ImmunoHorizons

RESEARCH ARTICLE

Infectious Disease

Level and Duration of IgG and Neutralizing Antibodies to SARS-CoV-2 in Children with Symptomatic or Asymptomatic SARS-CoV-2 Infection

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ABSTRACT

There are conflicting data about level and duration of Abs to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in children after symptomatic or asymptomatic infection. In this human population, we enrolled adults and children in a prospective 6-mo study in the following categories: 1) symptomatic, SARS-CoV-2 PCR$^+$ (SP$^+$; children, $n = 8$; adults, $n = 16$), 2) symptomatic, PCR$^-$, or untested (children, $n = 27$), 3) asymptomatic exposed (children, $n = 13$), and 4) asymptomatic, no known exposure (children, $n = 19$). Neutralizing Abs (nAbs) and IgG Abs to SARS-CoV-2 Ags and spike protein variants were measured by multiplex serological assay. All SP$^+$ children developed nAb, whereas 81% of SP$^+$ adults developed nAb. Decline in the presence of nAb over 6 mo was not significant in symptomatic children (100 to 87.5%; $p = 0.32$) in contrast to adults (81.3 to 50.0%; $p = 0.03$). Among children with nAb ($n = 22$), nAb titers and change in titers over 6 mo were similar in symptomatic and asymptomatic children. In children and adults, nAb levels postinfection were 10-fold lower than those reported after SARS-CoV-2 mRNA vaccination. Levels of IgG Abs in children to SARS-CoV-2 Ags and spike protein variants were similar to those in adults. IgG levels to primary Ags decreased over time in children and adults, but levels to three spike variants decreased only in children. Children with asymptomatic or symptomatic SARS-CoV-2 infection develop nAbs that remain present longer than in adults but wane in titer over time and broad IgG Abs that also wane in level over time. However, nAb levels were lower postinfection than those reported after immunization. *ImmunoHorizons*, 2022, 6: 408–415.

INTRODUCTION

Since the onset of the COVID-19 pandemic, 56.9 million children were infected with COVID-19 with ~17,200 pediatric deaths globally (1). The latest COVID-19 surges increasingly affected children, yet COVID-19 vaccines were only recently approved for children over the age of 5 y, and no vaccine is available for children under 5. Understanding the breadth and durability of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunity in children after natural infection is critical to guide future protective measures for children.

Children typically have milder symptoms and less severe disease with SARS-CoV-2 infection than adults. One mechanism thought to mediate protection is the development of neutralizing Abs (nAbs) to SARS-CoV-2, which have been shown to be predictive of protection from symptomatic COVID-19

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Abbreviations used in this article: AE, asymptomatic exposed; ANE, asymptomatic, no known exposure; nAb, neutralizing Ab; NCP, nucleocapsid protein; NTD, N-terminal domain; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SP$^-$, symptomatic PCR$^-$ or untested; SP$^+$, symptomatic PCR$^+$.

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infection (2) and are often used as a correlate of protective immunity to SARS-CoV-2. Although there are numerous studies of humoral immunity in adults, studies in children are limited, with conflicting results (3–17). Two recent and large studies in children demonstrated that children develop nAbs at similar levels and with similar duration to those seen in adults postinfection (8, 12). Yet in earlier studies, children with severe or mild to severe COVID-19 had lower nAb levels and duration than adults (4, 11, 17). It is not clear whether age, severity of COVID-19 infection, presence of specific SARS-CoV-2 variants, or other factors are responsible for the different study findings.

To evaluate how level and duration of nAbs and IgG Abs to SARS-CoV-2 differ in children versus adults and whether symptomatic versus asymptomatic infection affects this course in children, we conducted a study in adults and children starting early in the pandemic in the United States (June 2020) and evaluated these responses over a 6-mo period of variable SARS-CoV-2 transmission.

MATERIALS AND METHODS

Study population, enrollment, and follow-up

From June 18 to December 29, 2020, children (<18 y old) and adults were enrolled in the Development of Immunity to SARS-CoV-2 after Exposure and Recovery (DISCOVER) study in Indianapolis, IN. Individuals were enrolled in four subject categories according to symptoms of and exposure to COVID-19 infection: 1) symptomatic PCR^+ (SP^+), 2) symptomatic PCR^- or untested (SP^-), 3) asymptomatic exposed (AE), and 4) asymptomatic, no known exposure (ANE). Symptomatic children included those who reported persistent cough, fever, chills, sore throat, shortness of breath, headache, new loss of taste or smell, muscle pain, or diarrhea 21 d to 3 mo prior to study enrollment. SP^+ individuals had a documented positive SARS-CoV-2 naso- or oropharyngeal PCR test, and SP^- had a negative PCR test or were not tested. Asymptomatic children reported no symptoms of COVID-19 in the 3 mo prior to study enrollment. AE children were in close contact with an individual with symptoms and a positive SARS-CoV-2 PCR. ANE children had no known close contact with an individual with confirmed or suspected COVID-19 requiring quarantine. Exclusion criteria included acute illness at study enrollment, immunocompromising conditions, or receipt of convalescent plasma.

Blood samples were collected at study enrollment and 6 mo after enrollment. Vaccination was available for adults but not children during the later follow-up period, and date of vaccination for those vaccinated was recorded at the 6-mo visit.

Serological assays

Luminex xMAP technology is a multiplex, flow cytometry-based platform that allows the simultaneous quantitation of many protein analytes in a single reaction (18). A custom Luminex-based assay was developed to measure serology and Ab ACE2–receptor-binding domain (RBD) binding inhibition in a single assay as previously described (19, 20).

Surrogate nAbs. Patient serum samples were titrated (1:20–1:4.3E8) in PBS–high-salt solution (0.01 M PBS, 1% BSA, 0.02% Tween, and 300 mM NaCl). Diluted serum samples were combined with Luminex MagPlex microspheres coupled with individual Ags and a recombinant, labeled RBD-PE protein and incubated for 60 min to allow endogenous Abs to bind to either recombinant RBD-PE or Ag-coated Luminex beads. The suspension was placed on a magnet, collecting the MagPlex beads, while the supernatant was transferred to a new plate. The transferred solution was combined with ACE2-coated beads and incubated for 60 min; remaining beads were washed and incubated for 60 min with anti–IgG-PE beads to detect bound Abs. All beads on both plates were washed and resuspended in a PBS-1% BSA solution and read using a Luminex FLEXMAP 3D System with xPONENT Software. The titer was evaluated from the median fluorescence intensity, and the ability of the endogenous Abs to inhibit RBD-ACE2 binding was calculated based on the IC_{50}, which represents the Ab titer at which the ACE2-RBD binding is reduced by half. The ACE2 binding inhibition potency was assessed using the inverse of IC_{50}.

IgG to SARS-CoV-2 Ags. Ag-coated microspheres were used to detect and quantitate endogenous IgG Abs against SARS-CoV-2 proteins, including spike–N-terminal domain (NTD) and several mutant RBD epitopes (Supplemental Table I). Testing was done before widespread delta and omicron variant infections, and Abs to these variants were not tested.

Statistical analysis

For the serial dilution-based serology assay, titer was calculated based on interpolating assay values that straddled the predetermined cut point (19). To calculate IC_{50} of data from the ACE2 neutralization component of the serology assay, a four-parameter logistic function was used to estimate the absolute IC_{50} based on 1/dilution factor. If a sample indicated no neutralization, the IC_{50} was imputed to 1 (20 times the maximum 1/dilution factor).

Pairwise comparisons in the same individual were done using the McNemar test for Ab prevalence and Wilcoxon matched-pairs signed-rank testing for titers. For comparisons across groups (children compared with adults or vaccine versus no vaccine in adults), a χ^2 test or rank-sum was used for comparisons of prevalence or titers, respectively. No adjustment for multiple comparisons was done in the primary analysis because only two comparisons (by time point or by group at each time point) were performed, and none was made for the secondary analysis because it was exploratory. Statistical analyses were conducted using Stata V16 (StataCorp LP, College Station, TX), and figures were created using GraphPad Prism 9.

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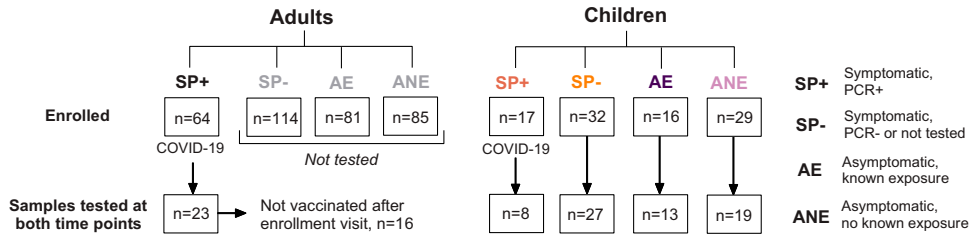

FIGURE 1. Study flowchart.

RESULTS

Demographic characteristics of children and adults in the study and SARS-CoV-2 epidemiology in Indiana at the time of the study

A total of 94 children and 344 adults were enrolled in the study (Fig. 1). For this study on duration of Abs, we included only children and adults who had samples collected at enrollment and 6 mo after enrollment, and with the focus on children and limited testing capacity, we tested children from all 4 groups but only adults with COVID-19 (SP^+). From the individuals with samples at both time points, all children with COVID-19 (SP^+; $n = 8$) and 23 of 58 SP^+ adults were tested, as well as 27 of 29, 13 of 13, and 19 of 27 children from the SP^-, AE, and ANE groups (Fig. 1). Local state and county case rates of COVID-19 during the study period relative to sample collection times are shown in Supplemental Fig. 1. Pediatric participants were 6 mo to 17 y old (Table I). ANE children were significantly older than AE children. There were no differences in sex between categories. A total of 13% of adults and 13% of children were Black, and 13% of adults and 6% of children were Hispanic. There were no Asian or Native American participants in the group tested.

Children with symptomatic SARS-CoV-2 infection develop nAbs more frequently than AE or ANE children, but titers among Ab-positive children do not differ in symptomatic versus asymptomatic children

One hundred percent of SP^+ children developed nAbs to SARS-CoV-2 compared with 30%, 39%, and 5% in the SP^-, AE, and ANE groups, respectively (Fig. 2A). Ab titers among nAb-positive symptomatic versus asymptomatic children did not differ (Fig. 2C).

nAb seroprevalence and titers decrease in children with symptomatic or asymptomatic SARS-CoV-2 infection after 6 mo

Among the 22 children with nAb at enrollment, 17 had nAb at 6 mo (Fig. 2B) ($p = 0.03$). Titers decreased significantly in children with symptomatic or asymptomatic infections (Fig. 2C).

Children with symptomatic SARS-CoV-2 infection develop nAbs to SARS-CoV-2 at a similar proportion and level to adults and for a longer duration

We next compared nAb presence titers and duration between SP^+ children and adults (unvaccinated between visits, $n = 16$). All children (100%) developed nAb, whereas only 81% of adults developed nAb (Fig. 3, Supplemental Table I). At 6 mo, 88% of SP^+ children had nAb, and only 50% of SP^+ adults had nAb present (Fig. 3, Supplemental Table II). Thus, a greater proportion of adults lost nAb by 6 mo. nAb titers decreased in both SP^+ children and adults over 6 mo, and the mean difference in nAb titers was greater in adults than children, but this difference was not statistically significant, nor did the slope of change in titer differ between children and adults (Fig. 3, Supplemental Table II).

Children with COVID-19 develop IgG Abs to SARS-CoV-2 Ags and variants at similar or higher levels to adults with COVID-19 and have similar decreases in Ab levels 6 mo after initial infection

We next compared IgG Abs to different SARS-CoV-2 proteins between adults and children with COVID-19 (the SP^+ group), specifically examining total spike protein (ST4), RBD1 and 2, nucleocapsid protein (NCP), and NTD (Supplemental Table I).

TABLE I. Demographic characteristics according to age and cohort

	Adults, Symptomatic, PCR^+ ($n = 23$)		Children ($n = 67$)			
Characteristic	No Vaccine ($n = 16$)	Vaccine after Visit 1 ($n = 7$)	Symptomatic, PCR^+ ($n = 8$)	Symptomatic, No Testing or PCR^- ($n = 27$)	Asymptomatic, Exposed ($n = 13$)	Asymptomatic, No Known Exposure ($n = 19$)
Age (y), mean (SD)	37.2 (11.7)	35.9 (8.2)	8.0 (7.1)	7.4 (4.5)	4.5 (4.4)	11.2 (3.4)[a]
Sex (female), *n* (%)	10 (62.5)	3 (42.9)	4 (50.0)	8 (29.6)	6 (46.2)	7 (36.8)

[a]Asymptomatic children with no known exposure were significantly older than asymptomatic, exposed children ($p = 0.001$, Tukey post hoc test).

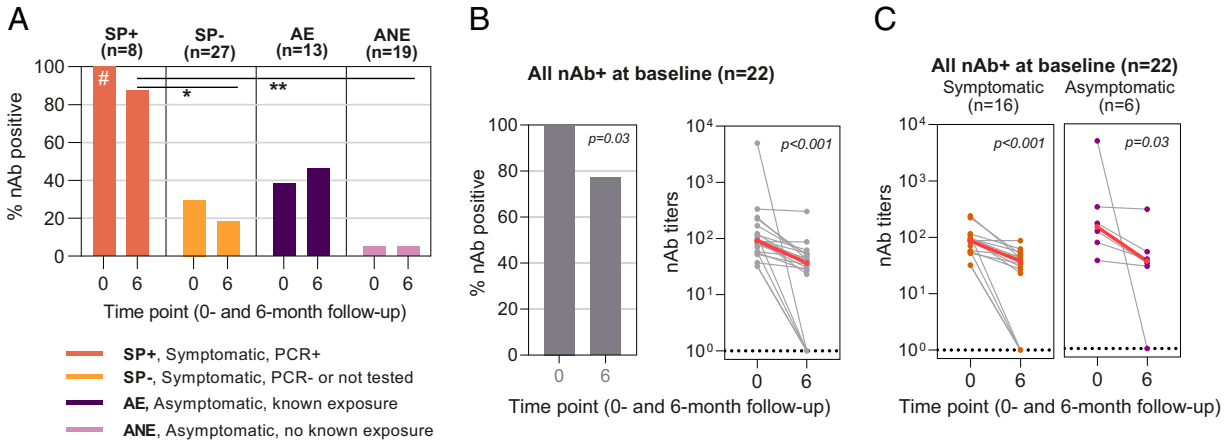

FIGURE 2. nAb prevalence and titers at baseline and 6-mo follow-up in children according to study group (A), all children with nAb at baseline (B), and comparing children who were in the symptomatic groups to asymptomatic groups (C).

Prevalence compared by visit using McNemar test and by study cohort using Fisher exact test, with Bonferroni correction for pairwise comparisons. Red dots and lines represent median of titers. Of the 22 children with positive nAb at baseline, 8 were SP^+, 8 SP–, 5 AE, and 1 ANE. Titers compared by visit using Wilcoxon matched-pairs signed-rank test and by study cohort using Wilcoxon rank-sum test. #Significantly different from all other groups: $*p < 0.05$, $**p < 0.001$.

At the initial visit, children had similar Ab levels as adults and higher levels of RBD1 IgG compared with adults (Fig. 4). Children and adults had significant decreases in Ab levels to RBD2 and NCP, whereas only children had significant decreases RBD1, and there were no significant differences in Ab levels to ST4 or NTD in children or adults (Fig. 4). SP^-, AE, and ANE had no changes in these IgG levels in the second visit (Supplemental Fig. 2).

Given the continual emergence of novel SARS-CoV-2 variants, we also evaluated IgG Abs to several spike protein variants (Supplemental Table I). These variants did not include the delta or omicron variants, which were not well established at the time of testing. SP^+ children developed robust IgG Abs to every variant tested and had higher levels of Abs to the E484Q and K417N variants than SP^+ adults (Fig. 4). IgG Abs to all spike variants decreased significantly in SP^+ children over time, but IgG Abs to three of the spike variants did not differ significantly over time in SP^+ adults (Fig. 4). We also examined changes in IgG Abs in SP^-, AE, and ANE. SP^- had decreases in levels of IgG to E484Q and delta 69-70 NTD, but asymptomatic children had no change in IgG levels at 6 mo, likely reflecting the large proportion of children who were nAb negative in these groups at baseline (Supplemental Fig. 1). Neither nAbs nor IgG Abs in any child group correlated with age (all $p > 0.05$).

Vaccinated adults develop nAb and IgG Ab titers to multiple SARS-CoV-2 Ags higher than those postinfection and have a slow linear decrease in nAb titers over time

Vaccines were unavailable to children during our study period, but 7 SP^+ adults received COVID-19 vaccine between the

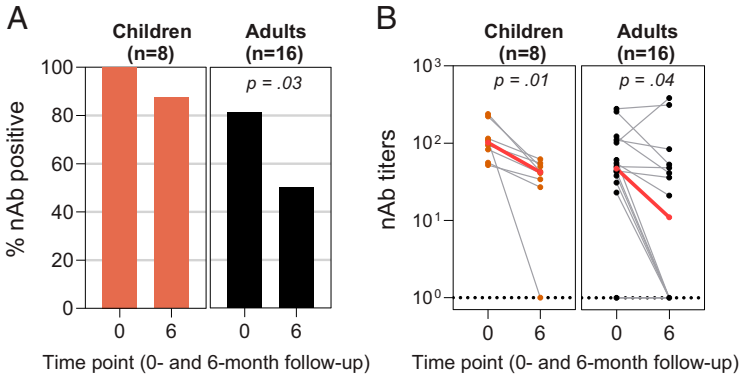

FIGURE 3. Prevalence (A) and titers (B) of nAbs at study baseline and 6-mo follow-up in SARS-CoV-2 PCR^+, symptomatic unvaccinated children and adults.

Prevalence compared by visit using McNemar test and by age group using χ^2 test. Red dots and lines represent median of titers. Titers compared by visit using Wilcoxon matched-pairs signed-rank test and by age group using Wilcoxon rank-sum test.

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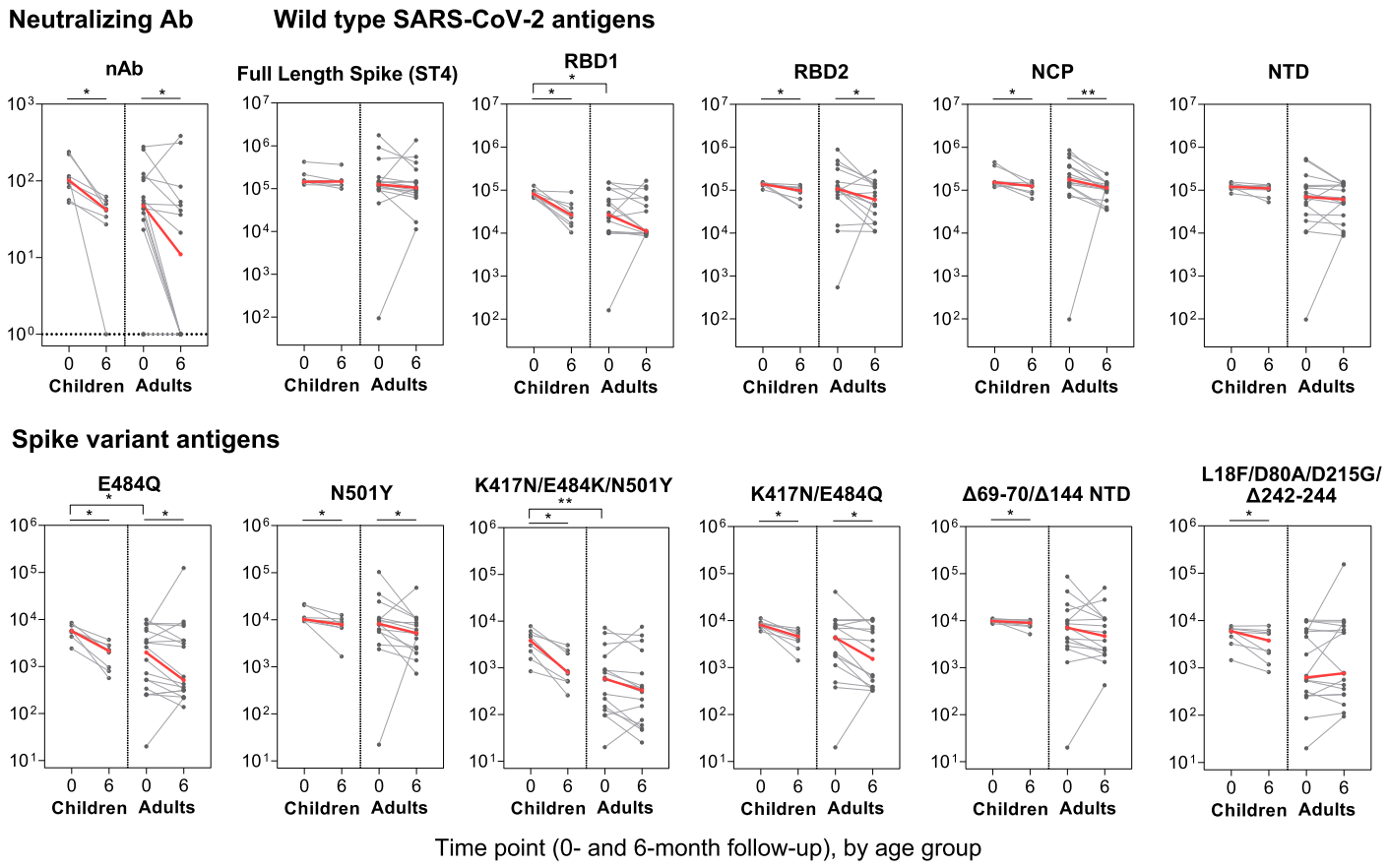

FIGURE 4. Titers at study baseline and 6-mo follow-up in SARS-CoV-2 PCR$^+$, symptomatic unvaccinated children (n = 8) and adults (n = 16). Red dots and lines represent median of titers. Titers compared by visit using Wilcoxon matched-pairs signed-rank test and by age group using Wilcoxon rank-sum test: *$p < 0.05$, **$p < 0.01$. Ag definitions included in *Materials and Methods*.

enrollment and 6-mo visits. We compared Ab titers at the 6-mo visit between vaccinated and unvaccinated adults. All vaccinated adults had nAb at the 6-mo visit, whereas only 50% of unvaccinated adults had nAb in the follow-up visit (Fig. 5A). In vaccinated adults, nAb titers rose to levels higher than those with natural infection (Fig. 5B). However, when we examined

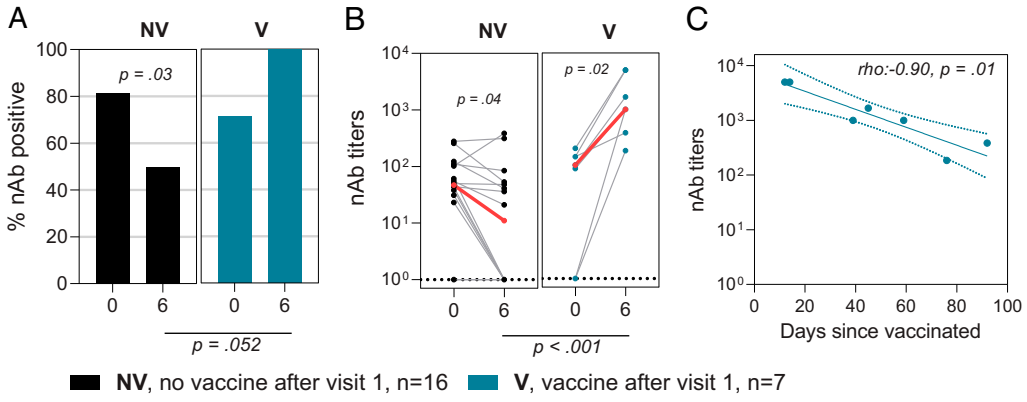

FIGURE 5. Prevalence (A) and titers (B) of nAbs at baseline and 6-mo follow-up in adults who are not vaccinated (NV; n = 16) and vaccinated after first visit (V; n = 7) and association between titers of nAb at 6-mo follow-up and days since first vaccination dose in adults who were vaccinated (C).

Prevalence compared by visit using McNemar test and by vaccination status using Fisher exact test. Red dots and lines represent median of titers. Titers compared by visit using Wilcoxon matched-pairs signed-rank test and by vaccination status using Wilcoxon rank-sum test. Mean (SD) days since first vaccine: 48 (30).

nAb titers relative to the time of vaccination, nAb titers decreased as the days postvaccination increased (Fig. 5C). Ab responses to SARS-CoV-2 spike, RBD 1 and 2 and NTD increased after vaccination, but Abs to NCP decreased in both vaccinated and unvaccinated adults, as expected because the vaccine targets spike protein. Ab levels to spike protein variants were also increased after vaccination, whereas unvaccinated adults had decreases in these Ab levels at the follow-up visit.

DISCUSSION

In this study, we show that children with symptomatic or asymptomatic SARS-CoV-2 infection develop robust nAbs and that these nAbs persist in children with symptomatic SARS-CoV-2 infection at a greater proportion than in adults at 6 mo postinfection, but titers wane over time in both children and adults. Children with symptomatic SARS-CoV-2 infection also develop IgG Abs to multiple SARS-CoV-2 Ags and spike Ag variants at similar or higher levels to adults, and with a generally similar decrease over 6 mo, though for some Ags, the decrease was greater in children than adults. Finally, we show that adults vaccinated against SARS-CoV-2 between the first and second visits develop levels of nAbs and IgG Abs significantly higher than with infection alone. The findings demonstrate the importance of evaluating nAbs in studies of the immune response to SARS-CoV-2 in children and adults, as the IgG Ab levels to some Ags showed a greater decrease in children than adults, whereas a higher proportion of children than adults retained nAbs to SARS-CoV-2 at 6 mo.

Our results support recent studies showing similar or higher level and greater duration of nAbs in children than adults after SARS-CoV-2 infection (8, 9, 12), but differ from other studies that showed lower levels of nAbs (17) or IgG Abs to the spike protein (4) and shorter duration of nAbs or IgG Abs in children compared with adults (4, 11). Our results suggest that children develop robust nAb responses as well as Abs to multiple Ags variants after initial SARS-CoV-2 infection. Importantly, these responses remain present for at least 6 mo, though titers decrease over time. Pediatric responses are at least as robust as adults, and a higher proportion of children maintain nAbs at 6 mo compared with adults. Children in this study were not vaccinated, so we could not compare titers after vaccine to titers postinfection. As in other studies, adults developed much higher IgG and nAb levels postinfection followed by vaccination (21). Pediatric vaccine studies also show significantly higher nAb levels after vaccination (Refs. 22, 23 and B. Girard, J. E. Tomassini, W. Deng, M. Maglinao, H. Zhou, A. Figueroa, S. S. Ghamloush, D. C. Montefiori, R. Das, and R. Pajon, manuscript posted on medRxiv, DOI: 10.1101/2022.01.24.22269666), ~10-fold higher than those seen in the current study postinfection, and demonstrate that vaccinated children have greater protection against severe COVID-19 than unvaccinated children (24). Together, the study findings support a robust immune response in children, but show waning of nAbs over time, supporting the need for vaccination and for studies on Ab kinetics in children after vaccination.

Potential reasons for differences in the pediatric studies may include the age of children, disease severity, various serological testing modalities, and the predominant SARS-CoV-2 variant in circulation at the time of the study. Regarding the effects of age, in a subanalysis, we found no correlations between age and levels of nAb or tested IgG. Our study showed, as did one prior study, a decrease in IgG Abs to some SARS-CoV-2 Ags in children but not adults (4), but the prior study did not evaluate nAbs, which we show decreased less frequently in children than adults. We used a surrogate neutralizing assay in this study that was previously been reported to be strongly correlated with levels of nAb measured with pseudovirus assays (20). Two prior studies showed lower nAb titers in children than adults with SARS-CoV-2 infection, one of which included only hospitalized children (11) and the other included hospitalized children and children with asymptomatic infection (17). In the second study, children also had a reduced breadth of Abs and predominantly generated Abs to the S but not N Ag (17). In contrast, the current study confirms the findings of three other studies that show children, whether symptomatic or asymptomatic, make nAbs at similar or higher titers to adults, also generate Abs to multiple SARS-CoV-2 Ags, and retain nAbs longer than adults (8, 9, 12). The present study adds to the prior studies in strongly supporting the robust acquisition of IgG and nAbs in children after symptomatic or asymptomatic SARS-CoV-2 infection.

Children in our cohort developed Abs to multiple SARS-CoV-2 variants, but we did not have the Ags for delta or omicron variants, so could not determine if IgG Abs developed against these variants. High nAb levels were shown to correlate with protection against these variants of concern, and children had relatively high nAb titers, but it is unclear if they would be high enough to protect against variants of concern, particularly against omicron. Recent data demonstrate that children develop nAb to omicron, but at significantly lower levels than wild-type (22, 23). Similarly, mRNA COVID-19 vaccines were shown to elicit nAbs against omicron in children and adolescents but at lower levels than nAb to wild-type (Ref. 22 and B. Girard et al., manuscript posted on medRxiv, DOI: 10.1101/2022.01.24.22269666). Based on the epidemiology of omicron infections in children, which showed substantial transmission in areas in which many children had been previously exposed to virus (Ref. 25 and L. Wang, N. A. Berger, D. C. Kaelber, P. B. Davis, N. D. Volkow, and R. Xu, manuscript posted on medRxiv, DOI: 10.1101/2022.01.12.22269179), it is unlikely that the nAbs generated by non-omicron variant infection would be sufficient to protect against omicron. T cell responses are likely to be important in the response to SARS-CoV-2 infection in children and adults, including to SARS-CoV-2 variants like omicron (26, 27). We did not have the resources to measure T cell responses in this study. Future studies assessing the immune response in children postinfection or vaccination should assess these

responses in addition to nAb and IgG Ab responses to SARS-CoV-2.

The relatively small sample size (22 children with SARS-CoV-2 infection and samples at enrollment and follow-up), absence of children with severe SARS-CoV-2 infection, lack of testing for IgG against delta or omicron variants, and lack of testing of cellular immunity, which plays a critical role in memory responses to COVID-19 infection (8, 28–31), are all study limitations. We also did not measure total IgG, IgG subclasses, or IgM levels in the children in this cohort.

However, the study strengths, notably the evaluation of Abs over time in children and adults, evaluation of nAbs and IgG Abs against multiple spike protein variants, and the inclusion of testing in asymptomatic children, provide valuable new data on level and duration of Ab response in children after SARS-CoV-2 infection.

In conclusion, our study shows that the nAb response to SARS-CoV-2 infection in children after symptomatic or asymptomatic infection is at least as strong as in adults and lasts longer in children than in adults but does wane over time in both children and adults. IgG Abs to some SARS-CoV-2 Ags showed a greater decrease in children than adults, but the more functionally important nAbs showed a greater decrease in adults than children, highlighting the importance of evaluating nAbs when assessing changes in immunity over time. Now that SARS-CoV-2 vaccines are available for children, studies of post-vaccination Ab responses in children will be important to determine the extent to which these vaccines augment and prolong the duration and breadth of Ab response, as they have in adults.

DISCLOSURES

The authors have no financial conflicts of interest.

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