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MAJOR ARTICLE

Delayed and Attenuated Antibody Responses to Coronavirus Disease 2019 Vaccination With Poor Cross-Variant Neutralization in Solid-Organ Transplant Recipients—A Prospective Longitudinal Study

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Background. Therapeutically immunosuppressed transplant recipients exhibit attenuated responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines. To elucidate the kinetics and variant cross-protection of vaccine-induced antibodies in this population, we conducted a prospective longitudinal study in heart and lung transplant recipients receiving the SARS-CoV-2 messenger RNA (mRNA) 3-dose vaccination series.

Methods. We measured longitudinal serum antibody and neutralization responses against the ancestral and major variants of SARS-CoV-2 in SARS-CoV-2-uninfected lung (n = 18) and heart (n = 17) transplant recipients, non–lung-transplanted patients with cystic fibrosis (n = 7), and healthy controls (n = 12) before, during, and after the primary mRNA vaccination series.

Results. Among healthy controls, strong anti-spike responses arose immediately following vaccination and displayed cross-neutralization against all variants. In contrast, among transplant recipients, after the first 2 vaccine doses, increases in antibody concentrations occurred gradually, and cross-neutralization was completely absent against the Omicron B.1.1.529 variant. However, most (73%) of the transplant recipients had a significant response to the third vaccine dose, reaching levels comparable to those of healthy controls, with improved but attenuated neutralization of immune evasive variants, particularly Beta, Gamma, and Omicron. Responses in non–lung-transplanted patients with cystic fibrosis paralleled those in healthy controls.

Conclusions. In this prospective, longitudinal analysis of variant-specific antibody responses, lung and heart transplant recipients display delayed and defective responses to the first 2 SARS-CoV-2 vaccine doses but significantly augmented responses to a third dose. Gaps in antibody-mediated immunity among transplant recipients are compounded by decreased neutralization against Omicron variants, leaving many patients with substantially weakened immunity against currently circulating variants.

Keywords. COVID-19 vaccination; cross-variant neutralization; cystic fibrosis; longitudinal antibody responses; solid-organ transplant recipients.

The human coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19) [1]. For immunocompromised individuals, data on the longitudinal kinetics of antibody responses to vaccines against SARS-CoV-2, including messenger RNA (mRNA) vaccines, are limited. Solid-organ transplant recipients, who take immunosuppressants to prevent organ rejection, as well as individuals with bone marrow transplants for hematologic malignancy, display reduced responses to 2 doses of SARS-CoV-2 vaccine compared with other immunocompromised hosts and healthy individuals [2–14]. Improved antibody responses develop in transplant recipients after a third dose [15–18]. The kinetics and long-term durability of antibody responses to the initial 3-dose vaccine series among immunocompromised patients are poorly understood.

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Lung and heart transplant recipients generally require more immunosuppression than other transplant recipients to prevent graft rejection. The combination of a calcineurin inhibitor (tacrolimus more often than cyclosporine), a DNA synthesis inhibitor (mycophenolate more often than azathioprine) and/or a mammalian target of rapamycin (mTOR) inhibitor (sirolimus), and low-dose glucocorticoids is expected to impair antibody responses through a variety of mechanisms. DNA synthesis inhibitors and calcineurin inhibitors block T-cell expansion. Tacrolimus may affect follicular helper T cells [19], which are critical for the affinity maturation and isotype switching of an effective antibody response. Loss of mTOR activity blunts T- and B-cell proliferation, impairs the development of $CD8^+$ T-cell memory [20], skews helper T-cell differentiation toward regulatory T cells [21], and disrupts B-cell progression through germinal center reactions, including class switching and somatic hypermutation [22–24]. Glucocorticoids interfere with function of virtually all immune cells.

To assess the strength, durability, and kinetics of responses among solid-organ transplant recipients, we conducted a longitudinal study of vaccine responses. As a comparison group, we include individuals with cystic fibrosis without lung transplant because they both experience a high-risk chronic condition and often require eventual lung transplantation. We compared antibody responses and cross-neutralization of multiple variants in these groups with those of healthy controls up to 3 months after the administration of the third vaccine dose or first booster. Our data provide insights into the durability of antibody responses, attenuation of cross-variant neutralization, the benefit of a third vaccine dose, and alterations in the kinetics of antibody responses in solid-organ transplant recipients, which may inform the study and implementation of future booster doses in immunocompromised individuals.

METHODS

Study Participants

Study participants included 18 lung and 17 heart transplant recipients and 7 nontransplanted patients with cystic fibrosis—aged >18 years, living in eastern Massachusetts (to enable rapid sample processing), and recruited from among individuals receiving care at Massachusetts General Hospital, Boston. In addition, 12 nonimmunosuppressed healthy volunteers, matched for age and also living in eastern Massachusetts, were recruited via a publicly accessible institutional website (https://rally.massgeneralbrigham.org/) and local newsletters. All participants were enrolled ≤2 weeks after administration of the second SARS-CoV-2 vaccine dose, except in participants with cystic fibrosis, who were enrolled up to 24 weeks after the first SARS-CoV-2 booster. If a participant received prophylactic or therapeutic anti-spike monoclonal antibodies (as did most transplant recipients), samples were not collected after their administration, because it was impossible to discriminate natural from monoclonal antibodies.

All study procedures involving human subjects were approved by the Mass General Brigham Human Research Committee, the governing institutional review board. Informed consent was received from participants before inclusion in the study, either in writing or by institutional review board–established verbal consent procedures used during the COVID-19 pandemic. Participant data were deidentified, with study identification numbers assigned.

Demographic and Clinical Data

Demographic and clinical data were collected from the electronic medical record and/or study participants. All participant samples were tested for evidence of SARS-CoV-2 infection by assaying for antibodies to SARS-CoV-2 nucleocapsid.

Sample Collection

Serial blood samples were collected immediately prior and 1 week after each vaccine dose; 2, 3, and 6 months after dose 2; and 1 and 3 months after the dose 3. Because non–lung-transplanted individuals with cystic fibrosis were enrolled late during the period of study, no baseline or early samples were obtained for this group (Supplementary Figure 1). Sample processing and analysis are described in Supplementary Methods.

RESULTS

Cohort

Two heart and 2 lung transplant recipients had evidence of prior SARS-CoV-2 infection at the time of vaccination and were excluded from analysis. In addition, 1 lung transplant recipient, 1 heart transplant, 1 healthy control, and 1 patient with cystic fibrosis had evidence of SARS-CoV-2 infection during the study, and samples collected after infection were excluded from analysis. The age and sex distributions of the transplant recipients and healthy controls were similar (Table 1).

Most heart and lung transplant recipients were maintained on a DNA synthesis inhibitor (77%), a calcineurin inhibitor (91%), and prednisone (94%); 10%–20% were also maintained on an mTOR inhibitor (Table 1). Reflecting institutional practice, lung transplant recipients were generally maintained on higher doses of prednisone than heart transplant recipients (Table 1). No other substantial medication differences were observed between heart and lung transplant recipients.

Impaired Serologic Response Among Transplant Recipients to Initial Two Vaccine Doses

As reported elsewhere [25], compared with healthy controls, solid-organ transplant recipients had significantly lower levels of anti–SARS-CoV-2 spike total immunoglobulin levels at all

Table 1. Demographic and Clinical Characteristics of Study Participants

Characteristic	Participants, No. (%)[a]				
	Transplant Recipients			Healthy Controls (n = 12)	Patients With Cystic Fibrosis (n = 7)
	Heart (n = 17)	Lung (n = 18)	Heart or Lung (n = 35)		
Age, mean (range), y	61 (41–76)	62 (35–76)	61 (35–76)	59 (37–74)	42 (30–58)
Female sex	9 (53)	10 (56)	19 (54)	5 (42)	5 (71)
Race/ethnicity					
Hispanic	1 (6)	1 (6)	2 (11)	0 (0)	0 (0)
African American	1 (6)	0 (0)	1 (3)	1 (8)	0 (0)
White	15 (88)	17 (94)	32 (91)	11 (92)	7 (100)
Time since transplant, y					
<1	3 (18)	2 (11)	5 (14)	NA	NA
1–<5	7 (41)	8 (44)	15 (43)	NA	NA
5–<10	1 (6)	4 (22)	5 (14)	NA	NA
10–<15	5 (29)	1 (6)	6 (17)	NA	NA
≥15	1 (6)	3 (17)	4 (11)	NA	NA
Prior or concurrent SARS-CoV-2 infection	2 (12)	2 (11)	4 (11)	0 (0)	0 (0)
Primary vaccine series					
BNT162b2 (Pfizer-BioNTech)	14 (82)	15 (83)	29 (83)	9 (75)	2 (29)
mRNA-1273 (Moderna)	2 (12)	3 (17)	5 (14)	3 (25)	4 (57)
Ad26.COV2.S (Janssen)	1 (6)	0 (0)	1 (3)	0 (0)	1 (14)
Immunosuppressive regimen					
≥3 Agents	14 (82)	13 (72)	27 (79)	NA	NA
DNA synthesis inhibitor[b]	13 (76)	14 (78)	27 (77)	NA	NA
Total dose, mean (range), mg/d	1488 (360–3000)	802 (360–1080)	1206 (360–3000)	NA	NA
Calcineurin inhibitor[c]	16 (94)	16 (89)	32 (91)	NA	NA
mTOR inhibitor[d]	3 (18)	2 (11)	5 (14)	NA	NA
Prednisone					
None	2 (12)	0 (0)	2 (6)	NA	6 (86)
Any dose	15 (88)	18 (100)	33 (94)	NA	1 (14)
1–5 mg/d	12 (71)	2 (11)	14 (40)	NA	1 (14)
>5 to <10 mg/d	2 (12)	4 (22)	6 (17)	NA	0 (0)
≥10 mg/d	1 (6)	12 (67)	13 (37)	NA	0 (0)

Abbreviations: mRNA, messenger RNA; mTOR, mammalian target of rapamycin; NA, not applicable; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Data represent no. (%) of participants unless otherwise specified.

[b]Mycophenolate or azathioprine.

[c]Tacrolimus or cyclosporine.

[d]Everolimus or sirolimus.

time points measured around the 2-dose vaccine series (Figure 1*A* and Supplementary Figure 2*A*). These defects in antibody responses of transplant recipients persisted through 5 weeks after the second dose; transplant recipients displayed substantial heterogeneity, with approximately 40% showing partial yet delayed increases in immunoglobulin levels. Among healthy controls, serologic responses showed a gradual decay after the 2-dose primary series and again after the booster dose (Supplementary Figure 3), consistent with published findings [26–32]. As a result of these 2 trends, the differences between transplant recipients and healthy controls decreased by 24 weeks after the second vaccine dose (Figure 1 and Supplementary Figure 2*A*–2*C*). For non–lung-transplanted participants with cystic fibrosis, anti-spike antibody concentrations did not differ significantly from those of healthy controls (Figure 2*A*).

The frequent reporting of antibody responses as immunoglobulin (Ig) G may miss early-phase responses. To better understand the kinetics of the composite immunoglobulin responses (Figure 1) and assess whether deficiencies in isotype switching may contribute to defective responses among transplant recipients, we measured isotype-specific anti–SARS-CoV-2 receptor-binding domain immunoglobulins. Similar to findings for total anti-spike immunoglobulin, transplant recipients displayed significantly lower levels of IgG, IgA, and IgM over the same time frame, although IgM levels in healthy controls decreased earlier ($P < .001$; Supplementary Figures 4–6). The correlation between total immunoglobulin levels and IgG-specific concentrations was strong (Supplementary Figure 7), suggesting that a predominant proportion of the total antibody response to the vaccine is IgG.

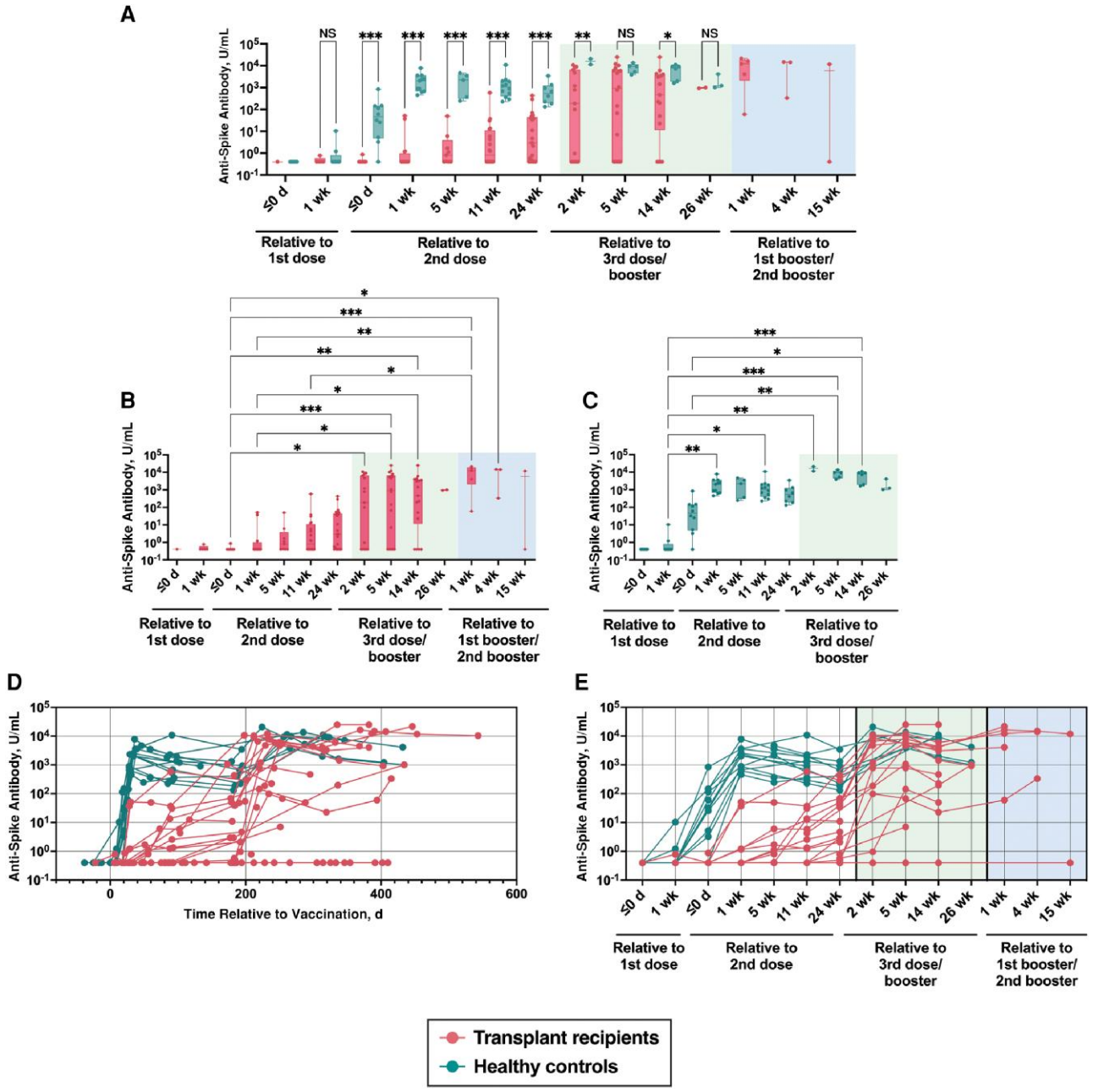

Figure 1. Anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike receptor-binding domain serum antibodies in lung and heart transplant recipients through to after the third vaccine dose. Serum antibody concentrations in lung (n = 16) and heart (n = 15) transplant recipients are compared with those in healthy controls (n = 12). *A*, Immunoglobulin levels in transplant recipients and healthy controls at each time point. Pairwise comparisons were performed using Mann-Whitney tests. *B, C*, Immunoglobulin levels in transplant recipients (*B*) or healthy controls (*C*) across time points. Box plots show 25th, 50th, and 75th percentiles; whiskers, maximum and minimum. Multiple-group comparisons were performed using Kruskal-Wallis tests. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$; NS, not significant. *D, E*, Longitudinal progression of antibody concentrations, displayed as line plots for each transplant recipient and healthy control as a function of days since receipt of first vaccine dose (*D*) or relative to each vaccine dose received (*E*). All data points are shown; y-axes are in logarithmic scale.

Neutralizing antibody titers were correlated with total anti-spike immunoglobulin (Supplementary Figure 8) and followed similar patterns, with similar differences between transplant recipients and healthy controls and similar levels in healthy controls and patients with cystic fibrosis (Figures 1, 2*A*, *D*, 3, and Supplementary Figure 2*D*–2*F*). Neutralizing activity and immunoglobulin levels 11 and 24 weeks after the second vaccine dose but before the third dose were worse among lung transplant than among heart transplant recipients (Supplementary Figure 2*A* and 2*D*); this best correlates with the higher levels of prednisone prescribed for the former.

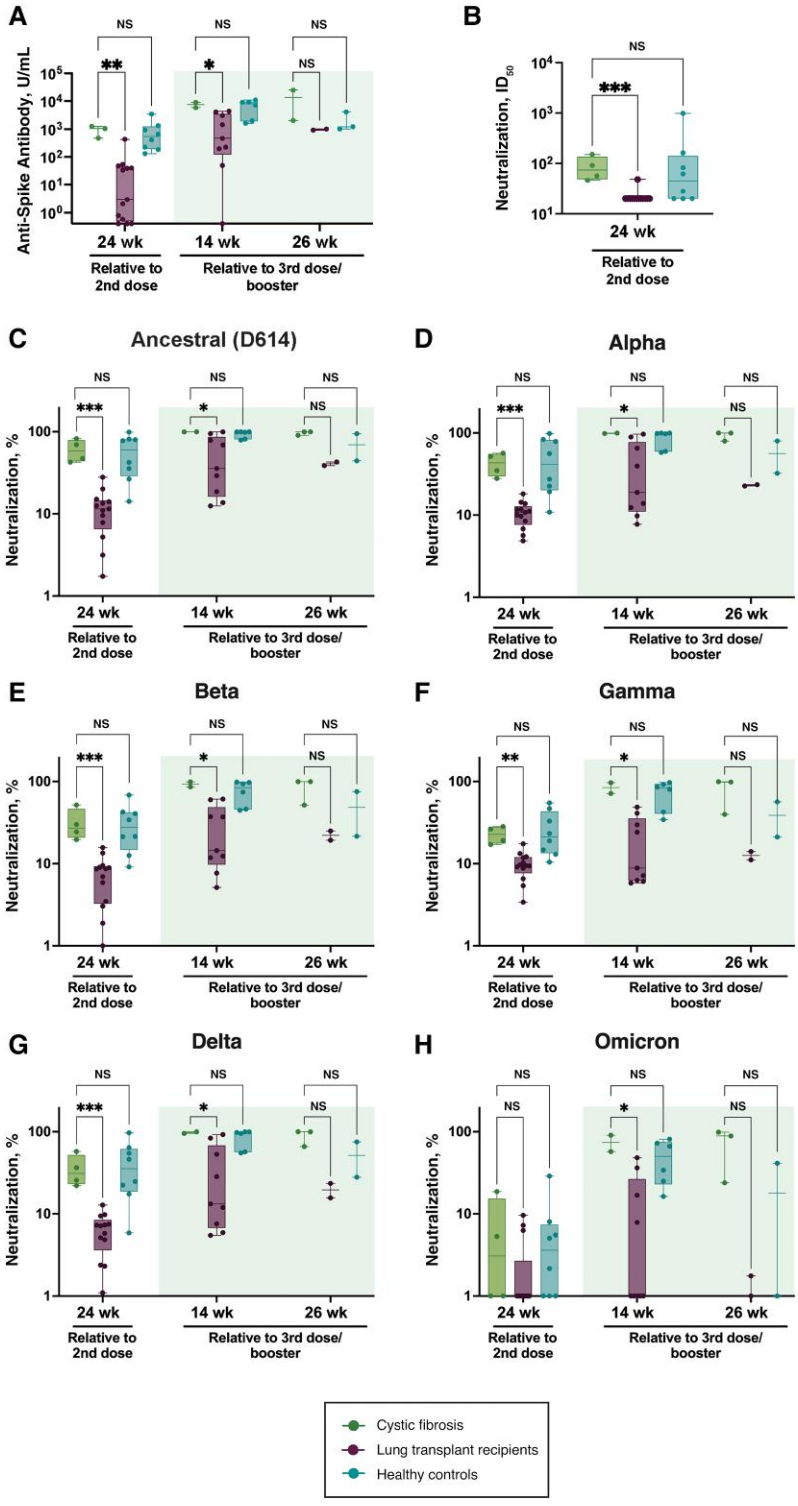

Figure 2. Anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike receptor-binding domain serum antibody responses and neutralizing antibody levels among participants with cystic fibrosis without lung transplantation (n = 5), lung transplant recipients (n = 16), and healthy controls (n = 9). *A*, Immunoglobulin levels in patients with cystic fibrosis, lung transplant, and healthy controls at each time point. *B*, Neutralization activity by pseudovirus neutralization assay of serum at 24 weeks after primary 2-dose vaccination series. Abbreviation: ID_{50}, median infective dose. *C–H*, Neutralization activity in patients with cystic fibrosis, lung transplant recipients, and healthy controls at each time point against the ancestral (D614) SARS-CoV-2 strain (*C*) and the variants Alpha (B.1.1.7) (*D*), Beta (B.1.351) (*E*), Gamma (P1) (*F*), Delta (B.1.617.2) (*G*), and Omicron (B.1.1.529) (*H*). Pairwise comparisons were performed using Mann-Whitney tests. Box plots show 25th, 50th, and 75th percentiles; whiskers, maximum and minimum. **P* < .05; ***P* < .01; ****P* < .001; NS, not significant. All data points are shown; y-axes are in logarithmic scale.

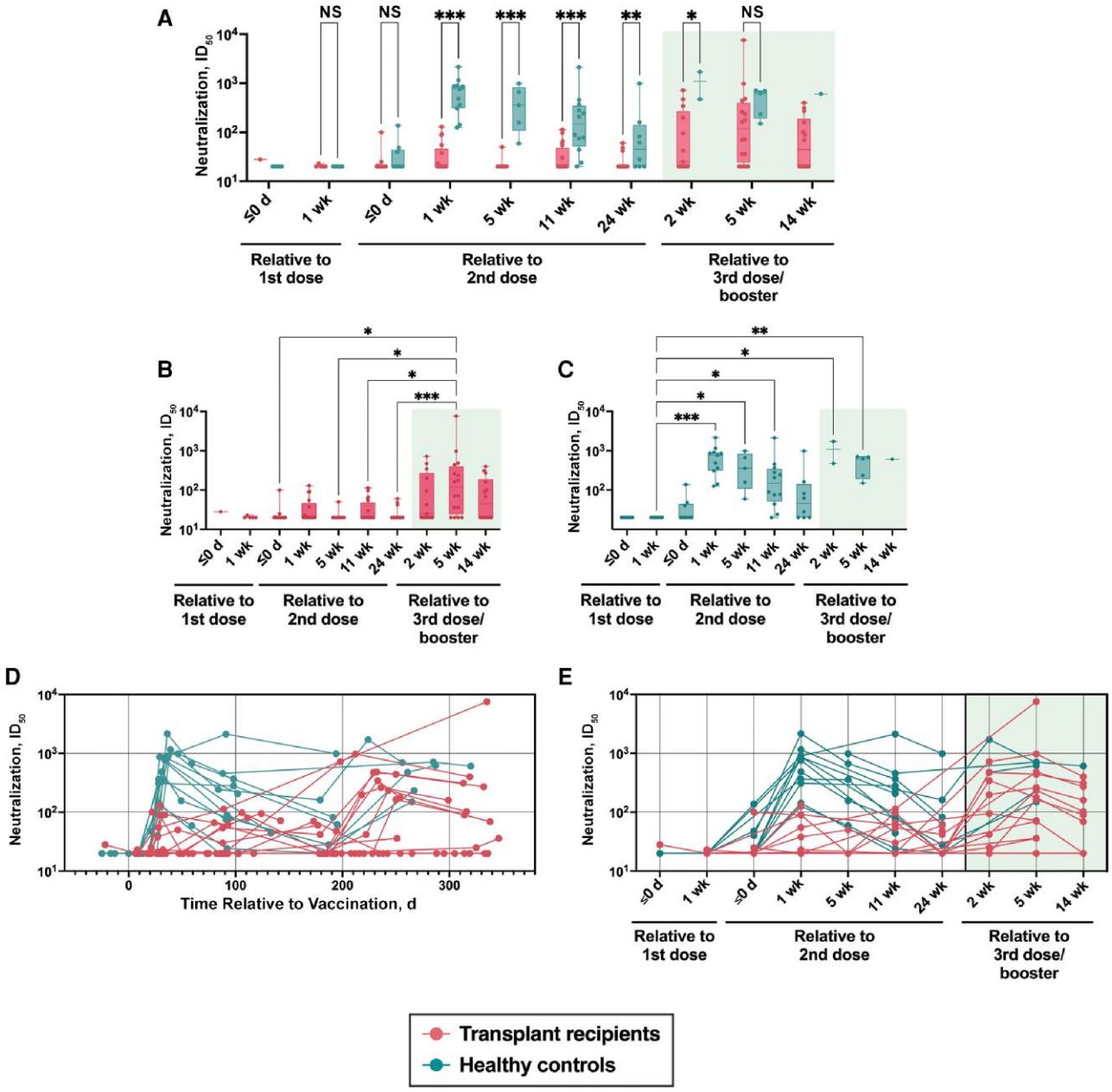

Figure 3. Neutralization activity of serum in lung and heart transplant recipients through to after the third vaccine dose. Pseudovirus neutralization assay was used to measure neutralization activity in serum samples from lung (n = 16) and heart (n = 15) transplant recipients compared with healthy controls (n = 12). *A*, Neutralization activity in transplant recipients and healthy controls at each time point. Pairwise comparisons were performed using Mann-Whitney tests. *B, C*, Neutralization activity in transplant recipients (*B*) and healthy controls (*C*) across time points. Box plots show 25th, 50th, and 75th percentiles; whiskers, maximum and minimum. Multiple-group comparisons were performed using Kruskal-Wallis tests. **P* < .05; ***P* < .01; ****P* < .001; NS, not significant. *D, E*, Longitudinal progression of neutralizing antibody titers as a function of days since receipt of first vaccine dose (*D*) or relative to each vaccine dose (*E*). All data points are shown; y-axes are in logarithmic scale. Abbreviation: ID_{50}, median infective dose.

Augmented Responses of Transplant Recipients to Third Vaccine Dose

During the time frame of this study, additional doses were recommended for both transplant recipients (a third dose and then a booster dose) and healthy controls (2 booster doses). For mRNA-1273 but not BNT162b2, the booster is a reduced dose. After the third vaccine dose, transplant recipients displayed significant increases in anti-spike antibodies and neutralization activity compared with baseline, which persisted after the booster dose (Figures 1 and 3). Total anti-spike immunoglobulin, IgG, and IgA among transplant recipients reached levels that were similar to those in healthy controls (Figure 1*A* and Supplementary Figures 4 and 5). Neutralization activity levels among transplant recipients also approached those of healthy controls, but not to the extent observed for total

immunoglobulin (Figures 1*A* and 3*A*). Heterogeneity of responses among transplant recipients persisted. Five transplant recipients (1 lung and 4 heart transplant recipients) who exhibited no response to the initial 2 doses also displayed no response to the third dose, with levels of anti-spike antibody and neutralization activity remaining at baseline throughout the sampled time points (Figures 1 and 3). These findings suggest that in a substantial subset of lung and heart transplant recipients, a third dose induces a heightened antibody response that is nearly comparable to the responses of healthy controls.

Association of IgM Response With Overall Response in Transplant Recipients

We classified participants who were sampled longitudinally through the third dose into 3 response groups as a function of whether their total anti-spike antibody levels surpassed the predefined 0.8 U/mL threshold for positivity (Cobas Elecsys): (1) participants with levels above threshold after the second dose, (2) those with levels above threshold after the third dose, and (3) nonresponders. The trajectories of isotype-specific antibody responses demonstrate that persistent nonresponsiveness (group 3) and response only after the third dose (group 2) were both associated with low IgM production throughout the vaccination series (Supplementary Figure 9), suggesting that production of IgM may be predictive of a robust antibody response.

Defects in Neutralization of Immune Evasive SARS-CoV-2 Variants

We assessed serum neutralizing antibody activity across all time points against the variants Alpha (B.1.1.7), Beta (B.1.351), Gamma (P1), Delta (B.1.617.2), and Omicron (B.1.1.529), along with the ancestral SARS-CoV-2 spike S1 protein using a bead-based binding assay (ProcartaPlex 6-plex Neutralizing Antibody Panel) that measures the loss in angiotensin-converting enzyme 2 binding due to neutralizing antibodies (Figure 4). Among healthy controls, significant immune escape was seen for Beta, Gamma, and particularly Omicron, as observed elsewhere [33]. Before the third vaccine dose among transplant recipients, cross-reactive protection was <10% for all variants, 3%–10% for Beta and Delta, and essentially undetectable for Omicron; the third dose was associated with variable increases in cross-neutralization activity, with improved but persistently attenuated neutralization of Beta, Delta, and Omicron.

Among healthy controls, neutralization activity of each variant displayed a strong correlation to that of the ancestral spike protein ($r > 0.9$, Figure 4). Non–lung-transplanted participants with cystic fibrosis displayed cross-protection that was similar to that in healthy controls and distinctly better than in lung transplant recipients (Figure 2*C*–2*H*). For all variants except Omicron, transplant recipients also displayed a pronounced relationship between the variant and the ancestral S1 (Alpha, Beta, and Delta, $r > 0.9$; Gamma, $r > 0.8$; Omicron, $r = 0.57$) (Figure 5). For Omicron, a significant number of samples clustered at values <0 neutralization (Figure 5*E*). The strong correlation observed between this neutralization activity to the ancestral spike protein and total and isotype specific immunoglobulin (measured on the other platforms used in this study) (Supplementary Figure 10) affirms the reliability of the competitive angiotensin-converting enzyme 2 binding assay as an efficient way to assess neutralization.

DISCUSSION

Our data demonstrate that although lung and heart transplant recipients display a diminished response to the first 2 doses of the initial 3-dose series of the COVID-19 vaccine, most exhibit a substantial increase in response after the third dose. The antibody responses of immunocompromised patients displayed altered kinetics, continuing to rise in a delayed manner in the absence of new antigenic stimulation following the first 2 doses, while responses of healthy controls waned. These differences in kinetics of the responses between immunocompromised and healthy patients raise questions about whether the timing of sentinel events in the germinal center in response to SARS-CoV-2 [34] may differ in immunocompromised patients. Immunosuppressive drugs taken by lung and heart transplant recipients hinder B- and T-cell development, maturation, and activation.

The initial poor and delayed responses are associated with the immunosuppressive medications administered to these individuals to prevent organ rejection, documented herein and elsewhere [25]. Although the precise mechanisms underlying the defective responses remain uncertain, possible contributing factors include mycophenolate-mediated deficiencies in T-cell help and mTOR inhibition of memory B cells, thus diminishing the proliferation of memory B cells [22–24, 35]. As plasma cells are differentiated from memory B cells, this decrease in memory B cells inhibits plasma cell-dependent secretion of IgA and large-scale production of IgG.

Our data demonstrate that in most lung and heart transplant recipients, the third dose promotes serologic antibody concentrations to levels nearly comparable to those observed after the second vaccine dose in healthy individuals. Together with other studies [15, 36–38], this observation reinforces the guidance for solid-organ transplant recipients to receive a full third dose. Moreover, among healthy participants, we observed a longitudinal decrease in antibody production after the primary 2-dose vaccination (Figure 1*C* and 3*C* and Supplementary Figure 3), as also reported elsewhere [39], highlighting the importance of administration of boosters, which provide clinical benefit [40–42].

Neutralizing activity is highly predictive of protection from symptomatic SARS-CoV-2 infection [43]. Unfortunately,

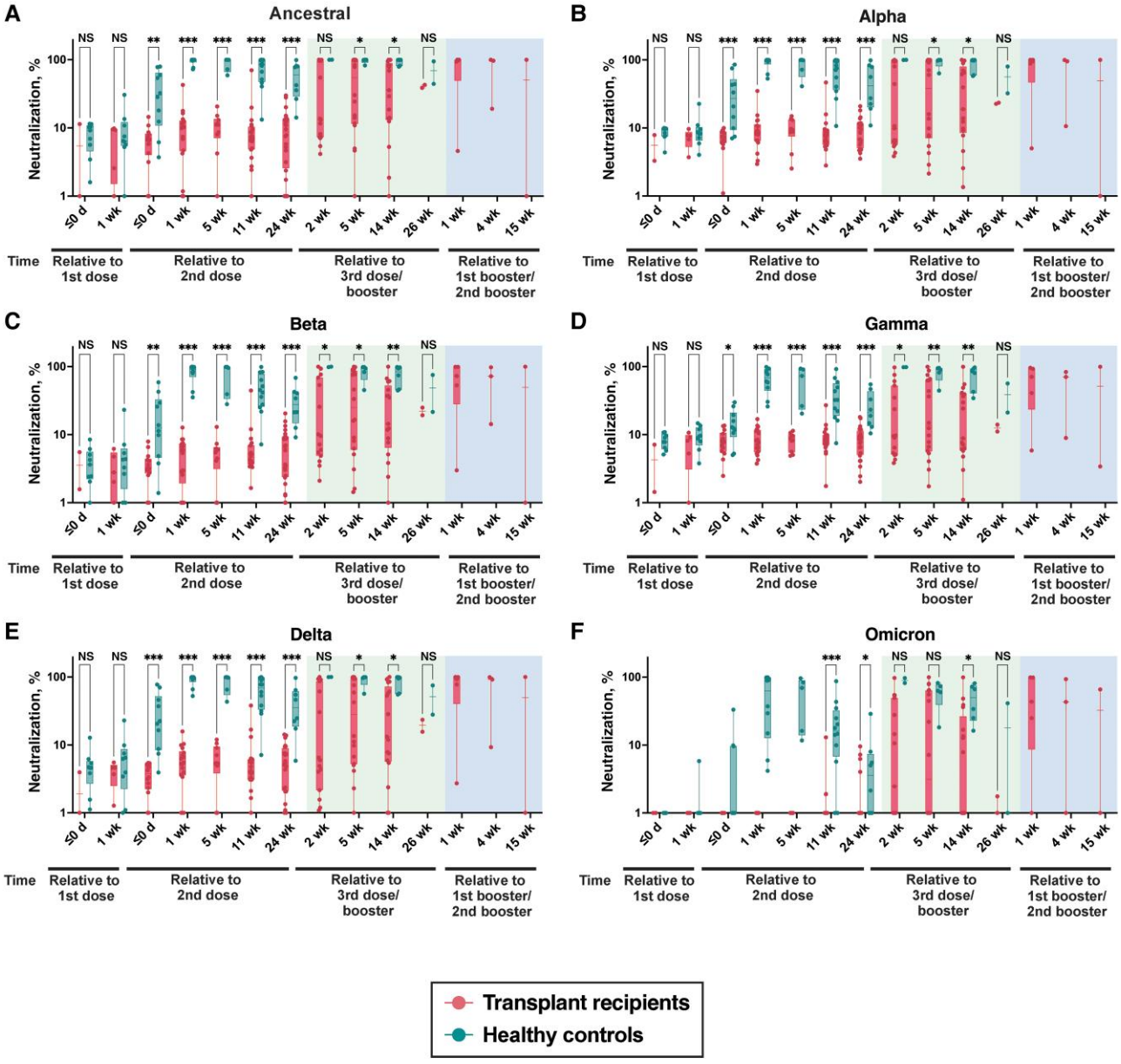

Figure 4. Neutralizing activity of antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike S1 protein in serum samples from lung and heart transplant recipients through to after the third vaccine dose. Neutralizing activity against the ancestral SARS-CoV-2 strain and 5 variants was measured in serum samples from lung (n = 16) and heart (n = 15) transplant recipients compared with healthy controls (n = 12). *A–F*, Neutralization activity in transplant recipients and healthy controls at each time point against the ancestral strain (*A*) and the variants Alpha (B.1.1.7) (*B*), Beta (B.1.351) (*C*), Gamma (P1) (*D*), Delta (B.1.617.2) (*E*), and Omicron (B.1.1.529) (*F*). Pairwise comparisons were performed using Mann-Whitney tests. Box plots show 25th, 50th, and 75th percentiles; whiskers, maximum and minimum. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$; NS, not significant. All data points are shown; y-axes are in logarithmic scale.

robust antibody responses produced by multidose vaccine series were undermined—in some cases markedly so—by variants. Variant-specific neutralization immunoassays demonstrated dramatic defects among transplant recipients in cross-protection before the third vaccine dose, with cross-reactive protection <10% for all variants, 3%–10% for Beta and Delta, and essentially undetectable for Omicron (Figure 4). As for antibody responses to the ancestral virus, cross-reactive neutralization activity improved in a delayed manner even before the third dose and was substantially increased after the third dose. Building on previous reports of broader groups of transplant recipients [38, 44], we show that variant-specific responses of lung and heart transplant recipients to the third vaccine dose were variable, with many individuals showing robust neutralization of Beta, Delta, and Omicron, whereas others possess neutralization activities

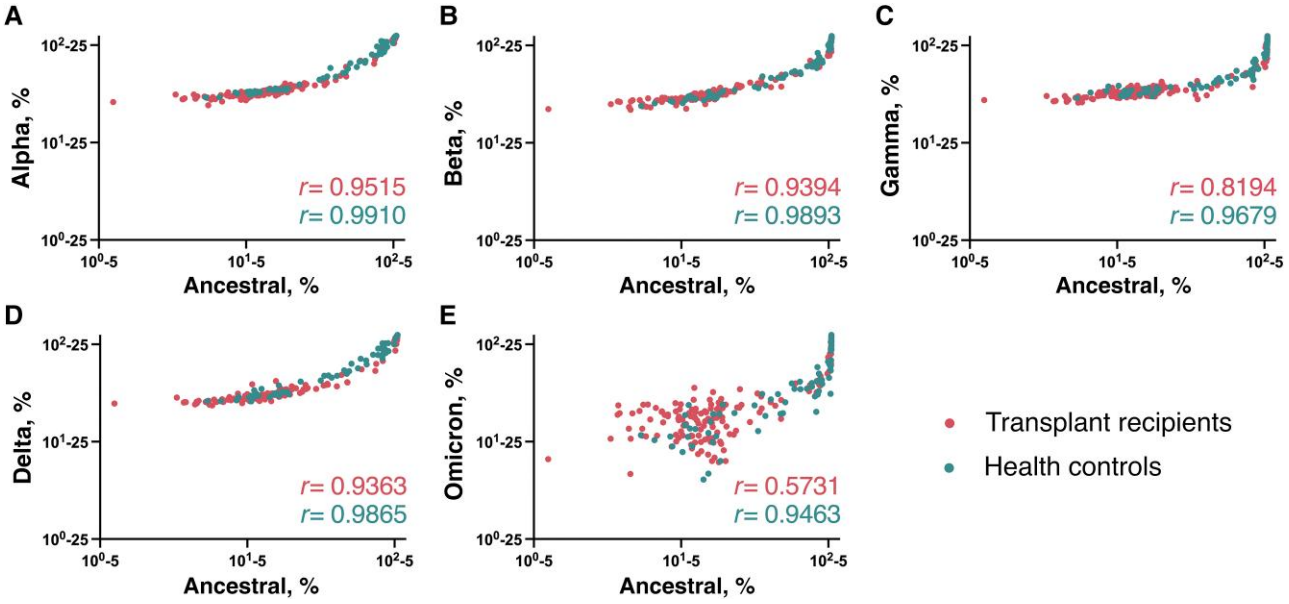

Figure 5. Neutralizing activity of antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike S1 protein in serum samples from lung and heart transplant recipients, for SARS-CoV-2 variants compared with the ancestral strain. Correlation plots compare neutralizing activity against the ancestral SARS-CoV-2 spike S1 protein (x-axes) with that against the variants Alpha (B.1.1.7) (*A*), Beta (B.1.351) (*B*), Gamma (P1) (*C*), Delta (B.1.617.2) (*D*), and Omicron (B.1.1.529) (*E*) (y-axes). Antibodies were measured in serum samples from lung (n = 16) and heart (n = 15) transplant recipients and healthy controls (n = 12). Spearman rank correlation coefficients are provided. All data points are shown; both axes are in logarithmic scale. As noted on the x-axis and y-axis scale bars, a standardized offset of each data point of 5% (x-axis) and 25% (y-axis) for clarity in plotting.

≤10%. In immunocompromised hosts, SARS-CoV-2 vaccination combined with Omicron BA.1 infection, but not infection with earlier variants, induces potent neutralization of Omicron variants [45, 46].

Limitations of our study include the relatively small sample size, missing or incomplete data points, and the lack of immunologic information characterizing the subsets of immune cells present, which might elucidate the pathways that contributed to the observed dynamics and specificity of antibody production over time.

In conclusion, our data, along with other published data, highlight the susceptibility of a large subset of immunocompromised hosts to currently circulating variants and the importance of continued exploration of approaches, such as heterologous boosting with currently circulating variants, for protecting lung and heart transplant recipients from clinically severe SARS-CoV-2 infection.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. All authors: No reported conflicts.

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