INTRODUCTION

Rapid vaccine developments and distributions have dramatically mitigated the disease burden of SARS-CoV-2. Children and adolescents, who were mostly spared from the initial surge of COVID-19, were excluded from early vaccine efforts. However, as the pandemic continued, it has become evident that children, too, can suffer from severe COVID-19 as well as long-term disease. The pandemic has continued, evolving towards the emergence of variant of concern (VOC), which have shown less epitope restriction, might be equally important to block transmission and disease. While SARS-CoV-2 vaccines are able to induce broad binding and functional antibodies in adults, particularly the most widely distributed BNT162b2 vaccine, and particularly the most widely distributed BNT162b2 vaccine, induce functional humoral immune responses in children, we comprehensively profiled vaccine-induced immune responses in 32 children (5–11 years) receiving two doses of BNT162b2 with the age-specific recommended 10 μg dose compared to adolescents (12–15 years, n = 30) and adults (16 + years, n = 9) receiving the adult 30 μg dose. Despite robust induction of SARS-CoV-2 Spike-specific antibodies, we observed differences in the humoral immune responses across the groups, marked by slower induction and lower antibody titers in the children, but the induction of more robust functionality. While lower in magnitude, VOC-specific immunity in children target VOCs broadly and exhibit enhanced functionality that may contribute to the attenuation of disease.

BNT162b2 induces robust cross-variant SARS-CoV-2 immunity in children

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breadth was comparable across the groups, albeit effector functions to omicron were lowest in the 10 µg dosed children. Collectively, our data point to age and dose-related differences in BNT162b2-induced humoral immunity that may underly differences in the viral breakthrough in the pediatric populations but point to the robust induction of functional humoral immune responses that may confer protection against severe disease across VOCs.

RESULTS

To begin to investigate whether age and dose-dependent differences exist in vaccine-induced antibody profiles across different age groups, we compared the humoral immune response in children 5–11 years old (n = 32, median age 9 years, 34% female) receiving the age recommended 10 µg dose to adolescent 12–15 years old (n = 31, median age 13 years, 65% female) or adults (n = 20, median age 23 years, 60% female) receiving the 30 µg dose BNT162b2. Plasma samples were collected before the first (V0) and second dose (V1) as well as 2–4 weeks after the second dose (V2).

BNT162b2 vaccination elicits robust SARS-CoV-2 specific isotype titers across the different age groups

To compare the humoral immune responses, we analyzed Spike and Receptor binding domain (RBD) specific IgM, IgG1, and IgA1 isotype titers (Fig. 1). All individuals 12 years and older vaccinated with the 30 µg dose seroconverted (marked by an increase in titer) after the first dose. Likewise, most younger individuals immunized with 10 µg BNT162b2 seroconverted after the first dose (V1). Overall, IgM and IgA Spike and RBD titers tended to be highest in adults, whereas IgG1 titers were higher in the 30 µg vaccinated adolescent group. Furthermore, after one dose, Spike and RBD-specific IgG1 titers were significantly lower in children compared to adolescents (p = 0.03) and adults (p < 0.01) and titers remained lower in young children after the second dose. In contrast, epitope-specific responses to the N-terminal domain (NTD) within S1 as well as responses to the S2 domain were more equally recognized across the groups (Supplementary Fig. 1) pointing to a selective deficit in RBD-specific immunity in the youngest group. Interestingly, we noted that some individuals had detectable, albeit low, Spike and RBD-specific IgA and IgG titers at the pre-vaccination (V0) timepoint. None of the participants reported a known previous SARS-CoV-2 infection and serological evidence of pre-exposure, marked by SARS-CoV-2 Nucleocapsid specific IgG1, was not observed (Supplementary Fig. 2). However, these pre-vaccine titers were highly correlated to Spike specific antibodies against the beta-coronavirus OC43 and HKU1, pointing to the existence of cross-coronavirus immunity, as previously reported by other groups. Importantly, the frequency of these responses did not differ across the age groups but they did correlate with the magnitude of the vaccine-induced responses after the 1st or 2nd dose of the vaccine (Supplementary Fig. 3). Collectively, these data argues that BNT162b2 vaccination can induce robust SARS-CoV-2 specific antibody responses in adolescent and adults but result in a more variable response in children 5-11 years old receiving the three-fold lower dose.

Fc effector activity is robust but delayed in children 5-11 years old

Emerging data point to an important role for antibody Fc-effector functions in natural, vaccine-induced, and therapeutic protection against COVID-19. Specifically, in addition to neutralization, antibodies are able to recruit additional effector functions via interactions between their Fc domains and Fc receptors found on all immune cells. Hence, we analyzed the Fc receptor (FcR) binding properties of SARS-CoV-2 Spike specific antibodies across the three immunized groups (Fig. 2a). After the first dose, more robust cross-FcR-binding profiles were observed in the adolescents and adults, both receiving the 30 µg vaccine dose.
Interestingly, adults raised FcγR3a (p < 0.001) and FcγR3b (p ≤ 0.03) responses more rapidly compared to adolescents who received the matching 30 μg dose. In contrast, after two doses adolescent Fc receptor binding was higher, albeit not significantly, compared to adults across nearly all FcγRs. Significantly higher binding to the IgG receptors FcγR2a (pV1 = 0.001; pV2 = 0.005), FcγR2b (pV1 = 0.001; pV2 = 0.002), FcγR3a (pV1 < 0.001; pV2 = 0.01) and FcγR3b (pV1 = 0.002; pV2 < 0.001) was observed in the adolescent group compared to the younger 5–11-year-old group after the first and second vaccination. However, 5–11-year-old children mounted similar levels of FcγR binding to adults across all FcγRs, albeit the responses were more variable in young children. Finally, similar binding of IgA to its FcγR receptor, FcγR, were noted across all 3 groups. Thus, adolescents clearly generate more Spike-specific FcγR-binding antibodies, with the capacity to bind to FcγRs more effectively than adults at the same dose. Conversely, 5-11 years old children induced comparable levels of FcγR-binding antibodies, at a lower antibody titer (Fig. 1) compared to adults, suggesting that children generate qualitatively superior functional antibodies compared to adults.

While antibody titers and FcγR-binding levels were more variable in younger children receiving the lower vaccine dose, we next analyzed whether antibody effector profiles differed across the groups (Fig. 2b). While young children clearly required 2 doses of the vaccine to induce robust neutralizing antibody levels, both groups of children raised comparable antibody levels after the 2nd dose, arguing that young children have the capacity to raise nearly equivalent neutralizing antibody titers to adolescents who received a higher dose of the vaccine. Of note, comparable to binding titers, some children and adolescents had pre-existing reactive antibodies that have been described previously in children.20–22 Conversely, more differences were noted across the 3 groups with respect to antibody effector functions. While all Fc effector functions were detected in all groups after the first dose (V0 to V1) or from the first to the second dose (V1 to V2), horizontal lines indicate the median and error bars the 95% confidence interval.

Fig. 2 Vaccination with BNT162b2 mRNA in children induces higher FcγR, complement deposition, phagocytic activity, and NK cell activation. (a) Binding of SARS-CoV-2 spike specific antibodies to FcγR2a, FcγR2b, FcγR3a, FcγR3b and FcαR determined by Luminex in children receiving 10 μg of BNT162b2 (ages 5-11 years old, yellow) or 30 μg BNT162b2 (ages 12-16 years old, blue and 16+ years old, red) before (V0: 10μg (5-11y): 32; V0: 30μg (12-16y): 29; V0: 30μg (16+y): 7), after the first dose (V1: 10μg (5-11y): 32; V1: 30μg (12-16y): 27; V1: 30μg (16+y): 8), or after the second dose (V2: 10μg (5-11y): 30; V2: 30μg (12-16y): 26; V2: 30μg (16+y): 11). (b) The ability of SARS-CoV-2 spike specific antibody Fc to induce neutralization, complement deposition (ADCD), neutrophil phagocytosis (ADNP), monocyte phagocytosis (ADCP) and NK cell activation by the frequency degranulated CD107+ NK cells. A two-way ANOVA (two-sided) was used to calculate statistically significant differences between the groups at each timepoint. Exact p-values for statistically significant differences after Benjamini-Hochberg correction for multiple testing are shown above the graph. (c–d) Heatmaps show the relative fold changes of Fc mediated function before to after the first to the second dose (V0 to V1) or from the first to the second dose (V1 to V2). Horizontal lines indicate the median and error bars the 95% confidence interval.

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year-old children (Supplementary Fig. 4). Specifically, adolescents and adults generated comparable antibody-dependent complement depositing (ADCD), antibody-dependent neutrophil phagocytic antibodies (ADNP) and antibody-dependent cellular monocyte phagocytosis (ADCP), adults generated superior NK cell activating antibodies (CD107a) after a single dose. Yet, after the second dose, young children who received the low vaccine-dose tended to generate superior ADNP-inducing antibodies and induced comparable ADCP- and ADNKA-inducing antibodies compared to adolescents and adults that received the higher vaccine dose. The relative fold-induction of Fc effector functions after the first dose (V0 to V1) were relatively uniformly enhanced across the groups (Fig. 2c). In contrast, fold-changes after the second dose for all Fc-mediated functions (except IFNγ expression of NK cells which was higher in adolescents) were considerably higher in younger children compared to the adolescent, adult, and young children group, and adults exhibited the weakest fold-increase in antibody function after the second dose (Fig. 2d). Given that the magnitude of these functional antibody responses was not significantly different across the groups after the second dose, these data further highlight the importance of the second dose in children, whose antibodies, despite significantly lower subclass/isotype titers, were equally able to induce robust SARS-CoV-2 specific effector functions. Thus, young children experience a unique functional maturation, even at a lower vaccine dose, that may be attributable to their younger, more flexible immune response.

Humoral immune responses are distinct across the groups

Given the various differences in antibody profiles across the age groups, we next aimed to define the specific differences in vaccine responses across age groups that may provide insights on differences in age-dependent immunogenicity profiles. Given that antibody levels, Fc-receptor binding, and function are all highly interrelated, we elected to use a multivariate strategy aimed at rigorously capturing the differences in antibody profiles that explained the greatest level of variance across age-groups. Specifically, focusing on peak immunogenicity, after the complete two-dose series, we exploited a Least Absolute Shrinkage and Selection Operator (LASSO) to initially define a minimal set of SARS-CoV-2 specific antibody features that could separate the groups. A minimal set of features was selected to avoid statistical overfitting and define the multivariate features that can explain the variation in antibody profiles across any 2 groups maximally. Using these features, we then used a Partial Least Square Discriminant Analysis (PLS-DA) to visualize the profiles across individual plasma samples (Fig. 3). Thereby, multivariate profiling, unlike univariate profiling, provides insights into the combined vaccine induced features that maximally differ across doses and/or ages. Vaccine-induced antibody Fc-profiles diverged across the children (10 μg dose) and adolescents (30 μg dose) (Fig. 3a), marked by elevated levels of Spike-specific IgM, IgA2, IgG1 and IgG3 titers as well as FcyR2a binding and IFNγ secretion by NK cells in adolescents. Conversely, vaccine-specific ADNP and NK cell MIP-1β secretion were selectively enriched in children. Similarly, a comparison of children and adults separated more distinctly in the PLS-DA, with NK cell features including MIP-1β and IFNγ production, enriched in children and IgG1, IgA and IgM titers, FcyR3b binding, ADCD, and ADPC enriched in adults (Fig. 3b). Interestingly, even adolescent and adult responses differed, despite the matching vaccine dose, marked by age-dependent induction of higher vaccine-specific IgM, IgA and ADCD in adults, but increased binding to FcyR2a and monocyte phagocytosis (ADCP) in adolescents (Fig. 3c). These data clearly illustrated that specific differences between children, adolescents and adults exist, where adolescents and adult immune responses were marked by higher antibody titers, but a unique expansion of functional responses was noted in children.

Variable responses to variants of concern after BNT162b2 vaccination

The emergence of variants of concern (VOCs) like the most recent omicron variant has led to continued waves of SARS-CoV-2 infections and breakthroughs globally. Dramatic antigenic changes particularly in the omicron Spike Receptor Binding Domain – important for viral entry – led to near complete loss of vaccine-induced neutralizing antibody titers.27,28 However, antibodies are still able to sense other parts of Spike VOCs, elicit Fc effector functions, and thereby potentially mediate protection from severe disease. However, whether vaccination in children leads to recognition of VOCs is unclear. Thus, we next analyzed the specific humoral response to different VOCs, including alpha (B.1.1.7), beta (B.1.351), gamma (P.1), delta (B.1.617.2) and omicron (B.1.1.529) at peak immunogenicity (Fig. 4). Despite the lower antibody titers in children, all 3 groups exhibited more stable IgA and IgG binding across the alpha, beta, gamma, and delta VOCs, but compromised recognition of omicron (Fig. 4a and Supplementary Fig. 5). IgM binding antibodies were significantly impaired in recognition of the omicron Spike, but exhibited enhanced recognition of other VOCs, pointing to an isotype-specific compromised response to this more mutated VOC; however, all 3 groups clearly adapted their more mature IgA and IgG responses to compensate for this deficit in IgM immunity. Unexpectedly, IgM reactivity to the Beta variant was improved in children and adolescents compared to the D614G response pointing to age-dependent differences in the evolution of breadth of binding across VOCs. Moreover, fold changes of binding over D614G across isotype and FcR-binding titers to different VOC full Spike and RBDs highlighted consistent patterns of breadth of binding across VOCs across the age groups (Fig. 4b), with the exception of IgM binding in children and adolescents that was superior across all VOCs, except omicron. These data point to a unique flexibility in cross-reactivity in IgM in children. Given the robust opsonophagocytic and complement-fixing function of IgM, these data point to a unique capability programmed in children that may help provide some level of persistent broad recognition of VOCs following vaccination.

While antibody binding to most VOC Spikes was largely preserved, there was an apparent reduction for the omicron variant recognition across all ages. We therefore profiled the ability of vaccine-induced antibodies to elicit neutralizing and Fc-mediated effector functions to omicron (Fig. 4c and Supplementary Fig. 5). As expected, omicron-specific antibody effector functions were detectable but reduced when compared to D614G Spike-specific responses after first and second dose of BNT162b2. Children receiving the 10 μg dose, showed lower omicron-specific reactivity at both timepoints compared to adult-dosed adolescent or adults. Interestingly, while the omicron-specific effector profiles expanded from the first to the second dose in the 10 μg dosed children and 30 μg dosed adolescents, ADNKA and ADCP to omicron were reduced from the first to the second dose in adults. Collectively, our data indicate that while adults produce sufficient effector responses after the first vaccine dose, adult immunity becomes narrower towards the vaccine insert antigen after the second dose. In contrast, children and adolescents may not be fully primed after the first dose but develop a broad and highly functional vaccine response after the full vaccine series.

DISCUSSION

Early in the pandemic, pediatric cases accounted for approximately 5% of the total COVID-19 cases in the US. Reasonably, initial vaccine campaigns focused on the protection of adults. Since then, pediatric cases of SARS-CoV-2 have exceeded 13 million with over 42,000 hospitalizations, resulting in the
emergency use authorization for mRNA vaccines for children 5 years of age and older. However, due to the delayed vaccine rollout and increased vaccine hesitancy by parents, vaccination rates in children still lag behind. Together with the occurrence of emerging variants like omicron accompanied by relaxed masking and social distance measurements, cases of COVID-19 have soared with up to 20% of the total SARS-CoV-2 infections occurring in children during the recent omicron wave. In addition to acute infection, children are at special risk of developing a severe multi-inflammatory syndrome (MIS-C) weeks after asymptomatic SARS-CoV-2 exposure. Importantly, data indicates that 30 μg BNT162b2 not only protects adolescents (12–18 years) from severe COVID-19 but also MIS-C29, underscoring the importance and effectiveness of vaccination across the ages.

As innate and adaptive immune systems mature during the first decades of life, it was not clear how the naïve, pediatric immune system would respond to mRNA vaccine technology. Furthermore, due to safety and tolerance concerns, doses were adjusted for the younger 5-11-year-old age group. Our data point to a more variable immune response in children 5–11 years of age receiving
a three-fold lower dose BNT162b2 compared to adolescents and adults. Analysis of a dose-down study of the Moderna mRNA-1273 vaccine suggests that children have an attenuated immune response after a two-fold lower dose, though, age-specific differences may have also accounted for this. Here, we noted a selective humoral deficiency in isotype and Fc receptor binding titers after one dose of BNT162b2 in children and adolescents compared to adults. Yet, humoral immunity expanded robustly after the second dose, raising functions to levels comparable or superior to those observed in adults, with titers in the adolescent
group exceeding those in adults. Moreover, after two doses, children had specifically elevated highest levels of opsonophagocytic and NK cell activating antibodies that have been previously associated with resolution of severe disease\textsuperscript{30,31}. This enhanced functionality could be attributable to a variation in epitope selection or post-translational modification of the antibody FC-domain that collectively could alter the quality of the pediatric immune response antibody quality. For example, emerging data point to striking differences in antibody FC-glycosylation across the ages resulting in the generation of more pro-inflammatory antibodies in the earliest years of life, which are associated with increased NK cell activation in COVID-19\textsuperscript{19,22,33}. While we were limited by the amount of available pediatric samples for antibody glycosylation analysis, future studies may provide enhanced insights on the specific vaccine-induced antibody changes that may permit children to generate more functional antibodies at a half-vaccine dose compared to adolescents or adults. Furthermore, antibody avidity was higher in adolescents and adults (Supplementary Fig. 3) pointing to qualitative differences in the response to mRNA vaccination in children, adolescents and adults. Additionally, whether a matched adult dose of mRNA or expanded response to mRNA vaccination in children, adolescents and adults. (Supplementary Fig. 3) pointing to qualitative differences in the more, antibody avidity was higher in adolescents and adults compared to adolescents or adults. Further-...
For the ADCP assays, biotinylated antigens were coupled to FluoSphere NeutrAvidin beads (ThermoFisher). Immune complexes were formed by incubating antigen coupled beads with 10 ul of 1:10 diluted plasma samples at 37 °C for 2 hours. Following incubation, non-specific antibodies were washed off followed by incubation of the immune complex with guinea pig complement in GVB++ buffer (Boston BioProducts) at 37 °C for 20 min. To stop the complement reaction, 15 mM EDTA (Corning) in PBS was added to the immune complex. C3 deposited on beads were stained with anti-guinea pig C3-FITC antibody (MP Biomedicals, 1:100, polyclonal) and analyzed on an iQue analyzer (IntelliCyt).

Antibody-dependent neutral phagocytosis (ADNP) assays
For the ADNP assays, biotinylated antigens were coupled to FluoSphere NeutrAvidin beads (ThermoFisher). Immune complexes were formed by incubating antigen coupled beads with 10 ul of 1:50 diluted plasma samples at 37 °C for 2 hours. Primary neutrophils were isolated as previously mentioned and 50,000 cells per well incubated with washed immune complexes at 37 °C for 1 hour. Following incubation, neutrophils were stained for surface CD66b (Biolegend, 1:100, clone: G10F5) expression and fixed with 4% para-formaldehyde. Analysis was done on an iQue analyzer (IntelliCyt).

Antibody-dependent cell phagocytosis (ADCP) assays
For the ADCP assays, biotinylated antigens were coupled to FluoSphere NeutrAvidin beads (ThermoFisher). Immune complexes were formed by incubating antigen coupled beads with 10 ul of 1:100 diluted plasma samples at 37 °C for 2 hours. THP-1 monocytes (25,000 cells per well) were then added to the beads then incubation at 37 °C for 16 hours. Following incubation, cells were fixed with 4% para-formaldehyde and analyzed on an iQue analyzer (IntelliCyt).

Antibody-dependent NK activation (ADNKA) assays
MaxiSorp ELISA plates (ThermoFisher) were coated with either 1 μg/mL SARS-CoV-2 wt spike or omicron variant spike at room temperature for 2 hours followed by blocking with 5% BSA (Sigma-Aldrich). 50 ul of 1:50 diluted plasma samples were added to the wells and incubated overnight at 4 °C. Primary NK cells were prepared as described above. Cells were added to the washed ELISA plate and incubated with anti-human CD107a (BD, 1:40, clone: HA43), brefeldin A (Sigma-Aldrich) and monensin (BD) at 37 °C for 5 hours. Following incubation, cells were surface stained for CD56 (BD, 1:200, clone: NCAM16.2) and CD3 (BD, 1:200, clone: UCHT1). The NK cells were then washed, fixed, and permeabilized using the Fix & Perm Cell Permeabilization Kit (ThermoFisher). Cells were then stained for intracellular markers MIP1β (BD, 1:50, clone: D21-1351) and IFNγ (BD, 1:17, clone: B27). NK cell populations were defined to be CD3-CD56+ and the frequency of degranulated cells marked by CD107a and the expression of intracellular IFNγ and MIP-1β were determined on an iQue analyzer (IntelliCyt).

Virus neutralization
Three-fold serial dilutions of plasma samples starting at 1:12 or 1:30 were performed before adding pseudovirus expressing either SARS-CoV-2 wild-type spike or omicron variant spike to HEK293T expressing ACE-2 cells for 1 hour. Pseudoviruses were produced by PEI transfection of lentiviral vector with CMV-Luciferase-IRES-ZsGreen, lentiviral helper plasmids, and either SARS-CoV-2 wt spike or omicron variant spike expression plasmid. Pseudovirus neutralization titers (pNT50) values were calculated by taking the inverse of 50% inhibitory concentration value for all samples with a pseudovirus neutralization value of 80% or higher at the highest concentration of serum.

Data analysis and statistics
Data analysis was performed on GraphPad Prism (v.9.3) and RStudio (v.1.3). A two-way ANOVA test with Benjamini-Hochberg correction for multiple testing in GraphPad Prism was used to determine statistically significant differences between the groups at each timepoint. Flower plots were generated with the ggplot package in R using Z-scored data. Multivariate classification models were built to discriminate between humoral profiles of vaccination arms. Feature selection was performed using the least absolute shrinkage and selection operator (LASSO) and classification and visualization were performed using partial least square discriminant analysis (PLS-DA). These analyses were performed using R package “ropls” version 1.20.043 and “glimnet” version 4.0.244. Co-correlate networks were built using Spearman method followed by Benjamini-Hochberg correction and the co-correlate network was generated using R package “network” version 1.16.0.5.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

DATA AVAILABILITY
All relevant data are included in this manuscript. No data was stored externally.

CODE AVAILABILITY
No custom code was used in this study.

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REFERENCES
1. Swann, O. V. et al. Clinical characteristics of children and young people admitted to hospital with covid-19 in United Kingdom: prospective multicentre observational cohort study. BMJ 370, m3249 (2020).
2. Stephenson, T. et al. Long COVID and the mental and physical health of children and young people: national matched cohort study protocol (theClock study). BMJ Open 11, e052838 (2021).
3. Donovan, C. V. et al. SARS-CoV-2 Incidence in K-12 School Districts with Mask-Required Versus Mask-Optional Policies - Arkansas, August-October 2021. MMWR Morb. Mortal. Wkly Rep. 71, 384–389 (2022).
4. Marks, K. J. et al. Hospitalizations of Children and Adolescents with Laboratory-Confirmed COVID-19 - COVID-NET, 14 States, July 2021-January 2022. MMWR Morb. Mortal. Wkly Rep. 71, 271–278 (2022).
5. Siegel, D. A. et al. Trends in COVID-19 Cases, Emergency Department Visits, and Hospital Admissions Among Children and Adolescents Aged 0-17 Years - United States, August 2020-August 2021. MMWR Morb. Mortal. Wkly Rep. 70, 1249–1254 (2021).
6. Feldstein, L. R. et al. Multisystem Inflammatory Syndrome in U.S. Children and Adolescents. N. Engl. J. Med 383, 334–346 (2020).
7. Zimmermann, P., Pittet, L. F. & Curtis, N. How Common is Long COVID in Children and Adolescents. Pediatr. Infect. Dis. J. 40, e482–e487 (2021).
36. Roltgen, K. et al. Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination. Cell 185, 1025–1040 e1014 (2022).

37. Gagne, M. et al. mRNA-1273 or mRNA-Omicron boost in vaccinated macaques elicits similar B cell expansion, neutralizing responses, and protection from Omicron. Cell 185, 1556–1571 e1518 (2022).

38. Brown, E. P. et al. High-throughput, multiplexed IgG subclassing of antigen-specific antibodies from clinical samples. J. Immunol. Methods 386, 117–123 (2012).

39. Fischinger, S. et al. A high-throughput, bead-based, antigen-specific assay to assess the ability of antibodies to induce complement activation. J. Immunol. Methods 473, 112630 (2019).

40. Ackerman, M. E. et al. A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples. J. Immunol. Methods 366, 8–19 (2011).

41. Garcia-Beltran, W. F. et al. COVID-19-neutralizing antibodies predict disease severity and survival. Cell 184, 476–488 e411 (2021).

42. Garcia-Beltran, W. F. et al. COVID-19-neutralizing antibodies predict disease severity and survival. Cell 184, 476–488 e411 (2021).

43. Thevenot, E. A., Roux, A., Xu, Y., Ezan, E. & Junot, C. Analysis of the Human Adult Urinary Metabolome Variations with Age, Body Mass Index, and Gender by Implementing a Comprehensive Workflow for Univariate and OPLS Statistical Analyses. J. Proteome Res 14, 3322–3335 (2015).

44. Friedman, J. H., Hastie, T. & Tibshirani, R. Regularization Paths for Generalized Linear Models via Coordinate Descent. J. Stat. Softw. 33, 22 (2010).

45. Butts, C. T. network: A Package for Managing Relational Data in R. J. Stat. Software 24 (2008).

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AUTHOR CONTRIBUTIONS
Y.C.B., J.W.G. and J.K. performed the serological experiments. K.J.D., M.L.S. and A.B.B. performed the neutralization assay. Y.C.B., L.M.Y. and G.A. analyzed and interpreted the data. M.D.B., J.P.D., A.G.E. and L.M.Y. supervised and managed the sample collection. G.A. supervised the project. Y.C.B., L.M.Y. and G.A. drafted the manuscript. All authors critically reviewed the manuscript.

COMPETING INTERESTS
G.A. is a founder of Seromyx Systems, a company developing a platform technology that describes the antibody immune response. G.A.’s interests were reviewed and are managed by Massachusetts General Hospital and Partners HealthCare in accordance with their conflict of interest policies. All other authors have declared that no conflicts of interest exist.

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