Kinetics of immune responses elicited after three mRNA COVID-19 vaccine doses in predominantly antibody-deficient individuals.

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Research Article

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Abstract

Purpose

Mass vaccination campaigns have reduced the incidence and severity of COVID-19. However, there is limited information about how patients with predominantly antibody-deficiencies (PAD) respond to COVID-19 vaccination. Here, we evaluated humoral and cellular responses developed in SARS-CoV-2-naïve PAD individuals after three mRNA-1273 vaccine doses.

Methods

Patients and healthy controls (HCs) were immunized at week 0 (w0) and w4. PAD individuals received an additional dose at w24. Blood samples were collected at w0, w4, w8, w24, and/or w28. We determined levels of anti-Spike and anti-RBD antibodies, Spike-specific IgG avidity, and neutralizing activity (Wuhan-Hu-1, Delta, and Omicron variants). Cellular responses were evaluated by IFN-γ ELISpot and flow cytometry.

Results

Unclassified primary antibody-deficiency patients (unPAD, n = 9) and HCs developed comparable vaccine-induced humoral responses. However, common variable immunodeficiency patients (CVID, n = 12) showed lower antibody responses than HCs. While the frequency of Spike-specific CD4 + T cells was similar between PAD patients and HCs, CD8 + T cells responses were reduced in CVID individuals. Both PAD groups showed lower levels of Spike-specific IFN-γ-producing T-cells. Combined immunodeficiency (CID, n = 1) and thymoma with immunodeficiency (TID, n = 1) patients developed cellular but not humoral responses after two immunizations. The third vaccine dose boosted humoral responses in most PAD patients, but had little effect on cellular immunity.

Conclusion

mRNA-1273 vaccine-induced immune responses in PAD individuals are heterogeneous, depend on the type and degree of antibody-deficiency, and should be immunomonitored to define a personalized vaccination strategy.

Introduction

As of March 2022, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected more than 481 million people worldwide, and reached an overall death toll of 6.12 million (https://covid19.who.int/). Fortunately, mass COVID-19 vaccination has drastically reduced the number of SARS-CoV-2-infected individuals that require hospitalization[1, 2].

Human inborn errors of immunity (IEI) encompass a diverse set of diseases characterized by monogenic germline mutations that result in increased susceptibility to infection, malignant phenotypes, autoimmune, autoinflammatory and allergic diseases[3, 4], mainly due to an impaired immune system and specific immunosuppressive treatment (e.g. B cell-depleting agents). IEI patients show high heterogenicity in their phenotype and clinical manifestation, even in those individuals showing identical genetic alterations[4]. Although these patients were initially considered at risk of severe COVID-19, SARS-CoV-2 seroprevalence and COVID-19-related fatality rate is similar to immunocompetent individuals, with most patients developing mild COVID-19[5]. However, median age of individuals requiring ICU admission, or COVID-19-related deaths are lower among IEI groups compared to the general population[5]. Severe COVID-19 illness in IEI individuals has also been associated with comorbidities, including autoimmune or inflammatory complications, lung disease, or higher proinflammatory responses[5, 6]. Since IEI comprise a highly heterogeneous disease group, severity and fatality rate differ among pathologies[7]. Particularly, severe combined immunodeficiency, autoimmune polyglandular syndrome type 1, innate immune defect, and Good syndrome are among those IEI groups with higher fatality rate and ICU admission after SARS-CoV-2 infection[8].

COVID-19 vaccine clinical trials were initially designed to exclude immunocompromised individuals or people receiving immunosuppressive treatment. Thus, there is limited data about COVID-19 vaccine efficacy in IEI patients. Recent studies determined that BNT162b2 vaccine is well tolerated in IEI patients[9–13], and that most patients mount heterogeneous humoral and cellular responses[9–12]. For example, whereas individuals with X-linked agammaglobulinemia (XLA) do not generate vaccine-induced antibodies, these patients develop potent T cell responses[9–11]. Conversely, SARS-CoV-2-specific immune responses in common variable immunodeficiency (CVID) patients remain controversial. Hagin et al.[10] and Bergman et al.[9] showed that more than 60% of CVID patients seroconverted after COVID-19 vaccination. However, Fernandez-Salina and colleagues[11] reported that 20% of CVID patients elicit an antigen-specific antibody response with reduced T cell frequency after vaccination[11]. Interestingly, CVID patients seroconversion rate is higher after natural infection than vaccination (82% vs 34%, respectively), and subsequent immunization of convalescent CVID patients can boost their humoral responses. Despite this, some patients remain non-responders[13].

While the Center of Disease Control (CDC) started recommending a third vaccine dose to immunocompromised people, its impact on the immune responses of IEI patients has only been partially clarified. Here, we characterized SARS-CoV-2-specific humoral and cellular responses in SARS-CoV-2-uninfected IEI patients with predominantly antibody deficiency (PAD) after three mRNA-1273 vaccine doses.

Materials And Methods

Study design

A prospective observational cohort-comparative study was conducted at the Hospital Universitari Germans Trias i Pujol (Badalona, Spain) with previous Institutional Review Board approval (PI-21-107). We included 27 PAD patients (age > 18 years) that had received immunoglobulin replacement therapy (IRT). Patients with the following conditions were excluded: previous SARS-CoV-2 vaccination, vaccine-induced anaphylactic reaction, allergy to polysorbate or polyetilenglycol, as well as pregnant or breastfeeding women. Samples from 10 COVID-19-vaccinated healthy controls (HCs, age and gender balanced) were included for comparative purposes. All participants provided written informed consent. Patients and HCs were administered with two doses of mRNA-1273 (Moderna) vaccine. A third dose was administered to PAD patients at week 24 (w24) after first immunization.

Samples collection

Blood samples were collected at w0, w4, w8, w24, and w28 post-first dose in EDTA tubes. Peripheral blood mononucleated cells (PBMCs) were isolated by standard density-gradient centrifugation using Ficoll-Paque (Atom Reactiva) and cryopreserved in liquid nitrogen. Plasma was obtained after blood centrifugation and stored at -80ºC until use.

SARS-CoV-2-specific IgG, IgA, and IgM ELISA

Anti-SARS-CoV-2 IgG, IgA, and IgM antibodies were quantified as previously described[14]. Plates were coated with an anti-6xHis antibody (clone HIS.H8; ThermoFisher Scientific) at 2 µg/mL, and blocked with PBS/1% BSA (Miltenyi Biotech). The following SARS-CoV-2 antigens (1µg/mL) were added to half plate: Spike, receptor binding domain (RBD) or Nucleocapsid protein (NP) (Sino-Biological). The other half plate received PBS/1%BSA. Heat-inactivated plasma samples were assessed in duplicate in both wells containing SARS-CoV-2 antigens or PBS/1%BSA. A serially-diluted positive plasma sample was used as standard, and a pool of pre-pandemic SARS-CoV-2-uninfected samples as negative control. Isotypes were detected using: HRP-goat anti-human IgG, HRP-goat anti-human IgM, and HRP-goat anti-human IgA (Jackson ImmunoResearch). O-phenylenediamine dihydrochloride (OPD, Sigma Aldrich) was used as a substrate. Enzymatic reaction was stopped with 2M H_2SO_4 (Sigma Aldrich). Signal was analyzed as optical density at 492 nm with noise correction at 620 nm. Antigen-specific signal was calculated by subtracting background obtained for each sample in antigen-free wells. Results are shown as arbitrary units (AU)/mL.

IgG Avidity ELISA

Plates were coated with Spike (1 µg/mL, Sino Biological) and blocked using PBS/1% BSA. Samples were diluted to 0.5 AU/mL, added to the corresponding wells, and evaluated in quadruplicate. Two wells were incubated with 2M guanidine HCl and PBS for 15 minutes. Plates were incubated with HRP-goat anti-human IgG. Bound antibodies were detected using OPD as described above. Avidity index was calculated as the ratio between mean signal obtained with and without guanidine treatment.

Pseudovirus production and neutralization assay

HIV reporter and replication-incompetent pseudoviruses expressing Wuhan-Hu-1 (WH1), Delta, or Omicron Spike proteins were produced as previously described[14, 15]. Neutralization was evaluated by incubating pseudovirus and heat-inactivated plasma samples before transferring them onto DEAE-dextran-treated HEK293T/hACE2 cells. Neutralization ID_{50} titers (reciprocal 50% inhibitory dilution) were calculated using non-linear fit of transformed data in GraphPad Prism v8.0.

IFN-γ ELISPOT

ELISpot was performed using the Human IFN-γ ELISpot kit (ALP) (Mabtech). ELISpot plates (Millipore) were coated with the anti-IFN-γ 1-D1K antibody (2 µg/mL). PBMCs were thawed and rested in RPMI-1640 media supplemented with 10% FBS, and 1% penicillin/streptomycin (R10) (ThermoFisher Scientific). PBMCs were stimulated for 16 hours with: 1) R10; 2) CytoStim (Miltenyi Biotech); or 3) Spike-S1 peptide pool (Miltenyi Biotech). Plates were incubated with the biotinylated anti-human IFN-γ 7-B6-1 antibody, and streptavidin-ALP. Wells were developed using BCIP/NBT-plus substrate (BioRad). Spots were enumerated using an ImmunoSpot reader (Cellular Technologies Limited). PBMCs from an unvaccinated SARS-CoV-2-negative donor, and from a BNT162b2-vaccinated individual were used as negative and positive controls, respectively.

Flow cytometry (FCM)

PBMCs were stimulated with anti-CD40 (0.5 µg/mL, HB14, Miltenyi Biotech), anti-CD49d (1 µg/mL, 9F10, ThermoFisher Scientific), and Spike-S1 peptides (Miltenyi Biotech) for 16 hours, as previously described[16–18]. Cells were incubated with live/dead fixable aqua (ThermoFisher Scientific). Fcγ receptors were blocked using human truStain FcX (BioLegend). Cells were stained with: CD5-R718 (UCHCT2), CD8-APC-H7 (SK1), CD4-BV605 (RPA-T4), CD25-BV421 (2A3), CD14-V500 (M5E2), CD19-V500 (Hib19), CD154-BB700 (Trap-1), CD137-APC (4B4-1) from BD Biosciences, and OX40-PE-Cy7 (Ver-ACT35) and CD69-PE (FN50) from BioLegend. Samples were acquired on a BD LSRII, and analyzed using FlowJo software (Treestar).

Statistical analysis

ELISA binding data, neutralizing titer, and IFN-γ spot-forming cells/10^5 cells are shown as mean±standard deviation. Differences between PAD and HC groups were established using Kruskal-Wallis test corrected for multiple comparisons using Dunn's test. Wilcoxon signed-rank test was used to identify significant differences elicited over time in one group. A significance threshold of 0.05 was used for each statistical test, and all *P* values reported were two-tailed. Dose-response neutralization curves were fit to a logistic equation by nonlinear regression analysis. Statistical analysis were performed using GraphPad Prism v8.0.

Results

Patient characteristics

Twenty-seven PAD adult patients under IRT were included in the current study. According to 2019 ESID criteria[19], patients were classified into four groups: combined immunodeficiency (CID) (1/27), common variable immunodeficiency (CVID) (14/27), thymoma and immunodeficiency (TID) (1/27), and unclassified primary antibody deficiency (unPAD) (11/27). Main patient characteristics are described in Table 1. No severe adverse effects were reported after mRNA-1273 vaccination (Table 2).

Table 1

Patient characteristics

Mean age (range)	53.52 years (29-73)	
Gender	Female	13 (48.1%)
	Male	14 (51.9%)
Average years since diagnosis (range)	8.67 years (1-31)	
Ig deficiency	IgG	6 (22.2%)
	IgG and IgA	4 (14.8%)
	IgG, IgA and IgM	15 (55.6%)
	IgG and IgM	2 (7.4%)
Underlying or related diseases	Lymphoma	2 (7.4 %)
	Solid cancer	7 (25.9%)
	Basocellular carcinoma	2 (7.4%)
	Breast carcinoma	2 (7.4%)
	Lung adenocarcinoma	1 (3.7%)
	Seminoma	1 (3.7%)
	Thymoma	1 (3.7%)
	Chronic liver disease	1 (3.7%)
	Autoimmune diseases	7 (25.9%)
	Thrombotic thrombocytopenic purpura	2 (7.4%)
	Celiac disease	1 (3.7%)
	Collagenous colitis	1 (3.7%)
	Chron's disease	1 (3.7%)
	Ulcerative proctitis	1 (3.7%)
	Autoimmune anemia	1 (3.7%)
	Lupus-like syndrome	3 (11.1%)
	GLILD	6 (22.2%)
	Asthma	8 (29.6%)

	Drug or food allergies	
Immunosuppressive agents	Azathioprine	1 (3.7%)
	Corticosteroids	1 (3.7%)
	Rituximab	5 (18.5%)
	Rituximab and corticosteroids	1 (3.7%)
Isohemagglutinin levels	Total number of patients evaluated	22 (81.5%)
	High rates	8 (29.6%)
	Low rates	12 (44.4%)
	Not evaluable	2 (7.4%)
Polysaccharide typhim Vi antibody response	Total number of patients evaluated	12 (44.4%)
	Adequate response	6 (22.2%)
	Not adequate response	6 (22.2%)
IRT administration	Subcutaneous	17 (63%)
	Intravenous	10 (37%)
Years on IRT	< 1 year	3 (11.1%)
	1-5 years	12 (44.4%)
	5-10 years	6 (22.2%)
	>10 years	6 (22.2%)
IgG mean trough levels (range)	807.37 mg/dL (598-1,112)	

GLILD: Granulomatous-lymphocytic interstitial lung disease

IRT: immunoglobulin replacement therapy

Table 2
Vaccine-induced adverse effects in IEI patients

Adverse events	First dose	Second dose	Third dose
Local pain	23 (85.2%)	25 (92.6%)	23 (85.2%)
Local blush	3 (11.1%)	5 (18.5%)	6 (22.2%)
Local inflammation	3 (11.1%)	5 (18.5%)	8 (29.6%)
Paresthesia	0 (0%)	1 (3.7%)	1 (3.7%)
Headache	8 (29.6%)	8 (29.6%)	9 (33.3%)
Shivers	6 (22.2%)	9 (33.3%)	11 (40.7%)
Arthromyalgia	6 (22.2%)	11 (40.7%)	11 (40.7%)
Asthenia	11 (40.7%)	14 (51.9%)	15 (55.6%)
Dizziness	1 (3.7%)	4 (14.8%)	4 (14.8%)
Syncope	0 (0%)	0 (0%)	0 (0%)
Nausea/vomiting	1 (3.7%)	2 (7.4%)	1 (3.7%)
Diarrhea	2 (7.4%)	1 (3.7%)	3 (11.1%)
Fever	3 (11.1%)	10 (37%)	11 (40.7%)
Local adenopathy	0 (0%)	0 (0%)	1 (3.7%)
Anaphylaxis	0 (0%)	0 (0%)	0 (0%)
Other adverse events	2 (7.4%)	5 (18.5%)	5 (18.5%)
Medical assistance	0 (0%)	1 (3.7%)	0 (0%)
Sick leave	1 (3.7%)	1 (3.7%)	0 (0%)
Days of sick leave	2 (7.4%)	3 (11.1%)	0 (0%)

To better define vaccine-induced immune responses in PAD subjects, we excluded four patients (two from unPAD, and two from CVID groups) that were diagnosed with SARS-CoV-2 infection either prior to vaccination or during the length of this study. As expected, none of the remaining 23 SARS-CoV-2-naïve PAD patients described below showed antibodies against Spike (Fig. 1 and Sup Fig. 1), RBD (Sup Fig. 2), or NP (not shown) before immunization, confirming their seronegative status.

Vaccine-induced SARS-CoV-2-specific humoral response in PAD patients

To determine how PAD individuals responded to COVID-19 mRNA vaccine, we analyzed the humoral response elicited against Spike (Fig. 1) and RBD (Sup Fig. 2) in 23 SARS-CoV-2-uninfected PAD vaccinees (Fig. 1A). Of note, HCs did not receive the third vaccine dose at w24. While all HCs developed Spike-specific IgG at w8 (Fig. 1B), IgG seroconversion was observed in 25% (3/12) and 67% (8/12) of CVID patients at w4 and w8, respectively (Fig. 1B). Average IgG levels in CVID responders at w8 (53±40 AU/mL) were still significantly lower than those observed in HCs (191±83 AU/mL, p = 0.007, Fig. 1C). Of the CVID patients that showed antigen-specific IgG at w8, 25% (2/8) became undetectable after six months, while 75% (6/8) were able to sustain their titers (Fig. 1B and D). Most CVID individuals that had a detectable antigen-specific IgG response at w8 (67%) showed a rise in antibody levels four weeks after the third immunization (w8: 53±40 AU/mL; w28: 104±85 AU/mL, p = 0.016, Fig. 1B). Despite this, 42% (5/12) of CVID individuals remained IgG seronegative at w28.

Conversely, 78% (7/9) and 100% (9/9) of unPAD individuals seroconverted at w4 and w8, respectively (Fig. 1B). Anti-Spike IgG levels in unPAD responders at w8 (181±149 AU/mL) were similar to those observed in HCs (191±83 AU/mL, p > 0.99), and significantly higher than in CVID responders (p = 0.04, Fig. 1C). A significant decrease in antibody levels was observed from w8 to w24 in both HC (191±83 AU/mL vs 100±81 AU/mL, p = 0.006) and unPAD groups (181±149 AU/mL vs 34±23 AU/mL, p = 0.004, Fig. 1B). Remarkably, and even though unPAD antibody levels were similar at w8 to the HC group (Fig. 1C), these individuals showed lower IgG levels at w24 (p = 0.02, Fig. 1D). Despite that, only 11% (1/9) of unPAD patients were below the detection limit at w24. Administration of the third vaccine dose boosted IgG levels in all unPAD patients (p = 0.008) to similar levels than those observed at w8 in the HCs (p > 0.99, Fig. 1E). We were unable to detect antigen-specific IgG in patients with CID or TID at w8 or w24 (Fig. 1B and Sup Fig. 2). However, the CID patient seroconverted after the third dose (60 AU/mL), showing the potential of this additional shot.

To characterize the humoral responses developed after vaccination in our cohort of PAD patients, we also assessed the presence of anti-Spike IgA and IgM in circulation (Sup Fig. 1). At w8, we identified anti-Spike IgA in 90% (9/10) of HCs, whose levels remained stable over time, 8% (1/12) of CVID, and 67% (6/9) of unPAD patients. While IgA levels were sustained in HCs, they decreased in PAD responders from w8 to w24 (w8: 12±19, w24: 4±6, p = 0.016). Interestingly, the third vaccine dose boosted IgA in these individuals (w28: 27±45; p = 0.031, Sup Fig. 1A). Nonetheless, the majority of CVID patients (92%) remained IgA negative during the length of this study. IgM responses were only observed in 33% (3/10) of HCs, 17% (2/12) of CVID, and 44% (4/9) of unPAD individuals (Sup Fig. 1A). Only 50% of IgM PAD responders were able to maintain these responses over time. The third vaccine dose had little effect on IgM titers (Sup Fig. 1A). It is noteworthy to mention that of the five CVID patients that had undetectable Spike-specific IgG at w28, one of them was IgM positive at w8 and w28, and another one elicited low levels of IgM at w28. We were unable to detect Spike-specific IgA in any of these patients throughout the course of this study.

Anti-Spike IgG responses correlated with RBD-specific IgG levels (Sup Fig. 2D). Since RBD is considered a major target of neutralizing antibodies (NAbs)[20, 21], we evaluated the capacity of our 23 SARS-CoV-2-

uninfected PAD vaccinees to neutralize the ancestral SARS-CoV-2 (WH1), and two additional variants of concern (VoC): Delta (B.1.617.2) and Omicron (B.1.1.529). We detected higher titers of NAbs against both WH1 and Delta VoC in HCs (WH1: 4047±3657; Delta: 762±376), and unPAD patients (WH1: 2892±2680; Delta: 786±1107) than in CVID individuals at w8 (WH1: 664±1247, Delta: 188±382, WH1: HC vs CVID p = 0.003, unPAD vs CVID p = 0.05, Delta: HC vs CVID p = 0.001, unPAD vs CVID p = 0.049, Fig. 2A). Low Nab titers against Omicron were measured in HCs at w8 (131±93, Fig. 2A). However, this neutralizing activity was hardly detected in CVID and unPAD individuals. According to our binding data (Fig. 1C), the Nab titers against WH1 waned over time in HC (p = 0.002) and unPAD groups (p = 0.004), even though they remained stable in CVID patients (p = 0.1, Fig. 2B). Despite that, HCs showed higher levels of neutralization against WH1 than CVID group (p = 0.001) and against Delta and Omicron than unPAD (p = 0.019, p = 0.035, respectively) and CVID groups (p = 0.001, p = 0.006, respectively) at w24 (Fig. 2C). Interestingly, while Nab titers decreased in unPAD individuals, and were sustained in CVID patients over time, a transient increase was observed in HCs at w24. After that, NAbs decreased in the absence of an additional vaccine dose (Fig. 2D). Anti-Omicron neutralization titers also waned over time, but were still detected in 50% (5/10) of HCs at w24 (Fig. 2C and E), becoming practically undetectable at w28.

The third vaccine dose increased neutralization levels against all variants in the unPAD group (WH1: p = 0.008, Delta: p = 0.04, Omicron: p = 0.03, Fig. 2B, D, and E), who recovered their WH1-specific Nab titers observed at w8. After boosting, unPAD patients showed higher Nab titers against all VoC than those elicited at w8 (Delta: 786±1107 vs 1626±1138, p = 0.03; Omicron: 96±120 vs 291±288, p = 0.03, Fig. 2D and E), and similar neutralizing activity to the ones observed in HCs (Fig. 2F). No impact on the Nab titers was observed in CVID (p > 0.1, Fig. 2B and F). In line with previous binding data (Fig. 1B), poor neutralizing activity was observed in the CID patient at w28, probably due to the presence of low IgG levels.

We then evaluated anti-Spike IgG avidity in a subset of PAD patients who responded to vaccination, and observed that IgG avidity significantly increased in both unPAD and HC groups over time (Fig. 3A). A similar positive trend was observed in CVID patients. Interestingly, although unPAD patients had similar levels of anti-Spike IgG to HCs (Fig. 1C), they showed reduced IgG avidity at w8 (0.26±0.07 vs 0.36±0.06, p = 0.044, Fig. 3B). Conversely, these patients developed higher IgG avidity than HCs at w24 (0.58±0.1 vs 0.48±0.06, p = 0.049, Fig. 3C). After the third dose, antigen-specific IgG avidity in CVID and unPAD patients continued to increase, reaching similar values (0.64±0.09 vs 0.66±0.14, p > 0.99, Fig. 3A and D).

Vaccine-induced SARS-CoV-2-specific cellular response in PAD patients

Next, we evaluated vaccine-induced cellular responses against Spike by IFN-γ ELISpot and FCM at w0, w8, w24, and w28 (Sup Fig. 3A). All HCs showed high levels of Spike-specific IFN-γ-producing cells at w8 (50±28 SFC/10^5 cells) and w28 (84±55 SFC/10^5 cells), indicating that these responses were stable and, in some cases, increased over time (Fig. 4A). Conversely, IFN-γ responses were detected in 67% (8/12) of CVID patients at w8 (23±20 SFC/10^5 cells, p = 0.01, Fig. 4A and B). Six months later, we observed IFN-γ

responses in only 33% (4/12) of CVID individuals. Interestingly, the administration of the third vaccine dose restored the frequency of CVID patients that showed IFN-γ-producing cells to those levels observed at w8 (15±17 SFC/10^5 cells, Fig. 4A and B). Similarly, 67% (6/9) of unPAD patients developed IFN-γ-producing cells after two doses (21±20 SFC/10^5 cells, p = 0.05, Fig. 4A and B), and five of them remained detectable at w24 (24±20 SFC/10^5 cells). The third vaccine dose had no effect in this group (p > 0.1, Fig. 4A and B). Of note, the magnitude of these responses in CVID and unPAD groups was lower than HCs at w8 (p = 0.007, p = 0.014, respectively), and w24 (compared with HCs at w28) (p = 0.001, p = 0.044, respectively). After the third vaccine boost (w28), these responses were still lower than those observed in HCs at w8 (p = 0.001, p = 0.025, respectively, Fig. 4C, D, E). While both CID and TID patients did not develop antigen-specific IgG after two doses, these individuals showed detectable IFN-γ-producing cells at w8 (Fig. 4A). Particularly, the CID patient showed a large IFN-γ-producing response at w8, which progressively declined until w24. No boost was observed in these patients after the third vaccine dose (Fig. 4A, B).

We then analyzed activation-induced markers by FCM in both CD4 + and CD8 + T cells after stimulation with S1 peptides (Sup Fig. 3). We observed a significant increase at w8 in the frequency of S1-specific CD69 + CD154 + CD4+ (Fig. 5A), CD69 + CD137 + CD4+ (Fig. 5B) and CD25 + OX40 + CD4 + T cells in CVID (p = 0.03, p = 0.0005, p = 0.005, respectively) and unPAD patients (p = 0.004, p = 0.004, p = 0.008, respectively, Fig. 5C). The magnitude of the CD4 + subsets analyzed at w8 in CVID and unPAD groups was similar to those observed in HCs (p > 0.3, Fig. 5D), and remained stable in the unPAD group until w24 (Fig. 5A, B, C). Similar results were observed in CID and TID patients. However, when the CVID group was analyzed, we observed a reduction in the frequency of CD69 + CD137 + CD4 + T cells (p = 0.02), and a decreasing trend in both CD69 + CD154 + CD4+ (p = 0.06) and CD25 + OX40 + CD4 + T cells (p = 0.08) from w8 to w24 (Fig. 5A, B, C). Remarkably, the third vaccine dose did not significantly boost CD4 + T cell responses in unPAD patients (p > 0.99, Fig. 5E). However, a frequency increase of CD69 + CD137 + CD4 + T cells was observed in CVID patients after the third vaccine dose (p = 0.02, Fig. 5B), reaching similar values than HCs at w8 (p > 0.99, Fig. 5E). Despite that, lower frequency of CD25 + OX40 + CD4 + T cells in CVID patients at w28 was observed when compared to HCs (p = 0.006, Fig. 5E). Interestingly, 92% of CVID (11/12, p = 0.001) and 100% of unPAD patients (9/9, p = 0.004) elicited CD25 + CD8 + T cells at w8, which remained stable in all groups (p > 0.5, Fig. 6A). Despite that, the magnitude of CD25 + CD8 + T cells in CVID patients was significantly lower than in HCs at w8 (p = 0.01, Fig. 6B). The administration of the third vaccine dose did not boost CD8 + T cells responses in CVID or unPAD individuals (p > 0.2, Fig. 6A), which remained significantly lower than in HCs at w8 (p = 0.002, Fig. 6C). Similar results were observed in CID and TID patients (Fig. 6A).

Discussion

Here, we characterized the immune responses elicited in 23 SARS-CoV-2-naïve PAD patients after receiving three mRNA-1273 vaccine doses. While our PAD cohort is mainly composed of unPAD and CVID patients, it includes one CID and TID patient, which might be interesting due to the limited information

about how these patients respond to COVID-19 vaccination[22, 23]. According with previous reports[10, 23], our results showed that immunization was safe, and most patients developed Spike-specific immune responses. However, while our results regarding humoral responses in PAD patients are similar to previous studies [10–12, 22, 24], there are several factors (i.e. patient heterogenicity, methodology, time points, and administered vaccine) that hinder direct comparison among studies.

PAD is a group of heterogenous disorders, which is reflected in the distinct immune responses elicited after vaccination. For example, the kinetics of vaccine-induced humoral responses in unPAD patients was similar to those elicited in HCs. However, unPAD individuals showed a faster decline in NAb titers over time, requiring a third vaccine dose to develop NAbs against Omicron, which was achieved in HCs after two doses. Thus, COVID-19 vaccination efficacy could be reduced in unPAD patients compared to HCs over time. In contrast, only 67% of CVID individuals seroconverted at w8, which developed lower levels of anti-Spike IgG and NAb titers against all VoC compared to HC and unPAD groups. Interestingly, antibody levels remained stable over time in most CVID responders. While vaccine-induced IgA responses were detected in most unPAD and HC individuals, only one CVID patient showed anti-Spike IgA. These results were not surprising, since most CVID individuals show an impaired IgA response[25]. Notably, we detected low levels of anti-Spike IgM in two CVID patients who had not developed IgG or IgA responses. One of them showed low NAb titers against WH1 and Delta VoC.

Differences in the humoral responses observed among groups may be due to the enrollment of different B cell subsets. Particularly, the generation of antibody-secreting cells (ASCs) with short to intermediate half-life could explain why antibody levels waned in unPAD and HC groups. Of note, the frequency of T and B cell subsets in unPAD fell within a normal range[26], and could explain the similarity with HCs. Although humoral responses in the CVID group were more heterogenous, their antibody levels suggests that long-lived ASCs might be generated in a fraction of CVID individuals. It has been described that CVID individuals show a dysregulated B cell compartment[25, 27], which could explain the lower humoral responses observed in this group. Additionally, CVID patients showed an increased frequency of atypical memory B cells (CD19 + CD27-CD21-IgM-IgD-) that could encompass most Spike-specific memory B cells after COVID-19 vaccination[11]. While the origin of these cells remain unclear, it has been postulated that they might derive from an extrafollicular B cell response, a T-independent response, or an early germinal center (GC) reaction[11, 28]. Our results showed that the avidity of anti-Spike IgG responses gradually increased after the second dose in CVID responders, which support antigen-specific B cell selection, and probably involvement of GCs. Accordingly, vaccination induces a persistent GC reaction in healthy individuals[29], and somatic hypermutations are indeed detected in CVID patients[30].

The CDC recently started recommending a third COVID-19 vaccine dose to immunocompromised individuals, whose efficacy remains to be clarified. Here, we have shown that while NAb titers against all variants, including Omicron, increased in unPAD individuals, only NAb titers against Delta rose in CVID patients.

We then defined the vaccine-induced cellular responses in our PAD cohort using two assays: IFN-γ ELISpot, and the detection of activation markers by FCM [17]. Both assays showed that cellular responses were sustained in unPAD and HC groups, and that the third vaccine dose had no effect on expanding the magnitude of previously-generated responses in unPAD individuals. Despite that CD4 + T cell responses decreased in CVID patients, they recovered after the third immunization. Compared to HCs, CVID patients developed lower levels of CD8 + T cells that remained stable over time. Intriguingly, a discrepancy was observed in the magnitude of the T cell responses detected by both techniques. Our results may suggest that although T cell responses could be generated after vaccination in unPAD and CVID individuals, their function (i.e. IFN-γ production) might be impaired in both PAD groups. Accordingly, Fernandez et al. described a lower proportion of IFN-γ responses after stimulation with Spike-derived peptides in COVID-19 vaccinated CVID patients[11], which could be a general particularity of CVID individuals[31]. Conversely, Hagin et al.[10] stated that vaccine-induced cellular responses in IEI patients and HCs were similar. While IEI patients in this latter study and ours are different, ELISpot data obtained in the CVID group from Hagin and colleagues, and the one presented herein are comparable. It is possible that the discrepancy lies in the IFN-γ-producing responses of our HCs, which showed greater levels than those described in Hagin et al.[10].

In addition to unPAD and CVID patients, we analyzed the vaccine-induced immune responses in one CID and one TID patients. While none of them developed antibodies against Spike, both patients developed Spike-specific cellular responses after two vaccine doses. Similarly, we identified detectable T cell responses in three of five CVID patients who did not elicit humoral responses. Indeed, it has been previously described that individuals that lack humoral responses after vaccination (e.g. XLA individuals[11] or patients treated with anti-CD20 antibodies[32]) can develop antigen-specific cellular responses. Interestingly, while the CID patient seroconverted after receiving a third dose, showing Spike-specific IgG and anti-WH1 NAbs, we were unable to detect Spike-specific immune responses in two CVID patients after three COVID-19 immunizations. Immunosuppressive therapies have been associated with the development of poor humoral responses in patients with multiple sclerosis[33], limiting efficacy of COVID-19 mRNA vaccines[34, 35]. However, of the two CVID subjects who did not elicit vaccine-induced immune responses, only one received immunosuppressive treatment. Thus, we cannot conclude the impact of these therapies in halting vaccine-induced immunity in our study.

Conclusions

Our study highlights the distinct response to COVID-19 vaccination elicited in PAD individuals. While most individuals mount Spike-specific immune responses, there is a fraction of subjects that remained non-responders even after three vaccine doses. Therefore, immunomonitoring of these patients could provide insights about their immune status and the need of additional vaccine doses or other prophylactic approaches.

Declarations

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CONFLICTS OF INTERESTS

The authors declare no competing interests. Unrelated to the submitted work, JB and JC are founders and shareholders of AlbaJuna Therapeutics, S.L. BC is founder and shareholder of AlbaJuna Therapeutics, S.L. and AELIX Therapeutics, S.L.

AVAILABILITY OF DATA AND DATA MATERIAL

All data generated from the current study will be available upon request.

AUTHOR CONTRIBUTION

JC, MLPB: Study conception, design, and funding

CB, EAE, NPL, MLPB, JC: Manuscript draft preparation

EAE, NPL, CB, CAN, MLRC, EP, BT, SM, CM, SG, EJM, RP, RT, MF, SB, TE, SC, JGP, BM, BC, NIU, JVA, JS, MM, RMB, AR, DMM, JB, BC, LM, MLPB, JC: Data acquisition, analysis, interpretation

EAE, NPL, CB, CAN, MLRC, EP, BT, SM, CM, SG, RT, MF, SB, TE, SC, JGP, BM, BC, NIU, JVA, JS, MM, RMB, AR, DMM, JB, BC, LM, MLPB, JC: Manuscript editing

ETHICS APPROVAL

Previous Institutional Review Board approval was granted by the Ethics Committee of the Germans Trias i Pujol Universitary Hospital (code: PI-21-107) according to local regulations.

CONSENT TO PARTICIPATE

All participants provided written informed consent prior to enrollment.

CONSENT FOR PUBLICATION

Consent form contains permission to publish data. All the authors approved the final version of this manuscript.

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Figures

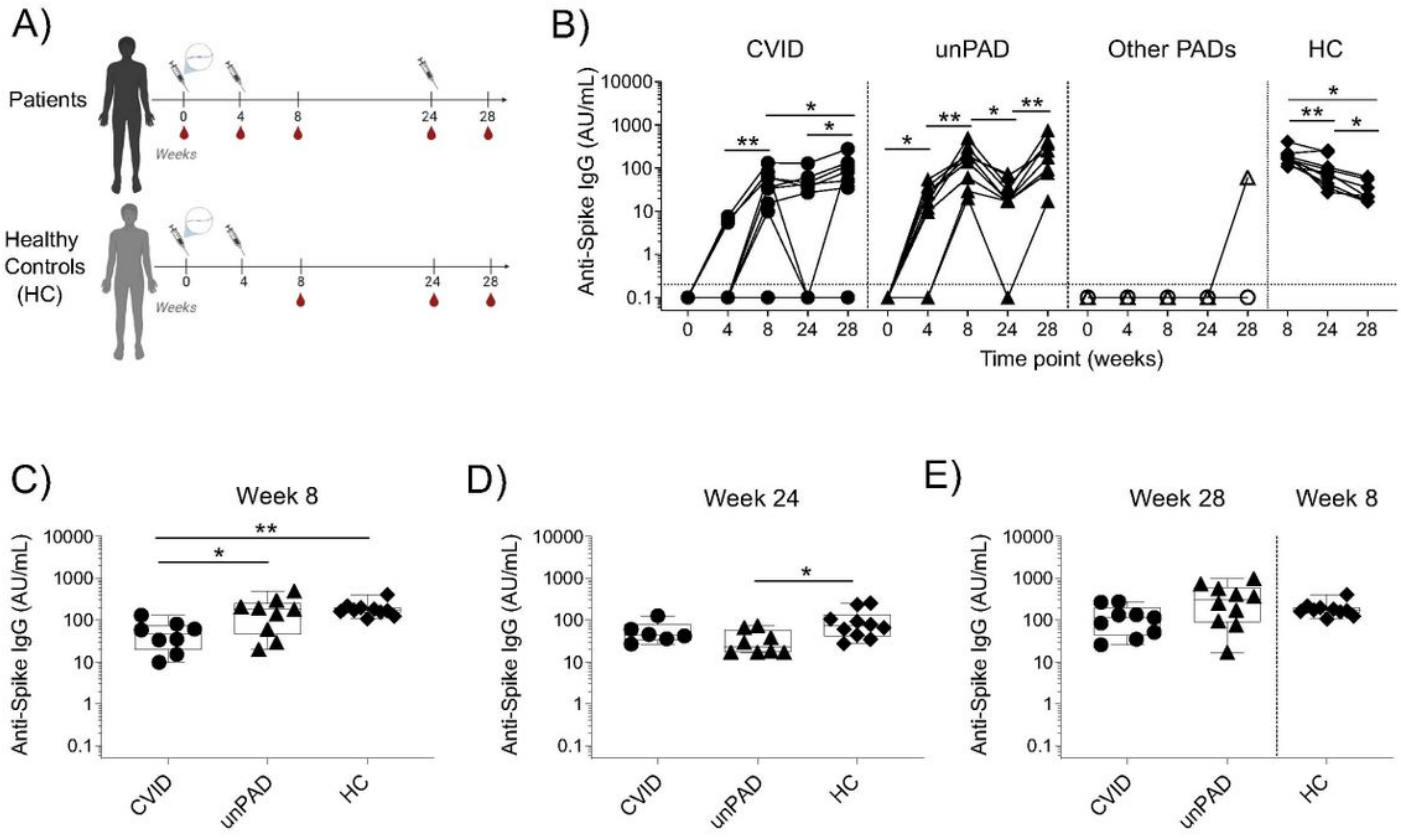

Figure 1

Kinetics of SARS-CoV-2 Spike-specific humoral immune response after vaccination. A, Vaccine regimen timeline and samples collection. **B**, Anti-Spike IgG levels (in AU/ml) over time. CVID (n=12, black circles), unPAD (n=9, black triangles), other PADs (CID: n=1, open triangles and TID: n=1, open circles), and HC (n=10, black diamonds) groups. Data were analyzed using Wilcoxon signed rank test. Vaccine-induced anti-Spike IgG titers in CVID, unPAD and HC at week 8 (**C**), and at week 24 (**D**), after the first vaccination. **E**, Vaccine-induced anti-Spike IgG titers in CVID and unPAD patients after 28 weeks compared to those elicited at week 8 in HCs. Dunn's multiple comparison test was utilized for detect differences among groups. * P < .05; ** P < .01; *** P < .001.

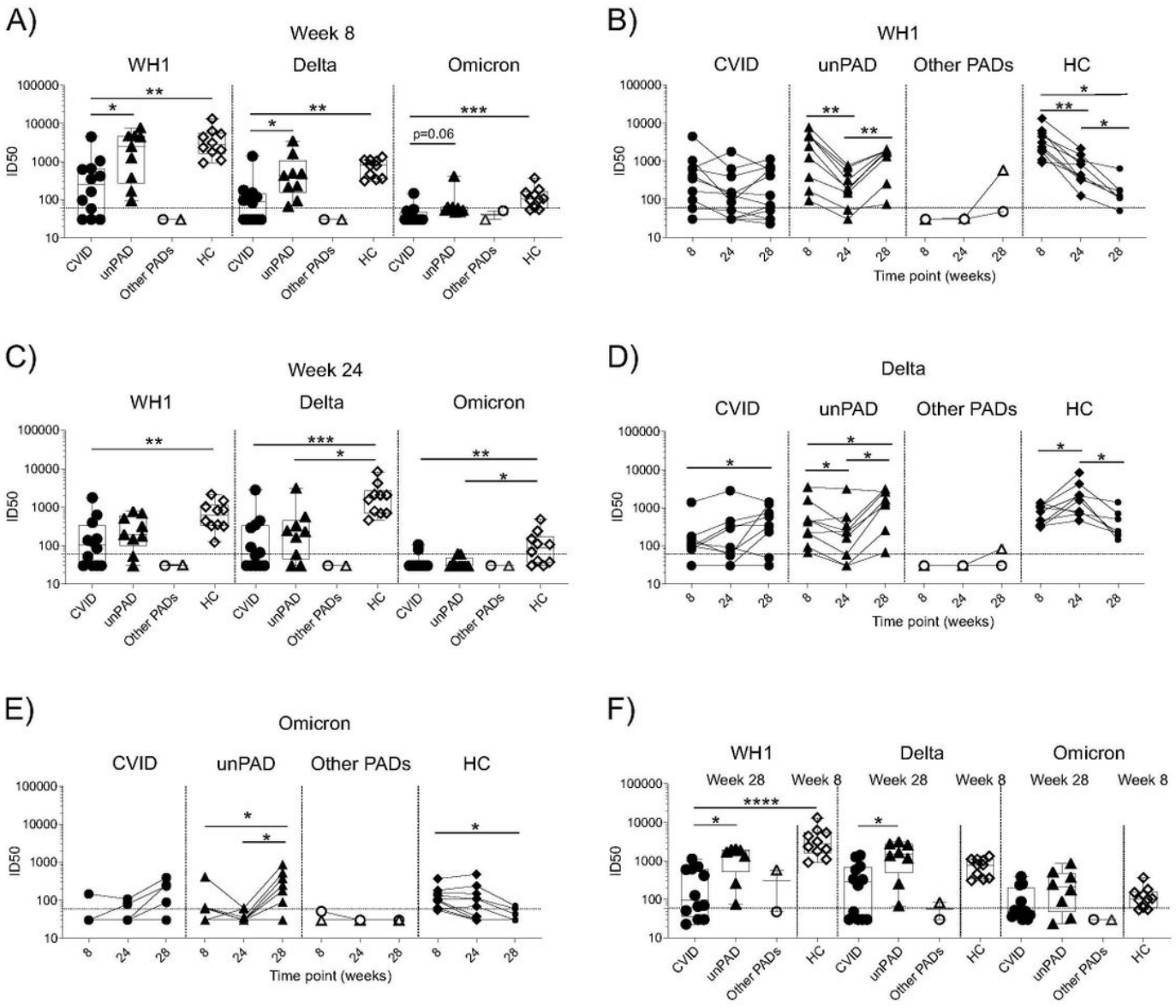

Figure 2

Vaccine-induced neutralizing activity against WH1, Delta, and Omicron in PAD patients. A, NAb ID50 titers elicited in CVID (n=12, black circles), unPAD (n=9, black triangles), other PADs (CID: n=1, open triangles and TID: n=1, open circles), and HC groups (n=10, black diamonds) against SARS-CoV-2 WH1, Delta, and Omicron variants at week 8. **B**, Time course of vaccine-induced neutralizing antibodies in all groups against WH1 variant. **C**, Levels of NAbs at week 24 in PAD patients and HC group against WHu-1, Delta, and Omicron variants, respectively. NAb titers against Delta (**D**) and Omicron (**E**) elicited in CVID, unPAD, other PADs, and HCs. **F**, NAb levels after three vaccine doses (w28) in PAD patients compared to those elicited after two doses (w8) in HCs. ID50: Half maximal inhibitory dilution. Data was analyzed using

Dunn's Multiple Comparison Test (A, C and F), and Wilcoxon signed rank test (B, D and E). * P < .05; ** P < .01; *** P < .001; ****P<.0001

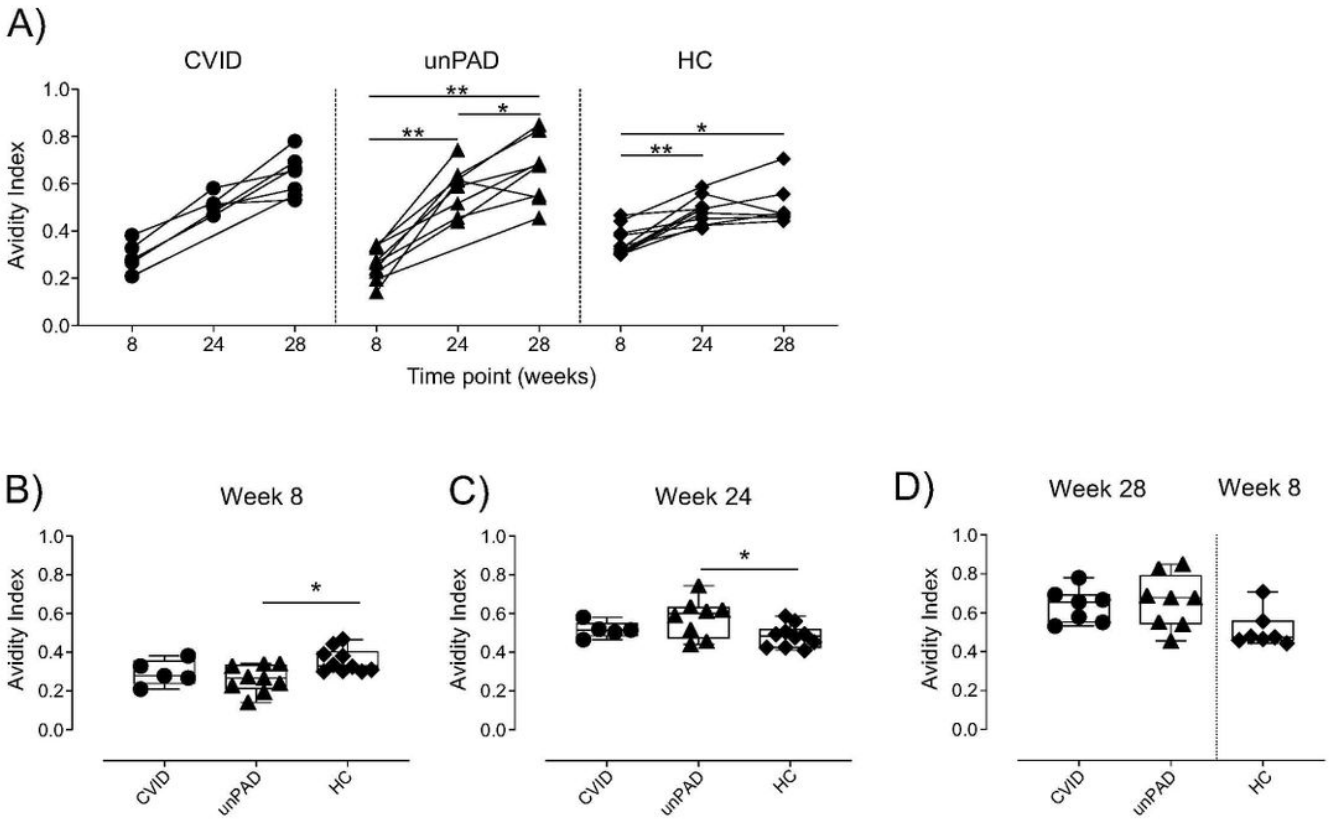

Figure 3

Avidity of Vaccine-induced Spike-specific IgG binding. A, Anti-Spike IgG avidity over time in vaccinated CVID (black circles, n=6), unPAD (black triangles, n=9), and HCs (black diamonds, n=10). Comparison of anti-Spike IgG avidity in vaccinated CVID, unPAD, and HCs at w8 (**B**), and w24 (**C**). **D,** Comparison of anti-Spike IgG avidity in vaccinated CVID, unPAD at week 28 and HCs at week 8. Data in A was analyzed using Wilcoxon signed rank test. Data in B-D were analyzed using Dunn's Multiple Comparison Test. * P < .05; ** P < .01.

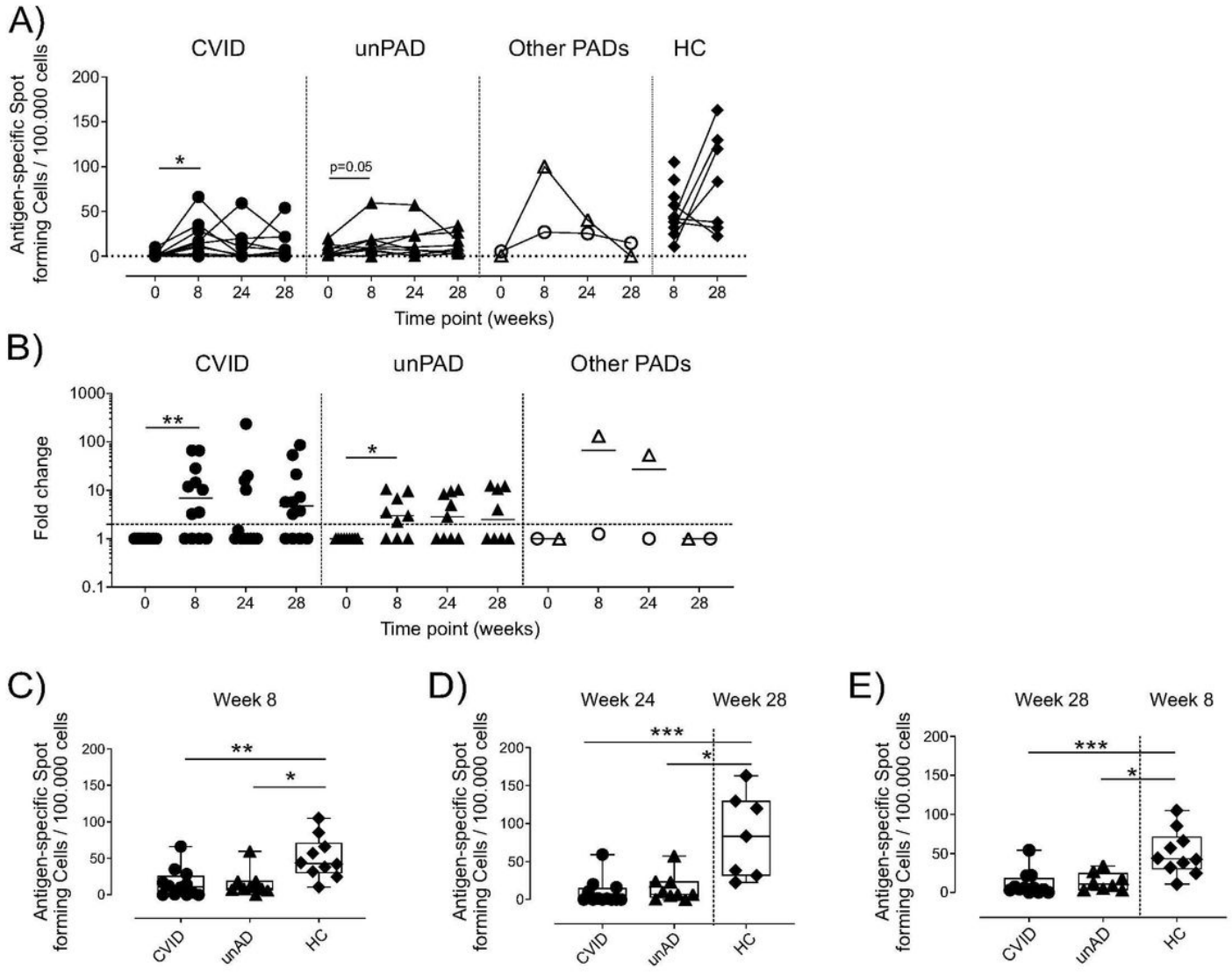

Figure 4

Vaccine-induced SARS-CoV-2-specific IFN- T cell responses. A, Number of antigen-specific IFN- -producing T cells in CVID (n=12, black circles), unPAD (n=9, black triangles), other PADs (CID: n=1, open triangles and TID: n=1, open circles), and HC groups (n=10, black diamonds) per 100.000 cells. **B,** Antigen-specific IFN- -producing T cells fold change respect to basal (w0). Comparison of Spike-specific spot-forming cells among CVID, unPAD, and HC groups at w8 (**C**), w24 vs w28 (**D**), and w28 vs w8 (**E**). Data in **A** and **B** were analyzed using Wilcoxon signed rank test. Data in **C-E** were analyzed using Dunn's Multiple Comparison Test. * P < .05; ** P < .01; ***P<.001.

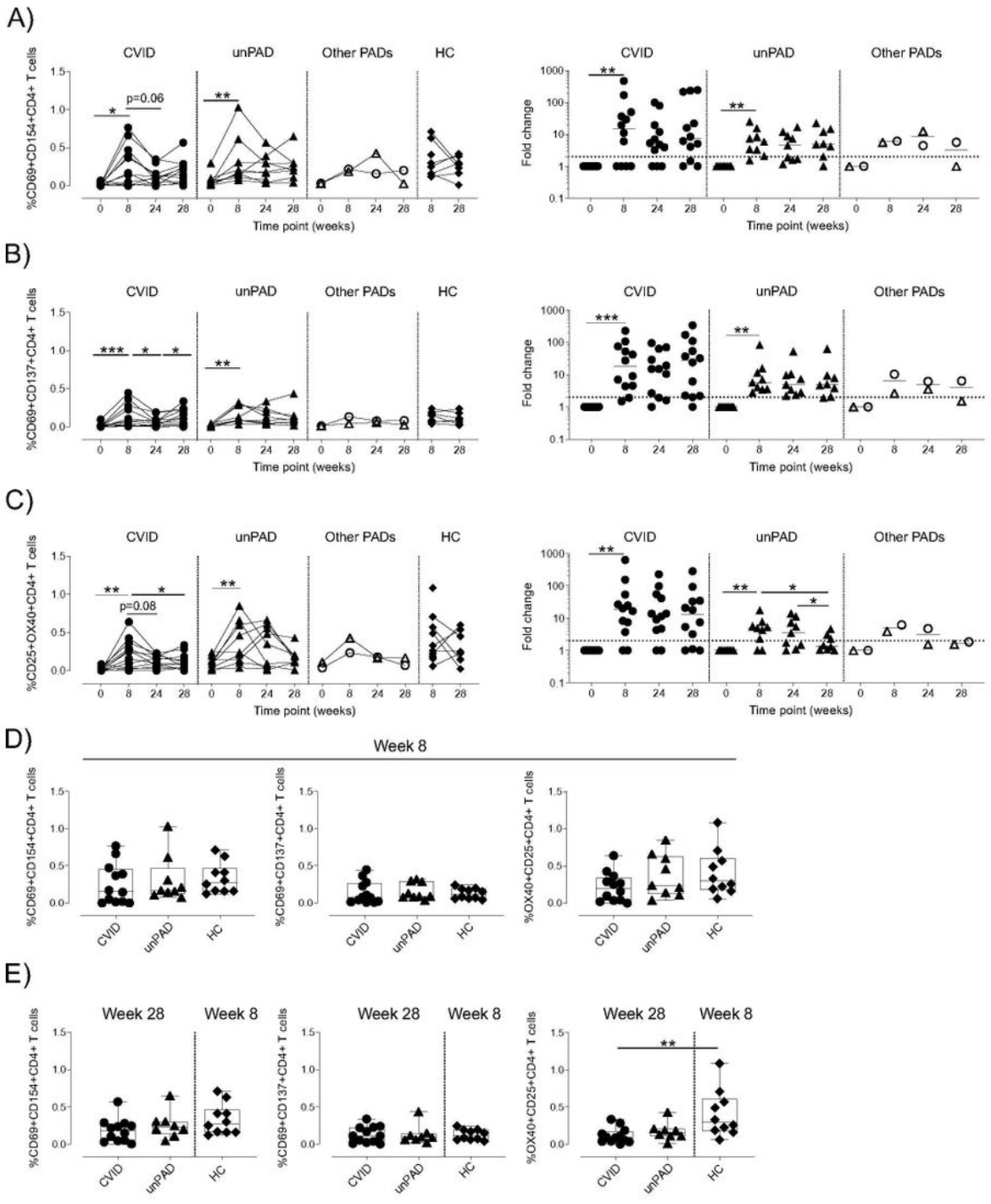

Figure 5

Frequency of Spike-specific CD4+ T cell using activation-induced markers. Frequency (left panel) or fold change respect to w0 (right panel) of Spike-specific CD4+ T cells expressing CD69+CD154+ (**A**), CD69+CD137+ (**B**) or CD25+OX40+ (**C**) in CVID (n=12, black circles), unPAD (n=9, black triangles), other PADs (CID: n=1, open triangles and TID: n=1, open circles), and HC groups (n=10, black diamonds) after vaccination. Comparison of Spike-specific CD4+ T cell subsets in CVID, unPAD and HC groups at w8 (**D**),

and w28 vs w8 (**E**). Data in **A-C** were analyzed using Wilcoxon signed rank test. Data in **D** and **E** were analyzed using Dunn's Multiple Comparison Test. * P < .05; ** P < .01; ***P<.001.

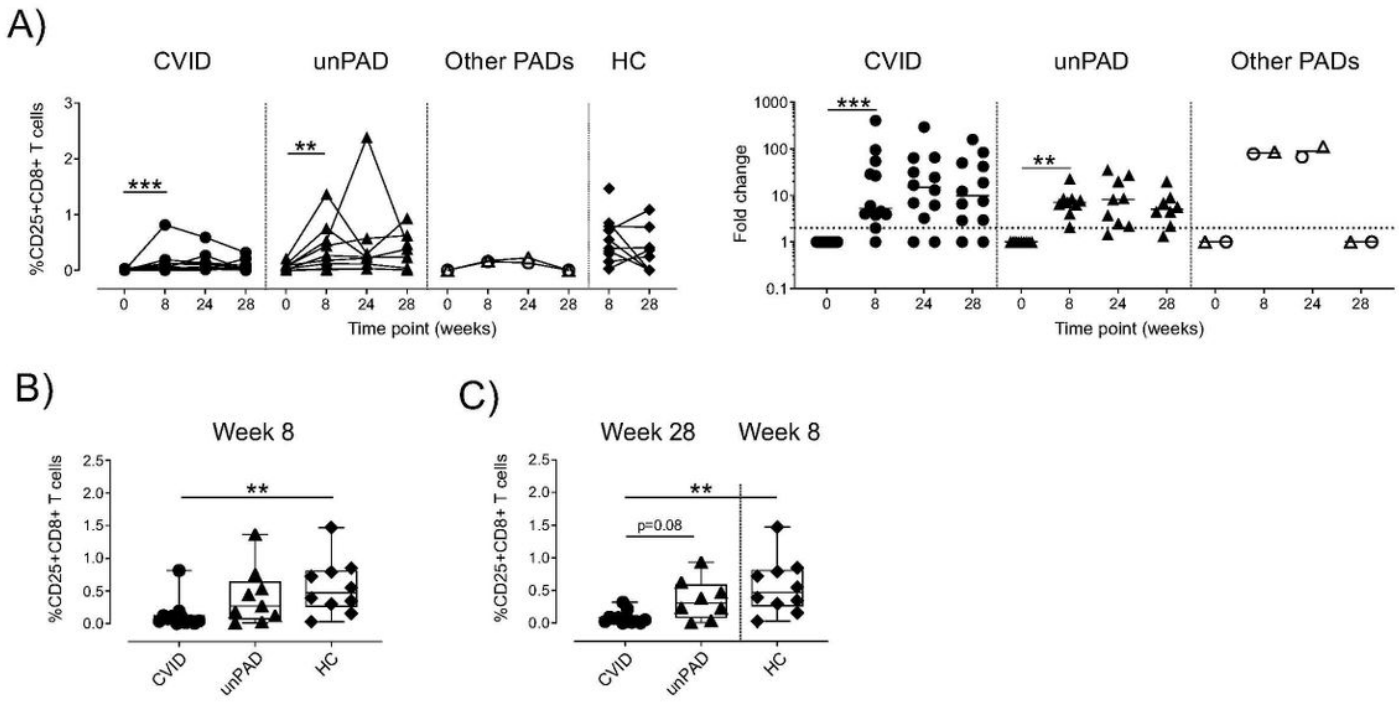

Figure 6

Vaccine-induced SARS-CoV-2-specific CD8+ T cells. A) Frequency of Spike-specific CD25+CD8+ T cell (left panel) and fold change (right panel) in CVID (n=12, black circles), unPAD (n=9, black triangles), other PADs (CID: n=1, open triangles and TID: n=1, open circles), and HC groups (n=10, black diamonds) after vaccination. Comparison of CD25+CD8+ T cell frequency among CVID (black circles, n=12), unPAD (black triangles, n=9), and HC groups (black diamonds, n=10) at w8 (**B**) and w28 vs w8 (**C**). Data in **A** was analyzed using Wilcoxon signed rank test. Data in **B** and **C** were analyzed using Dunn's Multiple Comparison Test. * P < .05; ** P < .01; ***P<.001.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalFigure1FINAL.pdf
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- SupplementalFigure3FINAL.pdf