Received: 27 January 2022 | Accepted: 2 April 2022

DOI: 10.1111/imm.13495

LETTER TO THE EDITOR

immunology
Insights into immunity in health and disease

Antibodies to SARS-CoV-2 in patients with primary immunodeficiencies treated with nonspecific immunoglobulins

To the Editor,

The World Health Organization declared the novel coronavirus SARS-CoV-2 outbreak as a global pandemic on March 2020. Since then, more than 250 million people have become infected worldwide exceeding 5 million deaths [1]. Although most people who fall sick with COVID-19 (the infectious disease caused by the virus) experience mild to moderate symptoms and recover easily, some become seriously ill and required intensive care. The risk of severe COVID-19 outcomes is mostly related to advanced age or the presence of comorbidities.

Different studies have demonstrated also a higher COVID-19 mortality rate in patients with primary immunodeficiencies (PIDs) [2]. However, PIDs are a heterogeneous group of diseases and both specific genetic defects and associated comorbidities can determine the variability and severity of COVID-19 presentation. In general, combined immunodeficiencies have the most severe course with frequent hospitalizations, while infections in patients with antibody deficiencies tend to be less severe [3].

In the present study, we have enrolled 19 adult patients with antibody deficiencies (14 of them with common variable immunodeficiency [CVID]) to investigate their response to SARS-CoV-2 stimulus. The patients' median age was 54 years (min: 30 max: 90 years). The cohort, recruited during the fifth wave of the pandemics in Spain, includes 11 men and 8 women. Most patients ($n = 15$) received immunoglobulin replacement therapy and antibiotic prophylaxis, while four received only antibiotics.

Neutralizing antibodies are considered a key element in controlling the spread of the virus. Strikingly, a common finding in the different series that have studied the impact of SARS-CoV-2 infection in patients with PID is the predominance of asymptomatic or mild courses among those patients with humoral deficiencies. Indeed, despite the increased risk of exposure to high-viral loads during their frequent visits to the hospital, we could only obtain microbiological evidence of a SARS-CoV-2 infection in two patients, one of whom required hospitalization.

During the first pandemic waves, local SARS-CoV-2 testing protocols did not prioritize people with PID, likely masking an accurate diagnosis rate. Considering possible previous exposures to the virus, we decided to study in these patients some humoral and cellular parameters related to the immunological memory.

The presence of anti-SARS-CoV-2 IgG neutralizing antibodies in the serum of the patients was determined using the LIAISON SARS-CoV-2 TrimericS IgG assay (Diasorin, Saluggia, Italy), a quantitative chemiluminescence immunoassay. Nucleocapside (N)-specific antibodies were determined qualitatively by electrochemiluminescence using the Elecsys® Anti-SARS-CoV-2 (Roche Diagnostics, Germany), and the study of T CD4+ and T CD8+ lymphocyte responses was done using the Quantiferon SARS CoV-2 assays (Qiagen, The Netherlands). As comparison, a previously-studied group of 50 healthy individuals was included [4].

We detected antibodies against the SARS-CoV-2 Nucleocapside, but not against the Spike, in the serum of eight of the 18 (44%) patients assessed despite the fact that none of them had any report of previous exposure to the virus (Table 1). N-reactive antibodies are readily increased upon infection [5] and, in convalescent patients, occasionally may even dominate the response [6].We reasoned that if these patients had recently become infected, they should have a prominent cellular response (mediated by T lymphocytes) against different viral antigens. Indeed, this response can be detected even in the absence of antibodies, particularly in those patients with mild or asymptomatic SARS-CoV-2 infection. However, when we studied the $CD4^+$ and $CD8^+$ T cell-mediated responses against epitopes from SARS-CoV-2 S1 and S2 subunits, we could not detect interferon gamma production in any of the patients ruling out a recent infection as a source of the antibodies.

All the patients tested as positive for anti-N antibodies received endovenous or subcutaneous immunoglobulin (Ig) infusions as life-long replacement therapy. These preparations are enriched in antibodies with multiple specificities including those reactive against SARS-CoV-2 [7]. Therefore, we investigated the presence of anti-S and anti-N antibodies

TABLE 1 Humoral and cellular response to the SARS-CoV-2 spike protein after full immunization with mRNA-based vaccines

Case	Diagnostic	Covid infection	Immunoglobulin infusion	IgG $Spike_{pre}$	IgG $Nucl_{pre}$	QFR_{pre}	IgG $Spike_{post}$ (AU/ml)	IgG $Nucl_{post}$	QFR_{post}
1	CVID	NOT	YES	NEG	POS	NEG	POS (59)	NEG	ND
2	CVID	NOT	YES	NEG	POS	NEG	POS (800)	POS	POS
3	CVID	NOT	YES	NEG	NEG	NEG	NEG	NEG	NEG
4	CVID	NOT	YES	ND	NEG	ND	NEG	POS	NEG
5	CVID	NOT	YES	NEG	POS	NEG	POS (73)	POS	ND
6	CVID	post-vacinne	YES	NEG	POS	NEG	POS (36)	POS	NEG
7	CVID	NOT	YES	NEG	POS	NEG	NEG	POS	NEG
8	CVID	NOT	YES	NEG	NEG	NEG	POS (745)	NEG	POS
9	CVID	NOT	YES	NEG	POS	NEG	POS (602)	POS	POS
10	CVID	NOT	YES	NEG	POS	NEG	POS (66)	POS	POS
11	CVID	NOT	YES	NEG	NEG	NEG	POS (231)	NEG	POS
12	CVID	NOT	YES	ND	NEG	ND	POS (20)	NEG	ND
13	CVID	post-vacinne	YES	NEG	POS	NEG	NEG	POS	ND
14	CVID	NOT	YES	NEG	NEG	NEG	POS (119)	NEG	POS
15	IgG subclass deficiency	NOT	YES	ND	ND	ND	POS (784)	NEG	POS
16	Specific antibody deficiency	NOT	NOT	NEG	NEG	NEG	POS (>800)	NEG	POS
17	Specific antibody deficiency	NOT	NOT	NEG	NEG	NEG	POS (>800)	NEG	POS
18	Hypogammaglobulinemia	NOT	NOT	NEG	NEG	NEG	POS (>800)	NEG	POS
19	Hypogammaglobulinemia	NOT	NOT	NEG	NEG	NEG	POS (>800)	NEG	POS

Abbreviations: CVID, common variable immunodeficiency; ND, not determined; NEG, negative; Nucl, Nucleocapside; POS, positive; QFR, Quantiferon.

TABLE 2 Presence of anti-nucleocapsid and anti-spike antibodies in immunoglobulin infusions that were used in the treatment of the patients

Batch 1	Product Flebogamma	Sample $n = 1$	Anti-nucleocapsid (signal to cut off ratio) NEG	0.25	Anti-spike (AU/ml) NEG	9.32
2	Flebogamma	$n = 1$	POS	1.69	POS	14.80
3	Flebogamma	$n = 1$	POS	5.72	POS	30.00
4	Flebogamma	$n = 1$	POS	1.77	NEG	12.50
5	Flebogamma	$n = 6$	POS	14.02 +/− 0.55	POS	32.28 +/− 1.49
6	Flebogamma	$n = 5$	POS	3.85 +/− 0.06	NEG	9.95 +/− 0.54
7	Flebogamma	$n = 1$	POS	13.60	POS	34.70
8	HyQvia	$n = 2$	POS	6.06 +/− 0.13	POS	21.60 +/− 2.83
9	Plangamma	$n = 6$	NEG	0.24 +/− 0.12	NEG	5.99 +/− 0.83
10	Plangamma	$n = 1$	POS	57.70	POS	5.01
11	Plangamma	$n = 1$	POS	55.80	POS	55.40
12	Flebogamma	$n = 1$	POS	5.59	POS	27.60

Note: Flebogamma and Plangamma (manufacterer: Instituto Grifols S.A, Barcelona, Spain) and HyQvia (manufacterer: Baxalta Belgium manufacturing, S.A, Lessines, Belgium).
Abbreviations: NEG, negative; POS, positive.

among them. We examined 27 samples corresponding to twelve batches from different products and vendors that were used in the treatment of the patients (Table 2). In 10 out of the 12 batches assessed it was possible to find measurable levels of antibodies against SARS-CoV-2 structures: in eight of these preparations, we could detect antibodies against the Nucleocapsid and the Spike proteins, while in the other two batches we only detect anti-Nucleocapsid antibodies. These results suggest that the Ig preparations are the probable origin of the anti-viral antibodies detected in the patients´ serum. Whether these antibodies might have had any protective role during the different pandemic waves is currently unknown.

The development and administration of safe and effective vaccines to counter the serious consequences of SARS-CoV-2 infection is the only way out to the current pandemic. However, there are still concerns regarding the efficacy of COVID-19 vaccines in immunocompromised individuals. There are studies reporting poorer humoral response to SARS-CoV2 mRNA vaccine in bone marrow or solid organ recipients, in patients with primary immunodeficiencies or autoimmune disorders [8, 9]. In other cases, the conclusion is just the opposite, with anti-S seropositivity rates in up to 85% of the immunodeficient patients after full immunization [10], which was not significantly different from the seroconversion observed in the immunocompetent volunteers. One possible explanation of these discrepancies may be the heterogeneity of the patients included in the cohorts.

We have also investigated the humoral and cellular response to the SARS-CoV-2 Spike protein after full immunization with mRNA-based vaccines in adults with defective production of antibodies (Table 1). With the exception of 4 cases, CVID patients responded to the vaccines by increasing the serum levels of anti-Spike antibodies, although with lower values than in the control group (median antibody levels of 170 [20–800] AU/ml in patients compared to 734 [532–1149] AU/ml in controls [4]). Reactivity against the nucleocapsid was unaltered respect the levels before immunization. Similarly, T cell responses were found well above the threshold of detection of the technique in most patients. In conclusion, this study shows that humoral and cellular immune responses were clearly detected in patients with primary antibody defects after mRNA vaccination, and that Ig infusions could provide them with an additional level of protection against SARS-CoV-2.

FUNDING INFORMATION

This study was supported by the Spanish National Health Institute Carlos III (ISCIII) through the project COV20_00508 grant (Co-funded by European Regional Development Fund/European Social Fund¨a way to make Europë) to RG.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ETHICS STATEMENT

This study was approved by the Ethical Committee for Clinical Investigation of the Institut Hospital del Mar d'Investigacions Mèdiques (CEIC-PSMAR 2021/9726/I and CEIC-PSMAR 2021/9851/I). Informed consent was obtained from all subjects enrolled in the study.

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SUPPORTING INFORMATION

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