Differential T-cell and antibody responses induced by mRNA versus adenoviral vectored COVID-19 vaccines in patients with immunodeficiencies

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Background: Immunodeficient patients (IDPs) are at higher risk of contracting severe coronavirus disease 2019 (COVID-19). Targeted vaccination strategies have been implemented to enhance vaccine-induced protection. In this population, however, clinical effectiveness is variable and the duration of protection unknown.

Objective: We sought to better understand the cellular and humoral immune responses to mRNA and adenoviral vectored COVID-19 vaccines in patients with immunodeficiency.

Methods: Immune responses to severe acute respiratory syndrome coronavirus 2 spike were assessed after 2 doses of homologous ChAdOx1-nCoV-19 or BNT162b2 vaccines in 112 infection-naive IDPs and 131 healthy health care workers as controls. Predictors of vaccine responsiveness were investigated.

Results: Immune responses to vaccination were low, and virus neutralization by antibody was not detected despite high titer binding responses in many IDPs. In those exhibiting response, the frequency of specific T-cell responses in IDPs was similar to controls, while antibody responses were lower. Sustained vaccine specific differences were identified: T-cell responses were greater in ChAdOx1-nCoV-19– compared to BNT162b2-immunized IDPs, and antibody binding and neutralization were greater in all cohorts immunized with BNT162b2. The positive correlation between T-cell and antibody responses was weak and increased with subsequent vaccination.

Conclusion: Immunodeficient patients have impaired immune responses to mRNA and viral vector COVID-19 vaccines that appear to be influenced by vaccine formulation. Understanding the relative roles of T-cell– and antibody-mediated protection as well as the potential of heterologous prime and boost immunization protocols is needed to optimize the vaccination approach in these high-risk groups. (J Allergy Clin Immunol Global 2023;2:100091.)

Key words: *COVID-19, SARS-CoV-2, vaccine, ChAdOx1-nCoV-19, BNT162b2, immunodeficiency, antibodies, T cells, immunoglobulins, health care workers*

Coronavirus disease 2019 (COVID-19) vaccines such as the nonreplicating adenovirus-based ChAdOx1-nCoV-19 and the mRNA-based BNT162b2 are effective against severe COVID-19.[1-3] Despite these successes, reinfection and emergence of new virus variants continues. Antibody responses wane over time,[4] and while up to 98% of double-vaccinated healthy individuals neutralize the original Wuhan virus strain,[5] severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants have emerged that evade neutralization in these cohorts.[6]

T cells are crucial players in protection from SARS-CoV-2 infection and disease, as supported by an increasing body of evidence. Studies in mice and rhesus macaques show that infection-induced specific T cells are particularly important for protection when specific antibodies are waning or low.[7,8] In humans, successful control of COVID-19 infection without hospitalization in individuals who produced little to no neutralizing antibody after infection but who had high T-cell responses has been reported,[9,10] as well as in individuals with agammaglobulinemia[11] and those receiving B-cell depletion therapy.[12,13] Vaccine-induced T-cell responses have been shown to be highly conserved against SARS-CoV-2 variants of concern that evade vaccine-induced neutralizing antibodies.[14] In addition, given that a hyperinflammatory, dysregulated T-cell response plays a key role in severe COVID-19,[9,15] understanding the role of infection and vaccine induced T cells in protection from disease is important.

Deficiency in T-cell responses, particularly $CD4^+$ T follicular helper cells, affects the development of high-affinity neutralizing antibody responses.[9,16] Deficiencies in antibody development and

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Abbreviations used

CEFT: Peptides pool from human cytomegalovirus, Epstein-Barr virus, influenza A virus, and *Clostridium tetani*
COVID-19: Coronavirus disease 2019
CVID: Common variable immunodeficiency
HCW: Health care worker
HCW-nPI: HCW with no prior COVID-19 infection
HCW-PI: HCW with prior COVID-19 infection
IDP: Immunodeficient patient
IgGRx: Immunoglobulin replacement therapy
PV1: Post first vaccine dose
PV2: Post second vaccine dose
RBD: Receptor binding domain
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

maturation may also affect antibody-dependent mechanisms of T-cell and natural killer cell killing of infected cells.[17] Patients with immunodeficiencies (IDPs) are a clinically vulnerable group at higher risk of severe COVID-19 disease[18,19] and have reduced responsiveness to vaccination.[20,21] Characterizing the immune response in IDPs provides an avenue for understanding the relative role and interaction of humoral and cellular immune responses in COVID-19 vaccination and in gaining a deeper understanding of immune correlates of protection in different populations, ensuring adjunctive therapies such as passive immunization are appropriately targeted.

Following our previous report of poor neutralizing antibody response after the first COVID-19 vaccine dose in immunodeficient and healthy individuals,[22] we present here analyses of circulating T-cell and humoral responses after double homologous doses of either ChAdOx1-nCoV-19 or BNT162b2 vaccines in an extended cohort of IDPs and health care workers (HCWs). These analyses highlight the importance of considering targeted booster vaccination regimens for individuals with different B- and T-cell immunodeficiencies.

METHODS

Ethics statement

The study was approved by Research Ethics Committee Wales (IRAS 96194 12/WA/0148, amendment 5). Written informed consent was provided by all participants before enrollment onto the study.

Study cohorts

A total of 112 SARS-CoV-2 infection-naive IDPs with diagnosed primary or secondary immunodeficiency under the Respiratory Immunology Service, Royal Papworth Hospital, were recruited for this study between March and July 2021. Immune diagnosis and treatment with immunoglobulin replacement therapy (IgGRx) were recorded. Inclusion criteria included clinical and laboratory evidence of immunodeficiency in accordance with European Society for Immunodeficiency criteria (esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria). Exclusion criteria included a history (by clinical features as well as virologic and/or serologic diagnosis) of prior SARS-CoV-2 infection.

A total of 131 Royal Papworth Hospital HCWs were recruited from the Humoral Immune Correlates for COVID-19 study (gtr.ukri.org/projects?ref=MC_PC_20016) as healthy controls. They were classified as not previously infected (infection naive, HCW-nPI) and previously infected (HCW-PI), as previously described.[23] HCW-PI served as the benchmark for wild-type virus–primed responses against which vaccination responses were assessed in infection-naive HCW-nPI and IDPs.

Participants received 2 doses of either the ChAdOx1-nCoV-19 (Vaxzevria, AstraZeneca) or the BioNTech 162b2 (BNT162b2, Tozinameran, Pfizer) vaccine in accordance with the UK vaccination schedule (https://www.gov.uk/government/publications/prioritizing-the-first-covid-19-vaccine-dose-jcvi-statement/optimizing-the-covid-19-vaccination-programme-for-maximum-short-term-impact). Age, sex, and SARS-CoV-2 vaccination history were recorded.

Sample collection and processing

Sera and peripheral blood mononuclear cells were isolated from venipuncture-drawn whole blood that was cryopreserved until experimental use. More details are available in this article's Methods section in the Online Repository at www.jaci-global.org.

T-cell response assay

Peripheral blood mononuclear cell T-cell responses to peptides were assessed using a Human IFN-γ Single-Colour Enzymatic ELISpot Assay kit (ImmunoSpot) according to the manufacturer's protocol. The full protocol is available in this article's Methods section in the Online Repository.

Multiplex microbead immunoassay

For multiplex microbead immunoassay, we followed the protocol originally described in the Luminex (xMAP) Cookbook (4th edition) and as previously published.[23] The full protocol is available in this article's Methods section in the Online Repository.

Pseudotype microneutralization assay

We followed the protocol previously published by Ferrara and Temperton[24] and applied it to SARS-CoV-2 as described by Di Genova et al.[25] The full protocol is available in this article's Methods section in the Online Repository.

Statistical analysis

Statistical analyses were performed by GraphPad Prism v9 software (GraphPad Software). Statistical difference was determined by the nonparametric Mann-Whitney test for cohort comparisons and the Wilcoxon test for pairwise comparisons, unless otherwise stated, with $*P < .05$, $**P < .01$, $***P < .001$, and $****P < .0001$. Spearman correlation was used for multivariate analyses. Graphs were produced by GraphPad Prism, and tables were created by Microsoft Excel and PowerPoint.

RESULTS

Study cohort description

The demographic features of the cohorts and for IDPs, clinical diagnoses, and receipt of IgGRx are shown in Table I and, in this article's Online Repository at www.jaci-global.org, Table E1. The numbers of samples tested are shown in Table E2 (also available in this article's Online Repository). The IDPs were older than the HCWs, and although female subjects dominated in all cohorts, a greater percentage of men were represented in the IDPs. The median interval between first and second vaccination doses was similar between all cohorts (75-77 days), and the interval between vaccination and blood sampling averaged 4 to 6 weeks.

The clinical diagnoses of members of the IDP cohort were diverse and included including X-linked agammaglobulinemia, common variable immunodeficiency (CVID), combined immunodeficiency, selective antibody deficiency, and hypogammaglobulinemia. Individuals with secondary immunodeficiencies

TABLE I. Demographic features of study cohorts

Characteristic	No. of participants	Vaccine group (ChAdOx1-nCoV-19/BNT162b2)	Age at first vaccination (years), median (95% confidence interval)	Male sex (%)
Total	243			
HCW-PI	41	21/20	49 (43-53)	29
HCW-nPI	90	43/47	48 (44-51)	20
IDP	112	66/46	62 (59-66)	40

due to cancer treatment or after solid organ transplant were represented. Fifteen individuals were receiving immunosuppressive treatment at the time of vaccination. Patients with primary immunodeficiency dominated (72%), and 65% of all IDPs received IgGRx.

Fewer IDPs generate IFN-γ–secreting virus-specific T cells after vaccination

Using direct *ex vivo* IFN-γ ELISpot assay, we assessed SARS-CoV-2 reactive T-cell responses. Noting the distinct roles of spike (S) 1 and S2 domains in receptor binding and membrane fusion, respectively,[26] as well as the induction of S2 domain–reactive T cells by seasonal coronaviruses in the prepandemic population,[15] we assessed spike S1- and S2-specific responses separately after single and double homologous doses of either the BNT162b2 or ChAdOx1-nCoV-19 vaccine. Although differential enhancement of N-terminal and S2 domain antibody responses have been reported after mRNA vaccination,[27] there was no difference in the frequency of S1 and S2 T-cell responses within our cohorts at each blood-sampling point (Fig 1, *A* and *B*), so aggregate S1 and S2 counts were used in subsequent analyses. While 74% of HCW-nPI had detectable T-cell responses at first vaccination (PV1), rising to 97% at second vaccination (PV2), in IDPs, only 54% at PV1 and 77% at PV2 had positive responses (Table II). Compared to HCW-nPI, these are significantly lower proportions of those with response in IDPs (Fisher exact test: PV1 $P = .0016$; PV2 $P = .0026$). Ninety-two percent and 100% of HCW-PI exhibited a positive response at PV1 and PV2, respectively (Fig 1, *C* and *D*).

Magnitude of responses are similar in IDPs and HCW-nPI. There was no difference in the magnitude (frequency of antigen-specific T cells) of responses between HCW-nPI and IDPs at both time points in the cohort overall (Fig 1, *C* and *D*) or when only those with positive responses were analyzed (data not shown). Pairwise comparison revealed a significant increase in magnitude of responses from PV1 to PV2 in both HCW-nPI and IDPs (HCW-nPI, $P = .0023$; IDP, $P < .0001$) (Fig 1, *E*). This was not seen in HCW-PI, suggesting that an upper threshold in T-cell response had been reached after infection and 1 dose vaccination (Fig 1, *E*).

ChAdOx1-nCoV-19 induces greater T-cell responses than BNT162b2 in IDPs. Distinct patterns in T-cell responses by vaccination group were seen that were different in IDPs compared to HCWs (Fig 1, *F* and *G*). IDPs had higher T-cell responses in ChAdOx1-nCoV-19 compared to BNT162b2 recipients—a difference that increased with second immunization ($P = .0024$ and $P = .0003$, respectively). Age did not confound this result; when we matched cohorts by age by removing the 5 outlying participants aged ≥80 years, the difference was sustained (PV1 $P = .004$, PV2 $P = .0011$). HCW-nPI also had higher T-cell responses in ChAdOx1-nCoV-19 compared to BNT162b2 vaccine recipients at PV1 ($P = .0334$); however, this was not sustained at PV2. HCW-PI showed no difference in T-cell response by vaccination at either time point.

To determine whether the poor T-cell responses in IDPs were specific to COVID-19 vaccine antigens to which the IDPs were naive at first immunization, we assessed T-cell responses to a pool of peptides from human cytomegalovirus, Epstein-Barr virus, influenza A virus, and *Clostridium tetani* (CEFT)[28] at PV2. We considered that most individuals in the United Kingdom have either been vaccinated against or exposed to these organisms and thus have had the opportunity to generate T-cell memory to at least some of the antigens in the CEFT peptide pool. Our results showed a wide range of responses, with undetectable responses in only 9 individuals (Fig 1, *H*). While T-cell responses to CEFT and SARS-CoV-2 spike antigens correlated ($R = 0.29$, $P < .005$) (data not shown), CEFT responses were similar between ChAdOx1-nCoV-19 and BNT162b2 vaccine recipients (Fig 1, *H*), suggesting that the weaker response to BNT162b2 by IDPs was vaccine specific.

Binding and neutralizing antibody responses are weak in IDPs

We have previously reported PV1 neutralization responses from our cohort,[22] showing low virus neutralization in IDPs and HCW-nPI compared to HCW-PI. Here we report neutralization responses after second vaccination and antibody binding responses to full-length spike and receptor binding domain (RBD) proteins. Our previous data set is necessarily included for comparison.

Most IDPs demonstrate a detectable total IgG binding response at PV2. The IgG binding responses to spike and RBD was greatest in HCW-PI and lowest in IDPs (Fig 2, *A* and *B*). This was maintained after second immunization. In both IDPs and HCW-nPI, there was a significant increase in IgG response from PV1 to PV2 ($P < .0001$) (Fig 2, *C*). However, while IgG binding responses increased after second immunization in the IDP cohort, spike IgG was only detected in 75% at PV2 compared to 98% positive response in HCW-nPI (Fisher exact test: $P < .0001$). Sixty-five percent of IDPs had RBD-binding IgG response at PV2 compared to 97% HCW-nPI (Table III). HCW-PI showed no increase in IgG binding at PV2 (Fig 2, *C*), consistent with previous studies[27] and similar to our T-cell response results.

IgG_1 is the dominant isotype generated in virus vaccine-induced antibody responses; however, IgG_3 plays a particular role in early control of viral infection.[29] The relative role and importance of vaccination in priming for IgG_3 responses is less clear. In contrast to the highest titer IgG, IgG_1, and IgA responses

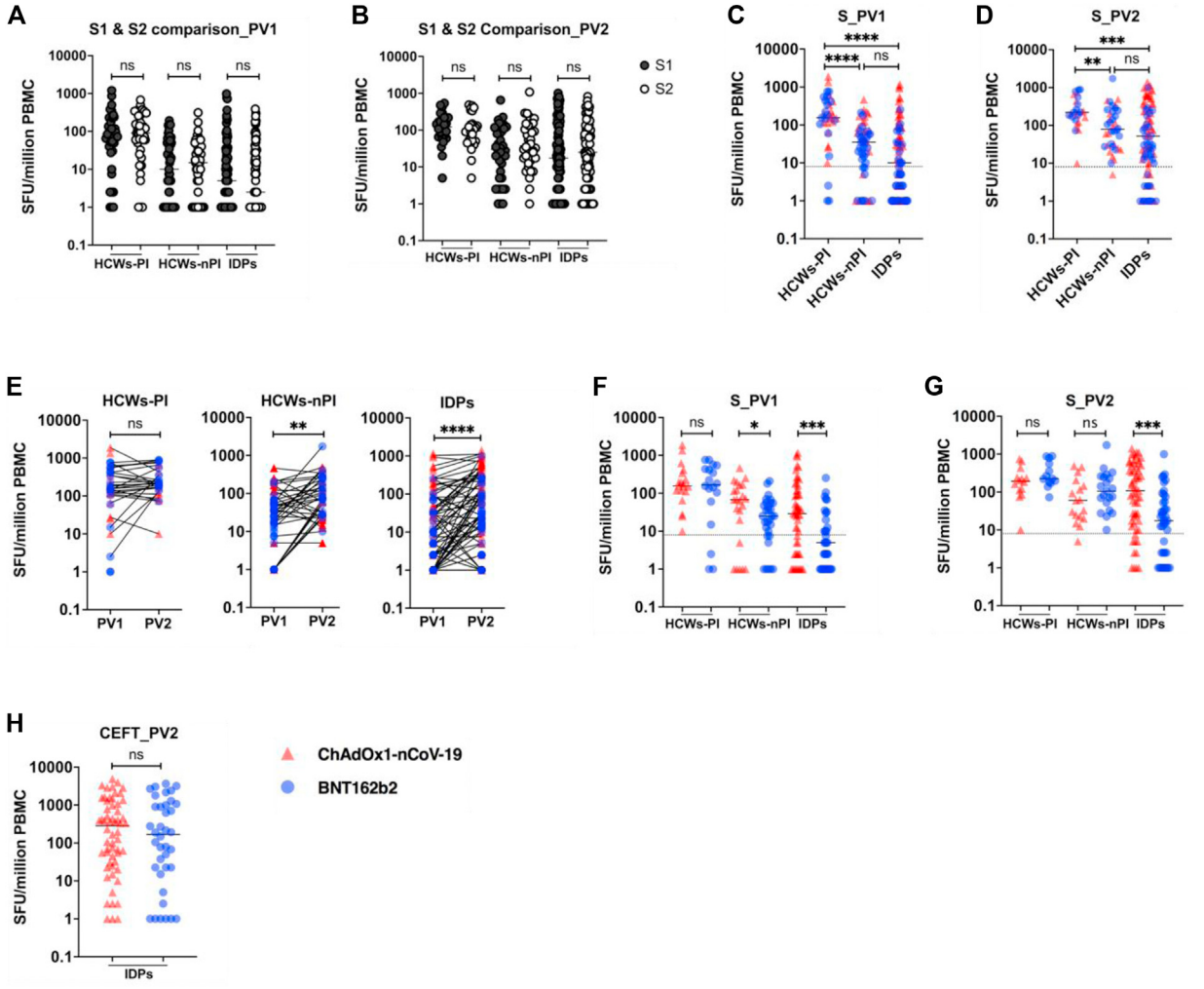

FIG 1. Frequency of IFN-γ–secreting spike (S)-specific T cells after PV1 and PV2. Comparison of S1- and S2-specific T-cell responses in each cohort after **(A)** PV1 and **(B)** PV2 measured as SFUs from PBMCs. Graphs of spike (sum of S1 and S2) SFU at **(C)** PV1 and **(D)** PV2 for different cohorts. **(E)** Pairwise comparison of S-specific responses after PV1 and PV2. (**F** and **G**) Comparison of spike T-cell frequency within each cohort based on vaccine received for PV1 and PV2. **(H)** T-cell response to CEFT-positive control peptide pool in IDPs. Data points are means of duplicate wells minus means of duplicate negative control wells. *Lines* within data set represent group medians; *dotted lines* indicate cutoffs for positive responses. $*P < .05$, $**P < .01$, $***P < .001$, $****P < .0001$, *ns,* not significant. *PBMC,* Peripheral blood mononuclear cell; *SFU,* spot-forming unit.

TABLE II. Cohorts with positive T-cell response

	PV1			PV2		
Cohort	**T-cell ELISpot response**	**Vaccine**	**Cohort response**	**T-cell ELISpot response**	**Vaccine**	**Cohort response**
HCW-PI	92%	ChAdOx1-nCoV-19	100% (19/19)	100%	ChAdOx1-nCoV-19	100% (14/14)
		BNT162b2	83% (15/18)		BNT162b2	100% (13/13)
HCW-nPI	74%	ChAdOx1-nCoV-19	74% (17/23)	97%	ChAdOx1-nCoV-19	94% (16/17)
		BNT162b2	73% (22/30)		BNT162b2	100% (21/21)
IDP	54%	ChAdOx1-nCoV-19	65% (34/52)	77%	ChAdOx1-nCoV-19	83% (49/59)
		BNT162b2	37% (13/35)		BNT162b2	67% (26/39)

being in HCW-PI, HCW-nPI generated the strongest IgG_3 responses, while 45% of IDPs had no detectable IgG_3 response at PV2 (see Fig E1 in this article's Online Repository at www.jaci-global.org).

Most IDPs lack IgA responses. IgA is important in mucosal defense, and IgA deficiency is relatively common.[30] The role of vaccination in inducing and boosting IgA responses remains to be fully elucidated. We found that

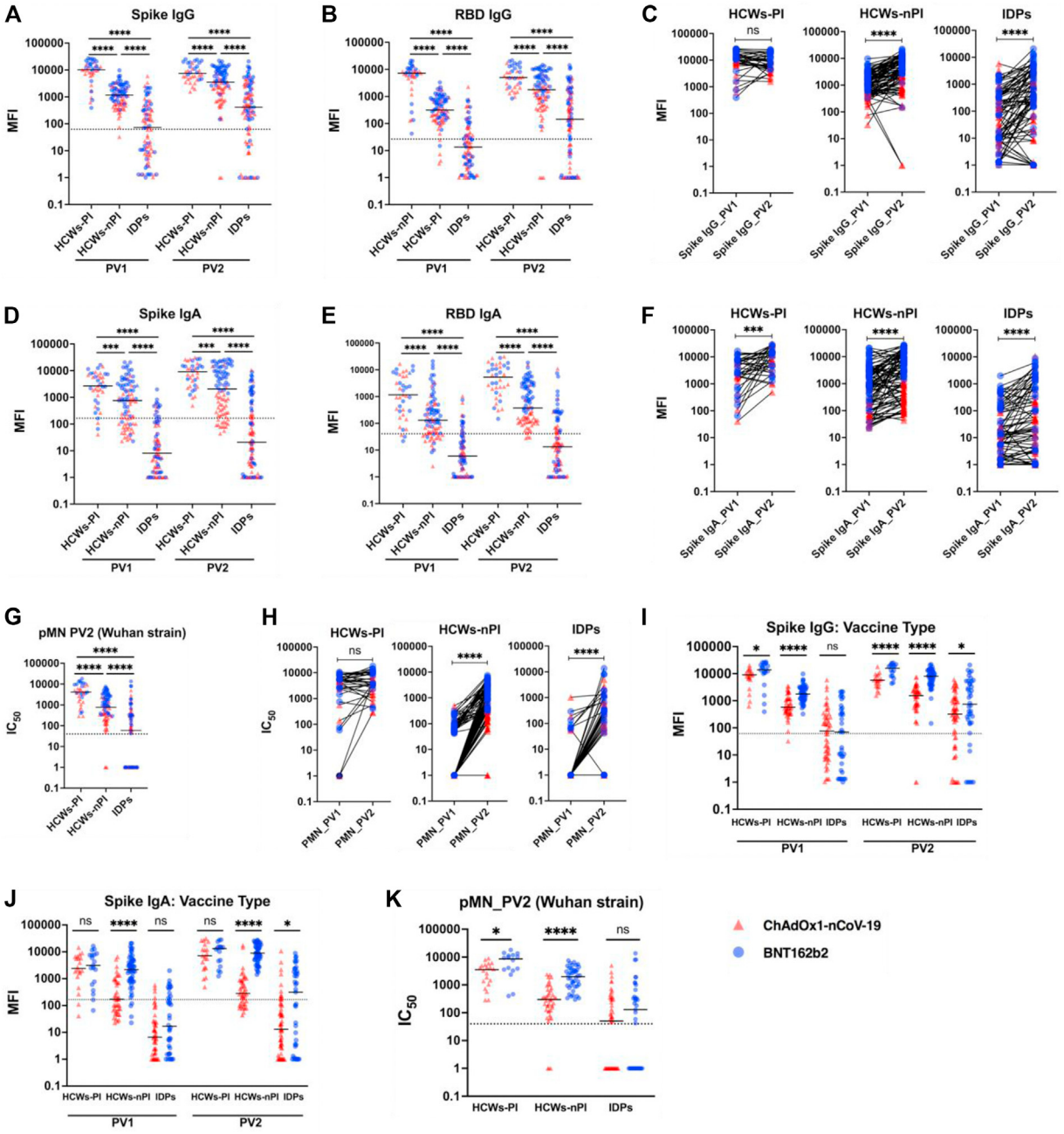

FIG 2. Binding and neutralizing antibody responses at PV1 and PV2. At PV1 and PV2, IgG binding antibody responses against **(A)** spike and **(B)** RBD were measured as MFI. **(C)** Pairwise comparisons of spike IgG responses after PV1 and PV2 in all cohorts. PV1 and PV2 IgA binding antibody responses against **(D)** spike and **(E)** RBD. **(F)** Pairwise comparisons of spike IgA responses after first and second immunization in all cohorts. **(G)** PV2 pMN titers against Wuhan strain of SARS-CoV-2. **(H)** Pairwise comparison of pMN titers against Wuhan strain after first and second immunization in all cohorts. **(I-K)** Vaccine group comparison of *(I)* spike-binding IgG titers, *(J)* spike-binding IgA titers at PV1 and PV2, and *(K)* pMN titers at PV2. *Dotted lines* represent cutoffs for positive response. *Lines* within data set represent group medians. $*P < .05$, $**P < .01$, $***P < .001$, $****P < .0001$, *ns,* not significant. *MFI,* mean fluorescence intensity; *pMN,* pseudovirus microneutralization.

SARS-CoV-2 vaccination induced IgA responses in all cohorts; however, fewer IDPs showed IgA binding compared to HCW-nPI (Table III), with 32% of IDPs clinically IgA deficient. When these individuals were excluded from the analysis, 47% of this subgroup of IDPs had detectable IgA binding responses, compared to 97% of HCW-nPI. Of those with response, the magnitude of response was also lower in IDPs (Fig 2, *D* and *E*). PV2 boosted IgA binding in all cohorts, including HCW-PI (Fig 2, *F*).

Fewer IDPs demonstrate neutralizing responses compared to binding responses. Antibody binding titer correlates with virus neutralization in most healthy individuals.[31]

However, the fine specificity of antibody binding is known to develop over time and may affect the quality of immune protection. We previously reported poor neutralization responses after the first dose in HCW-nPI and IDPs compared to HCW-PI.[22] Here we report increased neutralization in all cohorts; however, neutralization was considerably lower in IDPs compared to HCW-nPI and was greatest in HCW-PI (Fig 2, *G* and *H*). Fewer IDPs neutralized SARS-CoV-2 (56%) compared to HCW-nPI (96%) (Table III). In contrast to the T-cell response, of those with a positive neutralization response, neutralization titers were lower in IDPs than in HCW-nPI ($P = .0013$) (data not shown).

BNT162b2 induced greater antibody binding and neutralizing responses than ChAdOx1-nCoV-19. When we compared antibody responses by vaccination type, we found that HCW-PI and HCW-nPI spike-binding IgG and neutralization titers were higher in recipients of BNT162b2 compared to ChAdOx1-nCoV-19 vaccines at both time points (Fig 2, *I* and *K*). A similar trend was seen in IDPs at PV2. BNT162b2 immunization also induced greater IgA responses in HCW-nPI at both time points ($P < .0001$) and in IDPs at PV2 ($P = .02$) compared to ChAdOx1-nCoV-19, but not in HCW-PI (Fig 2, *J*). This difference between vaccines was further pronounced in IDPs when we removed clinically IgA-deficient subjects ($P = .005$). Overall, despite lower T-cell responses in IDPs, the BNT162b2 vaccine induced better binding and neutralizing antibody responses across all cohorts.

Interassay relationships

While antibody binding and neutralization responses, and their fold change, broadly correlated in all cohorts (Fig 3, *A-I,* and see Fig E2 in this article's Online Repository at www.jaci-global.org), the relationship was weaker in IDPs, with some individuals showing high binding titers but no evidence of neutralization, indicating a qualitative difference in response between IDPs and HCWs (Fig 3, *H*). When receiver operating characteristic (ROC) curves were generated to evaluate the antibody binding concentration that predicted neutralization in IDPs at PV2, an antibody titer of 8752.8 mean fluorescence intensity was generated—considerably higher than the 3914.8 mean fluorescence intensity in HCW-nPI predicting a similar neutralization response (Fig 3, *J* and *K*). This suggests the neutralization quality is poorer in antibodies in IDPs. The correlation between spike-specific T-cell numbers and antibody response was weakly positive, particularly in HCWs, and became stronger in all cohorts after the second immunization (see Fig E2 in this article's Online Repository).

Correlation of immune response with age and IDP clinical profile

Immune responses were lower in older patients with immunodeficiency. In IDPs, there was a significant negative correlation between age and IFN-γ producing T-cell counts, which increased between first ($R = -0.246$, $P = .0248$; data not shown) and second immunizations (PV2 $R = -0.36$, $P = .0002$) (Fig 4, *A*). There was no correlation between age and CEFT response (Fig 4, *B*), suggesting that older age is associated with reduced response to novel antigens rather than recall responses in this cohort. The IgG binding and neutralization responses also correlated inversely with age in IDPs (Fig 4, *C* and *D*). However, this relationship was weaker than that seen in the T-cell responses and did not reach significance for IgG binding. The age demographic was younger in HCWs, confounding comparison of response patterns with IDPs, and no relationship was observed between age and T-cell response, antibody binding, or neutralization in HCWs. There was no relationship between gender and immune responses in all cohorts (data not shown).

Immune responses are highly variable in IDP populations

Specific T-cell frequency, and antibody binding and neutralization titres varied widely across the IDP cohort (Fig 4, *E-G*). Firstly, there was no difference in antibody or T-cell response profiles in patients diagnosed with common variable immunodeficiency (CVID) or with a diagnosis of selective antibody deficiency with hypogammaglobulinaemia (ie, not meeting CVID diagnostic criteria). Secondly, patients with agammaglobulinemia had no detectable IgG responses, as expected; however, 2 of these individuals had positive T-cell responses. Thirdly, more patients with secondary immunodeficiency due to rheumatologic disease and its treatment had low antibody-binding and neutralization responses ($P = .062$ and $P = .0025$) compared to other patients with secondary immunodeficiency with previous lymphoma treatment, although T-cell responses were similar between these groups.

Further analyses based on broad diagnoses of primary or secondary immunodeficiency, on whether regular IgGRx was being provided, and whether the patient was taking active immunosuppression treatment, were performed considering only IDPs and HCW-nPI with detectable responses. T-cell responses remained similar to those of HCW-nPI, irrespective of broad diagnoses or IgGRx (Fig 4, *H* and *J*). However, while neutralization titers were comparable between primary and secondary IDPs (Fig 4, *I*), those who received IgGRx had slightly lower neutralization titers than those who did not receive this treatment ($P = .0162$, Fig 4, *K*), and the difference in titers between the latter and HCW-nPI was not significant. Of the 15 individuals receiving immunosuppressive treatment at the time of vaccination, 2 were receiving ibrutinib for chronic lymphocytic leukemia, 2 were receiving calcineurin inhibitors to manage lung transplant rejection, and 1 was receiving mycophenolate mofetil and 1 low-dose prednisolone (5 mg) to manage interstitial lung disease associated with CVID. The remaining 9 patients were rheumatology patients receiving prednisolone (5-15 milligrams once daily, n = 6; maintenance rituximab, n = 1; abatacept, n = 1; secukinumab, n = 1). While immune responses tended to be lower in this group (Fig 4, *L* and *M*), with only 4 of 15 having detectable neutralization, numbers were small, and larger-cohort studies are needed to determine whether particular immunosuppressant regimens are independent predictors of poor vaccine response.

DISCUSSION

In this study, we have shown that while most immunodeficient people respond to COVID-19 immunization with a significant boost in their spike protein–reactive T cells and antibody responses, their response is diverse, with evidence of age dependency. A considerable proportion of IDPs do not have evidence of neutralizing antibodies even with high titers of binding antibodies. Low or no neutralization by IDPs despite

TABLE III. Cohorts with positive binding and neutralizing antibody responses

		PV1			PV2		
Cohort	**Immune response**	**Response**	**Vaccine**	**Cohort response**	**Response**	**Vaccine**	**Cohort response**
IgG response							
HCW-PI	S	100%	ChAdOx1-nCoV-19	100% (20/20)	100%	ChAdOx1-nCoV-19	100% (20/20)
			BNT162b2	100% (19/19)		BNT162b2	100% (16/16)
	RBD	100%	ChAdOx1-nCoV-19	100% (20/20)	100%	ChAdOx1-nCoV-19	100% (20/20)
			BNT162b2	100% (19/19)		BNT162b2	100% (16/16)
HCW-nPI	S	99%	ChAdOx1-nCoV-19	98% (41/42)	98%	ChAdOx1-nCoV-19	95% (41/43)
			BNT162b2	100% (46/46)		BNT162b2	100% (43/43)
	RBD	94%	ChAdOx1-nCoV-19	91% (38/42)	97%	ChAdOx1-nCoV-19	93% (40/43)
			BNT162b2	98% (45/46)		BNT162b2	100% (43/43)
IDP	S	51%	ChAdOx1-nCoV-19	55% (28/51)	75%	ChAdOx1-nCoV-19	70% (43/61)
			BNT162b2	46% (19/41)		BNT162b2	83% (33/40)
	RBD	79%	ChAdOx1-nCoV-19	80% (41/51)	65%	ChAdOx1-nCoV-19	62% (38/61)
			BNT162b2	78% (32/41)		BNT162b2	70% (28/40)
IgA response							
HCW-PI	S	90%	ChAdOx1-nCoV-19	90% (18/20)	100%	ChAdOx1-nCoV-19	100% (20/20)
			BNT162b2	89% (17/19)		BNT162b2	100% (16/16)
	RBD	92%	ChAdOx1-nCoV-19	95% (19/20)	100%	ChAdOx1-nCoV-19	100% (20/20)
			BNT162b2	89% (17/19)		BNT162b2	100% (16/16)
HCW-nPI	S	73%	ChAdOx1-nCoV-19	52% (22/42)	84%	ChAdOx1-nCoV-19	67% (29/43)
			BNT162b2	91% (42/46)		BNT162b2	100% (43/43)
	RBD	77%	ChAdOx1-nCoV-19	64% (27/42)	97%	ChAdOx1-nCoV-19	93% (40/43)
			BNT162b2	89% (41/46)		BNT162b2	100% (43/43)
IDP	S	5%	ChAdOx1-nCoV-19	3% (2/60)	32%	ChAdOx1-nCoV-19	20% (12/61)
			BNT162b2	7% (3/39)		BNT162b2	51% (20/39)
	RBD	12%	ChAdOx1-nCoV-19	8% (5/60)	34%	ChAdOx1-nCoV-19	21% (13/61)
			BNT162b2	18% (7/39)		BNT162b2	54% (21/39)
Neutralization response (Wuhan strain)							
HCW-PI		90%	ChAdOx1-nCoV-19	86% (18/21)	100%	ChAdOx1-nCoV-19	100% (20/20)
			BNT162b2	94% (17/18)		BNT162b2	100% (16/16)
HCW-nPI		42%	ChAdOx1-nCoV-19	28% (12/43)	96%	ChAdOx1-nCoV-19	93% (40/43)
			BNT162b2	54% (25/46)		BNT162b2	100% (42/42)
IDP		16%	ChAdOx1-nCoV-19	15% (5/34)	56%	ChAdOx1-nCoV-19	54% (29/54)
			BNT162b2	17% (6/35)		BNT162b2	60% (21/35)

S, Spike.

RBD binding suggests impaired antibody development compared to healthy individuals. While the lower neutralization titers in IDPs may be attributed to fewer IDPs reaching a binding threshold to achieve virus neutralization, it is important to acknowledge potential qualitative differences in the antibodies generated in IDPs, including in the breadth of neutralization, compared to controls. We recommend the use of neutralization rather than antibody binding assays as a proxy for protective immunity and a predictor of which patients may benefit from passive immunization. Notably, immunization boosted IgA responses in all cohorts.

Affinity maturation of SARS-CoV-2 antibody response is understood to continue for at least 6 months after immunization[27] and is associated with both improved virus neutralization of the Wuhan strain and emerging variants in healthy individuals.[32] Interestingly, while this process is clearly impaired in most immunodeficient individuals, encouraging data have emerged from the COV-AD study[21] that third-dose priming (by mRNA booster) immunization improves virus neutralization responses in a small cohort of immunodeficient patients, suggesting that response maturation is sluggish but may occur if enhanced priming is used.

IgG_3 titers were greatest in HCW-nPI, likely due to the temporal kinetics of IgG subclass responses after antigenic exposure. This supports a model of an early IgG_3 peak response, followed by a steady decline after exposure to either infection or vaccination.[29,33] Moreover, IgG_3 has a shorter half-life than IgG_1 in serum.[34] The clinical significance of this difference in IgG_3 responses in HCW cohorts is unclear. IgG_3 responses were low in all cohorts, and while the mechanism of viral clearance differs between IgG_3 and IgG_1, IgG_1 concentration is much greater, and this would likely diminish the impact of variations in IgG_3-mediated responses.

Important differences in vaccine immunogenicity were observed, as in previous studies,[5,35,36] and including in older adults.[37,38] Immunization with mRNA-based vaccines induced greater antibody responses in all our cohorts, while the adenoviral vector vaccine induced higher and sustained T-cell responses in IDPs. The comparable response of the vaccine groups to a positive control peptide pool suggests this difference is not accounted for by a bias in the intrinsic T-cell responsiveness between cohorts; rather, it suggests a vaccine-specific affect in IDPs. Our data suggest that the adenovirus-based COVID-19 vaccine preferentially induces higher T-cell responses in individuals with suboptimal immunity. This may be due to bystander enhancement from higher levels of adenovirus-specific memory T-cell activation after immunization with the adeno-vectored ChAdOx1-nCoV-19

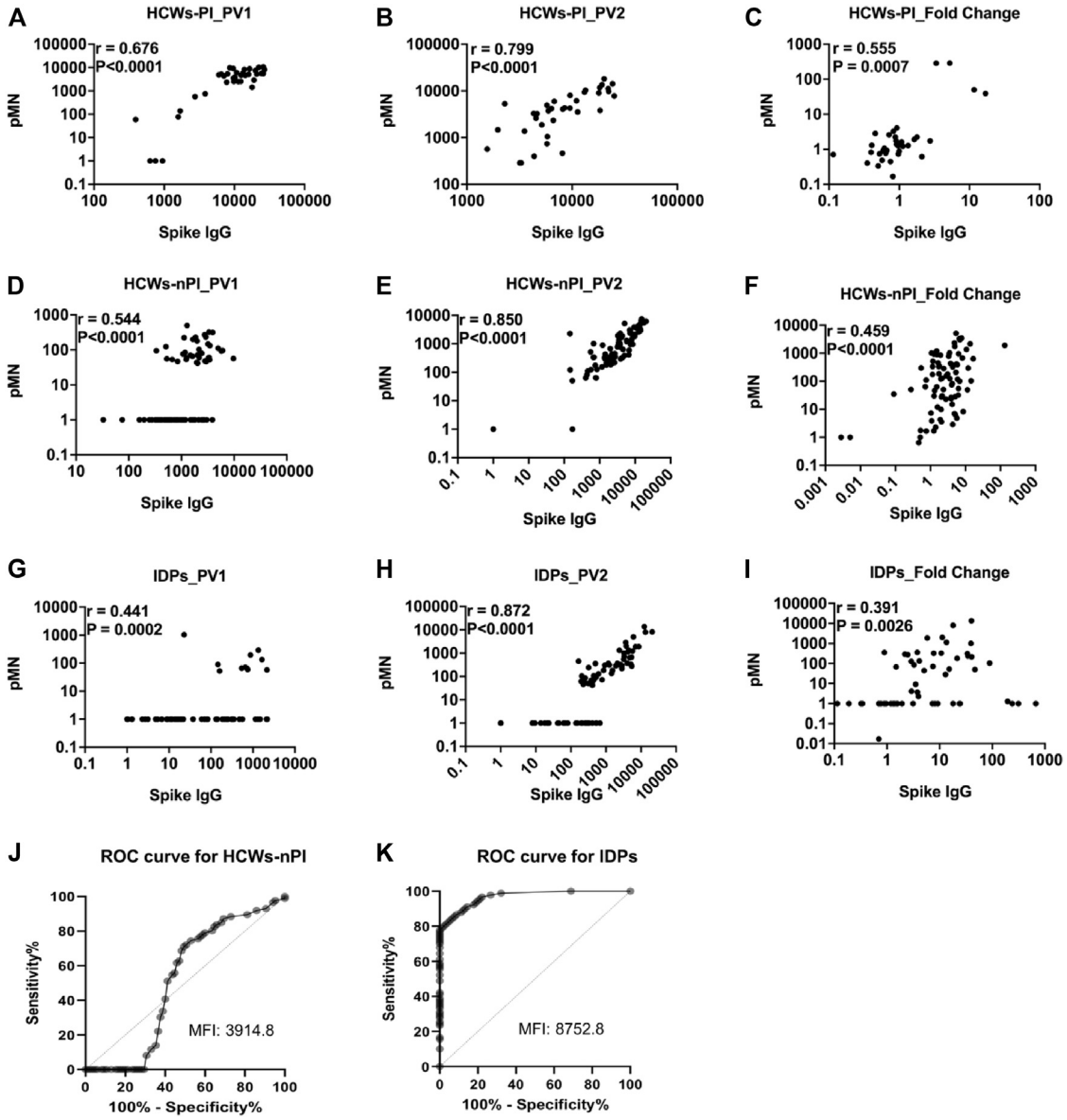

FIG 3. Relationship between antibody responses in IDPs and HCWs. Correlation between spike-binding IgG titers (spike IgG) and neutralizing antibody titer (pMN) at PV1 and PV2, along with their fold changes in, respectively, **(A-C)** HCW-PI, **(D-F)** HCW-nPI, and **(G-I)** IDPs. (**J** and **K**) ROC curve showing higher binding threshold predicting neutralization in IDP compared to HCW-nPI and indicating qualitative difference between cohorts. For ROC curves, drug concentration causing 50% inhibition was at lower end of HCW-nPI PV2's 95% confidence interval, with a value of 389. *pMN,* Pseudovirus microneutralization; *ROC,* receiver operating characteristic.

vaccine[39] as a result of defects in immune control compared to healthy individuals. This has been described for other viral infections.[40] Adenovirus-driven bystander enhancement may be a feature in individuals who develop less effective antibody binding and neutralization responses. Studies are underway to test this hypothesis.

There are some limitations to our study. First, the HCW and IDP cohorts were not well matched for age or sex, raising potential confounding demographic factors that may be relevant. As a result of infection control constraints, healthy controls were recruited from hospital staff, so the demographic of this cohort reflected this, with younger female subjects dominating. In contrast, immunodeficient outpatients were older, and more were male (in part reflecting the X-linked nature of many primary immunodeficiency disorders). Because immune response to SARS-CoV-2 is impaired in the elderly,[41] age may account for some of the differences in immune responses between HCWs and the older IDPs. However, IDPs were heterogeneous in terms of both age and clinical profile, and response ranges were large. Despite these caveats, many of the findings we have reported corroborate those of other groups, which, taken together, could better inform management of highly vulnerable groups.

In conclusion, we have demonstrated that compared to healthy controls, immunodeficient patients are at greater risk of failing to generate T-cell and antibody responses to COVID-19 immunizations. Our data also show that BNT162b2 induces higher binding and neutralizing antibody titers in healthy individuals, and to a lesser extent in immunodeficient patients, compared to

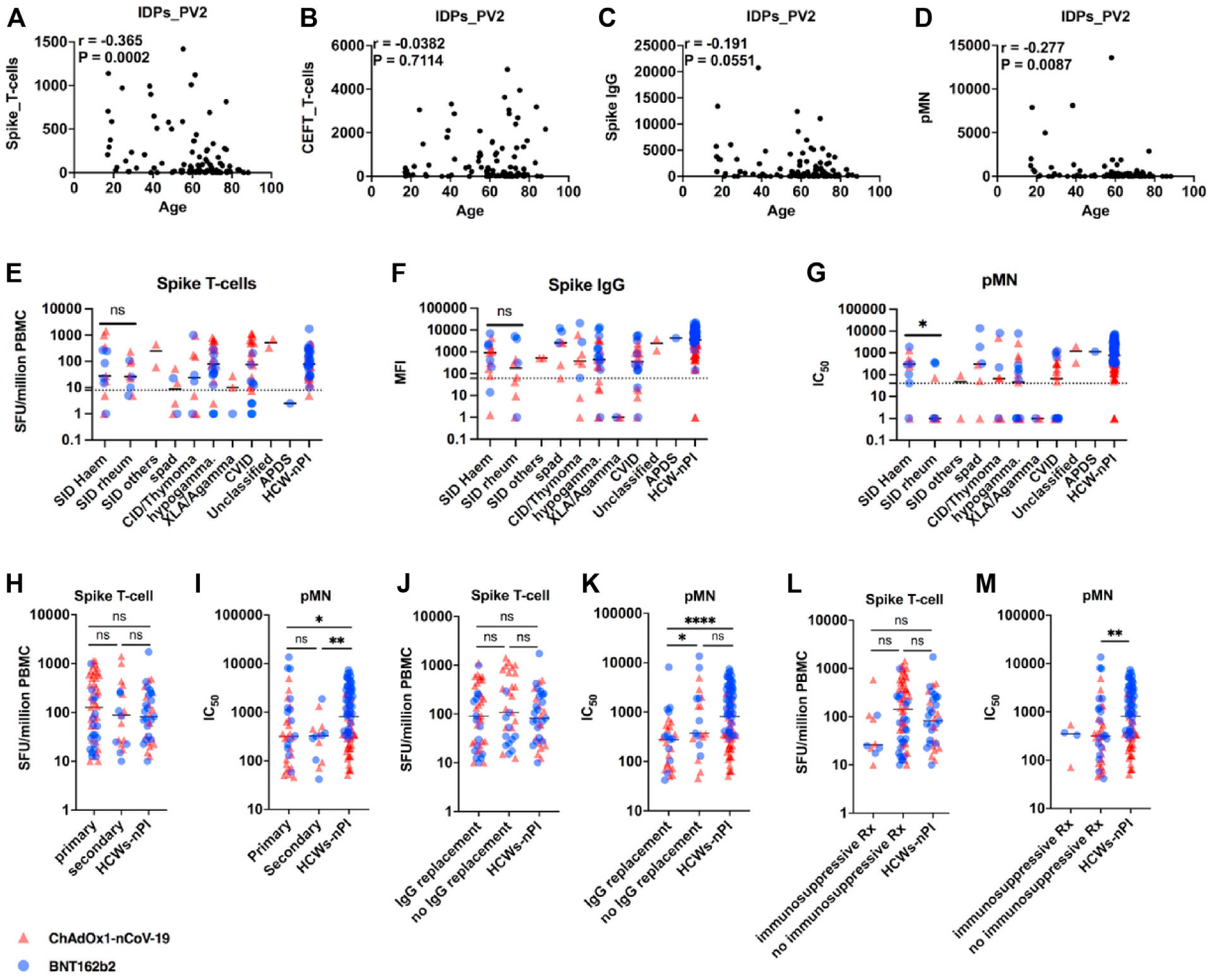

FIG 4. Relationship between immunodeficient patients' clinical profile and vaccine response. Correlation between age of immunodeficient patients and **(A)** SARS-CoV-2 spike-specific T cells, **(B)** CEFT-positive control peptide pool, **(C)** spike-binding IgG titers, and **(D)** pMN titers. Comparison of **(E)** spike-specific T-cell counts, **(F)** spike IgG binding antibody, and **(G)** pMN titers based on underlying immunodeficiency. (**H** and **I**) Spike-specific T-cell counts and pMN titers of those with positive response compared by broad immunodeficiency classification (primary or secondary immunodeficiency). (**J** and **K**) Spike-specific T-cell counts and pMN titers of positive response compared based on IgG replacement therapy. (**L** and **M**) Spike-specific T-cell counts and pMN titers of positive response according to immunosuppression therapy. $*P < .05$, $**P < .01$, $****P < .0001$, *ns,* not significant. *pMN,* Pseudovirus microneutralization.

ChAdOx1-nCoV-19, while the latter induces better T-cell response in immunodeficient patients. Studying the extent to which waning levels or absence of neutralizing antibodies are compensated for by strong protective T-cell responses in immunodeficient individuals would help define the role of T cells as a correlate of protection and may support the implementation of a heterologous vaccine regimen in immunosuppressed individuals for broader immune responses.[42,43] Our data highlight the need for special consideration in designing vaccine regimens to optimize immune responses in high-risk immunodeficient patients.

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Key messages

- **There are varied and impaired immune responses in immunodeficient individuals and poor correlation of antibody binding with functional neutralization.**
- **Modified vaccination approaches, including repeated booster doses of vaccination and mix-and-match schedules designed to recruit both cell-mediated and humoral responses, may be implemented.**
- **Functional (virus neutralization) rather than antibody binding assays act as a surrogate for protective immunity and help predict which patients may benefit from passive immunization.**

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