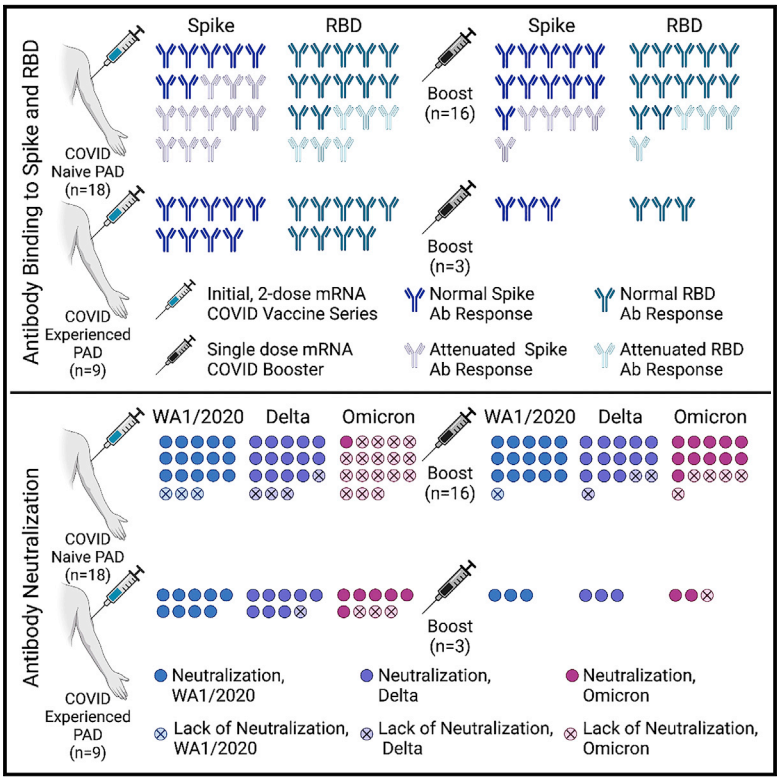
Cell Reports
Medicine

Article

mRNA vaccine boosting enhances antibody responses against SARS-CoV-2 Omicron variant in individuals with antibody deficiency syndromes

Graphical abstract



Highlights

- Most tested individuals with PAD have a measurable antibody response to mRNA vaccines
- Immunoglobulin replacement products have low levels of anti-SARS-CoV-2 antibody
- Individuals with PAD with a history of COVID-19 infection have higher vaccine responses
- A booster induces neutralizing antibodies against Omicron in most individuals with PAD

Authors

Ofer Zimmerman, Alexa Michelle Altman Doss, Paulina Kaplonek, ..., Ali H. Ellebedy, Galit Alter, Michael S. Diamond

Correspondence

zimmero@wustl.edu (O.Z.), mdiamond@wustl.edu (M.S.D.)

In brief

Zimmerman et al. evaluate the immune response of individuals with primary antibody deficiency (PAD) syndromes to COVID-19 mRNA vaccination and boosting. Although the response after a two-dose primary series is relatively transient, boosting induces higher levels of neutralizing antibody against the ancestral strain and variants, including Omicron.

Zimmerman et al., 2022, Cell Reports Medicine *3*, 100653
June 21, 2022
https://doi.org/10.1016/j.xcrm.2022.100653

CellPress

Cell Reports Medicine

CellPress
OPEN ACCESS

Article

mRNA vaccine boosting enhances antibody responses against SARS-CoV-2 Omicron variant in individuals with antibody deficiency syndromes

Ofer Zimmerman,[1,9,10,*] Alexa Michelle Altman Doss,[2,9] Paulina Kaplonek,[3] Chieh-Yu Liang,[1,4] Laura A. VanBlargan,[1] Rita E. Chen,[1,4] Jennifer Marie Monroy,[1] H. James Wedner,[1] Anthony Kulczycki, Jr.,[1,6] Tarisa L. Mantia,[1] Caitlin C. O'Shaughnessy,[1] Hannah G. Davis-Adams,[1] Harry L. Bertera,[3] Lucas J. Adams,[4] Saravanan Raju,[4] Fang R. Zhao,[1] Christopher J. Rigell,[1] Tiffany Biason Dy,[1] Andrew L. Kau,[1,5] Zhen Ren,[1] Jackson S. Turner,[4] Jane A. O'Halloran,[1] Rachel M. Presti,[1,7,8] Daved H. Fremont,[4] Peggy L. Kendall,[1,4] Ali H. Ellebedy,[4,6,7,8] Galit Alter,[3] and Michael S. Diamond[1,4,6,7,8,*]

[1]Department of Medicine, Washington University in St. Louis, St. Louis, MO 63110, USA
[2]Department of Pediatrics, Washington University in St. Louis, St. Louis, MO 63110, USA
[3]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA 02139, USA
[4]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA
[5]Center for Women's Infectious Disease Research, Washington University School of Medicine, St. Louis, MO 63110, USA
[6]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110, USA
[7]The Andrew M. and Jane M. Bursky Center for Human Immunology & Immunotherapy Programs, Washington University School of Medicine, St. Louis, MO 63110, USA
[8]Center for Vaccines and Immunity to Microbial Pathogens, Washington University School of Medicine, Saint Louis, MO 63110, USA
[9]These authors contributed equally
[10]Lead contact
*Correspondence: zimmero@wustl.edu (O.Z.), mdiamond@wustl.edu (M.S.D.)
https://doi.org/10.1016/j.xcrm.2022.100653

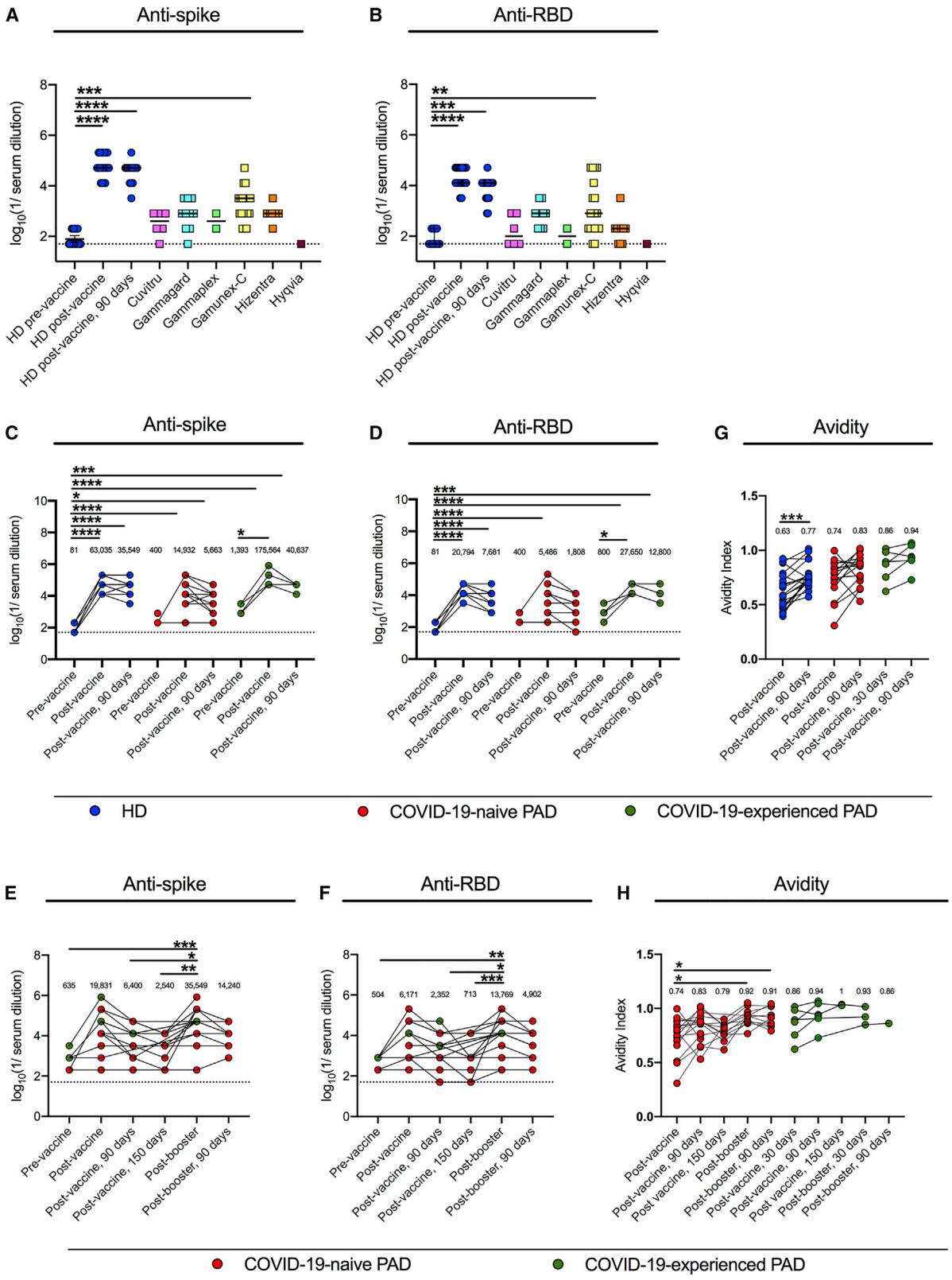
SUMMARY

Individuals with primary antibody deficiency (PAD) syndromes have poor humoral immune responses requiring immunoglobulin replacement therapy. We followed individuals with PAD after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination by evaluating their immunoglobulin replacement products and serum for anti-spike binding, Fcγ receptor (FcγR) binding, and neutralizing activities. The immunoglobulin replacement products tested have low anti-spike and receptor-binding domain (RBD) titers and neutralizing activity. In coronavirus disease 2019 (COVID-19)-naive individuals with PAD, anti-spike and RBD titers increase after mRNA vaccination but wane by 90 days. Those vaccinated after SARS-CoV-2 infection develop higher and more sustained responses comparable with healthy donors. Most vaccinated individuals with PAD have serum-neutralizing antibody titers above an estimated correlate of protection against ancestral SARS-CoV-2 and Delta virus but not against Omicron virus, although this is improved by boosting. Thus, some immunoglobulin replacement products likely have limited protective activity, and immunization and boosting of individuals with PAD with mRNA vaccines should confer at least short-term immunity against SARS-CoV-2 variants, including Omicron.

INTRODUCTION

Common variable immune deficiency (CVID) and other primary antibody deficiency (PAD) syndromes are associated with low immunoglobulin levels and impaired antibody responses to pathogens and vaccines.[1,2] Individuals with these immune disorders suffer from severe and recurrent infections and autoimmunity and are at increased risk for malignancies.[3] CVID has a prevalence of 1 in 25,000[4–6] and is the most common primary immunodeficiency in registries, with more than 20% of individuals suffering from this condition. CVID is not a single disease, but rather a collection of hypogammaglobulinemia syndromes resulting from multiple genetic defects.[7–11] Most individuals with PAD require intravenous or subcutaneous immunoglobulin replacement therapy, which decreases their risk for infection.[12–14] There are more than 15 commercially available immunoglobulin products in the United States. Production of immunoglobulin replacement products takes up to 1 year from sample donation to distribution.[15,16] Each vial contains immunoglobulins pooled from thousands of donors,[16,17] and each manufacturer has its own plasma donors. In individuals with PAD, immunoglobulin replacement therapy is dosed every 1 to 4 weeks, depending on the route of administration.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the global coronavirus disease 2019 (COVID-19) pandemic. From November 2019 until now,



(legend on next page)

the virus has caused more than 6 million deaths. In the United States, emergency use authorization has been granted for one COVID-19 vaccine (Ad26.COV2.S, Johnson & Johnson/Janssen), and full approval has been given to two mRNA vaccines (BNT162b2, Pfizer-BioNTech and mRNA-1273, Moderna). Presently, there is limited data regarding the effectiveness of mRNA or adenoviral vector vaccination against COVID-19 in individuals with PAD. Several studies have shown variable seroconversion rates with detection of anti-spike, S1, or receptor-binding domain (RBD) antibodies in 20%–90% of individuals with PAD after vaccination with BNT162b2, mRNA-1273, or ChAdOx1 (Oxford-AstraZeneca),[18–21] with better responses in those with a history of SARS-CoV-2 infection.[20,22] Available data are limited to the initial vaccine response with no information on durability or the effect of boosting in individuals with PAD. No data have been published on the ability of serum from individuals with PAD to neutralize authentic SARS-CoV-2 strains, including the currently dominant Omicron variants. Finally, no study has reported the anti-spike, anti-RBD, or neutralization activity of immunoglobulin replacement products individuals with PAD have received to rule out the possibility that their anti-SARS-CoV-2 antibodies originated from passive immunoglobulin therapy. To address these gaps, we evaluated the effect of mRNA vaccination and boosting on serum antibody responses in individuals with PAD against ancestral SARS-CoV-2 strains and key circulating variants.

RESULTS

Antibody binding to spike and the RBD

To begin to determine the baseline immunity afforded by antibody replacement therapy for our cohort of affected individuals, we tested 48 distinct lots of 6 different immunoglobulin products (Table S1) for binding to ancestral spike and RBD proteins and compared these results with serum from 20 healthy donors (HDs) before or 14 and 90 days after completion of 2 doses of BNT162b2 mRNA vaccine (Figures 1A and 1B). Only one immunoglobulin product, Gamunex-C, showed anti-spike and anti-RBD titers higher than unvaccinated HDs, and these values were lower than those from HDs after immunization ($p < 0.001$) (Figures 1A and 1B). Thus, most antibody replacement products in clinical use at the time of this study (May 2021–February 2022) had low levels of anti-SARS-CoV-2 antibody and likely were derived from donations obtained before or soon after onset of the pandemic.

We next compared anti-spike and anti-RBD titers of 27 individuals with PAD (Tables 1, S2, and S3) who completed immunization with mRNA vaccines (BNT162b2, n = 19; mRNA-1273, n = 8) with those of 20 HDs immunized with BNT162b2. Nineteen PAD patients had CVID, and the others had hypogammaglobulinemia (n = 4) or specific antibody deficiency (n = 4) diagnoses. Nine of the 27 individuals with PAD had a confirmed history of COVID-19 infection by RT-PCR and were convalescent or recovered (COVID-19 experienced; range, 36–276 days from infection to vaccination; median, 117 days; mean, 141 days). Because of the study design, we obtained pre-vaccination serum samples from only a subset (9 of 30) of individuals in the PAD cohort. Notwithstanding this limitation, our analysis of immunoglobulin replacement samples (Figures 1A and 1B) suggests that individuals without a history of SARS-CoV-2 infection should have low, if any, levels of pre-existing anti-spike or RBD antibodies. For all groups (COVID-19-naive individuals with PAD, COVID-19-experienced individuals with PAD, and HDs), anti-spike titers 14 and 90 days after completion of a two-dose mRNA vaccination series were higher than HD pre-vaccination titers (Figure 1C). Fourteen days after the second immunization, the mean anti-spike titers trended higher in COVID-19-naive and -experienced individuals with PAD as well as HDs compared with their respective pre-vaccination levels (37-fold, COVID-19-naive individuals with PAD; 126-fold, COVID-19-experienced individuals with PAD; 778-fold, HDs) (Figure 1C); these differences were statically significant for COVID-19-experienced individuals with PAD and HDs but not for COVID-19-naive individuals with PAD. 90 days after vaccination, individuals with PAD with a history of SARS-CoV-2 infection showed waning anti-spike titers (Figure 1C). The loss in anti-spike titers between days 14 and 90 after two-dose vaccination in individuals with PAD appeared to be greater than in HDs. The intragroup variation was high so that the differences between HDs and COVID-19-naive and COVID-19-experienced individuals with PAD were not significant (Figure S1A). Similar findings were observed with anti-RBD titers. For all groups, anti-RBD titers were higher 14 days after completion of a two-dose vaccination series than pre-vaccination titers (Figure 1D). However, 90 days after vaccination, only HDs and

Figure 1. Anti-spike and anti-RBD titers after primary vaccination and boosting in individuals with PAD

(A and B) Anti-spike (A) and RBD (B) (ancestral strain) endpoint titers in 48 lots of 6 different immunoglobulin replacement products (squares) compared with 20 HD (blue circles) before and 14 and 90 days after completion of the BNT162b2 vaccine series.

(C and D) Anti-spike (C) and RBD (D) endpoint titers in HDs (n = 20; blue circles), COVID-19-naive individuals with PAD (n = 18, red circles), and COVID-19-experienced individuals with PAD (n = 9, green circles) before or 14 and 90 days after completion of a mRNA vaccination series (BNT162b2, n = 19; mRNA-1273, n = 8).

(E and F) Anti-spike (E) and RBD (F) endpoint titers in COVID-19-naive individuals with PAD (n = 16, red circles) and COVID-19-experienced individuals with PAD (n = 3, green circles) before (n = 6) and 14 or 28 (n = 19), 90 (n = 18), and 150 (n = 12) days after completion of a primary mRNA (BNT162b2, n = 14; mRNA-1273, n = 3) or Ad26.COV2.S (n = 2) vaccine series and 14 (n = 19) days and 90 (n = 13) days after booster with a mRNA vaccine (BNT162b2, n = 16; mRNA-1273, n = 3). A dotted black line represents the limit of detection (1/50).

(G and H) Anti-spike avidity index in HDs (n = 19, blue circles), COVID-19-naive individuals with PAD (red circles), and COVID-19-experienced individuals with PAD (green circles) 14 and 90 days after primary vaccination (n = 16, COVID-19-naive; n = 6, COVID-19-experienced), 150 days after primary vaccination (n = 10, COVID-19-naive; n = 2, COVID-19-experienced), 14 days after boosting (n = 12, COVID-19-naive; n = 3, COVID-19-experienced), and 90 days after boosting (n = 10, COVID-19-naive; n = 1, COVID-19-experienced).

Numbers above graphed data (C–F) represent the geometric mean titer (GMT) for each time point and average avidity index (G and H). Bars indicate median (A and B) values. Kruskal-Wallis with Dunn's post-test (A–F), paired t test (G), and one-way ANOVA with Dunnett's post-test (H). Only significant differences are shown: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. See also Figure S1 and Tables S1–S3.

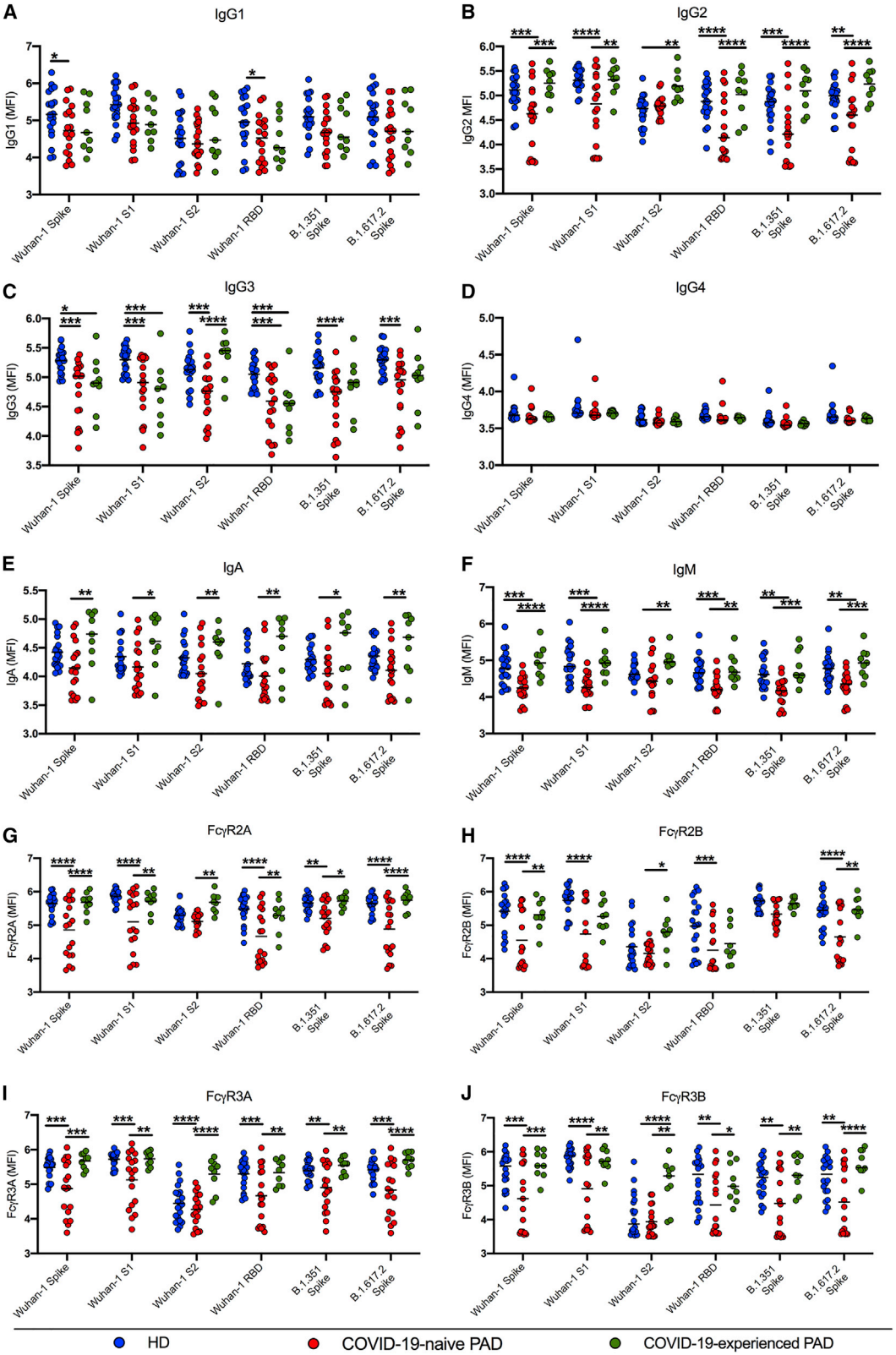
Table 1. Patient characteristics

Patient number	Age (years)	Sex	Diagnosis	Immunoglobulin replacement product	Vaccine	COVID-19 infection to first vaccine (days)	Vaccine completion to booster (days)	Booster
1	59	F	CVID	Gammagard	Pfizer	–	152	Pfizer
2	25	F	CVID	Hizentra	Pfizer	–	117	Pfizer
3	56	F	CVID	Gammagard	Pfizer	–	–	–
4	41	F	CVID	Gammaplex	Pfizer	–	150	Pfizer
5	30	M	CVID	Gammagard	Pfizer	–	143	Pfizer
6	61	F	CVID	Gamunex-C	Pfizer	–	–	–
7	73	F	SAB	none	Pfizer	96	134	Pfizer
8	61	F	CVID	Hizentra	Pfizer	–	128	Pfizer
9	37	F	SAB	HyQvia	J&J	–	162	Pfizer
10	46	F	CVID	Gammagard	Pfizer	–	138	Pfizer
11	59	F	CVID	Hizentra	Pfizer	–	163	Pfizer
12	44	M	CVID	Gamunex-C	Moderna	–	150	Moderna
14	34	F	SAB	Xembify	Pfizer	90	–	–
15	20	F	CVID	Gamunex-C	Moderna	181	–	–
16	26	F	SAB	none	J&J	–	–	–
17	82	F	CVID	Gamunex-C	Moderna	–	–	–
18	61	F	CVID	Cuvitru	Moderna	–	–	–
19	21	F	hypogam	Octagam	Pfizer	276	154	Pfizer
20	41	F	CVID	Gamunex-C	Moderna	–	196	Moderna
21	70	F	SAB	Cuvitru	Pfizer	–	185	Pfizer
22	49	F	CVID	Gamunex-C	Pfizer	117	–	–
23	70	F	hypogam	Xembify	Pfizer	–	108	Pfizer
24	54	F	CVID	Privigen	Moderna	222	–	–
25	56	F	hypogam	none	Moderna	106	–	–
26	57	M	CVID	Gamunex-C	J&J	–	65	Pfizer
27	56	F	CVID	Gammagard	Pfizer	–	162	Pfizer
28	63	F	hypogam	Gamunex-C	Pfizer	–	158	Pfizer
29	37	F	CVID	Hizentra	Pfizer	–	104	Pfizer
30	48	F	SAB	Gamunex-C	Moderna	144	104	Moderna
31	29	F	CVID	Gamunex-C	Pfizer	36	–	–
Mean (days)						141	141	

F, female; M, male; CVID, common variable immune deficiency; hypogam, hypogammaglobulinemia; SAB, specific antibody deficiency disorder.

COVID-19-experienced individuals with PAD had anti-RBD titers that were significantly higher than pre-vaccination titers. 14 days after completion of the two-dose immunization series, anti-RBD titers trended higher than respective pre-vaccination titers (14-fold, COVID-19-naive individuals with PAD; 35-fold, COVID-19-experienced individuals with PAD; 257-fold, HDs); these differences were statistically significant for COVID-19-experienced individuals with PAD and HDs but not for COVID-19-naive individuals with PAD (Figure 1D). 90 days after vaccination, anti-RBD titers of COVID-19-naive and COVID-19-experienced individuals with PAD were not statistically higher than pre-vaccination titers (Figure 1D), whereas titers of HDs had decreased but remained higher than before immunization (95-fold, HDs) (Figure 1D). The fold decrease in anti-RBD titers between days 14 and 90 after vaccination was not substantially different among the three groups (Figure S1B). Thus, vaccinated HDs and COVID-19-experienced individuals with PAD had more sustained anti-spike and anti-RBD responses than vaccinated individuals with PAD lacking a history of SARS-CoV-2 infection (Figures 1C and 1D).

Nineteen individuals with PAD received a booster (third) dose with an mRNA vaccine (range, 65–196 days from initial vaccine completion to booster; median, 150 days; mean, 141 days) (Table 1), and samples were obtained 1–4 weeks later (n = 18; range, 7–27 days; median, 17 days; mean, 18 days; one individual had a post-booster sample drawn on day 35). Serum anti-spike and RBD titers were higher after boosting than those obtained pre-booster (5- to 6-fold compared with day 90 after primary vaccination series and 14- to 19-fold compared with day 150 after primary vaccination series; Figures 1E and 1F). Although the anti-spike and RBD titers after boosting trended higher than those obtained 14 days after the primary two-dose



(legend on next page)

immunization series, these differences did not reach statistical significance ($p > 0.9$). Ninety days after boosting, serum titers against spike and the RBD from individuals with PAD had decreased to levels comparable with 14 days after the primary vaccination series (Figures 1E and 1F).

We also measured antibody avidity to the spike protein in 19 HDs and 22 individuals with PAD at 30 and 90 days after primary-series vaccination using a published urea wash-based ELISA.[23] In these studies, we excluded the two individuals with PAD who lacked responses after both primary two-dose vaccination series and third-dose boosting. Although the antibody avidity against the spike protein trended higher on day 90 compared with day 30 in serum from COVID-19-naive and COVID-19-experienced PAD patients, only HDs showed a statistically significant increase (Figure 1G). Boosting (third dose) resulted in higher avidity of anti-spike antibodies in COVID-19-naive PAD patients at 30 and 90 days later (Figure 1H). In comparison, COVID-19-experienced individuals with PAD already had relatively high-avidity antibodies before boosting. These findings are consistent with published data from convalescent HDs.[24] Thus, most individuals with PAD showed evidence of antibody avidity maturation after primary mRNA vaccination series or boosting, with differences associated with whether they had experienced natural infection.

Immunoglobulin subclass and Fc-γ receptor binding

Recent studies have suggested that immunoglobulin subclass and antibody interactions with Fcγ receptors (FcγR) can contribute to protective immunity against SARS-CoV-2.[25,26] Accordingly, we evaluated serum from immunized individuals with PAD for their immunoglobulin subclasses (immunoglobulin G1 [IgG1], IgG2, IgG3, and IgG4), IgA, and IgM that bind spike proteins and domains (S, S1, S2, and/or RBD) from ancestral, B.1.351 (Beta), and B.1.617.2 (Delta) SARS-CoV-2 strains (Figures 2A–2F and S2). Fourteen days after primary-series immunization, COVID-19-naive individuals with PAD had lower IgG2, IgG3, and IgM levels against spike and RBD proteins of all 3 tested virus strains than vaccinated HDs (Figures 2B, 2C, and 2F). COVID-19-naive individuals with PAD also had lower IgG1 levels against the ancestral SARS-CoV-2 spike and RBD than HDs (Figure 2A). In comparison, vaccinated COVID-19-naive individuals with PAD had IgA titers against spike and RBD proteins that were similar to those of HDs (Figure 2E). Vaccinated COVID-19-experienced individuals with PAD had lower IgG3 levels against the ancestral spike, S1, and RBD (Figure 2C) but similar levels of IgG1, IgG2, IgA, and IgM against the ancestral spike, S1, S2, and RBD and variant spike proteins compared with immunized HDs (Figures 2A, 2B, 2E, and 2F). Because our cohort of HDs did not include subjects with a history of COVID-19 prior to vaccination, we could not assess whether COVID-19-experienced HDs and individuals with PAD had comparable immune responses. Vaccinated, COVID-19-experienced individuals with PAD had higher levels of IgG2, IgA, and IgM against the ancestral spike and RBD than vaccinated, COVID-19-naive individuals with PAD. COVID-19-experienced individuals with PAD had higher IgG3, IgA, and IgM titers against S2 protein than COVID-19-naive individuals with PAD (Figures 2C, 2E, and 2F). The levels of IgG4 anti-spike or RBD protein in all groups were near the limit of detection (Figure 2D). Although prior infection with COVID-19 in individuals with PAD was associated with a better vaccine response, COVID-19-naive and -experienced individuals with PAD had lower IgG3 responses than HDs (Figures 2C, S2A, and S2B). This result suggests that class switching to IgG3 is impaired in individuals with PAD after infection or vaccination.

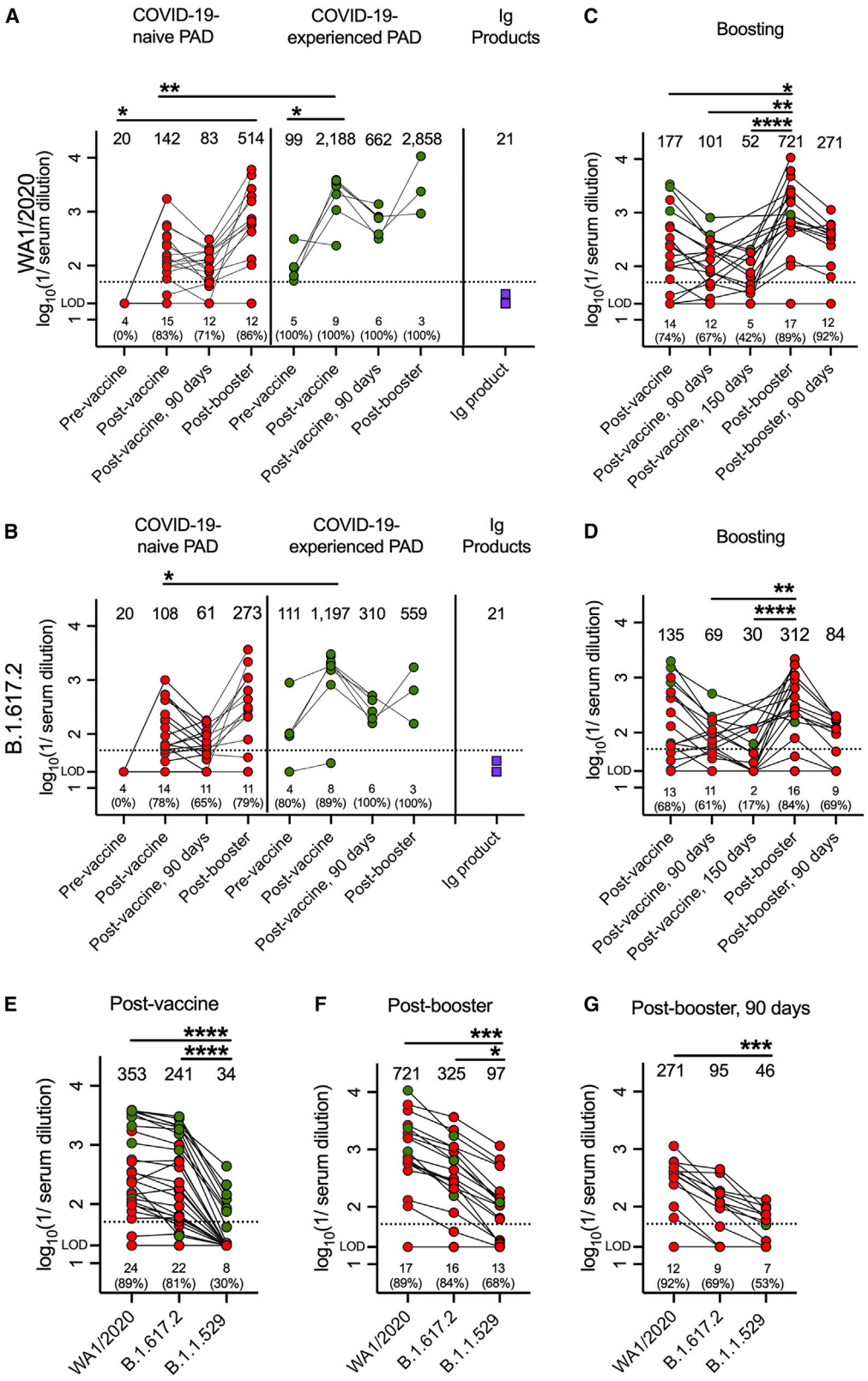
Given these results, we next evaluated anti-spike and anti-RBD antibody binding to FcγRs (FcγR2A, FcγR2B, FcγR3A, and FcγR3B) using a systems serology platform.[27] Vaccinated, COVID-19-naive individuals with PAD had lower levels of FcγR binding to anti-spike and RBD antibodies than vaccinated, COVID-19-experienced individuals with PAD or HDs (Figures 2G–2J). FcγR2A, 3A, and 3B binding was higher in serum from vaccinated, COVID-19-experienced individuals with PAD than vaccinated, COVID-19-naive individuals with PAD for all viral antigens tested (Figures 2G, 2I, 2J, and S2). Binding in serum from COVID-19-experienced individuals with PAD to FcγR2B was higher than that of COVID-19-naive individuals with PAD for ancestral and B.1.617.2 spike proteins (Figure 2H). FcγR2A, FcγR2B, FcγR3A, and FcγR3B binding was higher in HDs than in COVID-19-naive individuals with PAD for most spike proteins (Figures 2G–2J, S2A, and S2B). Serum anti-S2 responses from COVID-19-experienced individuals with PAD were higher than those of COVID-19-naive individuals with PAD for all tested FcγRs (Figures 2G–2J). The higher levels of FcγR binding by anti-spike and anti-RBD antibodies in COVID-19-experienced compared to COVID-19-naive individuals with PAD after vaccination suggest that individuals with PAD do not have an inherent defect in producing antibodies that mediate Fc effector functions.

Serum-neutralizing antibody responses

We evaluated the functional activity of antibody preparations by performing focus reduction neutralization tests (FRNTs) with authentic SARS-CoV-2 strains and variants (WA1/2020, B.1.617.2, and B.1.1.529). We used the model from Khoury et al.,[28] which predicts that a pre-existing serum neutralization titer of 54 is protective against symptomatic SARS-CoV-2 infection in 50% of vaccinated individuals. We tested the neutralizing activity of the commercial immunoglobulin products ($n = 17$), serum from COVID-19-naive individuals with PAD after vaccination ($n = 18$), and serum from COVID-19-experienced individuals after vaccination ($n = 9$) (Figures 3A and 3B). Fourteen of 17 different lots of immunoglobulin products tested had no appreciable neutralizing activity against WA1/2020 or B.1.617.2 at

Figure 2. IgG subclasses and FcγR-binding activity of anti-spike antibodies in serum from vaccinated individuals with PAD

(A–J) Levels of IgG1(A), IgG2 (B), IgG3 (C), IgG4 (D), IgA (E), IgM (F), FcγR2A-binding (G), FcγR2B-binding (H), FcγR3A-binding (I), and FcγR3B-binding (J) ancestral (S, S1, S2, and RBD), B.1.351, and B.1.617.2 spike antibodies in HDs ($n = 20$, blue circles), COVID-19-naive individuals with PAD ($n = 18$, red circles), and COVID-19-experienced individuals with PAD ($n = 9$, green circles) 14 days after completion of the second dose of the mRNA vaccine series. Two-way ANOVA with Tukey post-test, mean. Only significant differences are shown: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). See also Figure S2 and Table S3.



(legend on next page)

500 μg/mL (Figures 3A, 3B, and S2), which is approximately a 1/20 dilution of the mean IgG concentration measured in our individuals with PAD on IgG replacement therapy (9.7 mg/mL) (Tables S1 and S2). We tested this concentration (rather than a neat sample) because it corresponds to the serum dilution that is the presumed cutoff for vaccine-mediated protection.[28] Two lots of Gamunex-C and one of Hizentra had limited neutralizing activity against the WA1/2020 strain at a 1/20 dilution, and only one lot (Hizentra) had inhibitory activity against B.1.617.2 at this dilution (Figures 3A, 3B, S3A, and S3B; Table S1).

As expected, serum of COVID-19-naive individuals with PAD had no neutralizing activity against WA1/2020 prior to vaccination, whereas that of COVID-19-experienced individuals did (Figure 3A). Fourteen days after immunization, 15 of 18 (83%) COVID-19-naive individuals with PAD had levels of serum neutralizing antibodies against WA1/2020 that are considered protective[28] (titer > 50). By 90 days, neutralizing activity had waned, with 12 of 17 (71%) individuals with PAD in this group still having titers above the presumed protective threshold. After boosting, neutralizing titers increased in 12 of 14 (86%) COVID-19-naive individuals with PAD to levels greater than 50 against WA1/2020 (Figure 3A). In the cohort of COVID-19-experienced individuals with PAD, serum neutralizing activity against WA1/2020 exceeded the protective cutoff at all tested time points in all subjects (Figure 3A). 14 days after the primary immunization series, COVID-19-experienced individuals with PAD had serum neutralizing titers against WA1/2020 that were 15-fold higher than those of COVID-19-naive individuals with PAD (Figure 3A).

We repeated FRNTs with serum from individuals with PAD and the B.1.617.2 Delta strain, which can evade neutralizing antibodies because of amino acid substitutions in the RBD.[29] Although pre-immunization serum of COVID-19-naive individuals lacked inhibitory activity against B.1.617.2, serum from 4 of 5 (80%) COVID-19-experienced individuals with PAD neutralized B.1.617.2 before vaccination (Figure 3B). Fourteen and 90 days after vaccination, 14 of 18 (78%) and 11 of 17 (65%) COVID-19-naive individuals, respectively, had serum neutralizing titers against B.1.617.2 that were above 50. Following boosting, 11 of 14 (79%) COVID-19-naive individuals with PAD had B.1.617.2 neutralizing titers above 50 (Figure 3B). In comparison, 8 of 9 (88%), 6 of 6 (100%), and 3 of 3 (100%) of COVID-19-experienced individuals with PAD had serum neutralizing titers against B.1.617.2 above the presumed protective threshold 14 and 90 days after vaccination and 14 days after boosting, respectively. 14 days after vaccination, COVID-19-experienced individuals with PAD had 10-fold higher serum neutralizing titers against B.1.617.2 than COVID-naive vaccinated individuals (Figure 3B).

We next analyzed individuals with PAD (n = 19) who received an mRNA vaccine booster (Figure 3C). We included in this analysis two individuals who initially received an Ad26.COV2.S vaccine (Table 1; Figures S1C–S1F). 14, 90, and 150 days after primary vaccination, 14 of 19 (74%), 12 of 18 (67%), and 5 of 12 (42%) individuals, respectively, had serum neutralization titers above 50 against WA1/2020. 14 days after boosting, 17 of 19 (89%) individuals had neutralizing titers against WA1/2020 that exceeded 50 (Figure 3C), and the highest titers (geometric mean titer [GMT], 721) showed a 4-fold increase over levels 14 days after the primary series, a 7-fold increase over level 90 days after the primary series, and a 14-fold increase over level 150 days after the primary series (Figure 3C). Ninety days after boosting, 12 of 13 (92%) individuals had neutralization titers above 50 (Figure 3C). Similar findings were observed against B.1.617.2, with 13 of 19 (68%), 11 of 18 (61%), 2 of 12 (17%), 16 of 19 (84%), and 9 of 13 (69%) individuals with PAD having neutralizing titers greater than 50 14, 90, and 150 days after the primary immunization series or 14 and 90 days after boosting, respectively (Figure 3D). Similar to WA1/2020, the highest neutralization titers against B.1.617.2 were detected 14 days after boosting (GMT, 312) (Figure 3D), this level was higher than the neutralization titers measured 90 and 150 days but not 14 days after the primary vaccination series.

The B.1.1.529 (BA.1) Omicron variant has more than 30 substitutions, deletions, and insertions in its spike protein, which jeopardizes the efficacy of vaccines designed against ancestral SARS-CoV-2 strains.[30–32] Accordingly, we evaluated serum neutralizing activity against B.1.1.529 in our cohort of affected individuals (Figures 3E and 3F). Fourteen days after completing the primary mRNA vaccination series, only 8 of 27 (30%) individuals with PAD had serum levels of neutralizing antibody above 50 against B.1.1.529 (Figure 3E), and only 1 of 8 individuals in this group was COVID-19 naive. The neutralization titers against B.1.1.529 of HDs 14 days after the primary vaccination series were also low; only 5 (25%) had neutralizing titers against B.1.1.529 above the assay level of detection, with only one

Figure 3. Neutralizing antibody responses in individuals with PAD after vaccination and boosting

(A and B) Serum neutralizing activity against WA1/2020 (A) or B.1.617.2 (Delta) (B) in COVID-19-naive (red circles) and COVID-19-experienced (green circles) individuals with PAD before (n = 4, COVID-19-naive; n = 5, COVID-19-experienced) and 14 (n = 18, COVID-19-naive; n = 9, COVID-19-experienced) or 90 (n = 17, COVID-19-naive; n = 6, COVID-19-experienced) days after mRNA vaccination and 14 days after mRNA booster (n = 14, COVID-19-naive; n = 3, COVID-19-experienced). Shown is the neutralizing activity of immunoglobulin replacement products (n = 17, purple squares).

(C and D) Effect of boosting on serum neutralization of WA1/2020 (C) and B.1.617.2 (D) in COVID-naive (n = 16, red circles) and COVID-19-experienced (n = 3, green circles) individuals with PAD after completion of the primary mRNA (14 or 90 days after vaccination; BNT162b2, n = 14; mRNA-1273, n = 3) or Ad26.COV2.S (28 or 90 days after vaccination, n = 2) vaccine series and 14 (n = 19) or 90 (n = 13) days after boosting with a mRNA vaccine (BNT162b2, n = 16; mRNA-1273, n = 3).

(E–G) Effect of variant strains on serum neutralizing activity of individuals with PAD 14 days after completion of mRNA vaccination (E) (n = 27 total; n = 18, COVID-19-naive, red circles; n = 9, COVID-19-experienced, green circles), 14 days after boosting (F) (n = 19 total; n = 16, COVID-19-naive, red circles; n = 3, COVID-19-experienced, green circles), and 90 days after boosting (G) (n = 13 total; n = 12 COVID-19-naive, red circles; n = 1, COVID-19-experienced, green circle).

LOD, limit of detection. A dotted black line represents the presumptive protective titer as described.[28] Numbers immediately above the x axis indicate the number and percentage of individuals with serum-neutralizing titers above 50 at each time point. Numbers above graphed data represent the GMT for each time point. Kruskal-Wallis with Dunn's post-test. Only significant differences are shown: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001). See also Figure S3 and Table S3.

(5%) above 50 (Figure S1G). Fourteen days after boosting, 13 of 19 (68%) individuals with PAD had neutralizing titers against B.1.1.529 that exceeded 50 (Figure 3F). Ninety days after boosting, 7 of 13 (53%) individuals with PAD had serum neutralization titers against B.1.1.529 that exceeded 50 (Figure 3G). The mean neutralization titer against B.1.1.529 was lower than against WA1/2020 and B.1.617.2 14 days after primary immunization (~8- to 10-fold, $p < 0.0001$), 14 days after boosting (~3- to 7-fold, $p < 0.05$ and 0.001), and 90 days after boosting (~6-fold, $p < 0.001$), which is consistent with recent studies in immunized healthy cohorts.[30–32]

DISCUSSION

Our findings highlight the importance of immunizing and boosting individuals with PAD with SARS-CoV-2 mRNA vaccines. Even though these individuals have defects in their humoral responses, vaccination is an important prevention option for those with PAD against COVID-19 because immunoglobulin replacement products in use at the time of this study had limited inhibitory activity, and several of the commercially available monoclonal antibody therapeutic agents lose substantial neutralizing capacity against variants, including B.1.1.529.[33–35] Although two doses of mRNA vaccines induce serum neutralizing antibodies in most individuals with PAD that presumably would protect against ancestral and the B.1.617.2 variant, individuals with PAD and HDs without a history of SARS-CoV-2 infection had little to no serum neutralizing activity against B.1.1.529 (Omicron) after completion of a primary vaccination series. However, an mRNA vaccine booster in those with PAD increased anti-B.1.1.529 responses in most individuals, although the serum levels of neutralizing antibodies waned over time. Our findings support Center for Disease Control and Prevention recommendations for a three-dose primary mRNA vaccine series that also includes a booster dose 5 months later in moderately or severely immune-suppressed indivdiuals.[36]

In individuals with no history of COVID-19 infection, the immune response to two doses of mRNA vaccine was lower in magnitude and less durable than in HDs or individuals with PAD with a history of infection. The increase in serum neutralizing titers after boosting was higher than the increase in anti-spike and anti-RBD titers (4.5-fold compared with 2-fold). This observation highlights the utility of performing antibody neutralization assays in addition to spike or RBD binding assays to assess the quality of humoral immune responses. Although further studies that sample and sequence B cells in blood from individuals with PAD are needed, the avidity assays we performed suggest that individuals with PAD undergo antibody maturation after infection, vaccination, and boosting. This analysis also highlights the importance of a third vaccination dose, which resulted an increase in anti-spike avidity in COVID-19-naive individuals with PAD. These findings may also explain the apparent discrepancy between the ELISA and neutralization results that showed a higher fold increase in neutralization compared with binding titers after boosting.

Many of our individuals with PAD who historically had poor immune responses to bacterial and other protein antigens (e.g., *Streptococcus pneumoniae* polysaccharides, tetanus toxoid, and diphtheria toxin) as part of their initial immune workup (Table S3) responded to mRNA vaccines. The basis of this difference remains unclear, although it could be due to the unique adjuvant properties of the lipid nanoparticles or *in vitro*-synthesized mRNA.[37–39] In comparison, although the numbers in our cohort are too small (n = 3) to generalize, we detected little to no antibody response 35, 60, or 90 days after immunization of COVID-naive individuals with PAD with the Ad26.COV2.S adenoviral-vectored vaccine (Figures S1C–S1F). Because PAD is a heterogeneous clinical entity, with many of the genetic defects unknown,[7–11] certain classes of adjuvants or antigens may overcome specific deficiencies and promote B cell responses, albeit at lower levels than in healthy counterparts. Our data suggest that the mRNA platform may have utility for vaccination of individuals with PAD. That said, their less durable response, lower level of anti-spike and anti-RBD IgG3, and lower levels of complement-fixing and FcγR-engaging antibodies suggest that more frequent boosters may be required to establish and maintain protective immunity.

Our study also showed that many of the immunoglobulin replacement products currently used have low levels of inhibitory anti-SARS-CoV-2 antibodies. This may be due to the long lead time required for collection from donors, purification, and testing. Neutralization assays performed by one manufacturer and by us showed low inhibitory activity against ancestral strains and less activity against SARS-CoV-2 variants.[40] The three products we identified with some activity against an ancestral strain and B.1.617.2 had titers that likely would not confer protection against B.1.1.529, given the more extensive antibody evasion by this strain.[33,34,41,42] Indeed, neutralizing titers were below the presumed protective cutoff in the 4 COVID-19-naive individuals with PAD who donated pre-vaccination blood samples, even though all had received immunoglobulin replacement every 3–4 weeks before study enrollment. It is unclear when commercially available products will have sufficient levels of specific and neutralizing anti-SARS-CoV-2 antibodies to protect individuals with PAD. Further binding and neutralization studies are warranted when anti-SARS-CoV-2 antibodies become more widespread in plasma pools. The lag between collection of donor plasma to distribution of products may make most available commercial immunoglobulin replacement products less effective against current circulating Omicron variants. Although many individuals with PAD might be eligible for long-acting combination monoclonal antibody prophylaxis (e.g., Evusheld [AZD7442]) against COVID-19, recent studies showed substantial (~33-fold) losses in potency against some lineages of Omicron virus (e.g., BA.1.1). Immunization of individuals with PAD with mRNA vaccines that include a booster may an effective way to induce a protective antibody response against SARS-CoV-2 and its variants.

Limitations of the study

One limitation of our study is the heterogeneity in the cohort of individuals with PAD, which included those with CVID, hypogammaglobulinemia, or specific antibody deficiency. Although this was an inherent limitation of the cohort available for study, we did not observe substantive differences between the subgroups of affected individuals. Instead, the most significant differences

in antibody response to mRNA vaccines were between those who had or lacked a history of SARS-CoV-2 infection. Another limitation is the lack of an available HD comparison cohort with a history of COVID-19 prior to vaccination. It is possible that individuals with PAD with history of COVID-19 have a blunted immune response to vaccination compared with HDs with history of infection prior to vaccination. The serum neutralization titer cutoff we used for correlates of protection is based on one model.[28] Other models exist with different cutoff values that could affect interpretation of our data with regard to likely susceptibility to symptomatic infection. For example, another model predicts that a neutralization titer of 50 would confer higher levels of protection against SARS-CoV-2 infection.[43] One advantage of the model we selected is that it is based on data from seven different studies and was corroborated by a subsequent meta-analysis.[28,44]

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAIL
  - Patients and samples
  - Healthy donor controls
  - SARS-CoV-2 spike and RBD protein expression
  - Cells
  - Viruses
- METHOD DETAILS
  - ELISA for anti-spike and anti-RBD binding
  - Avidity assay
  - Luminex profiling
  - **Focus reduction neutralization test**
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2022.100653.

ACKNOWLEDGMENTS

This study was supported by grants and contracts from the NIH (R01 AI157155, U01 AI151810, and 75N93019C00051 to M.S.D.; U01AI141990, U01AI150747, HHSN272201400006C, HHSN272201400008C, and 75N93019C00051 to A.H.E.; and R01 DK084242 to P.L.K.). The study was also supported by a VA Merit Award (BX002882 to P.L.K.). This study utilized samples obtained from the Washington University School of Medicine COVID-19 biorepository, which is supported by the NIH/National Center for Advancing Translational Sciences (UL1 TR002345). The WU353 and WU368 studies were reviewed and approved by the Washington University Institutional Review Board (approval nos. 202003186 and 202012081, respectively). The authors also express their gratitude to all study participants.

AUTHOR CONTRIBUTIONS

O.Z. designed the study, wrote the study protocol, performed experiments, analyzed the data, and supervised the project. A.M.A.D. enrolled subjects, collected demographic and clinical data, performed experiments, and analyzed data. L.A.V., C.-Y.L., and R.E.C. designed and performed neutralization experiments and analyzed data. P.K. and H.L.B. performed Luminex profiling and analyzed data. H.J.W., A.K., Jr., T.B.D., A.L.K., and Z.R. provided patient care. T.L.M. wrote the study protocol, managed institutional review board compliance, enrolled individuals, and processed samples. J.M.M., C.C.O., and C.J.R. collected demographic and clinical data, enrolled individuals, and processed samples. H.G.D.-A. performed SARS ELISA CoV-2 spike and RBD protein expression experiments. L.J.A. and D.H.F. generated crucial reagents. S.R. planned experiments and analyzed data. F.R.Z. contributed to the design of the study and analyzed data. A.H.E. and J.S.T. contributed samples from the healthy donor cohort. J.A.O. and R.M.P. wrote and maintained the institutional review board protocol, recruited and phlebotomized participants, and coordinated sample collection of healthy donors. P.L.K. contributed to supervision of the project. G.A. designed experiments and analyzed data. M.S.D. planned experiments and analyzed data. O.Z. and M.S.D. wrote the initial draft with detailed comments from A.M.A.D. All other authors provided editorial comments after the first draft.

DECLARATION OF INTERESTS

M.S.D. is a consultant for Inbios, Vir Biotechnology, Senda Biosciences, and Carnival Corporation and on the Scientific Advisory Boards of Moderna and Immunome. The Diamond laboratory has received unrelated funding support in sponsored research agreements from Moderna, Vir Biotechnology, Immunome, and Emergent BioSolutions. O.Z. and family own Moderna stock. G.A. is a founder and equity holder for Seromyx Systems Inc. and an equity holder for Leyden Labs. The Ellebedy laboratory received unrelated funding support from Emergent BioSolutions and AbbVie. A.H.E. is a consultant for Mubadala Investment Company and the founder of ImmuneBio Consulting. J.S.T. is a consultant for Gerson Lehrman Group. J.S.T. and A.H.E. are recipients of a licensing agreement with Abbvie that is unrelated to this manuscript.

Received: January 25, 2022
Revised: April 26, 2022
Accepted: May 13, 2022
Published: June 9, 2022

REFERENCES

1. Bonilla, F.A., Barlan, I., Chapel, H., Costa-Carvalho, B.T., Cunningham-Rundles, C., de la Morena, M.T., Espinosa-Rosales, F.J., Hammarstrom, L., Nonoyama, S., Quinti, I., et al. (2016). International consensus document (ICON): common variable immunodeficiency disorders. J. Allergy Clin. Immunol. Pract. *4*, 38–59. https://doi.org/10.1016/j.jaip.2015.07.025.
2. Picard, C., Bobby Gaspar, H., Al-Herz, W., Bousfiha, A., Casanova, J.L., Chatila, T., Crow, Y.J., Cunningham-Rundles, C., Etzioni, A., Franco, J.L., et al. (2018). International union of immunological societies: 2017 primary immunodeficiency diseases committee report on inborn errors of immunity. J. Clin. Immunol. *38*, 96–128. https://doi.org/10.1007/s10875-017-0464-9.
3. Resnick, E.S., Moshier, E.L., Godbold, J.H., and Cunningham-Rundles, C. (2012). Morbidity and mortality in common variable immune deficiency over 4 decades. Blood *119*, 1650–1657. https://doi.org/10.1182/blood-2011-09-377945.
4. Hammarstrom, L., Vorechovsky, I., and Webster, D. (2001). Selective IgA deficiency (SIgAD) and common variable immunodeficiency (CVID). Clin. Exp. Immunol. *120*, 225–231. https://doi.org/10.1046/j.1365-2249.2000.01131.x.
5. Hermaszewski, R.A., and Webster, A.D. (1993). Primary hypogammaglobulinaemia: a survey of clinical manifestations and complications. Q. J. Med. *86*, 31–42.

6. Gathmann, B., Mahlaoui, N., Ceredih, Gerard, L., Oksenhendler, E., Warnatz, K., Schulze, I., Kindle, G., Kuijpers, T.W., van Beem, R.T., et al. (2014). Clinical picture and treatment of 2212 patients with common variable immunodeficiency. J. Allergy Clin. Immunol. *134*, 116–126. https://doi.org/10.1016/j.jaci.2013.12.1077.

7. Bogaert, D.J.A., Dullaers, M., Lambrecht, B.N., Vermaelen, K.Y., De Baere, E., and Haerynck, F. (2016). Genes associated with common variable immunodeficiency: one diagnosis to rule them all? J. Med. Genet. *53*, 575–590. https://doi.org/10.1136/jmedgenet-2015-103690.

8. Maffucci, P., Filion, C.A., Boisson, B., Itan, Y., Shang, L., Casanova, J.L., and Cunningham-Rundles, C. (2016). Genetic diagnosis using whole exome sequencing in common variable immunodeficiency. Front. Immunol. *7*, 220. https://doi.org/10.3389/fimmu.2016.00220.

9. de Valles-Ibanez, G., Esteve-Sole, A., Piquer, M., Gonzalez-Navarro, E.A., Hernandez-Rodriguez, J., Laayouni, H., Gonzalez-Roca, E., Plaza-Martin, A.M., Deya-Martinez, A., Martin-Nalda, A., et al. (2018). Evaluating the genetics of common variable immunodeficiency: monogenetic model and beyond. Front. Immunol. *9*, 636. https://doi.org/10.3389/fimmu.2018.00636.

10. Tuijnenburg, P., Lango Allen, H., Burns, S.O., Greene, D., Jansen, M.H., Staples, E., Stephens, J., Carss, K.J., Biasci, D., Baxendale, H., et al. (2018). Loss-of-function nuclear factor κB subunit 1 (NFKB1) variants are the most common monogenic cause of common variable immunodeficiency in Europeans. J. Allergy Clin. Immunol. *142*, 1285–1296. https://doi.org/10.1016/j.jaci.2018.01.039.

11. Abolhassani, H., Hammarstrom, L., and Cunningham-Rundles, C. (2020). Current genetic landscape in common variable immune deficiency. Blood *135*, 656–667. https://doi.org/10.1182/blood.2019000929.

12. Ellis, E.F., and Henney, C.S. (1969). Adverse reactions following administration of human gamma globulin. J. Allergy *43*, 45–54. https://doi.org/10.1016/0021-8707(69)90019-7.

13. Friedli, H.R. (1987). Methodology and safety considerations in the production of an intravenous immunoglobulin preparation. Pharmacotherapy *7*, S36–S40. https://doi.org/10.1002/j.1875-9114.1987.tb03512.x.

14. Busse, P.J., Razvi, S., and Cunningham-Rundles, C. (2002). Efficacy of intravenous immunoglobulin in the prevention of pneumonia in patients with common variable immunodeficiency. J. Allergy Clin. Immunol. *109*, 1001–1004. https://doi.org/10.1067/mai.2002.124999.

15. Barahona Afonso, A.F., and Joao, C.M. (2016). The production processes and biological effects of intravenous immunoglobulin. Biomolecules *6*, 15. https://doi.org/10.3390/biom6010015.

16. Grifols (2022). Plasma Journey. https://www.grifols.com/en/plasma-journey.

17. Perez, E.E., Orange, J.S., Bonilla, F., Chinen, J., Chinn, I.K., Dorsey, M., El-Gamal, Y., Harville, T.O., Hossny, E., Mazer, B., et al. (2017). Update on the use of immunoglobulin in human disease: a review of evidence. J. Allergy Clin. Immunol. *139*, S1–S46. https://doi.org/10.1016/j.jaci.2016.09.023.

18. Hagin, D., Freund, T., Navon, M., Halperin, T., Adir, D., Marom, R., Levi, I., Benor, S., Alcalay, Y., and Freund, N.T. (2021). Immunogenicity of Pfizer-BioNTech COVID-19 vaccine in patients with inborn errors of immunity. J. Allergy Clin. Immunol. *148*, 739–749. https://doi.org/10.1016/j.jaci.2021.05.029.

19. Arroyo-Sanchez, D., Cabrera-Marante, O., Laguna-Goya, R., Almendro-Vázquez, P., Carretero, O., Gil-Etayo, F.J., Suàrez-Fernández, P., Pérez-Romero, P., Rodríguez de Frías, E., Serrano, A., et al. (2021). Immunogenicity of anti-SARS-CoV-2 vaccines in common variable immunodeficiency. J. Clin. Immunol. *42*, 240–252.

20. Salinas, A.F., Mortari, E.P., Terreri, S., Quintarelli, C., Pulvirenti, F., Di Cecca, S., Guercio, M., Milito, C., Bonanni, L., Auria, S., et al. (2021). SARS-CoV-2 vaccine induced atypical immune responses in antibody defects: everybody does their best. J. Clin. Immunol. *41*, 1709–1722. https://doi.org/10.1007/s10875-021-01133-0.

21. Abo-Helo, N., Muhammad, E., Ghaben-Amara, S., Panasoff, J., and Cohen, S. (2021). Specific antibody response of patients with common variable immunodeficiency to BNT162b2 coronavirus disease 2019 vaccination. Ann. Allergy Asthma Immunol. *127*, 501–503. https://doi.org/10.1016/j.anai.2021.07.021.

22. Pulvirenti, F., Fernandez Salinas, A., Milito, C., Terreri, S., Piano Mortari, E., Quintarelli, C., Di Cecca, S., Lagnese, G., Punziano, A., Guercio, M., et al. (2021). B cell response induced by SARS-CoV-2 infection is boosted by the BNT162b2 vaccine in primary antibody deficiencies. Cells *10*, 2915. https://doi.org/10.3390/cells10112915.

23. Davis, C.W., Jackson, K.J.L., McElroy, A.K., Halfmann, P., Huang, J., Chennareddy, C., Piper, A.E., Leung, Y., Albarino, C.G., Crozier, I., et al. (2019). Longitudinal analysis of the human B cell response to ebola virus infection. Cell *177*, 1566–1582.

24. Kim, W., Zhou, J.Q., Horvath, S.C., Schmitz, A.J., Sturtz, A.J., Lei, T., Liu, Z., Kalaidina, E., Thapa, M., Alsoussi, W.B., et al. (2022). Germinal centre-driven maturation of B cell response to mRNA vaccination. Nature *604*, 141–145. https://doi.org/10.1038/s41586-022-04527-1.

25. Bartsch, Y.C., Wang, C., Zohar, T., Fischinger, S., Atyeo, C., Burke, J.S., Kang, J., Edlow, A.G., Fasano, A., Baden, L.R., et al. (2021). Humoral signatures of protective and pathological SARS-CoV-2 infection in children. Nat. Med. *27*, 454–462. https://doi.org/10.1038/s41591-021-01263-3.

26. Atyeo, C., Fischinger, S., Zohar, T., Slein, M.D., Burke, J., Loos, C., McCulloch, D.J., Newman, K.L., Wolf, C., Yu, J., et al. (2020). Distinct early serological signatures track with SARS-CoV-2 survival. Immunity *53*, 524–532.e4.

27. Ackerman, M.E., Barouch, D.H., and Alter, G. (2017). Systems serology for evaluation of HIV vaccine trials. Immunol. Rev. *275*, 262–270. https://doi.org/10.1111/imr.12503.

28. Khoury, D.S., Cromer, D., Reynaldi, A., Schlub, T.E., Wheatley, A.K., Juno, J.A., Subbarao, K., Kent, S.J., Triccas, J.A., and Davenport, M.P. (2021). Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat. Med. *27*, 1205–1211. https://doi.org/10.1038/s41591-021-01377-8.

29. Dhar, M.S., Marwal, R., Vs, R., Ponnusamy, K., Jolly, B., Bhoyar, R.C., Sardana, V., Naushin, S., Rophina, M., Mellan, T.A., et al.; Indian SARS-CoV-2 Genomics Consortium INSACOG‡ (2021). Genomic characterization and epidemiology of an emerging SARS-CoV-2 variant in Delhi, India. Science *374*, 995–999. https://doi.org/10.1126/science.abj9932.

30. Cele, S., Jackson, L., Khoury, D.S., Khan, K., Moyo-Gwete, T., Tegally, H., San, J.E., Cromer, D., Scheepers, C., Amoako, D., et al. (2021). Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. Nature *602*, 654–656. https://doi.org/10.1038/d41586-021-03824-5.

31. Lu, L., Mok, B.W.Y., Chen, L.L., Chan, J.M.C., Tsang, O.T.Y., Lam, B.H.S., Chuang, V.W.M., Chu, A.W.H., Chan, W.M., Ip, J.D., et al. (2021). Neutralization of SARS-CoV-2 Omicron variant by sera from BNT162b2 or Coronavac vaccine recipients. Clin. Infect. Dis., ciab1041. https://doi.org/10.1093/cid/ciab1041.

32. Collie, S., Champion, J., Moultrie, H., Bekker, L.G., and Gray, G. (2021). Effectiveness of BNT162b2 vaccine against Omicron variant in South Africa. N. Engl. J. Med. *386*, 494–496.

33. VanBlargan, L.A., Errico, J.M., Halfmann, P.J., Zost, S.J., Crowe, J.E., Purcell, L.A., Kawaoka, Y., Corti, D., Fremont, D.H., and Diamond, M.S. (2022). An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. Nat. Med. *28*, 490–495. https://doi.org/10.1038/s41591-021-01678-y.

34. Liu, L., Iketani, S., Guo, Y., Chan, J.F.W., Wang, M., Luo, Y., Chu, H., Huang, Y., Nair, M.S., Yu, J., et al. (2021). Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. Nature *602*, 676–681. https://doi.org/10.1038/d41586-021-03826-3.

35. Dejnirattisai, W., Zhou, D., Ginn, H.M., Duyvesteyn, H.M.E., Supasa, P., Case, J.B., Zhao, Y., Walter, T.S., Mentzer, A.J., Liu, C., et al. (2021). The antigenic anatomy of SARS-CoV-2 receptor binding domain. Cell *184*, 2183–2200.e22.

36. CDC (2022). COVID-19 Vaccines for Moderately or Severely Immunocompromised People. https://www.cdc.gov/coronavirus/2019-ncov/vaccines/recommendations/immuno.html.

37. Hou, X., Zaks, T., Langer, R., and Dong, Y. (2021). Lipid nanoparticles for mRNA delivery. Nat. Rev. Mater. *6*, 1078–1094. https://doi.org/10.1038/s41578-021-00358-0.

38. Alameh, M.G., Tombacz, I., Bettini, E., Lederer, K., Sittplangkoon, C., Wilmore, J.R., Gaudette, B.T., Soliman, O.Y., Pine, M., Hicks, P., et al. (2021). Lipid nanoparticles enhance the efficacy of mRNA and protein subunit vaccines by inducing robust T follicular helper cell and humoral responses. Immunity *54*, 2877–2892.e7. https://doi.org/10.1016/j.immuni.2021.11.001.

39. Ndeupen, S., Qin, Z., Jacobsen, S., Bouteau, A., Estanbouli, H., and Igyarto, B.Z. (2021). The mRNA-LNP platform's lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory. iScience *24*, 103479. https://doi.org/10.1016/j.isci.2021.103479.

40. Romero, C., Diez, J.M., and Gajardo, R. (2022). Anti-SARS-CoV-2 antibodies in healthy donor plasma pools and IVIG products-an update. Lancet Infect. Dis. *22*, 19. https://doi.org/10.1016/s1473-3099(21)00755-6.

41. Cao, Y., Wang, J., Jian, F., Xiao, T., Song, W., Yisimayi, A., Huang, W., Li, Q., Wang, P., An, R., et al. (2021). Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. Nature *602*, 657–663. https://doi.org/10.1038/d41586-021-03796-6.

42. Dejnirattisai, W., Huo, J., Zhou, D., Zahradník, J., Supasa, P., Liu, C., Duyvesteyn, H.M.E., Ginn, H.M., Mentzer, A.J., Tuekprakhon, A., et al. (2021). Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. Preprint at bioRxiv. https://doi.org/10.1101/2021.12.03.471045.

43. Gilbert, P.B., Montefiori, D.C., McDermott, A.B., Fong, Y., Benkeser, D., Deng, W., Zhou, H., Houchens, C.R., Martins, K., Jayashankar, L., et al.; Immune Assays Team§; Moderna, Inc. Team; Coronavirus Vaccine Prevention Network CoVPN/Coronavirus Efficacy COVE Team; United States Government USG/CoVPN Biostatistics Team (2022). Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. Science *375*, 43–50. https://doi.org/10.1126/science.abm3425.

44. Cromer, D., Steain, M., Reynaldi, A., Schlub, T.E., Wheatley, A.K., Juno, J.A., Kent, S.J., Triccas, J.A., Khoury, D.S., and Davenport, M.P. (2022). Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: a meta-analysis. Lancet Microbe *3*, e52–e61. https://doi.org/10.1016/s2666-5247(21)00267-6.

45. Turner, J.S., O'Halloran, J.A., Kalaidina, E., Kim, W., Schmitz, A.J., Zhou, J.Q., Lei, T., Thapa, M., Chen, R.E., Case, J.B., et al. (2021). SARS-CoV-2 mRNA vaccines induce persistent human germinal centre responses. Nature *596*, 109–113. https://doi.org/10.1038/s41586-021-03738-2.

46. Hsieh, C.L., Goldsmith, J.A., Schaub, J.M., DiVenere, A.M., Kuo, H.C., Javanmardi, K., Le, K.C., Wrapp, D., Lee, A.G., Liu, Y., et al. (2020). Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. Science *369*, 1501–1505. https://doi.org/10.1126/science.abd0826.

47. Tao, Y., Strelkov, S.V., Mesyanzhinov, V.V., and Rossmann, M.G. (1997). Structure of bacteriophage T4 fibritin: a segmented coiled coil and the role of the C-terminal domain. Structure *5*, 789–798. https://doi.org/10.1016/s0969-2126(97)00233-5.

48. Alsoussi, W.B., Turner, J.S., Case, J.B., Zhao, H., Schmitz, A.J., Zhou, J.Q., Chen, R.E., Lei, T., Rizk, A.A., McIntire, K.M., et al. (2020). A potently neutralizing antibody protects mice against SARS-CoV-2 infection. J. Immunol. *205*, 915–922. https://doi.org/10.4049/jimmunol.2000583.

49. Hassan, A.O., Case, J.B., Winkler, E.S., Thackray, L.B., Kafai, N.M., Bailey, A.L., McCune, B.T., Fox, J.M., Chen, R.E., Alsoussi, W.B., et al. (2020). A SARS-CoV-2 infection model in mice demonstrates protection by neutralizing antibodies. Cell *182*, 744–753. https://doi.org/10.1016/j.cell.2020.06.011.

50. Zang, R., Castro, M.F.G., McCune, B.T., Zeng, Q., Rothlauf, P.W., Sonnek, N.M., Liu, Z., Brulois, K.F., Wang, X., Greenberg, H.B., et al. (2020). TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. Sci Immunol *5*, eabc3582. https://doi.org/10.1126/sciimmunol.abc3582.

51. Plante, J.A., Liu, Y., Liu, J., Xia, H., Johnson, B.A., Lokugamage, K.G., Zhang, X., Muruato, A.E., Zou, J., Fontes-Garfias, C.R., et al. (2021). Author Correction: spike mutation D614G alters SARS-CoV-2 fitness. Nature *595*, E1–E121. https://doi.org/10.1038/s41586-021-03657-2.

52. Brown, E.P., Dowell, K.G., Boesch, A.W., Normandin, E., Mahan, A.E., Chu, T., Barouch, D.H., Bailey-Kellogg, C., Alter, G., and Ackerman, M.E. (2017). Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles. J. Immunol. Methods *443*, 33–44. https://doi.org/10.1016/j.jim.2017.01.010.

53. Brown, E.P., Licht, A.F., Dugast, A.S., Choi, I., Bailey-Kellogg, C., Alter, G., and Ackerman, M.E. (2012). High-throughput, multiplexed IgG subclassing of antigen-specific antibodies from clinical samples. J. Immunol. Methods *386*, 117–123. https://doi.org/10.1016/j.jim.2012.09.007.

54. Liu, Z., VanBlargan, L.A., Bloyet, L.M., Rothlauf, P.W., Chen, R.E., Stumpf, S., Zhao, H., Errico, J.M., Theel, E.S., Liebeskind, M.J., et al. (2021). Identification of SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization. Cell Host Microbe *29*, 477–488.e4.

55. VanBlargan, L.A., Adams, L.J., Liu, Z., Chen, R.E., Gilchuk, P., Raju, S., Smith, B.K., Zhao, H., Case, J.B., Winkler, E.S., et al. (2021). A potently neutralizing SARS-CoV-2 antibody inhibits variants of concern by utilizing unique binding residues in a highly conserved epitope. Immunity *54*, 2399–2416.e6.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
(HRP)-conjugated goat anti-human IgG (H + L)	Jackson ImmunoResearch	Cat #109-035-003
FRNT staining mix (SARS2-02, SARS2-11, SARS2-16, SARS2-31, SARS2-38, SARS2-57, and SARS2-71)	VanBlargan et al., 2022	N/A
Anti-Mouse IgG (whole molecule)–Peroxidase antibody	Sigma-Aldrich	Cat #A5278
Recombinant biotinylated human FcγR2a, FcγR2b, FcγR3a, FcγR2b	Duke Protein Production	Cat #NA, custom order
Mouse Anti-Human IgG1-Fc PE	Southern Biotech	Cat #9054-09 RRID: AB_2796628
Mouse Anti-Human IgG2-Fc PE	Southern Biotech	Cat #9060-09; RRID: AB_2796635
Mouse Anti-Human IgG3-Fc PE	Southern Biotech	Cat #9210-09; RRID: AB_2796701
Mouse Anti-Human IgM-Fc PE	Southern Biotech	Cat #9020-09 RRID: AB_2796577
Mouse Anti-Human IgA1-Fc PE	Southern Biotech	Cat #9130-09 RRID: AB_2796656
Chemicals, peptides, and recombinant proteins		
Dulbecco's Phosphate Buffered Saline	Gibco	Cat #14190-136
Dulbecco's Modified Eagle Medium	Gibco	Cat #11995-040
Fetal Bovine Serum Defined	HyClone	Cat #SH30070.03
GlutaMAX Supplement	Gibco	Cat #35050-061
HEPES (1M)	Gibco	Cat #15630-080
KPL TrueBlue Peroxidase Substrate	SeraCare	Cat #5510-0050
Methylcellulose	Sigma-Aldrich	Cat #M0512
Minimum Essential Medium Eagle	Sigma-Aldrich	Cat #M0275
Nonfat dry milk for western blotting applications	Bio-Rad	Cat #1706404
Paraformaldehyde 20% Solution	EMS	Cat #15713-S
PE-Streptavidin	Agilent Technologies, CA, USA	Cat # PJRS25-1
Penicillin Streptomycin	Gibco	Cat #15140-122
Saponin	Sigma-Aldrich	Cat #47036
Sodium bicarbonate	Sigma-Aldrich	Cat #S5761
SARS-CoV-2 spike protein (6P)	VanBlargan et al., 2021	N/A
SARS-CoV-2 spike RBD	VanBlargan et al., 2021	N/A
SARS-CoV-2 D614G Spike	Sino Biological	40589-V08B4
SARS-CoV-2 D614G S1	Sino Biological	40591-V08H
SARS-CoV-2 D614G S2	Sino Biological	40590-V08B
SARS-CoV-2 D614G RBD	Sino Biological	40592-V08H
SARS-CoV-2 B.1.351 Spike	Sino Biological	40589-V08B7
SARS-CoV-2 B.1.617.2 Spike	Sino Biological	40589-V08B16
Tetramethylbenzidine substrate	Thermo Fisher	Cat #34029
Tween-20	Thermo Fisher	Cat #BP337-500
Bacterial and virus strains		
SARS-CoV-2 WA/20 D614G	Chen et al., 2021	N/A
SARS-CoV-2 B.1.1.529	VanBlargan et al., 2021	N/A
SARS-CoV-2 B.1.617.2	R. Webby laboratory (St Jude Children Research Hospital)	N/A

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Cell lines		
Vero-TMPRSS2	Chen et al., 2021	N/A
Vero-TMPRSS2-human ACE2	Chen et al., 2021	N/A
Software and algorithms		
GraphPad Prism	GraphPad	V9
ImageJ	National Institute of Health	V1.53
Nanozoomer Digital Pathology	Hamamatsu	v2
Other		
MagPlex microspheres	Luminex corporation	Cat #MC12001-01

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to the lead contact, Ofer Zimmerman (zimmero@wustl.edu).

Materials availability

All requests for resources and reagents should be directed to the lead contact author. This includes viruses. All reagents will be made available on request after completion of a Materials Transfer Agreement.

Data and code availability

(a) Data. All serological results described in this study are available within the body of the paper. All data (including raw data used to generate neutralizing and binding curves) reported in this paper will be shared by the lead contact upon request
(b) Code. This paper does not report original code.
(c) Additional information. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAIL

Patients and samples

The study was approved by the Institutional Review Board of Washington University School of Medicine (Approval # 202104138). Patients were identified by a medical record search for PAD, and their records were reviewed to confirm their diagnosis and verify they met the inclusion criteria. COVID-19 vaccination status was reviewed, and subjects were contacted if they were within the vaccination window or not yet immunized. Laboratory values (Tables S2 and S3) were acquired based on review of patient history and records and were not performed as part of this study. Reference values and ranges of specific tests are based on data obtained at the time of original sampling from patient records.

Inclusion criteria included males and females over 18 years of age, health care provider-documented PAD syndrome including common variable immunodeficiency (CVID), specific antibody deficiency, or hypogammaglobulinemia, and the ability to give informed consent. Entry criteria also included receipt of a SARS-CoV-2 vaccine within 14 days of enrollment, receipt of the second dose of mRNA vaccine (Moderna mRNA-1273 or Pfizer BNT162b2) within 28 days of the first visit, or receipt of one dose of adenoviral-vector vaccine (J&J Ad26.COV2.S) within 35 days of initial visit. Exclusion criteria included participation in an investigational study of SARS-CoV-2 vaccines within the past year, history of HIV infection, an active cancer diagnosis, treatment with immunosuppressive medications, history of hematologic malignancy, treatment with anti-CD20 monoclonal antibody, receipt of live-attenuated vaccine within 30 days or any inactivated vaccine within 14 days of SARS-CoV-2 vaccination, blood or blood product donation within 30 days prior to study vaccination, and planned blood donation at any time during or 30 days after the duration of subject study participation.

469 charts were reviewed, and 160 subjects were contacted. A total of 30 adults (27 females, 3 males) with PAD met eligibility requirements and agreed to enroll in the study (see Table 1); we note a gender-bias in the enrollees from our PAD cohort, which is not typical for the disease itself. Ages ranged from 20 to 82, with an average age of 48.4 years old. Twenty PAD patients had CVID, six had specific antibody deficiency, and four had hypogammaglobulinemia. Twenty-seven of these subjects had received immunoglobulin replacement therapy before and during the study period from 9 different products. Nineteen subjects received the BNT162b2, 8 received mRNA-1273, and three received Ad26.COV2.S vaccines. Of the 30 subjects, 9 were diagnosed with a prior SARS-CoV-2

infection with a positive nasal swab RT-PCR test, and one received treatment with an anti-SARS-CoV-2 monoclonal antibody (bamlanivimab) 90 days prior to study enrollment.

All subjects had one mandatory post-vaccine blood sample collection with optional pre-vaccine and follow-up visits at days 60, 90, and 150 (±14 days) after vaccination. The optional pre-vaccination blood sample was collected up to 14 days before receiving vaccine. For subjects who received a two-dose series of mRNA vaccines, the first post-vaccination blood collection occurred 7–28 days after the second dose. For subjects receiving the Ad26.COV2.S single-dose vaccine, the first post-vaccination blood sample was collected 21–35 days after immunization. Since the study was non-interventional, patients were informed if they mounted an immune response to the vaccine, but the decision to receive a booster was made between the patient and their health care provider. Subjects who opted for boosting provided a blood sample up to 14 days prior to receiving the booster dose, unless the subject previously provided a sample within 2 weeks as part of the optional post-vaccine assessments. Subjects returned for an additional sample 7–28 days after receiving the booster (range 7-27 days, median 17 days, mean 17 days. One patient had her post-booster sample drawn at day 35), with a second post-booster visit and sample collection at 90 ± 14 days. Immunoglobulin replacement product vials that were used in PAD patients were collected at each study visit and/or post-infusion at the Washington University Allergy and Immunology Division infusion centers.

Healthy donor controls

Immunocompetent healthy donor volunteer blood samples were obtained as previously described.[45]

SARS-CoV-2 spike and RBD protein expression

Genes encoding SARS-CoV-2 spike protein (residues 1–1213, GenBank: MN908947.3) and RBD (residues 319–514) were cloned into a pCAGGS mammalian expression vector with a C-terminal hexahistidine tag. The spike protein was stabilized in a prefusion form using six proline substitutions (F817P, A892P, A899P, A942P, K986P, V987P),[46] and expression was optimized with a disrupted S1/S2 furin cleavage site and a C-terminal foldon trimerization motif (YIPEAPRDGQAYVRKDGEWVLLSTFL).[47] Expi293F cells were transiently transfected, and proteins were purified by cobalt-affinity chromatography (G-Biosciences) as previously described.[48,49]

Cells

Vero-TMPRSS2 cells[50] were cultured at 37°C in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10 mM HEPES pH 7.3, 1 mM sodium pyruvate, 1× non-essential amino acids, and 100 U/mL of penicillin–streptomycin, and 5 μg/mL of blasticidin. Expi293F cells were cultured at 37°C, Expi29™ media, on a shaker at 225 RPM.

Viruses

The WA1/2020 D614G recombinant strain was obtained from an infectious cDNA clone of the 2019n-CoV/USA_WA1/2020 strain as described previously.[51] The B.1.617.2 isolate was obtained a gift from R. Webby (Memphis, TN). All viruses were passaged once in Vero-TMPRSS2 cells and subjected to next-generation sequencing after RNA extraction to confirm the introduction and stability of substitutions. All virus experiments were performed in an approved Biosafety level 3 (BSL-3) facility.

METHOD DETAILS

ELISA for anti-spike and anti-RBD binding

Maxisorp ELISA (Thermo Fisher) plates were coated with SARS-CoV-2 ancestral spike or RBD (2 μg/mL) overnight in sodium bicarbonate buffer, pH 9.3. All plates were coated with spike and RBD from the same expression and purification batch. Plates were washed four times with PBS and 0.05% Tween-20 and blocked with 3% non-fat milk (reconstituted from powder) in PBS 0.05% Tween-20 for 1 h at 25°C. Plates were then incubated with 50 μL of patient and healthy donor serially-diluted samples (eight 4-fold dilutions, starting at 1/50) in 1% non-fat milk PBS 0.05% Tween-20, for 2 h at 25°C on a shaker. Immunoglobulin replacement products were diluted to 10 mg/mL (average patient IgG level) and then treated as described above. Plates were washed with PBS and 0.05% Tween-20 and incubated with horseradish peroxide (HRP)-conjugated goat anti-human IgG (H + L) (1:2000 dilution, Jackson ImmunoResearch) for 1 h at room temperature. After washing, plates were developed with 100 μL of 3,3′-5,5′ tetramethylbenzidine substrate (Thermo Fisher) for 90 s and fixed with 50 μL of 2N H_2SO_4. Plates were read at 450 nM using a microplate reader (Synergy H1; BioTek). Patient samples from different days of collection were run on the same plate. All plates were run with the same positive control sample (a healthy donor vaccinated with three doses of mRNA vaccine). End point titers were calculated using the average optical density as a cutoff. A specific well was considered positive if optical density signal was two times higher than average optical density of blank wells.

Avidity assay

Plasma antibody avidity was measured as previously described.[23] In brief, plasma dilutions that give an optical density reading of 1 were calculated from the serial dilution series performed. Plates were coated with spike protein overnight, washed and blocked with PBS 3% non-fat milk (reconstituted from powder), incubated with a specific plasma dilution as described above for 2 h and then washed one time for 5 min with either PBS or 8 M urea in PBS, followed by 4 washes with PBS 0.05% Tween-20. After assay

development as described above, the avidity index was calculated for each sample as the optical density ratio of the urea-washed to PBS-washed wells.

Luminex profiling

Serum samples were analyzed by a customized Luminex assay to quantify the levels of antigen-specific antibody subclasses and FcγR binding profiles, as previously described.[52,53] Briefly, SARS-CoV-2 antigens were coupled to magnetic Luminex beads (Luminex Corp) by carbodiimide-NHS ester-coupling (Thermo Fisher). Antigen-coupled microspheres were washed and incubated with plasma samples at an appropriate sample dilution (1:100 for antibody isotyping and 1:1000 for all low-affinity FcγRs) overnight in 384-well plates (Greiner Bio-One). Unbound antibodies were washed away, and antigen-bound antibodies were detected by using a PE-coupled detection antibody for each subclass and isotype (IgG1, IgG2, IgG3, IgA1, and IgM; Southern Biotech), and FcγRs were fluorescently labeled with PE before addition to immune complexes (FcγR2a, FcγR2b, FcγR3a, FcγR2b; Duke Protein Production facility). After 1 h of incubation, plates were washed, and flow cytometry was performed with an iQue (Intellicyt), and analysis was performed on IntelliCyt ForeCyt (v8.1). PE median fluorescent intensity (MFI) is reported as a readout for antigen-specific antibody titers.

Focus reduction neutralization test

Serial dilutions of immunoglobulins products or sera were incubated with 10^2 focus-forming units (FFU) of different strains of SARS-CoV-2 for 1 h at 37°C. Antibody-virus complexes were added to Vero-TMPRSS2 cell monolayers in 96-well plates and incubated at 37°C for 1 h. Subsequently, cells were overlaid with 1% (w/v) methylcellulose in MEM supplemented with 2% FBS. Plates were harvested 30 h later by removing overlays and fixed with 4% PFA in PBS for 20 min at room temperature. Plates were washed and sequentially incubated with an oligoclonal pool of SARS2-2, SARS2-11, SARS2-16, SARS2-31, SARS2-38, SARS2-57, and SARS2-71[54,55] anti-spike antibodies and HRP-conjugated goat anti-mouse IgG (Sigma) in PBS supplemented with 0.1% saponin and 0.1% BSA. SARS-CoV-2-infected cell foci were visualized using TrueBlue peroxidase substrate (KPL) and quantitated on an ImmunoSpot microanalyzer (Cellular Technologies).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical significance was assigned using Prism Version 9 (GraphPad) when $p < 0.05$. Statistical analysis was determined by one-way ANOVA with Dunnett's post-test, two-way ANOVA with Tukey post-test, paired t-tests or a Kruskal-Wallis with Dunn's post-test. The statistical tests, number of independent experiments, and number of experimental replicates are indicated in the Figure legends.