Passive antibody transfer from pregnant women to their fetus are maximized after SARS-CoV-2 vaccination irrespective of prior infection

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Background: Pregnancy is associated with a higher risk of adverse symptoms and outcomes for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection for both mother and neonate. Antibodies can provide protection against SARS-CoV-2 infection and are induced in pregnant women after vaccination or infection. Passive transfer of these antibodies from mother to fetus *in utero* may provide protection to the neonate against infection. However, it is unclear whether the magnitude or quality and kinetics of maternally derived fetal antibodies differs in the context of maternal infection or vaccination.

Objective: We aimed to determine whether antibodies transferred from maternal to fetus differed in quality or quantity between infection- or vaccination-induced humoral immune responses.

Methods: We evaluated 93 paired maternal and neonatal umbilical cord blood plasma samples collected between October 2020 and February 2022 from a birth cohort of pregnant women from New Orleans, Louisiana, with histories of SARS-CoV-2 infection and/or vaccination. Plasma was profiled for the levels of spike-specific antibodies and induction of antiviral humoral immune functions, including neutralization and Fc-mediated innate immune effector functions. Responses were compared between 4 groups according to maternal infection and vaccination.

Results: We found that SARS-CoV-2 vaccination or infection during pregnancy increased the levels of antiviral antibodies compared to naive subjects. Vaccinated mothers and cord samples had the highest anti-spike antibody levels and antiviral function independent of the time of vaccination during pregnancy.

Conclusions: These results show that the most effective passive transfer of functional antibodies against SARS-CoV-2 *in utero* is achieved through vaccination, highlighting the importance of vaccination in pregnant women. (J Allergy Clin Immunol Global 2024;3:100189.)

Key words: *SARS-CoV-2, COVID-19, antibody, placental transfer, maternal infection, maternal vaccination, neutralization, antibody-dependent cellular cytotoxicity, antibody-dependent phagocytosis, antibody-dependent complement activation*

Pregnancy is associated with altered immunity and increased risk of infections. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has a higher rate of adverse events in pregnant women, and infants of mothers infected during pregnancy have a higher risk of life-threatening complications.[1] These complications include higher incidence of preterm birth and stillbirth. As a result, the US Centers for Disease Control and Prevention recommends that all pregnant women, including those who are breast-feeding or trying to become pregnant, receive vaccination and boosters against SARS-CoV-2. A recent study showed that the infants of expecting mothers who received 2 doses of either Pfizer-BioNTech or Moderna mRNA coronavirus disease 2019 (COVID-19) vaccine had a lower hospitalization rate for infants <6 months of age.[1] This lower hospitalization rate in infants born to vaccinated mothers is likely mediated by transplacental transfer of maternal antiviral antibodies that protect the offspring against infection and/or disease.

Placental transfer of antibodies is vital for neonatal immunity against viral infections,[2] and SARS-CoV-2 vaccine–induced antibodies have been detected in umbilical cord sera collected at birth, demonstrating maternal transfer of SARS-CoV-2–specific antibodies,[1,3,4] and these antibodies are detectable out to 6 months in the offspring.[2] Antibody responses are essential to vaccine-induced immunity because they provide critical immune defenses against viral infections. Neutralization and Fc-mediated innate immune effector functions, including phagocytosis, complement deposition (ADCD), and antibody-dependent cellular cytotoxicity (ADCC), contribute to rapid clearance of infected cells and virus.[5] The function and transfer of functional antibodies

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Abbreviations used
ADCC: Antibody-dependent cellular cytotoxicity
ADCD: Antibody-dependent complement deposition
COVID-19: Coronavirus disease 2019
I + V: Infected plus vaccinated
I: Infected
LASSO: Least absolute shrinkage and selection operator
NK: Natural killer
RBD: Receptor binding domain
REDCap: Research Electronic Data Capture
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2
sPLS-DA: Sparse partial least squares discriminant analysis
U: Uninfected
V: Vaccinated

are likely a critical component of newborn and fetal protection. This study aimed to characterize the transplacental transfer of antibodies against SARS-CoV-2 that could provide antiviral protection to neonates.

METHODS

Study design and population

Maternal–infant dyads were recruited in the greater New Orleans area between November 2020 and February 2022. Inclusion criteria were as follows: age ≥18 years; pregnant for ≥12 weeks with single gestation; fluent in English or Spanish at the time of recruitment; and delivering at ≥34 weeks' gestation in the labor and delivery facilities at Tulane Lakeside Hospital or Ochsner Baptist Medical Center. Study participants were recruited for reporting ≥2 respiratory symptoms or testing positive for COVID-19, or both (95 dyads), or an additional control arm that did not (6 dyads). Exclusion criteria included positive human immunodeficiency virus test; multiple gestation; and any prenatally diagnosed congenital abnormality of the fetus. Eligible and interested women consented or e-consented (via Adobe software) to accommodate for COVID-19 restrictions. Standard questionnaires were used to collect demographic information, socioeconomic data, history of respiratory illness, personal history, vaccination history, and family history of atopy during the mother's hospital admission for childbirth.

Sample collection

Deidentified maternal blood and cord blood samples were collected during clinic visits or at the time of delivery and transported to Tulane University School of Medicine labs for processing and storage. Maternal blood samples were collected at the time of admission to the labor and delivery unit before childbirth, or after birth (after delivery and before discharge from the hospital). Cord blood samples were collected into sodium–heparin or CPT Vacutainers by hospital nurses via venipuncture of the umbilical cord vessels and placental vessels. Plasma samples were stored at −80°C. All samples were heat inactivated at 56°C for 30 minutes before antibody evaluations. As a result of biosafety protocols, we do not have parallel samples without heat inactivation. However, we have not observed an impact of heat inactivation on the immunogenicity or functional capacity of antibodies in prior studies analyzing Ebola-specific IgG responses.[6,7]

Data protection

All research data were securely kept on a computer connected to the Tulane University's firewall-protected server into Research Electronic Data Capture (REDCap). REDCap was protected by login and encryption. All study participants were assigned a deidentified ID to go on all the source documents and biological samples collected for this study. A key with each participant's deidentified ID, name, and medical record number was kept securely in REDCap.

Measurement of antibody levels

Levels of antibodies against SARS-CoV-2 spike, receptor binding domain, N protein, and seasonal coronaviruses were determined by ELISA and multiplexed bead-based assay. Experimental details are provided in the Methods section in this article's Online Repository at www.jaci-global.org.

Measurement of antibody antiviral functions

Four different assays were used to determine the levels of antibody-mediated antiviral functions, including neutralization using a lentivirus-based pseudovirus assay, measurement of antibody-dependent cellular phagocytosis by human monocytes, antibody-dependent activation of natural killer (NK) cells, and ADCD. Experimental details are provided in the Methods section in the Online Repository.

Statistical analysis

Distributions of continuous variables were assessed for normality, and data were $\log_{10}$ transformed to better represent standard distribution. Descriptive statistics for continuous variables are summarized as means ± SDs, and categorical variables were summarized using proportions. For the univariate tests, we used 1-way ANOVAs, and multiple *post hoc* comparisons were performed by the Tukey multiple comparison test. We used the Levene test to test for differences in variance between groups. Statistical analysis was performed by GraphPad Prism v9.3.1 software (GraphPad Software) or SAS v9.4 software (SAS Institute). Chi-square analysis was performed by Microsoft Excel.

For sparse partial least squares discriminant analyses (sPLS-DA), we used a multivariate analysis approach, sPLS-DA, which is an extension of the PLS-DA algorithm that utilizes the LASSO (least absolute shrinkage and selection operator) penalization to achieve a sparse solution, providing a tool for predictive and descriptive modeling with feature selection.[8,9] Data from the different groups were centered and scaled such that each column had mean 0 and variance 1. Missing values were imputed with the NIPALS (nonlinear iterative partial least square) algorithm before cross-validation. Tuning was performed using 3-fold cross-validation with 100 repeats. Additional methods used to estimate classification error rate and feature selection are detailed in the Methods section in the Online Repository. All sPLS-DA analyses were performed using the 'mixomics'[10] v6.24.0 package for R v4.3.1 (R Project; www.r-project.org).

RESULTS

To assess the transfer of antiviral immunity after maternal infection or vaccination during pregnancy, maternal and cord

blood samples were collected at birth from a cohort of pregnant women from the greater New Orleans area in Louisiana between November 2020 and February 2022. Of the 272 samples (158 maternal blood and 114 cord blood), 86 samples were excluded for incomplete maternal–dyad pairs, missing sample collection at birth, or missing clinical information, resulting in 93 mother–fetus dyads included in this study (Fig 1, *A*). Vaccination status was determined by vaccination records verified through the Louisiana department of health. Infection history was defined as either a positive PCR test and/or serologic evidence of infection obtained by detection of anti-N protein IgG. Within these 93 dyads, 12 dyads were infection and vaccination naive (uninfected; U); 32 dyads reported maternal infection during pregnancy but were not vaccinated (infected; I); 23 dyads reported maternal vaccination during pregnancy but did not report COVID-19 infection (vaccinated; V); and 24 dyads reported COVID-19 during pregnancy and were subsequently vaccinated (infected and vaccinated, I + V). Two dyads could not be classified because of conflicting vaccine records and/or inconclusive serology. The clinical demographic information for each group is shown in Table I. Of those who were vaccinated, 36 mothers received a 2-dose regimen of mRNA vaccine (n = 26 Pfizer BNT162b2; n = 10 Moderna mRNA-1273) and 2 received the Johnson & Johnson Ad26-vectored vaccine (Table I). Because the timing of maternal vaccination and/or infection to birth affects the magnitude and kinetics of humoral immune responses, time from maternal vaccination and/or infection to time of sample collection was considered. We calculated the mean number of days between maternal infection and/or vaccination to birth (sample collection) and compared the infected and vaccinated groups and found no significant differences between groups (Fig 1, *B*).

Transfer of virus-specific IgG from mother to fetus is equivalent between infection and vaccination, with higher IgG levels observed with infection plus vaccination

To characterize the humoral immune response transferred from mother to fetus, we analyzed maternal plasma and cord blood samples using a systems serology approach to determine both the quantity and antiviral quality of virus-specific antibodies, including neutralization and Fc-mediated innate immune effector functions (phagocytosis, NK cell activation, and complement deposition), against the SARS-CoV-2 spike protein to enable comparisons across vaccination and infection.

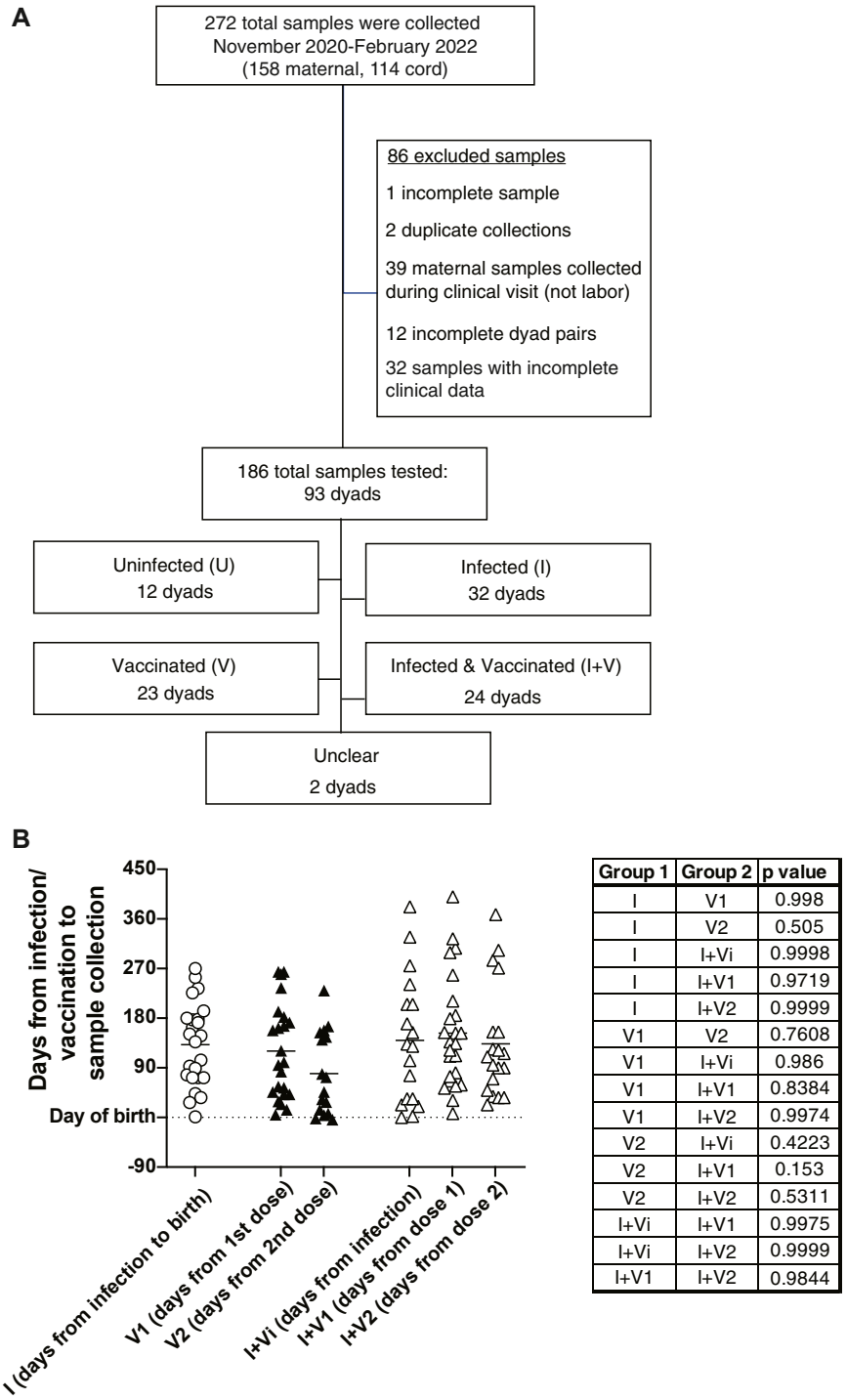
We first determined the levels of SARS-CoV-2 spike, receptor binding domain (RBD), and N-specific antibodies in plasma samples. Antibody responses against the N protein were only observed in the context of infection (see Fig E1 in the Online Repository at www.jaci-global.org), as expected, given that all the vaccine responses analyzed in this study are directed against the spike antigen. Across the maternal and fetal samples, I, V, and I + V samples had significantly higher levels of spike-specific IgG1 compared to the naive group. Importantly, although there was no significant difference between levels of spike-specific IgG1 between mothers with prior infection and those who had only been vaccinated, these antibodies were significantly elevated in both maternal and fetal plasma when the mother had been both infected and vaccinated during pregnancy compared to infection alone (Fig 2, *A*). With respect to the other IgG subclasses, while infection and/or vaccination induced significantly higher levels of spike-specific IgG2, IgG3, and IgG4 compared to uninfected mothers, we did not observe differences between infection and vaccination groups (Fig 2, *B,* and see Fig E2, *A* and *B,* in the Online Repository). Thus, these data suggest that vaccination of mothers after SARS-CoV-2 infection predominantly boosted levels of spike-specific IgG1.

Transfer of maternal antibodies across the placenta is mediated by the neonatal Fc receptor, FcRn, which has an affinity for all human IgG subclasses (IgG1, IgG2, IgG3, IgG4) but not for IgA or IgM.[11-13] To determine whether infection-induced or vaccine-induced antibodies differed in transfer across the placenta, we determined the percent of fetal response relative to the maternal response for IgG, IgA, and IgM levels between groups. A fetal response <90% of the maternal response was considered reduced, and the percentage of dyads with reduced fetal responses is shown in Fig 2. Comparisons across SARS-CoV-2 naive, I, V, and I + V dyads showed equivalent transfer of spike-specific IgG subclasses from mother to fetus (Fig 2, *A* and *B,* and Fig E2, *A* and *B*). In contrast, we did not observe any transfer of spike-specific IgM or IgA (Fig E2, *C-E*), consistent with FcRn-mediated transfer of IgG, but not IgM or IgA.

Transfer of neutralizing and functional virus-specific IgG from mother to fetus is elevated with vaccination

Neutralizing antibodies have been shown to play a critical role in the prevention of fatal COVID-19 outcomes,[14] and they have further been associated with vaccine-mediated protection.[15] We determined neutralizing antibody titers using a lentivirus-based pseudovirus expressing the SARS-CoV-2 D614G spike protein. Comparison of neutralizing antibody titers across I, V, and I + V dyads showed a significantly higher neutralizing antibody response in samples from mothers who were vaccinated compared to those who were infected (Fig 2, *C*). Importantly, this elevation in neutralizing antibody levels in the context of vaccination was mirrored in the corresponding cord plasma (Fig 2, *C*). Because we did not observe a significant difference in neutralizing antibody response between maternal and cord blood, these data suggest that neutralizing antibodies are efficiently transferred *in utero*. Moreover, we observed a significantly higher neutralizing antibody response in the V group compared to the I group. Because this response was observed in both the maternal and cord plasma, these data suggest that vaccination induces significantly higher neutralizing antibody response compared to natural infection (Fig 2, *C*).

In addition to mediating neutralization, antibodies can leverage innate immune cells to limit infection and dissemination,[16] including antibody-dependent activation of NK cells, antibody-dependent phagocytosis, and ADCD. Thus, we measured the activation of human NK cells, induction of phagocytosis in monocytes, and induction of complement deposition by spike-specific antibodies across the cohort (Fig 2, *D-F*). Surface expression of CD107a was used to measure antibody-mediated induction of NK cell degranulation and is a surrogate marker of ADCC.[17] Significantly higher levels of NK cell degranulation were observed in plasma from V dyads compared to I dyads (Fig 2, *D*). Similarly, ADCD was elevated in V dyads compared to I dyads. (Fig 2, *E*). In contrast, antibody-mediated phagocytic activity was not significantly different between I and V groups (Fig 2, *F*).



Group 1	Group 2	p value
I	V1	0.998
I	V2	0.505
I	I+Vi	0.9998
I	I+V1	0.9719
I	I+V2	0.9999
V1	V2	0.7608
V1	I+Vi	0.986
V1	I+V1	0.8384
V1	I+V2	0.9974
V2	I+Vi	0.4223
V2	I+V1	0.153
V2	I+V2	0.5311
I+Vi	I+V1	0.9975
I+Vi	I+V2	0.9999
I+V1	I+V2	0.9844

FIG 1. Schematic of samples included in this study. **(A)** Flowchart showing number of samples collected, number of dyads excluded for incomplete clinical data or paired sampling, and groupings of final dyads included. **(B)** Days from infection or vaccination (first dose [V1] or second dose [V2]) to sample collection were compared between groups by 1-way ANOVA with corrections for multiple comparisons. Table at *right* shows *P* values between comparisons.

We determined the percentage of maternal and cord samples that were above the positive threshold for each assay. Importantly, we found that 100% of maternal and fetal samples from vaccinated groups had neutralizing activity, 100% of fetal samples from vaccinated mothers had ADCD activity and spike-specific IgG1, and 100% of fetal samples from I + V mothers had NK cell activation (Table II). Thus, vaccination of both infected and uninfected mothers increased the proportion of cord blood samples containing antiviral antibodies mediating neutralization, NK cell

TABLE I. Clinical demographic data

Characteristic	COVID-19$^-$ and unvaccinated	COVID-19$^+$ and unvaccinated	COVID-19$^-$ and vaccinated	COVID-19$^+$ and vaccinated
No. (%)	12 (13.19)	32 (35.16)	23 (25.27)	24 (26.37)
Maternal age (years), mean (SD)	29.00 (4.43)	29.78 (5.29)	33.04 (5.10)	30.71 (4.90)
Maternal race				
White	6 (50.00)	23 (74.19)	16 (80.00)	12 (50.00)
Black	5 (41.67)	6 (19.35)	2 (10.00)	11 (45.83)
Asian	0	0	0	0
Other	1 (8.33)	2 (6.45)	2 (10.00)	1 (4.17)
Missing	0	1	3	0
Maternal ethnicity				
Hispanic	1 (8.33)	17 (53.13)	3 (13.04)	2 (8.33)
Non-Hispanic	11 (91.67)	13 (40.63)	18 (78.26)	20 (83.33)
Not applicable	0	1 (3.13)	2 (8.70)	2 (8.33)
Unknown/not reported	0	1 (3.13)	0	0
Delivery season				
Nov-Dec 2020	0	0	0	0
Jan-Mar 2021	2 (16.67)	13 (43.33)	1 (4.35)	0
Apr-Jun 2021	5 (41.67)	5 (16.67)	4 (17.39)	1 (4.17)
Jul-Sep 2021	3 (25.00)	8 (26.67)	4 (17.39)	8 (33.33)
Oct-Dec 2021	2 (16.67)	3 (10.00)	12 (52.17)	6 (25.00)
Jan-Feb 2022	0	1 (3.33)	2 (8.70)	9 (37.50)
Missing	0	2	0	0
Mode of delivery				
Vaginal	7 (58.33)	20 (62.50)	19 (82.61)	17 (70.83)
Total C-section	5 (41.67)	12 (37.50)	4 (17.39)	7 (29.17)
Emergency C-section	2 (40.00)	5 (45.45)	3 (100.00)	3 (60.00)
Scheduled C-section	3 (60.00)	6 (54.55)	0	2 (40.00)
Missing type of C-section	0	1	1	2
Missing mode of delivery	0	0	0	0
Gestational age (weeks), mean (SD)	37.54 (3.17)	38.63 (2.17)	38.69 (1.36)	38.26 (2.80)
Missing	0	3	1	0
Sex of infant				
Male	5 (41.67)	11 (34.38)	15 (65.22)	7 (29.17)
Female	7 (58.33)	21 (65.63)	8 (34.78)	17 (70.83)
Vaccination date (first dose)				
Vaccinated <20 gestational weeks	NA	NA	10 (47.62)	13 (54.17)
Vaccinated >20 gestational weeks	NA	NA	11 (52.38)	11 (45.83)
Missing			2	0
Time from vaccination to sample collection				
First dose (days), mean (SD)	NA	NA	130.9 (80.61)	152.8 (102.1)
Second dose (days), mean (SD)	NA	NA	79.5 (75.15)	133.5 (101.2)
Vaccination type				
Pfizer	NA	NA	14 (66.67)	16 (72.73)
Moderna	NA	NA	5 (23.81)	5 (22.73)
Johnson & Johnson	NA	NA	1 (4.76)	1 (4.55)
Unknown	NA	NA	1 (4.76)	0
Missing			2	2
Infection date, COVID-19 positivity date				
Infected <20 gestational weeks	NA	11 (50.00)	NA	9 (52.94)
Infected >20 gestational weeks	NA	11 (50.00)	NA	8 (47.06)
Missing		10		7
Time from infection to sample collection (days), mean (SD)	NA	132 (75.13)	NA	139.6 (115.4)
Infection date, symptom date				
Infected <20 gestational weeks	NA	5 (22.73)	NA	7 (43.75)
Infected >20 gestational weeks	NA	9 (40.91)	NA	6 (37.50)
No symptoms	NA	8 (36.36)	NA	3 (18.75)
Missing		10		8

Numbers of dyads within clinical or demographic categories are indicated. Percentage of dyads in each category within COVID-19–negative and COVID-19–positive categories are indicated after number of dyads. *NA,* Not applicable.

activation, and complement deposition compared to cord blood samples from mothers who had been infected but not vaccinated.

Vaccination within the last 20 weeks of pregnancy has been associated with increased vaccine efficacy against infant hospitalization.[1] Thus, we next determined if antibody quality or

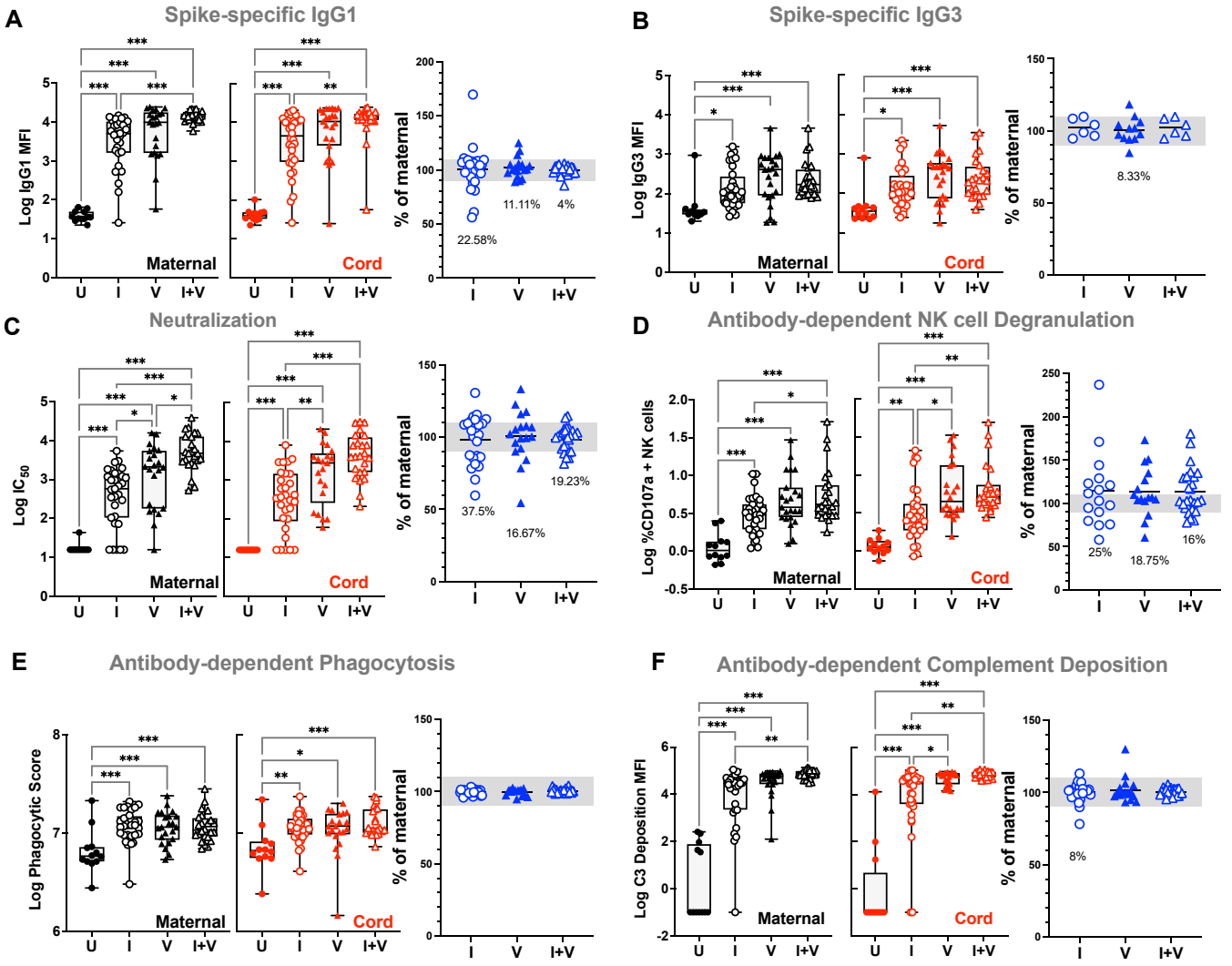


FIG 2. Transfer of antiviral virus-specific antibodies from mother to fetus is elevated with vaccination. Maternal *(black)* and cord *(red)* plasma samples obtained at birth/labor within U, I, V, and I + V groups were analyzed for levels of: **(A)** IgG_1 spike-specific antibodies, **(B)** IgG_3 spike-specific antibodies, **(C)** neutralizing antibodies, **(D)** antibody-dependent NK cell degranulation, **(E)** ADCD, and **(F)** antibody-dependent cellular phagocytosis. Graphs at *left* show log_{10} values; graphs at *right,* ratio of maternal antiviral antibodies transferred to fetus, expressed as fetal response as percentage of corresponding maternal sample for samples with response above positive cutoff for measurement. Cord sample responses between 90% to 110% of maternal responses were considered equivalent *(gray box),* and percentage of samples <90% are indicated. Significance was determined by ordinary 1-way ANOVA and multiple comparisons by Tukey multiple comparisons test. $*P < .05$, $**P < .001$, $***P < .0001$.

quantity in cord blood differed by time of vaccination. Although spike-specific IgG1 levels were not significantly different in maternal or cord samples between those vaccinated before or after 20 weeks of gestation (Fig 3, *A*), spike-specific IgG3 levels were elevated in maternal samples from those vaccinated after 20 weeks (Fig 3, *B*). Qualitatively, we did not observe statistically significant differences in antiviral functionality in antibodies induced via vaccination before or after 20 weeks (Fig 3, *C-F*). We performed a similar analysis within our cohort to determine if antibody transfer differed depending on when SARS-CoV-2 infection occurred during pregnancy, but we did not observe differences in the quality or quantity of antibodies between infection periods (see Fig E3 in the Online Repository at www.jaci-global.org). Thus, these data suggest that vaccination during pregnancy boosts the production of antiviral antibodies, which are equally transferred to the fetus, irrespective of the stage of pregnancy during which vaccination or infection occurs.

Because IgG3 is considered the most functional IgG subclass,[16] given the increase in spike-specific IgG3 in maternal samples if vaccinated in the last 20 weeks of pregnancy, we performed correlation analyses to determine if IgG3 levels were associated with an increase in antiviral effector functions (see Fig E4 in the Online Repository at www.jaci-global.org). Within maternal samples, only infection induced IgG3 correlated with neutralization and NK cell activation, whereas IgG1 induced by either infection or vaccination was strongly correlated with all functions (Fig E4, *A*). Within cord samples, infection-induced IgG3 correlated only NK cell activation, and both infection- and

TABLE II. Proportion of subjects positive for each humoral measurement

	Neutralizing		ADNK		ADCP		ADCD		IgG_1 spike		IgG_2 spike		IgG_3 spike		IgG_4 spike	
Assessment	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
% Positive																
U	8.3	0.0	0.0	0.0	16.7	16.7	0.0	8.3	0.0	8.3	0.0	8.3	8.3	8.3	8.3	0.0
I	83.9	78.1	64.5	51.6	81.3	78.1	77.4	80.6	96.9	96.9	25.0	12.5	21.9	25.0	6.3	15.6
V	95.5	100.0	82.6	95.5	68.2	81.0	91.3	100.0	95.5	95.7	39.1	34.8	56.5	56.5	34.8	39.1
I + V	100.0	100.0	91.7	100.0	70.8	91.7	100.0	100.0	100.0	95.8	20.8	20.8	25.0	33.3	29.2	33.3
Chi-square test, *P* value																
I vs V	.190	.021	.142	.001	.270	.804	.176	.028	.786	.811	.263	.048	.008	.018	.026	.048
I vs I + V	.039	.014	.019	.0001	.361	.172	.013	.022	.382	.835	.715	.401	.784	.495	.021	.120
I vs V	NS	*	NS	**	NS	NS	NS	*	NS	NS	NS	*	**	*	*	*
I vs I + V	*	*	*	***	NS	NS	*	*	NS	NS	NS	NS	NS	NS	*	NS

Percentage of subjects with increased humoral immune response was compared to naive group. Statistically significant differences in frequency between I only and either V or I + V was determined by chi-square test. *$P < .05$, **$P < .001$, ***$P < .0001$, *NS,* not significant.
ADCP, Antibody-dependent cellular phagocytosis; *ADNK,* antibody-dependent NK.

vaccine-induced IgG1 levels correlated with neutralization and complement activation (Fig E4, *B*). Taken together, these data indicate that while IgG3 may contribute to NK cell activation in infection-induced responses, higher antiviral antibody functionality activity is highly associated with spike-specific IgG1 induced in the context of infection or vaccination.

We next used multivariate partial least squares discriminant analysis to specifically identify the antibody features that are differentially associated with infection or vaccination. Fifty-seven variables were used in the analysis and included SARS-CoV-2 spike-specific antibody features and seasonal coronaviruses because there is increasing evidence that cross-reactive responses from seasonal coronaviruses may affect immunity to SARS-CoV-2 (Fig 4, and see Table E1 in the Online Repository at www.jaci-global.org).[18-20] We used a LASSO feature selection and dimensionality reduction method to define the minimal number of features needed to predict classification into infection or vaccination (Fig 4, *A*). Within the maternal samples, we found that the features that best classified vaccination-induced antibodies compared to infection-induced antibodies were overall higher levels of spike- and RBD-specific antibodies, consistent with the univariate analysis, followed by measures of antibody-mediated effector function and neutralization. Of note, IgA2 against RBD was selectively enriched in infection-induced antibodies compared to vaccination, potentially reflecting a mucosal response to infection. Interestingly, the model also selected several seasonal coronavirus antibody responses to classify infection-induced responses, including IgG against 229E and NL63, which were also present in the cord blood samples (Fig E4, *A*). To ensure that time from infection or vaccination relative to birth did not affect classification predictions or features differentially associated with infection or vaccination (see Fig E5 in the Online Repository), we determined if the samples within predicted classification differed in time from infection/vaccination to sample collection. We did not observe any differences in time between the predicted classifications, indicating that the models were classifying according to differences in antibody features rather than time from infection or vaccination.

We next identified features that were associated with I + V compared to I alone or V alone to determine if the hybrid immunity reflected responses more like vaccine- or infection-induced immunity (Fig 4, *B* and *C*). Consistent with the univariate analyses, I + V had elevated levels of SARS-CoV-2 spike antibodies compared to either V or I. Interestingly some of features associated with infection in the prior analysis (Fig 4, *A*) remained distinctly associated with infection only, including IgA2 against SARS-CoV-2 RBD and IgG4 levels against some seasonal coronaviruses, suggesting that vaccination shifted or reverted those antibody features that may have been initially induced after infection (Fig 4, *B*). Other infection-induced features remained sustained even after vaccination, including IgG1 levels against NL63 and 229E (Fig 4, *D*). Thus, SARS-CoV-2 maternal infection may have distinct impacts on immunity against seasonal coronaviruses that are transferred to the fetus.

DISCUSSION

The current study found that maternal vaccination during pregnancy enhances the quantity and quality of antiviral antibodies that are transferred to the fetus *in utero* compared to infection. We observed transfer of SARS-CoV-2–specific IgG, but not IgA or IgM, to the fetus, independent of prior COVID-19 infection or vaccination, consistent with the observations from others.[4,12-14] Importantly, we also observed that vaccination irrespective of prior SARS-CoV-2 infection increases the quality of antiviral antibodies in both maternal and fetal samples, with increased neutralizing activity and antibody-mediated activation of NK cells and complement that are associated with enhanced protection against SARS-CoV-2 infection.[15,21-24] Studies like ours are necessary to achieve increased precision in the strategies used to vaccinate pregnant women, which in turn increases the protection of the fetus and newborn against this common and potentially severe infection. Protection of newborns within 6 months of birth is increased when mothers are vaccinated in the last 20 weeks of pregnancy, and we observed elevated levels of the highly functional IgG3 in both maternal and fetal samples in the context of vaccination in the absence of prior COVID-19 infection, which is in line with other studies.[25,26] Although we did not observe increased antiviral functions when vaccination occurred after 20 weeks of pregnancy, the elevated levels of spike-specific IgG_3 may provide protection in infants through additional mechanisms not measured here. Moreover, reduced protection of infants in mothers vaccinated earlier during pregnancy may reflect the waning of maternal antibody titers after vaccination before the placenta is mature enough to allow the active transport of IgG to the fetus, which typically occurs only in the last trimester of

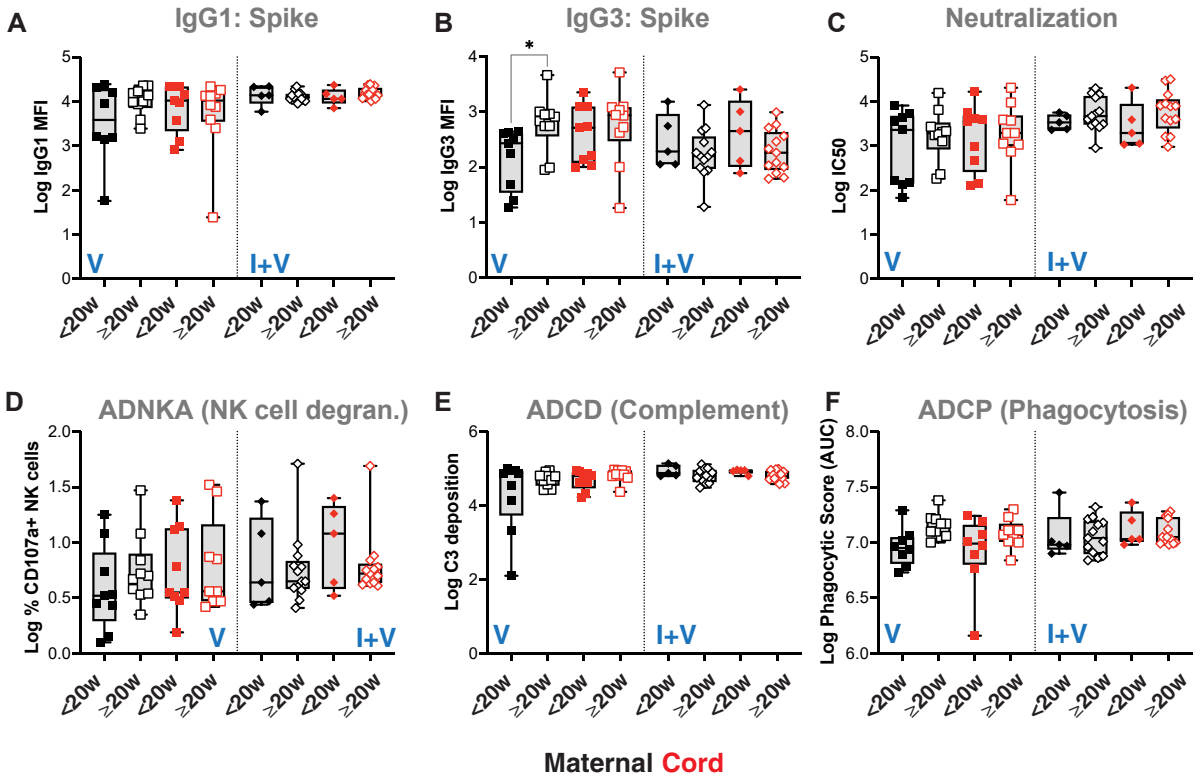


FIG 3. Vaccination during second 20 weeks of pregnancy increased spike-specific IgG_3 response in maternal samples. Maternal *(black)* and cord *(red)* responses for **(A)** IgG_1 spike-specific antibodies, **(B)** IgG_3 spike-specific antibodies, **(C)** neutralizing antibodies, **(D)** antibody-dependent NK cell degranulation, **(E)** ADCD, and **(F)** antibody-dependent cellular phagocytosis were compared between individuals vaccinated within first 20 weeks of pregnancy or last 20 weeks of pregnancy in V or I + V groups. Significance was determined by ordinary 1-way ANOVA and multiple comparisons by Tukey multiple comparisons test. $*P < .05$.

gestation. Also, variability among different dyads with similar vaccination status may reflect individual differences in placental function vis-à-vis the active systems necessary for the transport of immunoglobulins to the fetus. Longitudinal samples from this birth cohort are planned and will be addressed in future studies, which are also going to be instrumental in determining possible correlations between immune protection at birth and the later occurrence of reinfections or development of respiratory symptoms and other clinical outcomes.

Fc effector function can be modified by the Fc glycan, and specific glycan structures greatly affects functional capacity. For example, afucosylation enhanced affinity for FcγRIII, thus boosting ADCC activity. Aging, pregnancy, obesity, and smoking have been shown to affect glycosylation, and elevated levels of afucosylated IgG have been observed in the context of human immunodeficiency virus, dengue virus, and influenza.[27] In the context of SARS-CoV-2, elevated levels of afucosylated antibodies have been observed in hospitalized individuals with severe COVID-19. While we did not measure the glycan structures of spike-specific antibodies in our subjects, none of our subjects was hospitalized, and none reported experiencing severe disease. However, differences in spike-specific IgG glycosylation induced between infection and vaccination may have affected the quality of humoral immune responses.[28-30]

Overall, the data from our study demonstrate the importance of vaccination in pregnancy as a highly effective method of protecting the fetus and newborn from COVID-19 infection. Importantly, the advantage provided by maternal vaccination is independent of the natural occurrence of infection during pregnancy. One obvious advantage of COVID-19 vaccination of pregnant women is the prevention of preterm delivery, the incidence of which is increased by infection and is associated with serious postnatal complications affecting virtually every organ of the offspring. Data obtained from the same birth cohort also show that SARS-CoV-2 can be vertically transmitted in pregnancy from an infected mother to her fetus in approximately 1 of 10 pregnancies (data not shown), which reinforces the importance of vaccination for fetal protection. Furthermore, a COVID-19 infection in a pregnant woman can affect fetal development even in the absence of physical transfer of virus through the placenta by triggering maternal immune activation, which in turn leads to maternal and placental inflammation with expression of proinflammatory cytokines during critical developmental windows. For example, data show that SARS-CoV-2 exposure *in utero* may be associated with neurodevelopmental sequelae in some offspring[26] similar to those consistently shown with other vertically transmitted respiratory viruses, such as influenza. Finally, it must be emphasized that many complications related to vertically transmitted infection, or to the consequences of maternal immune activation, might require a long time to manifest, and therefore may become clinically evident only in late childhood or even early adulthood. Thus, only prospective studies

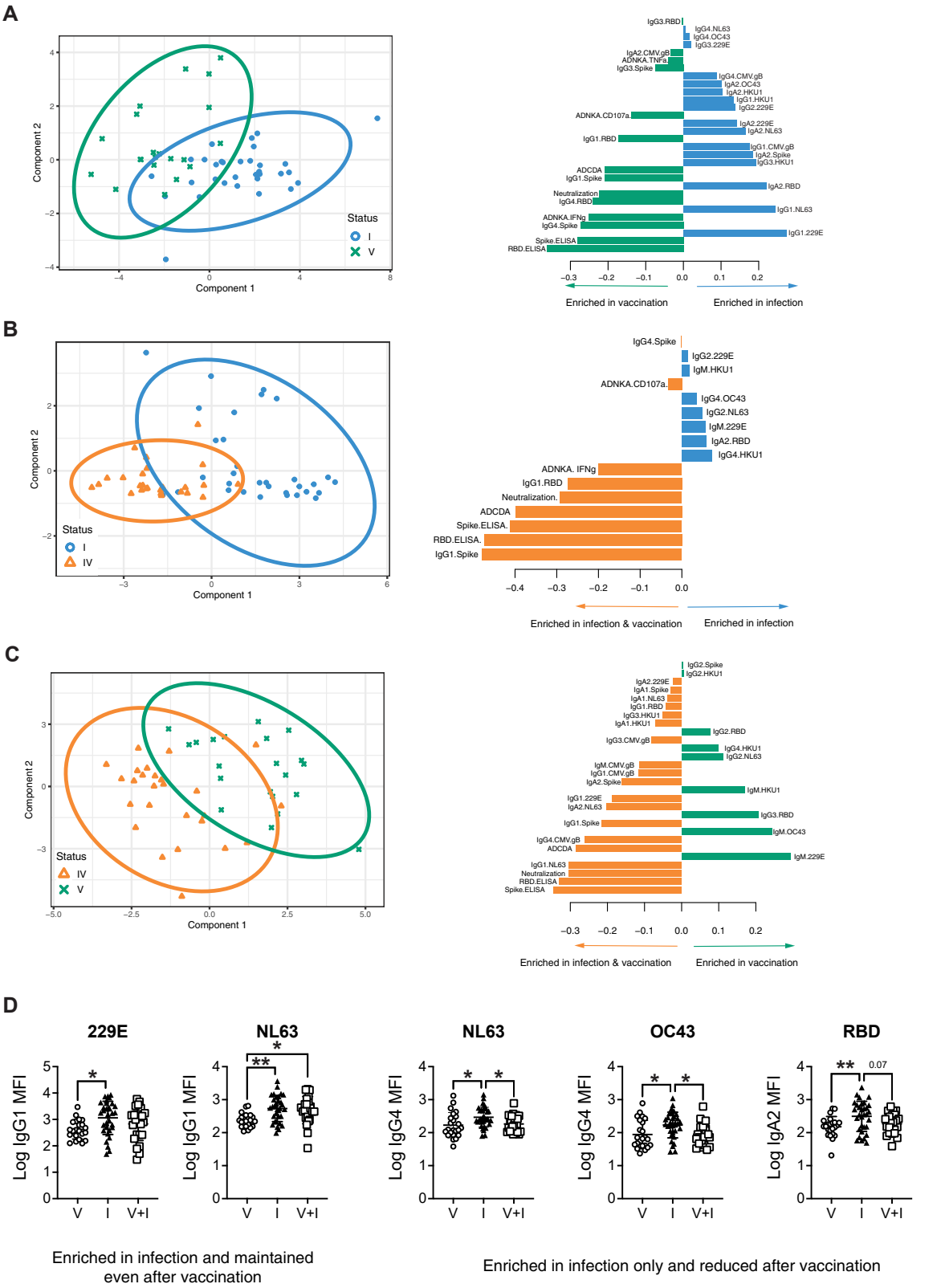


FIG 4. Distinct humoral profiles against seasonal coronaviruses are induced and sustained after infection despite subsequent vaccination. sPLS-DA was used to define minimal features needed to discriminate between **(A)** I and V, **(B)** I and I + V, and **(C)** V and I + V maternal antibody profiles. Loading plot *(left)* and variable importance in projection (VIP; *right*) are shown. **(D)** Features elevated and sustained after infection are at *left;* features elevated only after infection are at *right* in univariate comparison plots between V, I, and I + V groups. Significance was determined by ordinary 1-way ANOVA and multiple comparisons by Tukey multiple comparisons test. $*P < .05$, $**P < .001$.

with long follow-up will be able to uncover the true dimensions of perinatal COVID-19 infections.

DISCLOSURE STATEMENT

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Clinical implications: The most effective passive transfer of functional antibodies against SARS-CoV-2 *in utero* is achieved through vaccination, highlighting the importance of vaccination in pregnant women.

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