

Original Article

Preserved Cellular Immunity Upon Influenza Vaccination in Most Patients with Common Variable Immunodeficiency

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What is already known about this topic? Annual influenza vaccination is recommended for patients with common variable immunodeficiency although reports about B- and T-cell responses are sparse and contradictory.

What does this article add to our knowledge? Antigen-specific T-cell responses are heterogeneous according to the assay used but present in most patients with CVID.

How does this study impact current management guidelines? In view of lacking data on clinical end points of T-cell immunity in influenza infection, influenza vaccination of all patients with common variable immunodeficiency is reasonable.

BACKGROUND: Vaccination against influenza is recommended for patients with common variable immunodeficiency (CVID), although humoral immune responses in these patients are impaired and the evidence of effective T-cell responses in CVID is not well established.

OBJECTIVE: To determine plasmablast and T-cellular vaccination responses against influenza in patients with CVID.

METHODS: Patients with CVID and healthy controls were vaccinated with the quadrivalent vaccine Influsplit Tetra 2018/2019. Before and 1 week after vaccination plasmablasts and circulating inducible costimulator-expressing T follicular helper cells were measured to determine positive vaccine responses in these patients. In addition, antigen-specific T cells were determined by their upregulation of CD25 and OX40 after *in vitro* restimulation with the vaccine.

RESULTS: Most healthy controls but only 1 patient with CVID mounted a positive humoral immune response, measured by an

increase in plasmablasts 1 week after vaccination. In contrast, most patients with CVID showed an increase in inducible costimulator⁺ T follicular helper cells and/or an increase in antigen-specific CD25⁺OX40⁺ T cells 1 week after vaccination, demonstrating a positive T-cellular immune response.

CONCLUSIONS: Despite the remaining challenge of accurately assessing the complexity of T-cell responses, the recommendation of vaccinating patients with CVID against influenza is reasonable. © 2020 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2020;■:■-■)

Key words: Common variable immunodeficiency; Influenza vaccination; Plasmablasts; T cell; T follicular helper cell; ICOS

INTRODUCTION

An insufficient antibody response to vaccines is one of the diagnostic criteria of common variable immunodeficiency (CVID),^{1,2} leading to the recommendation of regular immunoglobulin replacement therapy. As a consequence, patients with CVID are often not routinely vaccinated, because it is believed that they will not respond with production of antigen-specific immunoglobulins. Nevertheless, several national committees recommend to immunize patients with CVID against seasonal influenza with inactivated vaccine,^{3,4} because of the high risk for respiratory infections^{5,6} and the insufficient coverage against seasonal influenza in the immunoglobulin preparations due to high mutation rates of the virus. This recommendation is based on the successful use of annually adapted quadri- or trivalent vaccines in the immunocompetent population⁷⁻⁹ and on the observation that some patients with CVID are able to mount residual humoral immune responses.^{10,11} Moreover, the induction of cell-mediated immunity by peptide vaccines has been proposed to support the protection against lytic viruses such as influenza.¹² Previous data have suggested that preserved

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Abbreviations used

CT-Computed tomography
 CVID-Common variable immunodeficiency
 CVIDc-Complex common variable immunodeficiency
 CVIDio-Infection-only common variable immunodeficiency
 GC-Germinal center
 HD-Healthy donor
 ICOS-Inducible costimulator
 SEB-Staphylococcal enterotoxin B
 smB-Switched memory B cell
 T_{FH} -T follicular helper
 TT-Tetanus toxoid

circulating switched memory B cells (EUROclass smB+) correlate directly with a better humoral immune response in patients with CVID.⁸ However, it is currently not known whether patients with CVID presenting with infections only (CVIDio) exhibit a better immune response than do patients with CVID who additionally suffer from symptoms of immune dysregulation (CVIDc) because patients with CVIDc often present with more severely reduced percentages of smBs.¹³

Currently, in the light of some encouraging results on T-cell responses against vaccines in patients with X-linked agammaglobulinemia and hyper-IgM syndrome,¹⁴⁻¹⁷ data about T-cell responses in patients with CVID are still rare and contradictory.¹⁸⁻²⁰

A report from Norway found a response to the pandemic A/California/7/2009 (H1N1)-like split virus (X179a) influenza vaccination in 2 of 3 patients with CVID. The response was determined by an increase in hemagglutination-inhibiting antibodies and vaccine-specific cytokine production by CD4⁺ T cells.¹⁸ Hanitsch et al¹⁹ studied influenza-specific antibody and T-cell responses in 8 patients with CVID, 8 unclassified antibody deficiencies, and 9 healthy controls. Although the vaccine-specific antibody responses were impaired in the hypogammaglobulinemic patients, their T-cell cytokine responses were comparable to those of healthy controls.¹⁹ In contrast, van Assen et al²⁰ showed in their cohort of 15 patients with CVID neither an increase in A/H1N1- or A/H3N2-specific antibodies in hemagglutination inhibition assay nor an adequate increase in IFN- γ -producing CD4⁺ T cells by flow cytometry in comparison to healthy controls.

Influenza vaccines provide protection by generating high-affinity antibodies against viral hemagglutinin,^{21,22} which depend on T follicular helper (T_{FH}) cell activity within the germinal center (GC) reaction.²³⁻²⁵ Therefore, Bentebibel et al²⁶ used elevated expression of the inducible costimulator (ICOS) on a subset of circulating T_{FH} cells 1 week after vaccination as readout for a positive influenza vaccine response. As an alternative readout for a T-cell response to vaccination, the detection of antigen-specific T cells has been described by evaluating the simultaneous upregulation of CD25 and CD134 (OX40) after *in vitro* (re-)stimulation with vaccine-derived peptides.²⁷

To assess these tests in comparison with the evaluation of plasmablast expansion 1 week after vaccination as recommended by Chovancova et al²⁸ and to determine the capacity of patients with hypogammaglobulinemia to respond to influenza vaccination, we investigated blood of 17 patients with CVID and controls before and 1 week after vaccination with quadrivalent influenza vaccine of the season 2018/2019.

METHODS**Patients and blood processing**

All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Informed consent was obtained from all individual participants before inclusion into the study. Peripheral blood samples were obtained from 17 patients with CVID (11 female and 6 male patients, age 21-67 years) and 17 healthy donor (HD) adult volunteers (14 females and 3 males).

The following clinical data were recorded: splenomegaly (defined as a diameter of $>11 \times 4.7$ cm proven by ultrasound or computed tomography [CT] scan); generalized lymphadenopathy (lymph nodes >1 cm in diameter in at least 2 different anatomical sites detected by clinical examination, ultrasound, or CT); granulomatous disease (suggested by CT or proven by histology); autoimmune cytopenias (autoimmune hemolytic anemia or immune thrombocytopenia), interstitial lung disease and respiratory disorders (based on CT morphology, bronchoalveolar lavage or biopsy, and pulmonary function tests), enteropathy (based on histology, biopsy, and endoscopic analysis), and liver disease (based on ultrasound and biopsy). Patients with only splenomegaly or lymphadenopathy were included in the "infection-only" CVID group ($n = 5$, CVIDio), whereas patients with any of the other complications built the "complex" CVID group ($n = 12$, CVIDc).¹³ Patients who never underwent the respective diagnostic workup were excluded for association studies. In addition, all patients were classified according to EUROclass classification,²⁹ considering the reduction of smBs and the expansion of CD21^{low} B cells.

Immunophenotyping by using flow cytometry

PBMCs were isolated by Ficoll density centrifugation (Pancoll, Pan-Biotech, Aidenbach, Germany). B-cell populations were characterized by staining for IgD, IgA, IgM, IgG, CD10, CD19, CD21, CD27, and CD38 expression and T-cell subsets by their expression of CD3, CD4, CD45, CD45RA, CCR6, CXCR3, CXCR5, PD-1, and ICOS. According to Zaunders et al,²⁷ antigen-specific CD3⁺CD4⁺CD45RA^{neg}CXCR5⁺ T cells were identified in a Heparin whole blood assay by detecting CD25 and CD134 (OX40) upregulation after 2-day stimulation with 5 μ L of the influenza vaccine Influsplit Tetra from GlaxoSmithKline when compared with a stimulation with staphylococcus enterotoxin B (SEB) (1 μ g/mL) (Sigma, St Louis, Mo) or tetanus toxoid (TT) (50 μ g/mL) (Statens Serum Institut, Copenhagen, Denmark).

All applied antibodies and their vendors are listed in Table E1 in this article's Online Repository at www.jaci-inpractice.org.

Data acquisition was performed on a Gallios flow-cytometer (Beckman Coulter, Miami, Fla) or LSR Fortessa (BD Biosciences, Franklin Lakes, NJ). Data were analyzed using FlowJo software (Treestar, Ashland, Ore).

Statistical analysis

Values were expressed as means $\pm$ SDs. Statistical significance was assessed by the paired *t* test for data sets with Gaussian distribution, or by the Wilcoxon test for data sets without Gaussian distribution. The unpaired *t* test or Mann-Whitney test was applied for the day 7 to day 0 ratio plots of HD versus CVID. Correlation data were assessed by linear regression. Contingency tables were analyzed by χ^2 or Fisher exact test.

Results were analyzed with the help of GraphPad Prism software (version 8.1.0; GraphPad Software, La Jolla, Calif), and *P* values of less than .05 were considered significant.

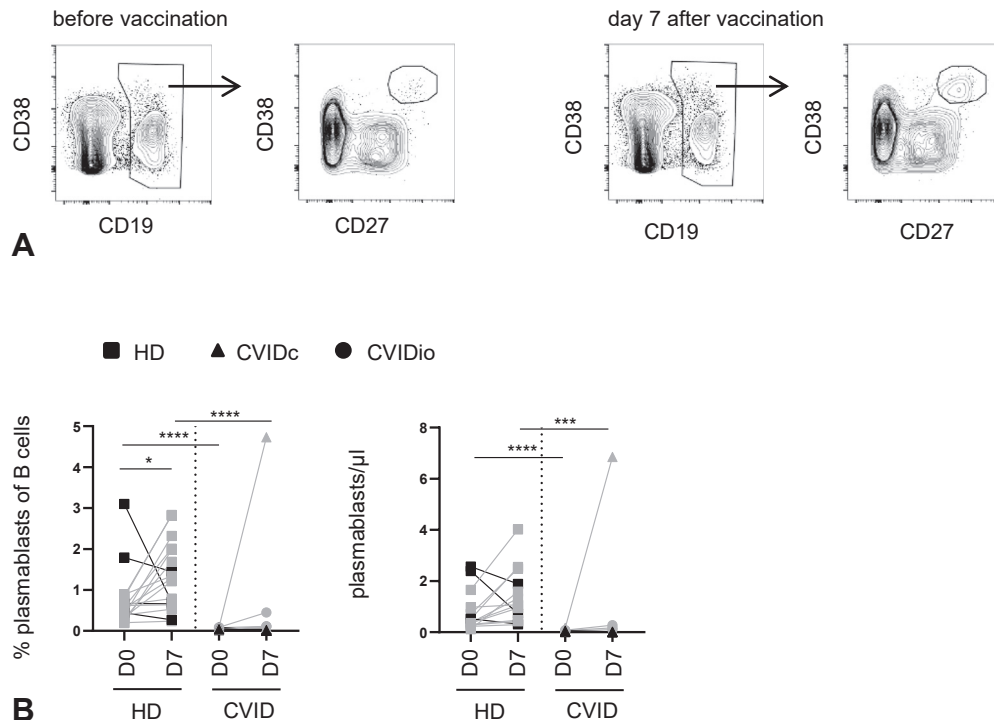


FIGURE 1. Seasonal influenza vaccination rarely induces plasmablast formation in patients with CVID. **(A)** Plasmablasts were determined via CD27 and CD38 expression as exemplified from peripheral blood of an HD before and 7 days after influenza vaccination. **(B)** Relative and absolute plasmablast counts of patients with CVID (patients with CVIDio in circles and patients with CVIDc in triangles) and HDs were compared before (D0) and 1 week after vaccination (D7) by Wilcoxon test. Responders with an increase in respective cell counts were depicted in gray lines and symbols. * $P < .05$; *** $P < .001$; **** $P < .0001$.

RESULTS

Influenza vaccination rarely induces plasmablast responses in patients with CVID

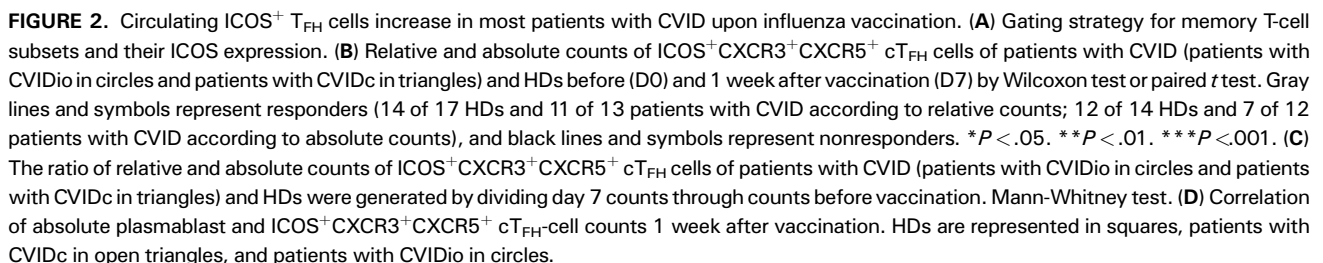
Immune phenotyping was performed before and 1 week after vaccination with Influsplit Tetra. Plasmablasts were identified by high CD38 and CD27 expression among CD19⁺ cells (Figure 1, A). As previously reported, patients with CVID had significantly lower percentage and absolute counts of plasmablasts before vaccination than HDs (Figure 1, B). In 79% of healthy controls, the absolute plasmablast counts increased 1 week after vaccination (1.1-13.7-fold, Figure 1, B), with 57% reaching absolute counts above 0.5/μL. In contrast, only 36% of the patients with CVID showed an increase in plasmablasts after vaccination (1.3-40 fold) and only 1 patient reached absolute counts greater than 0.5/μL (Figure 1, B).

The only responder belonged to the CVIDc subgroup, was characterized by EUROclass B+smB-21lowTrnorm with low smBs, a baseline serum IgG above 4 g/L, detectable but low serum IgA levels, and a positive vaccine response to tetanus and pneumococcal polysaccharide vaccination. Specific antibody titers against tetanus were available in 40% of the other patients and among these, only 1 patient had a detectable vaccine response to tetanus. Data about anti-pneumococcal polysaccharide vaccine response were too rare to draw any conclusions.

Most patients with CVID respond with a relative expansion of ICOS⁺CXCR3⁺ T cells upon influenza vaccination

Comparing the percentage of total CD4⁺ T cells, CD45RA⁺ memory CD4⁺ T cells, CD45RA⁺CXCR5⁺ and CD45RA⁺CXCR5⁺ memory CD4⁺ T cells, and their different T_H subsets in peripheral blood (for gating, see Figure 2, A) confirmed the relative increase in memory CD4 T cells with a skewing toward increased T_H1-like circulating T_{FH} cells (CXCR5⁺CXCR3⁺) in patients with CVID when compared with healthy controls as previously published.³⁰ The proportions of the different CD4⁺ subpopulations did not significantly change after vaccination (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org) in patients with CVID or HD controls.

When investigating the expansion of ICOS⁺CXCR3⁺ cells among CXCR5⁺ T_{FH} cells as suggested by Bentebibel et al,²⁶ the relative counts increased significantly in HDs as well as in patients with CVID, whereas a significant increase in the absolute counts was seen only in the healthy cohort (Figure 2, B). On the basis of the ratio of the absolute counts on day 7 and day 0, 86% of HDs and 58% of patients with CVID responded to the vaccination (Figure 2, C). The response of CXCR3⁺ T_{FH} cells was independent of the initial percentage or absolute counts of circulating ICOS⁺CXCR3⁺ T_{FH} cells. There was no difference



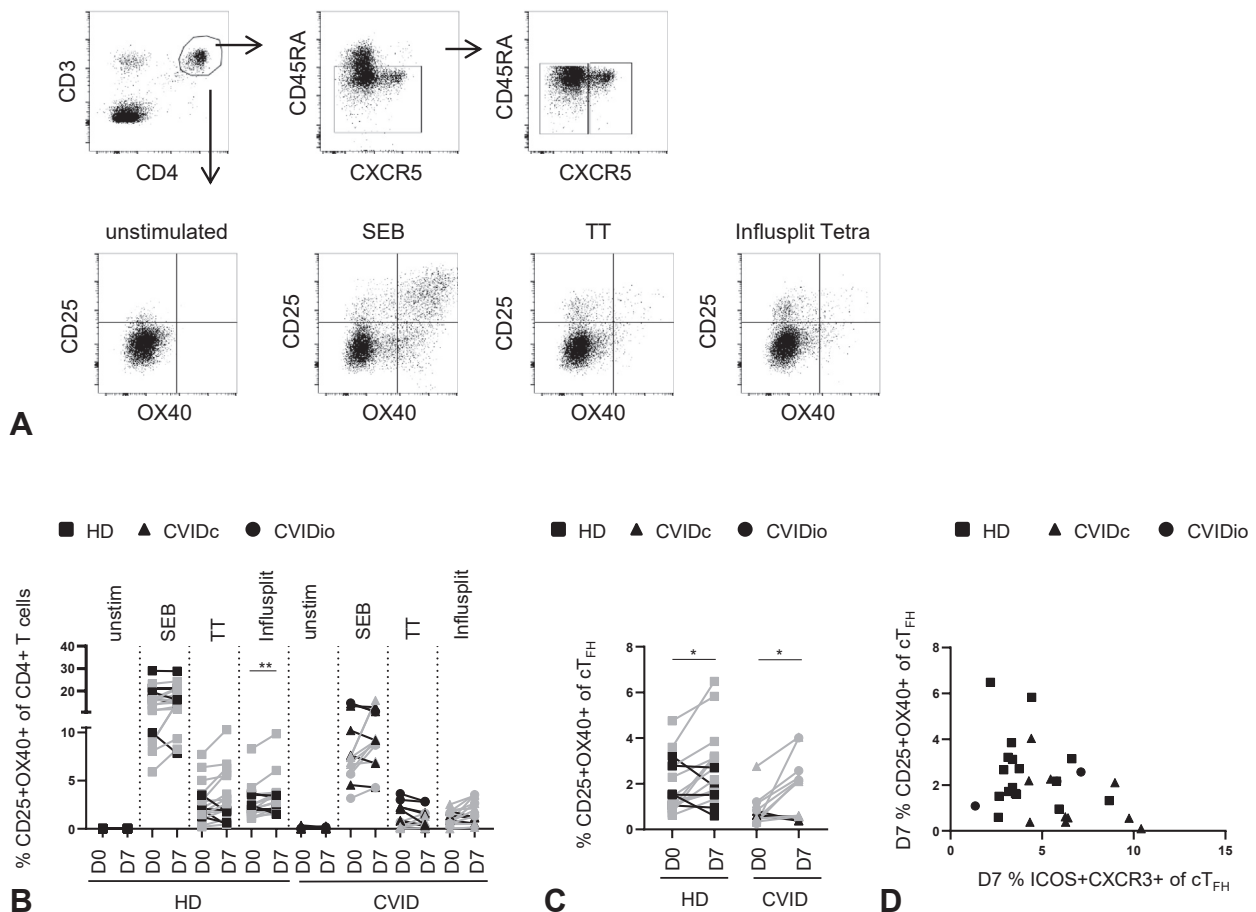


FIGURE 3. Antigen-specific induction of CD25⁺OX40⁺ T cells *in vitro* confirms antigen-specific cellular immune response in a subgroup of patients with CVID. **(A)** Gating strategy for CD25 and OX40 double-positive CD4⁺ T cells after *in vitro* stimulation with SEB, TT, or Influsplit Tetra. **(B)** Comparison of CD25⁺OX40⁺ antigen-induced CD4⁺ T cells before (D0) and 1 week after vaccination (D7) of HDs and patients with CVID (including patients with CVIDio in circles and patients with CVIDc in triangles). Responders with an increase in antigen-specific cells were marked in gray lines and symbols. **(C)** Comparison of relative counts of Influsplit Tetra-specific cells of cT_{FH} cells of HDs and patients with CVID (including patients with CVIDio in circles and patients with CVIDc in triangles) before (D0) and 1 week after vaccination (D7). Responders with an increase in antigen-specific cells were marked in gray lines and symbols (12 of 17 HDs and 9 of 12 patients with CVID), whereas nonresponders are represented in black lines and symbols. Wilcoxon test, * $P < .05$; ** $P < .01$. **(D)** Correlation of relative counts of ICOS⁺CXCR3⁺CXCR5⁺ cT_{FH} cells and of CD25⁺OX40⁺ Influsplit Tetra-specific cT_{FH} cells 1 week after vaccination for HDs (squares) and patients with CVID (including patients with CVIDio in circles and patients with CVIDc in triangles).

between the response ratios of HDs and patients with CVID except for a drop in absolute counts in 4 patients with CVID (Figure 2, C). Remarkable, however, was that 2 of them belonged to the CVIDio subgroup, demonstrating a failure to respond in 3 of 3 patients with CVIDio whereas 7 of 9 patients with CVIDc were able to show some response in regard to the absolute expansion of ICOS⁺CXCR3⁺ cells among CXCR5⁺ T_{FH} cells.

In contrast to Bentebibel et al,²⁶ we also saw an expansion of ICOS⁺ cells among circulating T_{FH1}, T_{FH1/17}, T_{FH17}, and T_{FH2} cells of HDs whereas in patients this expansion was significant only for the first 2 populations (see Figure E2, A, in this article's Online Repository at www.jaci-inpractice.org). Looking only at patients with CVID who responded by an increase in ICOS⁺ T_{FH1} cells, there was a similar increase in ICOS-expressing cells in all cT_{FH} subsets as seen in healthy controls.

In agreement with the reported positive correlation of ICOS⁺CXCR3⁺CXCR5⁺CD4⁺ T-cell expansion with the fold

increase in global antibody titers and plasmablast expansion for healthy individuals,²⁶ we also found a trend toward a positive correlation of the absolute counts at day 7 for healthy controls (Figure 2, D), but not for patients with CVID.

Thus, the expansion of ICOS-expressing CXCR3⁺ T_{FH} cells 1 week after vaccination was comparable between most patients with CVID and HDs and reflected the plasmablast response in HDs but not in patients with CVID. Still, the only patient with a positive plasmablast response was also identified as a responder in this test.

However, we could neither confirm the observed correlation between the increase in blood plasmablasts with the increase in ICOS⁺CXCR3⁺CXCR5⁺CD4⁺ T cells (see Figure E2, B) nor the positive correlation of increased CXCR3-expressing CXCR5⁺ T cells with increased ICOS expression on these cells (see Figure E2, C) for HDs or patients with CVID.

When comparing responders and nonresponders in this assay, responders were exclusively patients with CVIDc (displayed in

triangles in Figure 2, B and C) and belonged to the smB— group (see Figure E2, G–I) but had highly variable IgG levels before substitution. Only 1 responder (14%) had a positive vaccine response to tetanus, whereas 2 (28%) had no detectable tetanus vaccine response and for the other 58% no further clinical data about antitetanus response were available. Fifty percent of the nonresponders were patients with CVIDio and most belonged to the smB— group (83%); only 1 of 3 patients had a detectable tetanus vaccine response, whereas for the other 50% these data were not available.

The induction of antigen-specific CD25⁺OX40⁺ T cells confirms a cellular immune response in a subgroup of patients with CVID

We additionally identified antigen-specific T cells by stimulating whole blood cells according to Zaunders et al²⁷ with Influsplit Tetra for 48 hours *in vitro* and measuring the combined upregulation of CD25 (IL2R α) and CD134 (OX40) (for gating, see Figure 3, A). CD25⁺OX40⁺ double-positive cells were rare in unstimulated samples, whereas SEB induced a distinct double-positive cell population in all samples. There was an increase in Influsplit Tetra–induced double-positive CD4⁺ T cells in 82% of HD samples at day 7 when compared with day 0, whereas the percentage of double-positive cells after SEB- and TT-stimulated samples before and after influenza vaccination did not change significantly (Figure 3, B).

When comparing the *in vitro* responses to SEB and TT, the CVID samples showed compared with HD samples lower percentages of CD25/OX40 double-positive CD4⁺ T cells before ($P = .0006$ for SEB; $P = .036$ for TT) and after vaccination ($P = .0003$ for SEB and $P = .008$ for TT). More importantly, the specific T-cell response to the influenza vaccine was significantly lower compared with HDs before and after vaccination ($P = .0008$ and $P = .028$, respectively) (Figure 3, B). Although 77% compared with 82% of HDs showed some increase 1 week after compared with before vaccination, this difference in response did not reach significance in the CVID cohort (Figure 3, B). However, when gating on cT_{FH} cells instead of total CD4⁺ T cells, the double-positive cT_{FH} cells increased significantly after vaccination in both HDs (81%) and patients with CVID (75% in total cohort) (Figure 3, C). Unlike the response in regard to the expansion of ICOS⁺ T_{FH} cells, in this assay there was no detectable difference between the percentage of responding patients with CVIDio (2 of 3) and patients with CVIDc (5 of 13).

A correlation of the percentages of CD25⁺OX40⁺ cT_{FH} with ICOS⁺CXCR3⁺ cT_{FH} cells (Figure 3, D) did not show any correlation for either HDs or patients with CVID, but there was a tendency of HDs responding stronger in the OX40 assay whereas patients with CVID did better in the ICOS⁺CXCR3 assay (Figure 3, D). The 5 responders to both T-cell–based assays included the only responder in the plasmablast assay and all of them belonged to the CVIDc subgroup.

Neither the current B- nor T-cell–based classification systems allowed for a reliable differentiation between Influsplit Tetra responders and nonresponders (see Figure E3 in this article's Online Repository at www.jaci-inpractice.org), but the clinical phenotype showed a significant correlation according to Fisher exact test with the responsiveness in the ICOS assay (see Figure E3, B; $P = .046$). Furthermore, it was noteworthy that patients with CVIDio showed an impaired expansion of absolute

ICOS⁺T_{FH}1-cell counts, while they were able to respond to antigenic restimulation *in vitro* with upregulation of CD25⁺OX40⁺ cT_{FH}.

DISCUSSION

Administration of seasonal influenza vaccines provides protection from influenza in about 60% to 90% of immune-competent vaccinees.^{21,31} Decades ago Hermans et al³² were the first to describe a lack of vaccine-specific antibody production in a cohort of patients with CVID. Subsequently, current definitions of CVID include an inadequate vaccine response as one of the diagnostic criteria.

Although most vaccine-preventable infectious diseases are covered by adequate IgG substitution therapy,^{33,34} immunization against seasonal influenza is recommended in these patients given the lack of sufficient specific antibody activity against the seasonal influenza strains in the IgG preparations.

Our study confirms the severely reduced B-cell response to influenza vaccination in CVID, with only a single patient mounting a measurable response,^{19,20} corroborating the lack of humoral vaccine response as a diagnostic criterion. This result is more representative of the CVID cohort than the response previously reported by Pedersen et al.¹⁸ However, it is not clear how well T cells respond to vaccination in patients with CVID.

Intriguingly, it has been shown in mice that a lack of mature B cells does not impair the induction of virus-specific CD4⁺ T-cell memory³⁵ and that memory CD4⁺ T cells can contribute to the protection against viral disease in B-cell–deficient and CD8⁺ T-cell–depleted mice.^{36,37} In agammaglobulinemic patients, a previous study has found that functional CD4⁺ T-cell responses to viral recall antigens are comparable to those of healthy controls.¹⁵

There is no defined consensus on how to determine vaccine-specific T-cell responses in humans. Typically, these assays include antigen-induced proliferative responses, cytokine production, or upregulation of specific activation markers such as CD69, CD40L, or OX40.^{18–20,38} More recently, also the general expansion of T_{FH} cells and their subsets in peripheral blood 1 week after vaccination has been suggested as a mean to detect the T-cell portion of the GC response.²⁶ It is unclear how much the different assays reflect the same aspects of a T-cell response.

The first studies regarding antigen-specific T-cell memory in patients with CVID had demonstrated reduced cell-mediated immune responses after vaccination with recall and neo-antigens.^{39,40} More recently, T-cell–mediated responses to seasonal influenza vaccination have been described with variable results even when comparing only the cytokine production after 21 days. van Assen et al²⁰ found no relevant increase in the proportion of IFN- γ spot-forming cells in 15 patients with CVID upon stimulation with A/H1N1 or A/H3N2, whereas Pedersen et al¹⁸ reported that stimulation with the influenza vaccine antigen (X179a) induced high frequencies of IFN- γ –producing CD4⁺ T cells in all 3 tested patients with CVID. Similarly, Hanitsch et al¹⁹ observed adequate vaccine-specific (A/California/7/2009, A/Texas/50/2012, and B/Massachusetts/02/2012) cytokine responses of activated CD4⁺CD40L⁺ T cells 21 days after vaccination in 7 of 8 patients with CVID. These different outcomes are not readily explained by the minor differences in the methodology and may reflect more the heterogeneity of the investigated CVID population.

Here, we set out to validate 2 recently suggested assays^{26,27} in 17 well-characterized patients with CVID and matched controls. One assay measures ICOS expression on circulating CXCR3⁺ T_{FH} cells as a more general readout for the induction of a GC response by the vaccine. The other test measures antigen-specific upregulation of CD25 and OX40 after antigen-specific (re-) stimulation *in vitro* as a readout of the increase in antigen-specific memory CD4⁺ T cells.

Interestingly, the first assay revealed a near-normal response in most patients, suggesting the presence of a GC response after vaccination in most patients. This is of special interest because the failure of the classical readout of a functional GC response, namely the appearance of class-smBs and a plasmablast response is a unifying feature of nearly all patients with CVID independent of the physical presence of GCs. This interpretation is corroborated by the severe reduction in CXCR5⁺ T_{FH} cells in ICOS and CD40L deficiency.⁴¹ This discrepancy between the B- and T-cell memory output of GCs may be explained by the finding of Bentebibel et al²⁶ that the increase in ICOS⁺CXCR3⁺CXCR5⁺CD4⁺ T cells in blood of a HD control cohort correlated with the increase in pre-existing antibody titers, but not with the induction of primary antibody responses. Consistently, purified ICOS⁺CXCR3⁺CXCR5⁺CD4⁺ T cells efficiently induced memory B cells, but not naive B cells, to differentiate into plasma cells that produce influenza-specific antibodies *ex vivo*. Thus, the emergence of peripheral ICOS⁺CXCR3⁺CXCR5⁺CD4⁺ T cells correlates with the development of protective antibody responses generated by memory B cells upon seasonal influenza (re-)vaccination.²⁶ Concerning patients with CVID, who often have a disturbed memory B-cell compartment and a reduced B-cell—intrinsic response to T-cell stimulation,⁴² it would be worth it to investigate by coculture assays the *in vitro* differentiation of B cells with the help of vaccination-induced circulating ICOS⁺ T_{FH} cells to see whether these T cells are potent helper cells for the induction of antigen-specific antibodies. Only 4 patients with CVID had a severe deficiency in expanding ICOS⁺CXCR3⁺ T_{FH} cells after vaccination *in vivo* compatible with a potential GC failure. It is intriguing that 3 of the 4 patients with severely disturbed expansion of ICOS⁺CXCR3⁺ T_{FH} cells belonged to the CVIDio group, whereas 7 of 9 of the patients with CVIDc were able to respond in this regard. It has been shown that circulating T_{FH} cells reflect the capacity of GCs to produce a memory T_{FH} population,⁴³ a process that is also dependent on ICOS.⁴⁴ The difference in the capacity of producing circulating ICOS⁺ T_{FH} cells 1 week after vaccination between patients with CVIDio and patients with CVIDc may mirror a higher prevalence of patients with a failure of GC formation among the former. This is compatible with the findings from our group and others that the immune dysregulation in most patients with CVIDc is not associated with a lack of GCs but rather lymphadenopathy containing large—although disturbed—GCs.⁴⁵ This, however, does not explain why this difference is not visible at baseline and becomes visible only by an absolute decrease in ICOS⁺ cT_{FH} after vaccination, a phenomenon possibly detectable also in a subgroup of especially elderly patients.⁴⁶

In contrast to the first assay, which does not measure an antigen-specific response, upregulation of CD25 and OX40 on memory T cells after *in vitro* restimulation with influenza antigen depends on the expansion of antigen-specific memory T cells *in vivo*. Interestingly, most patients with CVID showed a detectable, although reduced response in this assay. It is noteworthy that patients with CVID responded even to control stimulants such as the

superantigen SEB and the TT antigen less strongly than healthy controls, suggesting a more generally impaired T-cell responsiveness in this assay. Eisenstein et al⁴⁷ reported that CD4⁺ T cells from patients with CVID proliferate normally in response to stimulation by PHA or SEB, but these stimuli induce significantly less IL-2 production than observed with healthy CD4⁺ T cells. Also, Thon et al⁴⁸ observed that CD3/CD28 stimulation of CVID T cells resulted in deficient IL-2 and IL-4 production, with deficient upregulation of CD40L on activated T cells, whereas CD69 was normally expressed, suggesting a partial activation defect of CVID patient—derived T cells, which may point toward different requirements for the upregulation of CD69 versus CD40L, CD25, or OX40. Although the heterogeneity of the response interferes with the significant antigen-specific increase in CD25⁺OX40⁺ T cells after vaccination in total CD4⁺ T cells, there is no detectable difference between HDs and patients with CVID when looking at the vaccine response among CXCR5⁺ T cells, possibly suggesting that there is a normal percentage of antigen-specific memory cells entering the circulating CXCR5⁺, but less the CXCR5^{neg} memory pool within the first week after influenza vaccination.

When establishing T-cell parameters as a readout for a relevant functional cellular response, it is important to recognize the many reported T-cell abnormalities in the blood of patients with CVID including reduced numbers of (naive) CD4⁺ and CD8⁺ T cells, decrease in circulating regulatory T cells, but increase in CXCR5⁺ CD4⁺ T cells, increased T-cell activation and exhaustion, altered cytokine production, altered CD4⁺ and CD8⁺ T-cell receptor repertoires, increased CD4⁺ and CD8⁺ T-cell turnover, and increased levels of spontaneous apoptosis,^{30,49–56} rendering the chance for 1 assay to reflect the complexity of predicting a vaccine-specific T-cell response nearly impossible.

CONCLUSIONS

Although the true readout of the impact of vaccination on clinically manifest influenza infections among patients with CVID is still missing, we could confirm other reports that most patients with CVID show at least a partial T-cell response to seasonal influenza vaccination. Although we cannot conclude on the longevity of this response from our data, it is of interest that T-cell—mediated immunity against influenza appears to be less affected by the annually occurring epitope changes. Studies of viral evolution over the years indicate that 15% of T-cell epitopes remain unchanged whereas only 2.7% of epitopes recognized by antibodies are conserved.⁵⁷ To our surprise, neither initial serum immunoglobulin levels nor the classification according to B-cell differentiation nor the clinical presentation allowed to distinguish between responders and non-responders. Considering that the heterogeneity of the response in the different T-cell assays may not allow a conclusive prediction with respect to a positive CD4 T-cell memory response and that only 3 patients failed to respond in both applied T-cell assays, we suggest that for the time being annual influenza vaccinations are justified in patients with CVID without prior extensive laboratory workup for selecting potential responders, if there is no contraindication for the vaccination.

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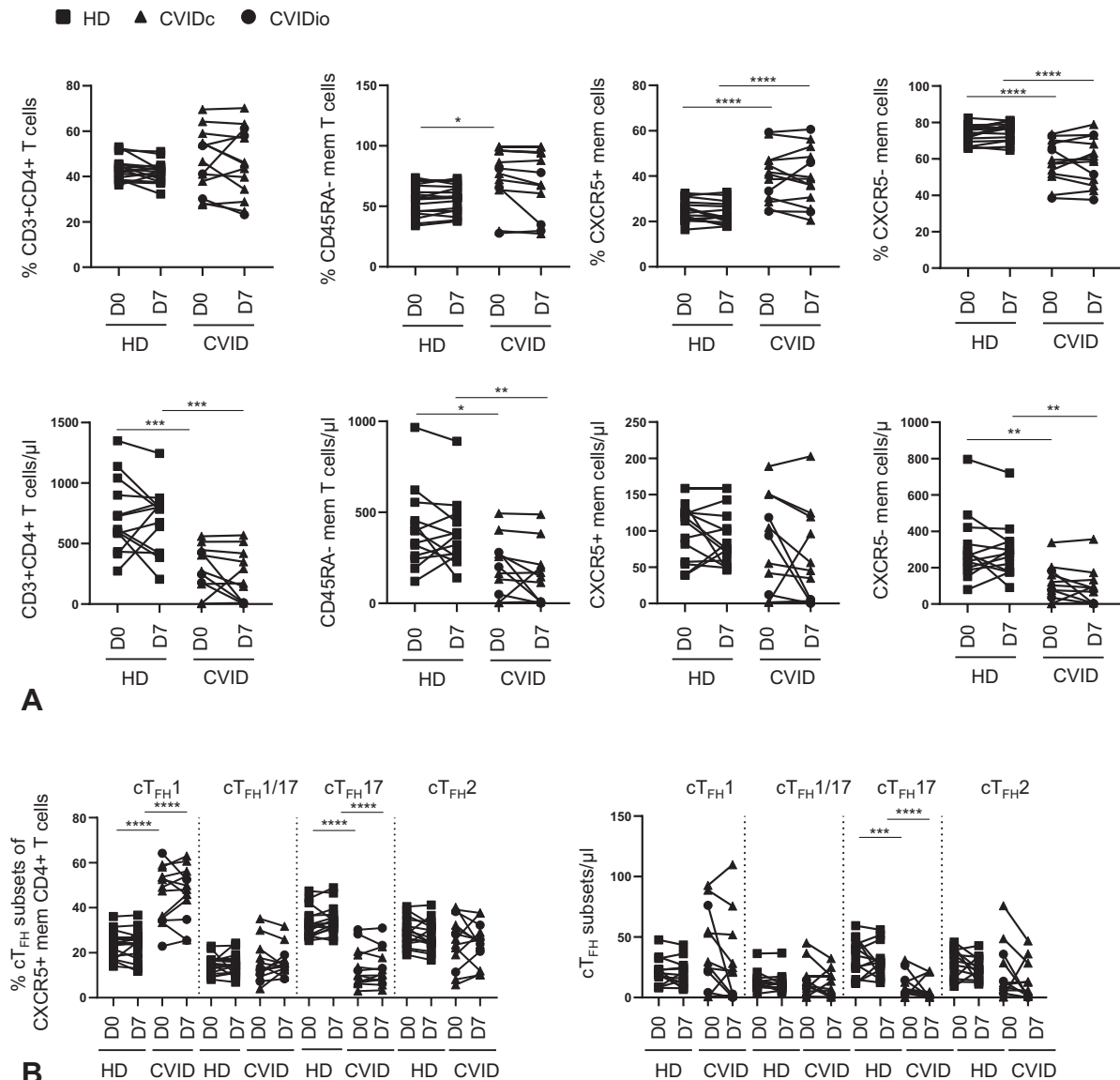


FIGURE E1. Stable T-cell compartment after influenza vaccination. **(A)** Total CD4 T cells, CD4 memory T cells, and CXCR5⁺ and CXCR5⁻ memory T cells of HDs and patients with CVID were compared before and 1 week after vaccination. HDs are represented by squares, patients with CVIDio by circles, and patients with CVIDc patients by triangles. The upper row shows relative counts and the lower row shows absolute counts of the indicated cell subsets. Paired *t* test, Wilcoxon test, and ANOVA were used for comparison. **P* < .05. ***P* < .01. ****P* < .001. *****P* < .0001. **(B)** cT_{FH}1, cT_{FH}1/17, cT_{FH}17, and cT_{FH}2 subsets of CXCR5⁺ memory T cells of HDs and patients with CVID before vaccination were compared with 1 week after vaccination. HDs are represented by squares, patients with CVIDio by circles, and patients with CVIDc by triangles. Paired *t* test, Wilcoxon test, and ANOVA were used for comparison. **P* < .05. ***P* < .01. ****P* < .001. *****P* < .0001.

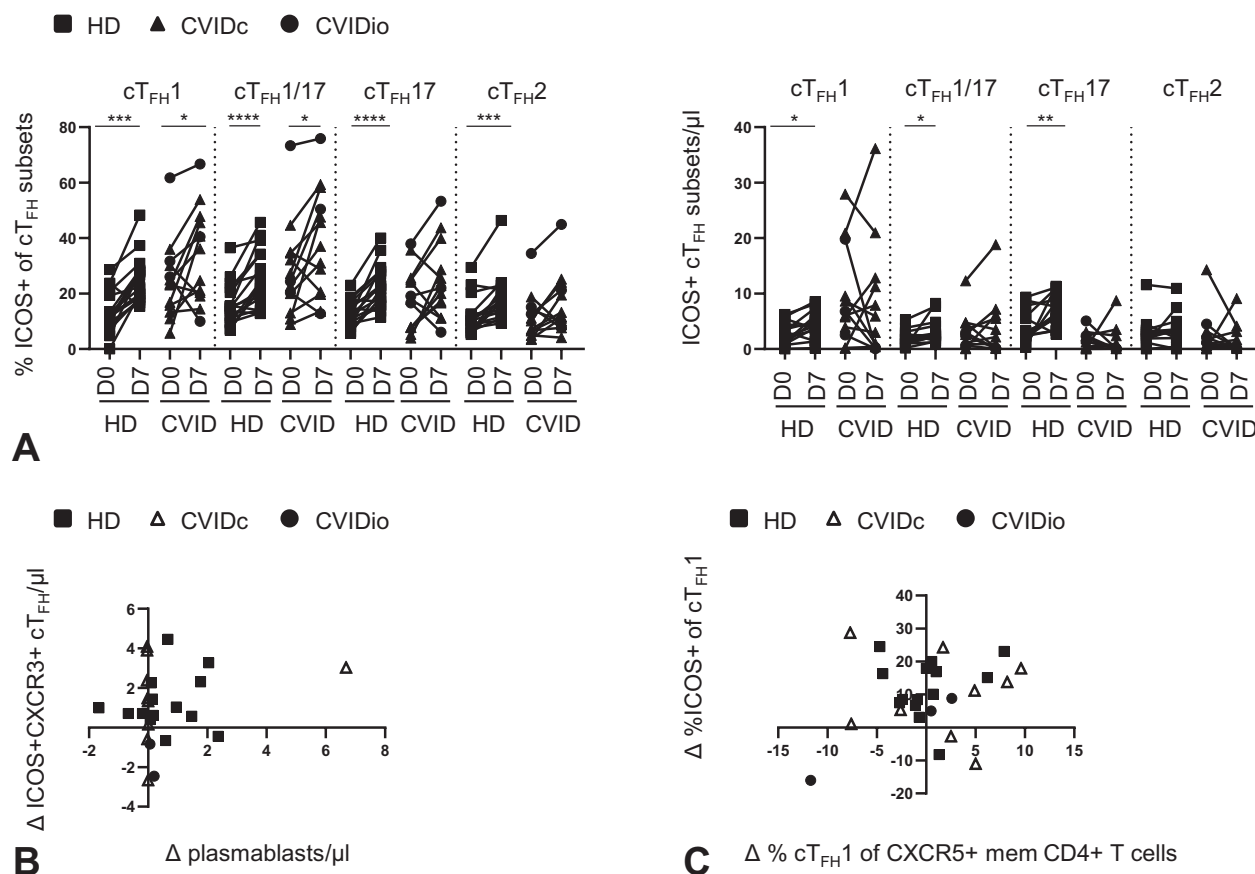


FIGURE E2. ICOS induction in different cT_{HH} subsets after influenza vaccination. **(A)** Percentages and absolute counts of ICOS⁺ cells among the indicated cell subsets of HDs and patients with CVID (including patients with CVIDio in circles and patients with CVIDc in triangles) were compared before and 1 week after vaccination. Paired *t* test or Wilcoxon test. **P* < .05. ***P* < .01. ****P* < .001. *****P* < .0001. **(B)** Correlation between the vaccination-induced change in absolute counts of ICOS⁺CXCR3⁺ cT_{HH}1 cells and plasmablasts for HDs (squares) and patients with CVIDio (open triangles) and patients with CVIDc (circles). **(C)** Correlation between the vaccination-induced change in percentages of ICOS⁺ cells within the cT_{HH}1 T-cell compartment and cT_{HH}1 cells among CXCR5⁺ memory CD4 T cells of HDs (squares) and patients with CVIDio (open triangles) and patients with CVIDc (circles).

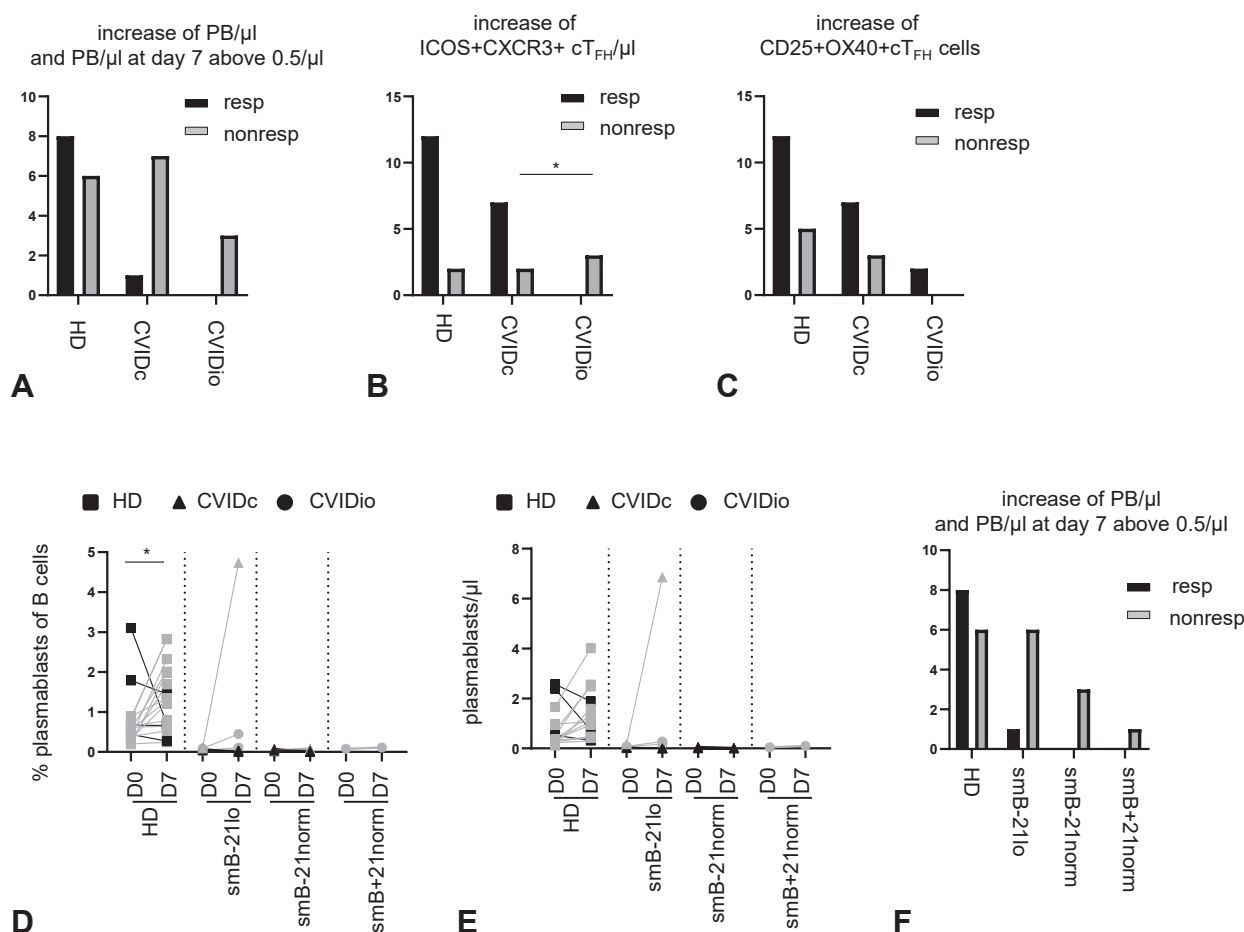


FIGURE E3. Stratifying responders and nonresponders according to common classification schemes. **(A)** Individuals with an increase in plasmablasts/ μ L and absolute plasmablast counts at day 7 above 0.5/ μ L were classified as responders and stratified according to patients with CVIDc or patients with CVIDio. **(B)** Individuals with an increase in ICOS⁺CXCR3⁺CXCR5⁺ cT_{FH} cells/ μ L were classified as responders and stratified according to patients with CVIDc or patients with CVIDio. **(C)** Individuals with an increase in CD25⁺OX40⁺ cT_{FH} cells were classified as responders and stratified according to patients with CVIDc or patients with CVIDio. **(D)** Relative plasmablast counts of HDs and smB-21lo, smB-21norm, and smB+21norm patients with CVID before vaccination (D0) were compared with counts 1 week after vaccination (D7) by Wilcoxon test. Individuals with an increase were depicted by gray lines and triangles. * $P < .05$. **(E)** Absolute plasmablast counts of HDs and smB-21lo, smB-21norm, and smB+21norm patients with CVID before vaccination (D0) were compared with counts 1 week after vaccination (D7) by Wilcoxon test. Individuals with an increase were depicted by gray lines and triangles. **(F)** Individuals with an increase in plasmablasts/ μ L and absolute plasmablast counts at day 7 above 0.5/ μ L were classified as responders and stratified according to smB-21lo, smB-21norm, and smB+21norm patients with CVID. **(G)** Relative ICOS⁺CXCR3⁺CXCR5⁺ cT_{FH}-cell counts of HDs and smB-21lo, smB-21norm, and smB+21norm patients with CVID before vaccination (D0) were compared with counts 1 week after vaccination (D7) by Wilcoxon test or paired t test. Individuals with an increase were depicted by gray lines and triangles. * $P < .05$. ** $P < .01$. *** $P < .001$. **(H)** Absolute ICOS⁺CXCR3⁺CXCR5⁺ cT_{FH}-cell counts of HDs and smB-21lo, smB-21norm, and smB+21norm patients with CVID before vaccination (D0) were compared with counts 1 week after vaccination (D7) by Wilcoxon test or paired t test. Individuals with an increase were depicted by gray lines and triangles. * $P < .05$, ** $P < .01$. **(I)** Individuals with an increase in ICOS⁺CXCR3⁺CXCR5⁺ cT_{FH} cells/ μ L were classified as responders and stratified according to smB-21lo, smB-21norm and smB+21norm patients with CVID. **(J)** Percentages of CD25⁺OX40⁺ antigen-specific cells of the cT_{FH} compartment of HDs and smB-21lo, smB-21norm, and smB+21norm patients with CVID before vaccination (D0) were compared with counts 1 week after vaccination (D7) by Wilcoxon test or paired t test. Individuals with an increase were depicted by gray lines and triangles. * $P < .05$. **(K)** Individuals with an increase in CD25⁺OX40⁺ cT_{FH} cells were classified as responders and stratified according to smB-21lo, smB-21norm, and smB+21norm patients with CVID. **(L)** Individuals with an increase in plasmablasts/ μ L and absolute plasmablast counts at day 7 above 0.5/ μ L were classified as responders and stratified according to naive T cells. **(M)** Individuals with an increase in ICOS⁺CXCR3⁺CXCR5⁺ cT_{FH} cells/ μ L were classified as responders and stratified according to naive T cells. **(N)** Individuals with an increase in CD25⁺OX40⁺ cT_{FH} cells were classified as responders and stratified according to naive T cells. *resp*, Responder; *nonresp*, nonresponder.

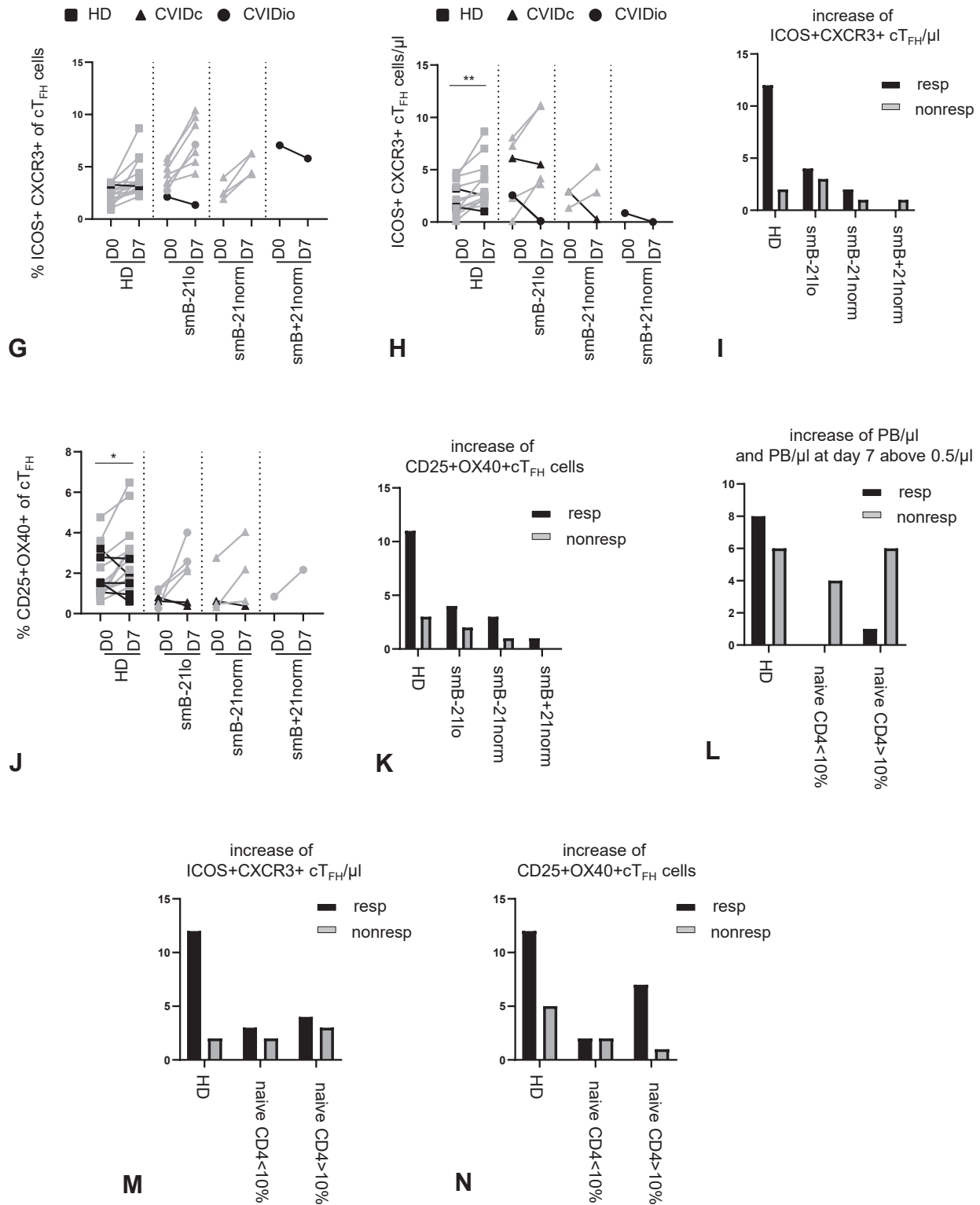


FIGURE E3. (CONTINUED).

TABLE E1. List of fluorescent antibodies and dyes used for staining

Antibody	Fluorochrom	Clone	Manufacturer
Anti-CD3	BV605	UCHT1	BioLegend, San Diego, Calif
Anti-CD3	PerCpCy5.5	SK7	BioLegend, San Diego, Calif
Anti-CD4	Pe-Cy7	RPA-T4	BioLegend, San Diego, Calif
Anti-CD10	BV605	HI10a	BioLegend, San Diego, Calif
Anti-CD19	APC-Cy7	HIB19	BioLegend, San Diego, Calif
anti-CD21	Pe-Cy7	Bu32	BioLegend, San Diego, Calif
Anti-CD25	PerCpCy5.5	M-A251	BioLegend, San Diego, Calif
Anti-CD27	BV421	M-T271	BioLegend, San Diego, Calif
Anti-CD38	PerCpCy5.5	HIT2	BioLegend, San Diego, Calif
Anti-CD45	BV785	HI30	BioLegend, San Diego, Calif
Anti-CD45RA	APC-Cy7	HI100	BioLegend, San Diego, Calif
Anti-CD134 (OX40)	PE	Ber-ACT35	BioLegend, San Diego, Calif
Anti-CD183 (CXCR3)	PE	WM59	R&D Systems, Minneapolis, Minn
Anti-CD185 (CXCR5)	AF488	RF8B2	BD Biosciences, San Jose, Calif
Anti-CD196 (CCR6)	BV605	G034E3	BioLegend, San Diego, Calif
Anti-CD278 (ICOS)	BV421	C398.4A	BioLegend, San Diego, Calif
Anti-CD279 (PD-1)	APC	EH12.2H7	BioLegend, San Diego, Calif
Anti-IgA	PE		SouthernBiotech, Birmingham, Ala
Anti-IgD	FITC		SouthernBiotech, Birmingham, Ala
Anti-IgG	AF700	G18-145	BD Biosciences, San Jose, Calif
Anti-IgM	AF647		Jackson ImmunoResearch Laboratories, Suffolk, United Kingdom
Zombie UV			BioLegend, San Diego, Calif

APC, Allophycocyanin; *FITC*, fluorescein isothiocyanate; *PE*, phycoerythrin.