Humoral profiles of toddlers and young children following SARS-CoV-2 mRNA vaccination

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Abstract

Although young children generally experience mild symptoms following infection with SARS-CoV-2, severe acute and long-term complications can occur. SARS-CoV-2 mRNA vaccines elicit robust immunoglobulin profiles in children ages 5 years and older, and in adults, corresponding with substantial protection against hospitalizations and severe disease. Whether similar immune responses and humoral protection can be observed in vaccinated infants and young children, who have a developing and vulnerable immune system, remains poorly understood. To study the impact of mRNA vaccination on the humoral immunity of infant, we used a system serology approach to comprehensively profile antibody responses in a cohort of children ages 6 months to 5 years who were vaccinated with the mRNA-1273 COVID-19 vaccine (25 μg). Responses were compared with vaccinated adults (100 μg), in addition to naturally infected toddlers and young children. Despite their lower vaccine dose, vaccinated toddlers elicited a stronger functional antibody response than adults, including against variant of concerns (VOCs), without report of side effects. Moreover, mRNA vaccination was associated with a higher IgG3-dependent humoral profile against SARS-CoV-2 compared to natural infection, supporting that mRNA vaccination is effective at eliciting a robust antibody response in toddlers and young children.

Introduction

Despite the early misconception that children were spared from COVID-19, children continue to account for approximately twenty percent of all documented cases of COVID-19 infection in the United States, with infants and children under 5 years of age disproportionately affected by high rates of hospitalization[1]. While most children experience mild symptoms with acute SARS-CoV-2 infection, severe complications can ensue, even in the youngest children, and myocarditis, cardiomyopathy, renal failure, as well as coagulation and hemorrhagic disorders occur at increased rates with COVID-19[2]. Concerningly, COVID-19 deaths in children far exceed deaths from influenza[3] and COVID-19 has now become one of the leading infectious cause of death in the Unites States[4].

SARS-CoV-2-targeting mRNA vaccines have become available for individuals six months of age and older[5, 6, 7, 8, 9, 10, 11]. These vaccines have provided substantial protection against hospitalizations and severe disease in children ages 5-17 years [5, 7, 10, 12]. Moreover, detailed humoral profiling of children and adolescents reveals that mRNA COVID-19 vaccines elicit robust, highly functional humoral immune responses in children in a dose-dependent manner[13], with strong cross-reactivity against variants of concerns (VOC)[13, 14]. In children under 5 years of age, mRNA vaccination results in neutralizing immunoglobulin titers comparable to vaccinated adults and vaccination protects against symptomatic infection[11]. Howerver, detailed humoral profiling in this age group has not yet been investigated. As an individual's humoral immune response evolves with age[15], age-related differences in mRNA vaccine responses must be fully characterized to fully understand the impact of mRNA vaccination in infants and toddlers.

In order to characterize the activation of humoral immunity in young children after SARS-CoV-2-specific mRNA vaccination, we used an unbiased system serology approach to analyze antibody levels and Fc-mediated functions in individuals ages 6 months through 5 years. We comprehensively profiled their antibody response following vaccination with the mRNA-1273 COVID-19 vaccine (25 μg) and compared it with antibody profiles of vaccinated adults (100 μg), as well as children infected with SARS-CoV-2. Our results reveal a strong activation of humoral immunity post-vaccination in these young children, with a highly functional and cross-reactive humoral immunity in comparison to adults and naturally infected infants.

Results

mRNA-vaccinated infants and toddlers generate robust Immunoglobulin G (IgG) responses

Our first objective was to profile vaccine-induced humoral immunity in infants and toddlers ages 6 months through 5 years (n = 18) after completion of the two doses of the pediatric mRNA-1273 vaccination series (vaccine dose: 25mcg mRNA-1273) and compare these serologic responses to those generated by fully vaccinated adults (n = 13; vaccine dose: 100mcg mRNA-1273). Demographics of participants are included in **Table 1**; mean age of vaccinated pediatric participants was 2.2 years (range 7 months- 4.5 years). None of the vaccinated adults or children reported SARS-CoV-2 infections prior to or during their vaccine series, which was supported by absence of elevated nucleocapsid responses (**Fig. S1**).

Our results demonstrate that despite their young age and receipt of only one quarter of the adult dose, total anti-Spike and anti-RBD IgG levels and IgG subclass in young children were similar to adults (**Fig. 1A, Fig. S2**). Interestingly, in contrast to IgG, this young population displayed lower

levels of vaccine-induced anti-Spike and anti-RBD IgM and IgA1, which shows the distinct isotype selection between adults and children (**Fig. 1A**). We then compared the binding of spike and RBD-specific antibodies to Fc receptors (FcR), as well as antibody effector functions, including antibody-dependent cellular (monocyte) phagocytosis (ADCP), antibody-dependent neutrophil phagocytosis (ADNP) and antibody-dependent complement deposition or activation (ADCD) in young children and adults. We saw that infants less than 5 years old were able to produce antibodies with strong FcγR2A, FcγR2B, FcγR3A, and FcγR3B binding at similar levels as adults, and remarkably, anti-RBD antibodies exhibited stronger ADCP and ADNP effector functions in young children than in adults (**Fig. 1B and 1C, Fig. S1**).

To determine cross-reactivity of the vaccine-induced humoral response against variants of concerns (VOCs), we quantified antibody levels and FcR binding against Spike and RBD for six different SARS-CoV-2 VOCs including wild type (WT), Alpha, Beta, Gamma, Delta, and Omicron. While IgM, IgA1, and the FcR for IgA1 (FcαR) were higher in adults across the different SARS-CoV-2 variants, IgG response was essentially indistinguishable between young children and adults. In fact, the only exceptions were total IgG against RBD Omicron and IgG4 against Spike Gamma, RBD Alpha, RBD Delta, and RBD Omicron, which were significantly increased in young children (**Fig. 1D**).

To further characterize the capacity of the pediatric population to generate a broad SARS-CoV-2-specific humoral response following mRNA-1273 vaccination, we calculated a Spike and RBD protein breadth score. The breadth score highlights that children less than 5 years old are able to induce a humoral response as robust as adults, with a strong recognition of different VOCs while

IgM- and IgA-specific immunity is higher in adults (**Fig. 1E**). Taken together, these results show specificities regarding isotypes selection between young children and adults, with overall similar to enhanced antibody functionality against SARS-CoV-2 proteins in infants and toddlers less than 5 years old compared to adults.

When looking more broadly at antibody responses against common respiratory infections, including non-SARS-CoV-2 human coronavirus (HCoV) HKU1 Spike (HKU1), HCoV-OC43 Spike (OC43), and Influenza haemagglutinin (HA), we see a strong age-related difference. In contrast to the robust SARS-CoV-2 vaccine-induced humoral immunity across the age spectrum, young children have significantly lower antibodies titers against HKU1, OC43, and HA. Multivariate analysis highlights a clear separation between the two age categories distributions, as attested by the Partial least squares discriminant analysis (PLS-DA) (**Fig. S3A**). The LASSO-selected features that were used to build the PLS-DA model revealed an enrichment of antibody levels and FcγR binding against HKU1, OC43, and HA in adults (**Fig. S3B**). Co-correlates analysis showed strong connections between isotypes and FcγR features against non-SARS-CoV-2 antigens (**Fig. S1C**), all of which being enriched in older individuals. These antibody profiles in adults reflect prior exposure to these respiratory viruses over their lifetime, while these young children may remain naïve. Alternatively, the lower antibodies could reflect lower total antigen-specific humoral responses to prior infection or the non-mRNA influenza vaccine, or more rapidly waning immunity in these young children.

mRNA-1273 vaccination induces lasting, cross-reactive immunity in young children

In order to evaluate the impact of mRNA-1273 vaccination on the evolution of humoral immunity in young children, we measured antibody levels and Fc functionality prior to vaccination (V0), one month after the first dose (V1), one month after the second dose (V2), six months after vaccination (V6), in addition to one month after boosting (Post-boost) (**Fig. 2, 3**). After just one vaccine dose, strong production of IgG, IgM, and IgA against Spike WT could be observed in these infants (**Fig. 2A**), with robust antibody binding to FcγR (**Fig. 2B**). Similarly, antibody effector function, characterized by ADCP, ADNP and ADCD, was significantly increased at V1 compared to V0 (**Fig. 2C**). Peak antibody responses were generally observed 2 months after the first dose of vaccination (**Fig. 2**), as attested by the high antibody levels and functionality (**Fig. 2C**). Of note, IgM levels started to wane after V1 **(Fig. 2A)**; In the setting of rising IgG and IgA1 titers, this supports antibody maturation with efficient class switching. Although the number of individuals that was included for the post-boost analysis was low, our results highlighted a strong activation of the immune system one month after boosting, especially for FcγR binding and antibody-induced neutrophil activation (**Fig. 2B, C**).

To evaluate the ability of mRNA-1273 vaccination to elicit broadly cross-reactive antibody responses and their durability over time in young children, we compared the antibody responses to Spike antigens from wild type through Omicron variants at each time point (**Fig. 3**). Total IgG responses to full-length Spike were similar across all variants. However, IgG responses to the Omicron RBD were consistently lower post-vaccination for all subclasses and FcγRs (**Fig. 3A, 3B**), which is not unexpected given the large number of mutations in the RBD of Omicron in comparison to other variants and is consistent with cross-reactivity seen in older individuals[13, 14].

To determine the breadth of antibody responses over time, breadth scores were calculated for each IgG subclass and each FcγR over the time as described above (**Fig. 3C**). Breadth was highest for IgG3, although this response did wane prior to boost. IgG2 and IgG3 responses both expanded with boosting, with minimal change in IgG1 responses. FcγR binding showed similar breadth for each FcγR tested, with a robust initial response, some waning in response at 6 months after vaccination, and increased breadth after boosting. Again, the breadth scores highlighted a broad anti-SARS-CoV-2 antibody response shortly after vaccination that wanes over 6 months, but then appears to re-expand to peak levels post-boost.

Vaccination produces greater IgG3 than natural infection in young children

To evaluate whether natural infection induces equivalent immunity compared to vaccination, we compared anti-Spike and anti-RBD titers, and Fc binding and effector function in serum collected from a group of 8 children (**Table 1**: mean age, 3.7 years; range: 1-5 years) one-month following acute SARS-CoV-2 infection, defined as symptomatic COVID-19 confirmed by PCR or rapid antigen test at the time of illness, and a second group of children one month after completion of their first dose of vaccine (V1). We did not detect a significant difference in total IgG levels (**Fig. 4A**), FcγR binding (**Fig. 4B**) and antibody functionality (**Fig. 4C**) between the naturally infected group and the vaccinated group. This suggests that the induction of humoral immunity following vaccination is as strong as the response induced by natural SARS-CoV-2 infection in infants and toddlers. Interestingly, the IgG3 response to both Spike and RBD was significantly higher in vaccinated young children compared to infected children (**Fig. 4A**). Levels of IgG3, a highly potent IgG subclass with the greatest levels of functionality[16, 17, 18], correlate strongly with neutralization [19, 20], suggesting that in young children vaccination induces a more mature and potent antibody

response than natural infection with SARS-CoV-2. Further, this robust vaccine-induced IgG3 response is consistently elevated across different VOCs (**Fig. 4D**) highlighting the benefit of cross-reactivity gained by vaccination compared to natural infection. As expected, anti-nucleocapsid antibody responses induced by natural infection were absent in children vaccinated with the mRNA-1273 vaccine as the vaccine does not encode for nucleocapsid (**Fig. 4D**). Of note, the elevated IgG1 levels against the Delta strain of Spike in the infected group reflect the VOC at time of infection based on the timing of samples collected (eight of ten of the children were infected with SARS-CoV-2 prior to the Omicron surge) highlighting the specificity of response in natural infection. Notably, vaccine-induced IgG3 levels still remained significantly higher than natural infection, even for the Delta strain. Taken together, these results show that mRNA-1273 vaccination in infants and children less than 5 years of age elicits strong humoral activation, with production of a highly mature and developed antibody response, suggesting a more effective humoral response following vaccination in comparison to natural infection with SARS-CoV-2.

Discussion

The availability of novel mRNA vaccine technology represented a key inflection point in the COVID-19 pandemic, dramatically reducing hospitalizations and deaths caused by SARS-CoV-2. However, with the novelty of the mRNA vaccine strategies, the impact on immune reponse in pediatric populations remains largy unknown. Thus, the risk/benefit ratio of vaccinating young children must be thoroughly analyzed; comprehensive profiling of the humoral immune response following vaccination, including characterization of antibody response profiles and cross-protective potential is critical. While in-depth antibody titers and effector function have been

described for mRNA-vaccinated adults and children ages 5 years and older[13, 14], limited information exists for younger age groups.

Humoral responses are known to vary with age [21], with the capacity to generate antibodies increasing over time, including following administration of SARS-CoV-2 mRNA vaccines [13, 14]. Remarkably, despite receiving only a quarter of the adult dose, our study suggests that infants and toddlers younger than 5 years are capable of generating titers of anti-SARS-CoV-2 IgG that are comparable to adults. The impact of diminished IgA and IgM in children in the setting of SARS-CoV-2 is not clear. In adults with COVID-19, elevated levels of anti-SARS-CoV-2 IgA immune complexes are associated with severe disease[22]and in children with MIS-C, anti-SARS-CoV-2 IgA immune complexes activate intravascular neutrophil extracellular traps which may contribute to endothelial damage[23]. However, IgA is also a key antibody for mucosal immunity, combating pathogens at the airway surface[24]. Additionally, circulating IgA does not directly correlate with mucosal IgA[25]; future studies will be needed to fully characterize mucosal immunity following vaccination and to compare mucosal responses in children and adults.

Fc binding capacity of anti-SARS-CoV-2 IgG may play important protective functions including enhanced activation of monocytes and neutrophils. Here we demonstrated that vaccinated young children display comparable Fc binding capacity as compared to vaccinated adults. The pediatric nasal passages contain higher quantities of neutrophils than adults and these pediatric neutrophils tend to be primed for anti-viral responses[26]. Thus, the combination of these activating antibodies and primed neutrophils may lead to efficient containment of the virus at the nasal surface. Direct humoral profiling of the pediatric mucosal surface may reveal important differences between

children and adults with potential implications for current vaccine strategies, as well as the development of mucosal vaccination strategies for COVID-19.

In addition to this strong and functional antibody response in young children two months after the first dose of vaccination, our results showed that this pediatric population was able to maintain functional humoral immunity for at least 6 months. We observed signs of efficient antibody class switching[27, 28], as IgM levels rapidly decreased 1 month after vaccination, when IgG and IgA continued to be produced, in addition to increasing FcR binding and Fc-mediated functionality. Moreover, the analysis of vaccine-induced humoral immunity against VOC highlighted a strong and sustained antibody response over time with Alpha, Beta, Gamma and Delta, while Omicron-specific immunity tended to be slightly lower, as reported previously [13]. It has been hypothesized that the naïve pediatric immune system facilitates the evolution and adaptation of immune response to allow broader immunity against future viral exposures[29, 30], which might explain the more robust VOCs-specific antibody response in infants compared to adults. Of the IgG subclasses, though, IgG3 declined the most by 6 months but responded well to boosting, highlighting the importance of boosters in maintaining effective protection against SARS-CoV-2 over time. Collectively, these data suggest that the vaccine can provide long-term immunoprotection against COVID-19 in young children, with likely efficacy against emerging VOCs.

Interestingly, our analysis suggests that mRNA vaccine series provides superior protection than viral exposure, as attested by the higher IgG3 response against different VOCs in the vaccinated group. With IgG3 being the most functional IgG subclass[16, 17, 18], these data show that vaccination in this young population elicits a stronger and more functional humoral immune response

compared to natural infection. We also observed that the antibody response against Delta, which is the strain that was circulating at the time of sample collection, was higher in the infected group. This suggests that adapting vaccine strategies to incorporate genetic variations that appear in emerging respiratory viruses will be an important strategy to maintain vaccine efficacy.

While our study is limited in size, the overall population of vaccinated young children is limited in part because of parental/guardian vaccine hesitancy. Additionally, routine phlebotomy presents numerous challenges in children. However, our data set advances the depth of understanding of antibody responses to the mRNA vaccine in young vaccine-eligible children and helps inform the risk/benefit ratio for providers and parents/guardians. As vaccines result in a dramatic improvement in morbidity and mortality of adults related to COVID-19 following mRNA vaccination[31], and we see comparable- if not improved- vaccine responses in young children, we expect vaccines will also reduce severe disease and long-term complications in this young population as well. As COVID-19 has become one of the leading infectious cause of death in children, and infected children can suffer from post-COVID complications[32], vaccination strategies for these young children, as well as their impact on the maturing immune system, need to be studied in depth. Overall, our data suggest that vaccination offers robust protection against future SARS-CoV-2 infections, potentially superior to natural infection, and thus support the notion that mRNA vaccination of this youngest group is highly effective. These results also provide insight for the design of the future mRNA-based vaccine technologies for this pediatric population.

Methods

Participant enrollment

Families with children 6 months to 5 years of age who were participating in the KidCOVE study, a phase two/three clinical trial to assess the safety and efficacy of the mRNA-1273 two-dose vaccine in healthy children (clinicaltrials.gov NCT04796896), at Massachusetts General Hospital (MGH) were approached for enrollment in the MGH Pediatric COVID-19 Biorepository (IRB: 2020P000955). Families with children who were infected with SARS-CoV-2 in the past 2-8 weeks and were presenting to MGH for a well-child visit or hospital visit were also approached and offered enrollment in the MGH Pediatric COVID-19 Biorepository. Parents/guardians provided informed consent prior to participation.

Sample collection

Blood was collected prior to vaccination (Pre-vaccine), one month following the first vaccination (V1), one month following the second vaccination, (V2), and six months following the second vaccination (V6). If a booster dose was received, blood was collected prior to receipt of the booster dose (if greater than six months from first vaccination), and one month following the booster (post-boost). Participants could opt out of providing blood at any of the time points. Blood was collected by venipuncture or by capillary microneedle device, processed for plasma, and stored at -80°C. All procedures were approved by the MGB IRB.

Banked samples from adults who had received the Moderna mRNA1273 vaccine were used for comparison. Detailed information regarding enrollment and specimen collection for this IRB-approved study were previously published [21].

295

296 **Antigens**

297 SARS-CoV-2 D614G or variants of concern Spike and RBD proteins, in addition to NTD, S1,
298 nucleocapsid, HCoV-HKU1 Spike (HKU1), and HCoV-OC43 Spike (OC43) antigens, were
299 expressed in mammalian HEK293 cells and purchased from Sino Biological. Influenza
300 haemagglutinin (HA) was obtained from Sino Biological. NHS-Sulfo-LC-LC kit was used for
301 antigen biotinylating, according to the manufacturer's instruction (Thermo Fisher Scientific).

302

303 **Antibody isotype, FcR binding and functions**

304 Antibody isotype and subclass levels (IgG, IgG1, IgG2, IgG3, IgG4, IgM, and IgA1), as well as
305 Fc-receptor (FcR) binding profiles (FcyR2A, FcyR2B, FcyR3A, FcyR3B, and FcαR) were
306 measured using a custom multiplex Luminex assay as described previously[33]. Plasma samples
307 were diluted between 1:50 and 1:500, depending on the secondary reagent. For functional analyses,
308 antibody-dependent complement deposition (ADCD), cellular phagocytosis (ADCP) and
309 neutrophil phagocytosis (ADNP) were performed as described previously[34,35,36] with 1:50 diluted
310 plasma samples. MFI values were analyzed by flow cytometry on an iQue analyzer (Intellicyt).

311

312 **VOC breadth score**

313 Spike and RBD protein breadth score were calculated by categorizing each antigen response as
314 positive or negative and calculating the percentage of Spike and RBD variant antigen responses
315 for each secondary (isotype or FcR) at each timepoint. We defined a positive response as six
316 standard deviations above the mean of the SARS-CoV-2-unexposed controls for the same antigen
317 and isotype or Fc receptor.

Statistical analysis

GraphPad Prism (v.9.2.0) and RStudio (v.1.3 and R v.4.0) were used to perform data analyses. We calculated breadth score by categorizing each antigen response as positive or negative and calculating the percentage of positive Spike and RBD variant antigen responses for each antibody feature at each timepoint. We defined a positive response as six standard deviations above the mean of the COVID-unexposed controls for the same antigen and isotype or Fc receptor.

Multivariate analyses to compare vaccinated adults and children were built as described previously[35, 37]. Data were normalized using z-scoring, then a least absolute shrinkage and selection operator (LASSO) approach was used for feature selection. For classification and visualization, partial least square discriminant analysis (PLS-DA) models were performed using LASSO-selected features, followed by a ten-fold cross-validation to assess model accuracy. Co-correlates of LASSO selected features were represented in a network format and identified using Spearman method followed by Benjamini-Hochberg correction.

References

1. Cox D. What do we know about covid-19 and children? *BMJ* **380**, 21 (2023).

2. Kompaniyets L*, et al.* Post-COVID-19 Symptoms and Conditions Among Children and Adolescents - United States, March 1, 2020-January 31, 2022. *MMWR Morb Mortal Wkly Rep* **71**, 993-999 (2022).

3. Faust JS, Del Rio C. Assessment of Deaths From COVID-19 and From Seasonal Influenza. *JAMA Intern Med* **180**, 1045-1046 (2020).

4. Flaxman S*, et al.* Assessment of COVID-19 as the Underlying Cause of Death Among Children and Young People Aged 0 to 19 Years in the US. *JAMA Netw Open* **6**, e2253590 (2023).

5. Ali K*, et al.* Evaluation of mRNA-1273 SARS-CoV-2 Vaccine in Adolescents. *N Engl J Med* **385**, 2241-2251 (2021).

6. Baden LR*, et al.* Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med* **384**, 403-416 (2021).

7. Creech CB*, et al.* Evaluation of mRNA-1273 Covid-19 Vaccine in Children 6 to 11 Years of Age. *N Engl J Med* **386**, 2011-2023 (2022).

8. Olson SM*, et al.* Effectiveness of BNT162b2 Vaccine against Critical Covid-19 in Adolescents. *N Engl J Med* **386**, 713-723 (2022).

9. Polack FP*, et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* **383**, 2603-2615 (2020).

10. Walter EB*, et al.* Evaluation of the BNT162b2 Covid-19 Vaccine in Children 5 to 11 Years of Age. *N Engl J Med* **386**, 35-46 (2022).

11. Anderson EJ*, et al.* Evaluation of mRNA-1273 Vaccine in Children 6 Months to 5 Years of Age. *N Engl J Med* **387**, 1673-1687 (2022).

12. Frenck RW, Jr.*, et al.* Safety, Immunogenicity, and Efficacy of the BNT162b2 Covid-19 Vaccine in Adolescents. *N Engl J Med* **385**, 239-250 (2021).

13. Bartsch YC*, et al.* SARS-CoV-2 mRNA vaccination elicits robust antibody responses in children. *Sci Transl Med*, eabn9237 (2022).

14. Bartsch YC*, et al.* BNT162b2 induces robust cross-variant SARS-CoV-2 immunity in children. *NPJ Vaccines* **7**, 158 (2022).

15. Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. *Proc Biol Sci* **282**, 20143085 (2015).

16. Irani V, Guy AJ, Andrew D, Beeson JG, Ramsland PA, Richards JS. Molecular properties of human IgG subclasses and their implications for designing therapeutic monoclonal antibodies against infectious diseases. *Mol Immunol* **67**, 171-182 (2015).

17. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol* **5**, 520 (2014).

18. Damelang T, Rogerson SJ, Kent SJ, Chung AW. Role of IgG3 in Infectious Diseases. *Trends Immunol* **40**, 197-211 (2019).

19. Richardson SI*, et al.* IgG3 enhances neutralization potency and Fc effector function of an HIV V2-specific broadly neutralizing antibody. *PLoS Pathog* **15**, e1008064 (2019).

20. Chen W*, et al.* The kinetics of IgG subclasses and contributions to neutralizing activity against SARS-CoV-2 wild-type strain and variants in healthy adults immunized with inactivated vaccine. *Immunology* **167**, 221-232 (2022).

21. Frasca D, Diaz A, Romero M, Landin AM, Blomberg BB. Age effects on B cells and humoral immunity in humans. *Ageing Res Rev* **10**, 330-335 (2011).

22. Bartsch YC*, et al.* Humoral signatures of protective and pathological SARS-CoV-2 infection in children. *Nat Med* **27**, 454-462 (2021).

23. Boribong BP*, et al.* Neutrophil Profiles of Pediatric COVID-19 and Multisystem Inflammatory Syndrome in Children. *Cell Reports Medicine*.

24. Li Y, Jin L, Chen T. The Effects of Secretory IgA in the Mucosal Immune System. *Biomed Res Int* **2020**, 2032057 (2020).

25. Pabst O, Slack E. IgA and the intestinal microbiota: the importance of being specific. *Mucosal Immunol* **13**, 12-21 (2020).

26. Loske J*, et al.* Pre-activated antiviral innate immunity in the upper airways controls early SARS-CoV-2 infection in children. *Nat Biotechnol*, (2021).

27. Stavnezer J, Schrader CE. IgH chain class switch recombination: mechanism and regulation. *J Immunol* **193**, 5370-5378 (2014).

28. Stavnezer J, Guikema JE, Schrader CE. Mechanism and regulation of class switch recombination. *Annu Rev Immunol* **26**, 261-292 (2008).

29. Vatti A, Monsalve DM, Pacheco Y, Chang C, Anaya JM, Gershwin ME. Original antigenic sin: A comprehensive review. *J Autoimmun* **83**, 12-21 (2017).

30. Goronzy JJ, Weyand CM. T cell development and receptor diversity during aging. *Curr Opin Immunol* **17**, 468-475 (2005).

31. Tenforde MW*, et al.* Association Between mRNA Vaccination and COVID-19 Hospitalization and Disease Severity. *JAMA* **326**, 2043-2054 (2021).

32. Woolf SH, Chapman DA, Lee JH. COVID-19 as the Leading Cause of Death in the United States. *JAMA* **325**, 123-124 (2021).

33. Brown EP*, et al.* High-throughput, multiplexed IgG subclassing of antigen-specific antibodies from clinical samples. *J Immunol Methods* **386**, 117-123 (2012).

34. 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).

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

36. Karsten CB*, et al.* A versatile high-throughput assay to characterize antibody-mediated neutrophil phagocytosis. *J Immunol Methods* **471**, 46-56 (2019).

37. Chung AW*, et al.* Dissecting Polyclonal Vaccine-Induced Humoral Immunity against HIV Using Systems Serology. *Cell* **163**, 988-998 (2015).

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Author contributions

N.N. and T.C. performed the serological experiments. N.N., Y.D., L.W., N.D., N.D.G., R.M., B.J., G.A. and L.Y. analyzed and interpreted the data. A.K., Z.S., J.D., M.D., A.C., A.F., A.E., N.J., B.H., W.S. and L.Y. managed the sample collection. N.N, G.A. and L.Y. drafted the manuscript. All authors critically reviewed the manuscript.

Competing interests

G.A. is a V.P. at Moderna, a founder and equity holder of Seromyx Systems, and an employee and equity holder of Leyden Labs. G.A.'s interests were reviewed and are managed by MGH and Partners HealthCare in accordance with their conflict-of-interest policies. All other authors declare that they have no competing interests.

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Figures

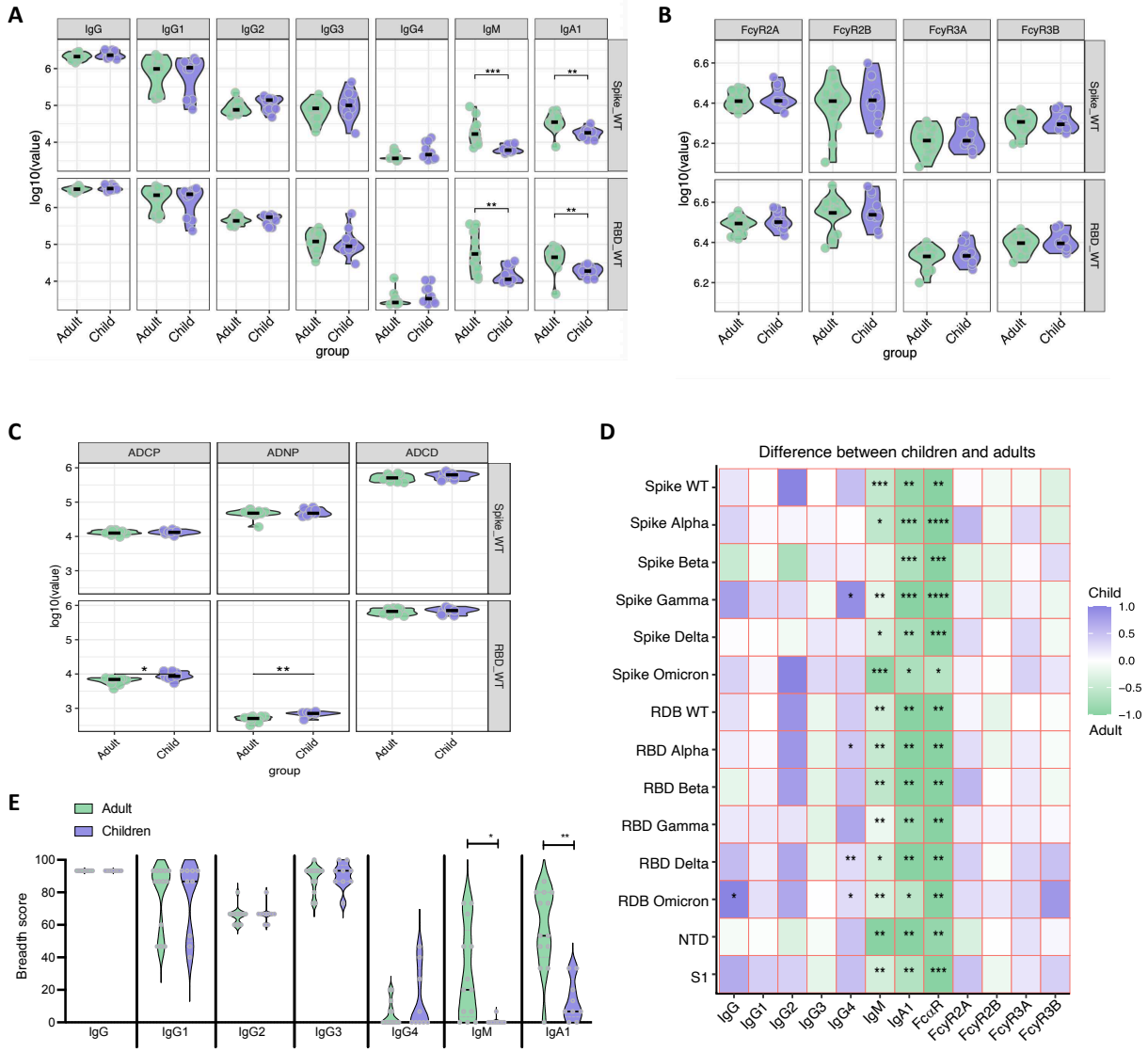
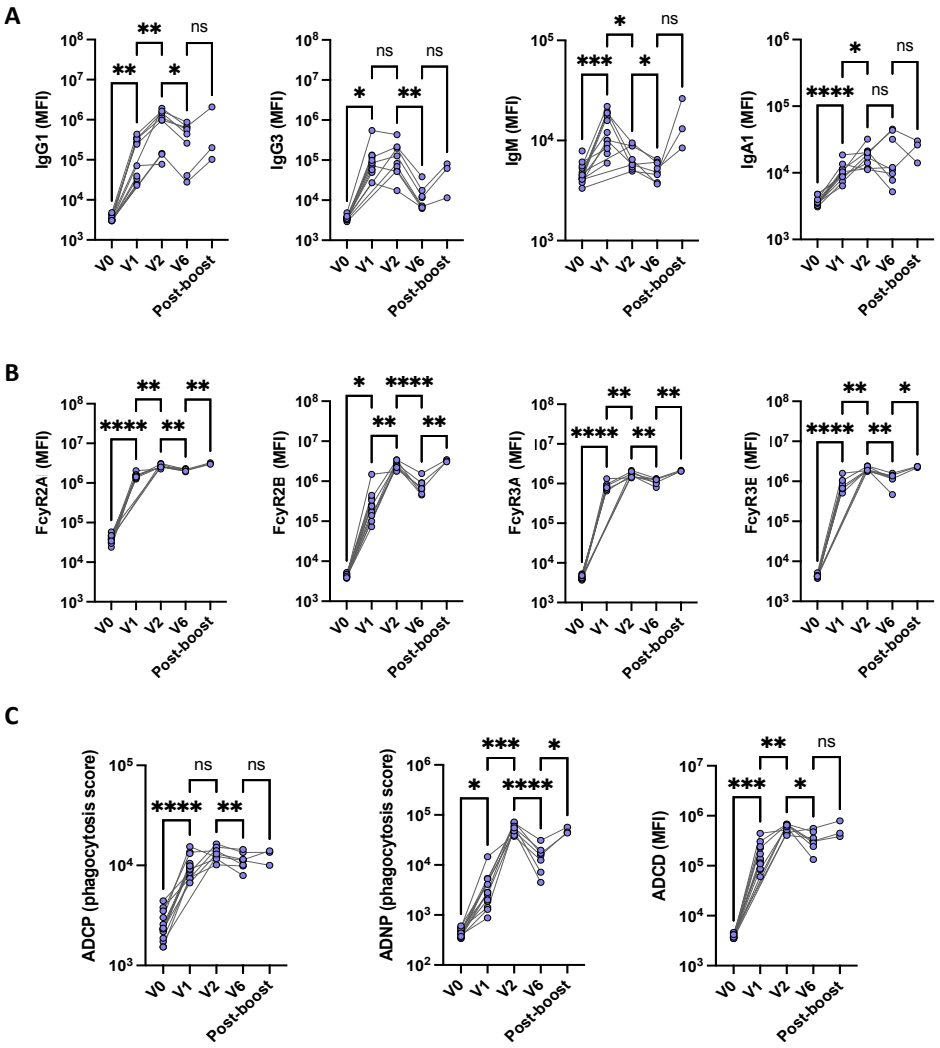


Fig. 1. mRNA-1273 vaccination induces a strong humoral immunity in children less than 5 years old. Antibody levels and functionality were measured in the plasma of children less than 5 years old and adults, 2 months after vaccination. (**A**) Total IgG, IgG1, IgG2, IgG4, IgG4, IgM and IgA1 against Spike and RBD WT. (**B**) FcyR2A, FcyR2B, FcyR3A and FcyR3B binding against Spike and RBD WT. (**C**) ADCP, ADNP and ADCD against Spike and RBD WT. (**D**) Heatmap shows the univariate comparison between SARS-CoV-2-specific antibody response in children

and adults. Difference between the median of Z-scored MFI data are represented, where the color corresponds to the group that has the highest antibody response. (**E**) Breadth score was calculated by categorizing each antigen response as positive or negative, with positive response defined as 6 standard deviations above the mean of the COVID-unexposed controls, then calculating the percentage of positive Spike and RBD variant antigen responses for each secondary. Non-parametric Mann-Whitney U-test was used to calculate statistical significance, followed by Benjamini-Hochberg correction for multiple testing. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.



493 **Fig. 2. Strong impact of mRNA-1273 vaccination on antibody response overtime.** Antibody
494 levels (**A**), binding to FcγR (**B**) and function (**C**) against Spike WT was analyzed in children at
495 different timepoints: before vaccination (V0), 1 month (V1) after first dose of mRNA-1273
496 vaccine, 2 months (V2) after the first dose of mRNA-1273 vaccine, in addition to 1 months after
497 the second dose, 6 months (V6) after the first mRNA-1273 vaccination in addition to 5 month after
498 the 2 doses, as well as 1 month after boosting (post-boost). Connecting lines represent identical
499 individuals that were followed over time, and statistical differences were calculated between 2
500 consecutive timepoints. Wilcoxon signed rank test was used to calculate differences between
501 timepoints for paired data. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

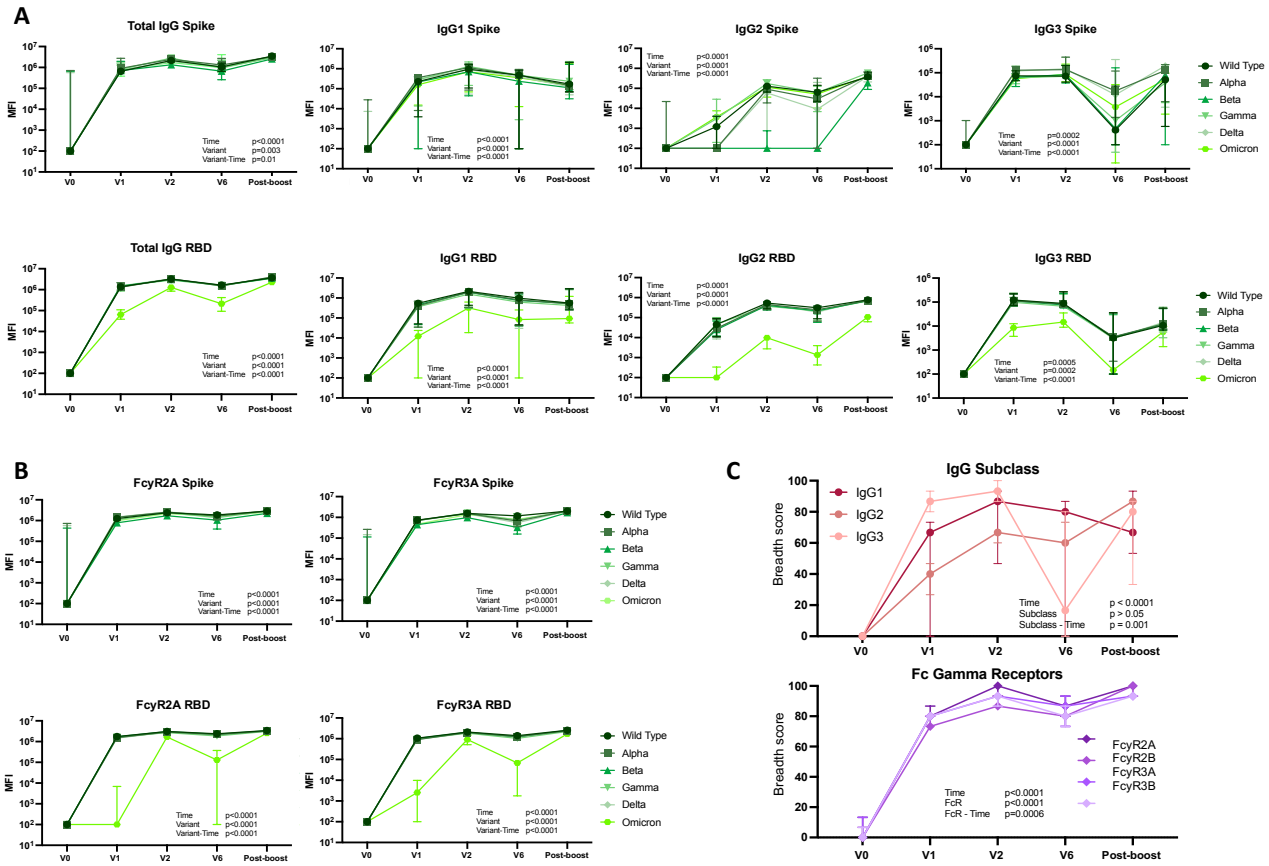


Fig. 3. Similar antibody responses across variant epitopes. Median and 95% confidence interval at each timepoint for (**A**) Total IgG, IgG1, IgG2 and IgG3, as well as (**B**) FcγR2A and FcγR3A binding against Spike and the RBD. Mixed effect models were run, and p values reported for time, variant and variant-time interaction coefficients. (**C**) Breadth score was calculated by defining positive responses to each antigen response as 6 standard deviations above the mean of the COVID-unexposed controls for the same antigen and subclass or FcR. We then calculated the percentage of Spike and RBD variant antigen responses for each secondary individual at each timepoint.

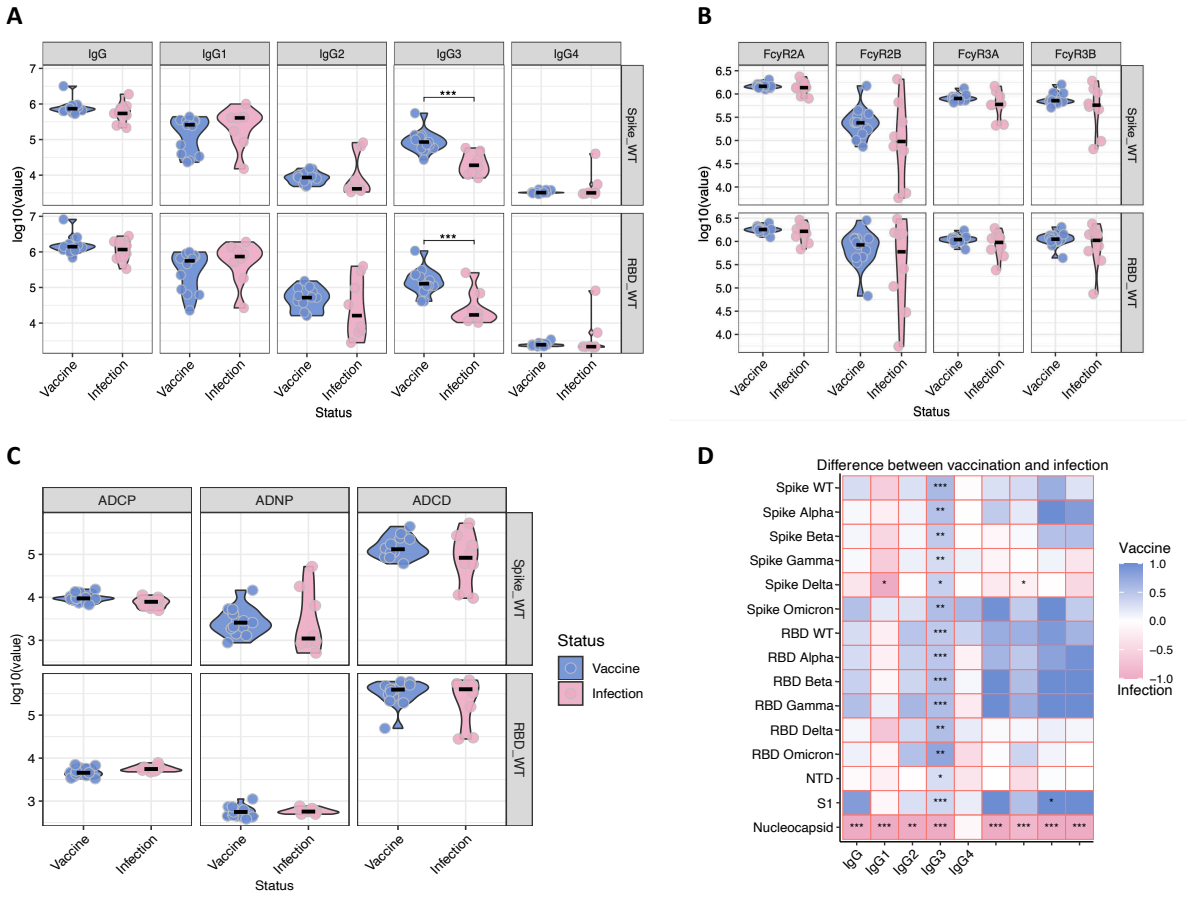


Fig. 4. Distinct antibody response between vaccination and natural infection in children under five. Antibody response was measured in the plasma of children 1 month after vaccination or diagnosis (on average). (**A**) Total IgG, IgG1, IgG2, IgG4 and IgG4 against Spike and RBD WT. (**B**) FcγR2A, FcγR2B, FcγR3A and FcγR3B binding against Spike and RBD WT. (**C**) ADCP, ADNP and ADCD against Spike and RBD WT. (**D**) Heatmap illustrates the difference between the median of Z-scored MFI data of the vaccinated and infected groups. Non-parametric Mann-Whitney U-test was used to calculate statistical significance, followed by Benjamini-Hochberg correction for multiple testing. *p < 0.05, **p < 0.01, ***p < 0.001.

Table 1. Demographics of mRNA-vaccinated and convalescent infants and children enrolled.

Patient Characteristics	mRNA-1273 vaccinated (n = 19)	COVID-Recovered (n = 5)	Total children enrolled (n = 24)
Age at Enrollment, mean (min, max)	2.2 (0.6, 4.5)	3.7 (1, 5)	2.5 (0.6, 5)
Male Sex, number (%)	7 (36.8)	3 (60)	10 (42)
Hispanic, number (%)	8 (42.1)	2 (40)	10 (42)
Race, number (%)			
American Indian/Native Alaskan	1 (5.3)	0 (0)	1 (4.2)
Asian	0 (0)	0 (0)	0 (0)
Black	2 (10.5)	0 (0)	2 (8.3)
Other	5 (26.3)	2 (40)	7 (29.2)
Unknown	2 (10.5)	0 (0)	2 (8.3)
White	9 (47.4)	3 (60)	12 (50)

Supplementary Files

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- CovidvaccineNatSupplementarymaterial.pdf