunodeficiency is a rare condition in which thymoma is associated with hypogammaglobulinemia. It is characterized by increased susceptibility to bacterial, viral, and fungal infections, as well as autoimmunity. Most patients have no circulating B cells. X-linked hyper IgM syndrome is a combined immunodeficiency that is characterized by antibody deficiency as well as an impaired ability of T cells to activate monocytes and dendritic cells. This disease is caused by mutations in CD40 ligand, a molecule that is expressed on the surface of activated T cells. CD40 ligand interacts with CD40 on the surface of B cells to activate immunoglobulin class switching (shifting antibody production from IgM to IgG, IgA, or IgE) and to establish B-cell memory. *PIK3R1* deficiency is also considered a predominantly antibody deficiency. Patients with X-linked agammaglobulinemia, CVID, Good syndrome, X-linked hyper IgM syndrome, *PIK3R1* deficiency, immunodeficiency 47, and CTLA-4 deficiency require uninterrupted immunoglobulin replacement therapy for the antibody deficiency component of their disease (see Table E1).

ELISA to detect anti–SARS-CoV-2 and anti–SARS-CoV antibodies in plasma samples was performed, with ELISA to detect antibodies blocking the binding of ACE2 to RBD. This assay (and its references) was performed at Stanford Health Care Clinical Virology Laboratory, a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. In brief, 96-well Corning Costar high binding plates (Thermo Fisher Scientific, Waltham, Mass, cat. 9018) were coated with SARS-CoV-2 spike RBD protein in phosphate-buffered saline (PBS) at a concentration of 0.1 μg per well overnight at 4°C. All competition ELISA steps were carried out on the next day at room temperature. Wells were washed 3× with PBS–Tween 20 (PBS-T) and blocked with PBS-T containing 3% nonfat milk powder for 1 hour. Wells were then incubated with plasma samples from our 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, 2 levels, cat. C58964, Beckman Coulter, Fullerton, Calif) and 2 blank wells incubated with PBS-T containing 1% nonfat milk were included on each plate. ACE2-mFc diluted to 0.5 μg/mL in 1% nonfat milk powder was added without washing steps and incubated for an additional 45 minutes. After washing 3× with PBS-T, horseradish peroxidase–conjugated goat anti-mouse IgG (Fc specific, cat. 31439, Invitrogen [Thermo Fisher Scientific], 1:10,000 dilution) in PBS-T containing 1% nonfat milk was added and incubated for 45 minutes. Wells were washed 3× with PBS-T and dried by vigorous tapping of plates on paper towels. TMB (3,3′,5,5′-tetramethylbenzidine) substrate solution was added, and the reaction was stopped after 12 minutes by addition of 0.16 mol sulfuric acid. The OD at 450 nanometers was measured with an EMax Plus microplate reader (Molecular Devices, San Jose, Calif). OD values were converted to percentage of blocking using the following formula: $100 \times [1 - (\text{sample OD} - 0.2)/(\text{QC1 OD} - 0.2)]$, taking into account the background noise of the assay of 0.2 as determined after testing prepandemic control plasma samples. A detailed description is provided in Roltgen et al.[E1]

The IGRA used here measured IFN-γ released by antigen-specific T cells after overnight stimulation with a commercially available peptide pool consisting of spike, S1, nucleocapsid, and membrane proteins (Miltenyi Biotec, San Diego, Calif).[E2] This assay (and its references) was performed at Stanford Health Care Clinical Virology Laboratory, a CLIA-certified laboratory. In brief, freshly collected blood in lithium heparin tube was (1) left unstimulated as negative control; stimulated with (2) peptide pool consisting of spike, S1, nucleocapsid, and membrane proteins (Miltenyi Biotec); or (3) mitogen as positive control. The IFN-γ concentration in the plasma fraction was measured with an automated ELISA instrument (IU/mL). IFN-γ response was defined as peptide stimulated minus unstimulated. The Mann-Whitney *U* test was used to compare median IFN-γ responses between groups. The Wilcoxon signed-rank test of medians was used to compare differences between paired results. The receiver operating characteristic curve was used to derive an IFN-γ response cutoff at the Youden maximum index value, which assigns equal weight to sensitivity and specificity.

The criteria to establish results were as follows:

- Criteria for positive: Nil is ≤8.0 and SARS-CoV-2 antigen minus nil is ≥0.35.
- Criteria for negative: Nil is ≤8.0 *and* SARS-CoV-2 antigen minus nil is <0.35 *and* mitogen-nil is ≥0.5.
- Criteria for indeterminate: (1) Nil is ≤8.0 *and* SARS-CoV-2 antigen minus nil is <0.35 *and* mitogen minus nil is <0.5; *or* (2) nil is >8.
- Criteria for borderline result: Although a borderline range has not been defined, various sources of variability can cause a positive result with SARS-CoV-2 antigen minus nil between 0.35 and 0.7.[E3]

The IFN-γ measured by IGRA is a signature cytokine for the T_H1 subpopulation, while mitogen assays measure a much wider panoply of T-cell–related responses. It is thus expected that only the T cells that can recognize specifically SARS-CoV-2 peptides will produce IFN-γ (~<1% of the T-cell population).[E4]

JMP v15 (SAS Institute, Cary, NC) and Microsoft Excel (Microsoft, Redmond, Wash) were used for data analysis and visualization. The chi-square test was performed by JMP v15. Normality of data was determined and established by the Kolmogorov-Smirnov test of normality (https://www.socscistatistics.com/tests/kolmogorov/default.aspx). Two-tailed unpaired *t* tests were performed by the GraphPad QuickCalcs website (https://www.graphpad.com/quickcalcs/ttest1/?format=C).

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TABLE E1. Subject characteristics

Subject no.	Age (years)	Sex	PID diagnosis	ALC	B cells	CD3 T cells	CD4 T cells	CD8 T cells	Immunosuppressant	SARS-CoV-2 spike protein IgG	SARS-CoV-2 ACE2 blocking activity	SARS-CoV-2 IGRA	Baseline IgG	Baseline IgM	Baseline IgA	IgG trough	Genetic information
1	21	M	Agammaglobulinemia	980	20	862	666	118	None	Negative	—	Positive	—	20	<8	738	PID Invitae panel negative
2	30	M	XLA	2158	0	2072	928	1079	None	Negative	—	Positive	—	8	<8	1040	*BTK* c.1349+2dup (splice site)
3	30	F	CVID	1008	71	796	504	131	None	Positive	50-60%	Positive	300	28.1	<8	876	PID: VUS in *PRKCD* and VSP13B
4	32	F	CVID	467	37	420	266	126	Azathioprine	Negative	—	Positive	—	26	0	1127	Negative WES
5	38	F	CVID	1788	215	1570	1091	411	Budesonide	Positive	40-50%	Positive	335	37	23	977	PID panel negative
6	40	M	CVID	1344	40	981	524	417	None	Positive	40-50%	Positive	542	75	90	90	—
7	41	F	CVID	1566	266	1237	626	407	None	Positive	<10%	Positive	—	—	—	926	CVID panel negative
8	53	M	CVID	774L	77L	642L	317	302	Adalimumab, ustekinumab	Negative	—	Positive	<60	<11	<15	968	—
9	56	M	CVID	3320	1162	1594	1328	199	Tocilizumab	Positive	<10%	Negative	288	15	<7	985	*NOD2* and VUS in *ODCK8*, *JAK3*, and *TERT*
10	58	F	CVID	1870	449	1253	804	411	None	Positive	<10%	Positive	361	26	14	818	—
11	59	M	CVID	1300	—	—	—	—	Budesonide, hydrocortisone, vedolizumab	Negative	—	Negative	132	17.4	23.3	1053	c.2104C>T (p.Arg702Trp) was identified in *NOD2*
12	60	F	CVID	1887	226	1189	774	396	None	Positive	30-40%	Positive	399	<1	32	1310	—
13	63	F	CVID	1822	109	1330	875	474	Budesonide	Positive	30-40%	Positive	413	52	67	1150	—
14	71	F	CVID	2982	209	2117	1700	388	None	Positive	20-30%	Positive	473	33	141	857	—
15	72	M	CVID	2242	157	1995	650	1300	None	Positive	—	Positive	150	<5	<5	1020	—
16	73	F	CVID	1495	194	912	628	254	Hydroxychloroquine	Positive	<10%	Positive	377	34	396	No Ig therapy	—
17	79	F	CVID	1066	11	831	725	117	None	Positive	<10%	Negative	374	1110	48	866	VUS in *RECQL4*
18	39	F	HGG	947	30	821	442	359	None	Positive	60-70	Positive	658	44	57	916	—
19	55	F	HGG	1670	117	1386	1052	251	Hydroxychloroquine, mycophenolate	Negative	—	Positive	573	21	115	958	PID panel: VUS *FOXP3, ATM, EPG5,* AND *TTC7A*
20	67	F	HGG	1359	82	1182	761	408	None	Positive	<10%	Positive	611	57	79	1900	—
21	75	M	HGG	1930	251	1583	656	965	None	Negative	—	Negative	661	—	—	1110	PID panel: VUS *ATM*
22	53	F	SAD	1749	175	1294	857	367	None	Positive	40-50%	Positive	1080	78	163	1410	—
23	74	F	SAD	1600	48	1312	1136	176	None	Positive	10-20%	Positive	1130	206	334	1200	—
24	43	M	GS with HGG	1579	0	1,392	199	1,132	Everolimus, prednisone	Negative	—	Negative	259	0	69	754	—
25	65	F	GS with HGG	1642	0	1412	755	903	None	Negative	—	Positive	118	22	11	656	—
26	68	F	GS with HGG	858	0	849	223	601	Tacrolimus	Negative	—	Negative	<8	705	<6	1034	—
27	70	F	GS with HGG	230	2	177	62	122	Cyclosporine, prednisone	Negative	—	Negative	—	—	—	1203	—
28	39	M	Hyper IgM syndrome	—	—	—	—	—	None	Negative	—	Positive	—	301	0	933	*CD40L*: c.491+1G>c (splice donor)

(Continued)

TABLE E1. *(Continued)*

Subject no.	Age (years)	Sex	PID diagnosis	ALC	B cells	CD3 T cells	CD4 T cells	CD8 T cells	Immunosuppressant	SARS-CoV-2 spike protein IgG	SARS-CoV-2 ACE2 blocking activity	SARS-CoV-2 IGRA	Baseline IgG	Baseline IgM	Baseline IgA	IgG trough	Genetic information
29	40	M	Hyper IgM syndrome	1340	107	1179	402	616	None	Negative	—	Positive	—	—	—	854	*CD40L*: 530 A>G
30	19	M	CTLA-4 deficiency	2502	500	1902	1001	626	Sirolimus	Negative	—	Positive	521	31	23	1030	*CTLA-4:* 567+5G>C
31	29	M	*PIK3R1*	795	215	517	231	278	None	Negative	—	—	—	305	0	805	*PIK3R1*:c.1425+1G.A (splice donor)
32	26	F	Ataxia telangiectasia	468	42	295	164	122	None	Negative	—	—	925	88	45	1250	—
33	20	M	*ATP6AP1* gene/ immunodeficiency 47	1376	537	743	523	179	None	Negative	—	Positive	40	7	12	708	*ATP6AP1*: p.E346K; (c.1036G>A)

ALC, Absolute lymphocyte count; *BTK,* Burton tyrosine kinase; *CD40L,* CD40 ligand; *GS,* Good syndrome; *HGG,* hypogammaglobulinemia; *VUS,* variant of unknown significance; *WES,* whole exome; *XLA,* X-linked agammaglobulinemia.