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Delayed Induction of Noninflammatory SARS-CoV-2 Spike-Specific IgG4 Antibodies Detected 1 Year After BNT162b2 Vaccination in Children

Robin Kobbe, MD,†‡‡ Cornelius Rau, MD, MPhil,*† Ulf Schulze-Sturm, MD,‡ Felix Stahl, MD,§ Luis Fonseca-Brito,§ Anke Diemert, MD,¶‖ Marc Lütgehetmann, MD,** Marylyn M. Addo, MD,*††‡‡ Petra Arck, MD,¶‖ and Leonie M. Weskamm, PhD*††‡‡*

Abstract: Humoral immune responses after BNT162b2 vaccination are predominantly composed of immunoglobulin (Ig) G1 and IgG3 subclass antibodies. As previously described in adults, S1-specific and receptor-binding domain–specific IgG4 levels increase significantly 1 year after the second BNT162b2 vaccination in children 5-11 years of age. Understanding mRNA vaccine–specific IgG4 responses in all age groups is crucial as more mRNA vaccines will reach licensure in the coming years.

Key Words: mRNA vaccine, IgG4, noninflammatory, SARS-CoV-2, IgG subclass, immune response, children, system immunology

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The new technology of mRNA-based vaccination proved to be one of the most important tools to fight the SARS-CoV-2 pandemic, allowing safe and effective mass vaccination, saving millions of lives and opening the possibility of developing wide-ranging future therapeutics.[1] The strong association of SARS-CoV-2 spike–specific antibodies with protection against COVID-19 and the detailed knowledge gathered by the research community about humoral immune responses after repeated mRNA vaccination has recently been reviewed in detail.[2] Meanwhile, some features induced by mRNA vaccination still need further investigation, among these the unusual induction of immunoglobulin (Ig) G4 antibodies.

In general, the protective humoral response after 2 doses of BNT162b2 or mRNA-1273 is composed predominantly of IgG1 and IgG3 subclass antibodies, both capable of mediating effector functions such as antibody-dependent cytotoxicity, phagocytosis and complement activation via their fragment crystallizable (Fc) region.[2,3] Real-life data and passive and active immunization studies in mice suggest that the engagement of the Fc region with Fc gamma receptors is required for vaccine-induced antibody-mediated protection against infection by antigenically distinct SARS-CoV-2 variants of concern, including Omicron strains.[3,4] Previously, it has been reported that 5- to 11-year-old children vaccinated with a 3-fold lower vaccine dose (BNT162b2, 10 µg) mount a lower magnitude of total antibodies at 2-4 weeks after the second vaccination compared to adults.[5] In the same study, the analysis of Fc receptor–binding properties by Luminex revealed that children mounted similar levels of Fc receptor–binding antibodies compared to adults. This finding suggests a qualitatively superior Fc antibody functionality that may contribute to the protection against variants of concern and attenuation of COVID-19 in this younger age group.[5]

Irrgang et al[6] were the first to report an increased proportion of SARS-CoV-2 spike–specific IgG4 in adults, starting after the second and increasing further after the third mRNA vaccine dose, resulting in up to 19.27% of total specific IgG levels. Furthermore, they observed a reduced capacity of spike-specific antibodies to mediate antibody-dependent cellular phagocytosis and complement deposition alongside substantial frequencies of IgG4-switched B cells. In adults, this mRNA-specific effect seems to be more pronounced in infection-naive individuals.[7] It was not observed after homologous vaccination with adenovirus-based and Modified Vaccinia virus Ankara (MVA)–based SARS-CoV-2 vaccines and, to a lesser extent, after heterologous immunization with adenovirus- and MVA-based followed by mRNA-based vaccines.[8–10] After the third mRNA vaccination, these studies also reported an atypical induction of IgG2 antibodies, which are usually not induced by protein but polysaccharide antigens and are known to have a lower affinity to Fc receptors.[6,7,10]

To determine IgG4 induction following BNT162b2 vaccination in children 5-11 years of age, we measured SARS-CoV-2 spike subunit 1 (S1)–specific and receptor-binding domain (RBD)–specific IgG subclasses by a bead-based multiplex immunoassay in 14 healthy children [median age, 8.5 (interquartile range [IQR], 6.4–10.0) years; 6 (43%)

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*From the Institute for Infection Research and Vaccine Development, ‡Department of Pediatrics-Kinder-UKE, §Institute of Immunology, ¶Division of Experimental Feto-Maternal Medicine, Department of Obstetrics and Fetal Medicine, ‖Hamburg Center for Translational Immunology, and **Institute of Microbiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; †Department of Infectious Disease Epidemiology and ††Department for Clinical Immunology of Infectious Diseases, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany; and ‡‡German Centre for Infection Research, Hamburg, Germany.

R.K. and C.R. contributed equally to this work.

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R.K. and L.M.W. conceived the study. R.K., L.M.W. and C.R. designed the study, cleaned and analyzed the data, constructed the figures, and wrote the first draft of the manuscript. R.K., U.S.-S., L.F.-B., A.D., M.L., M.M.A., P.A. and L.M.W. were involved in the study procedures, collected samples or performed laboratory work. All authors contributed data to the study, contributed to the data interpretation, critically reviewed the manuscript and approved the final manuscript for submission. Sharing of data will be possible on individual request, while sharing with third parties is not allowed. Requests for data access can be submitted to the corresponding author.

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Address for correspondence: Robin Kobbe, MD, Institute for Infection Research and Vaccine Development (IIRVD), University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany. E-mail: r.kobbe@uke.de.

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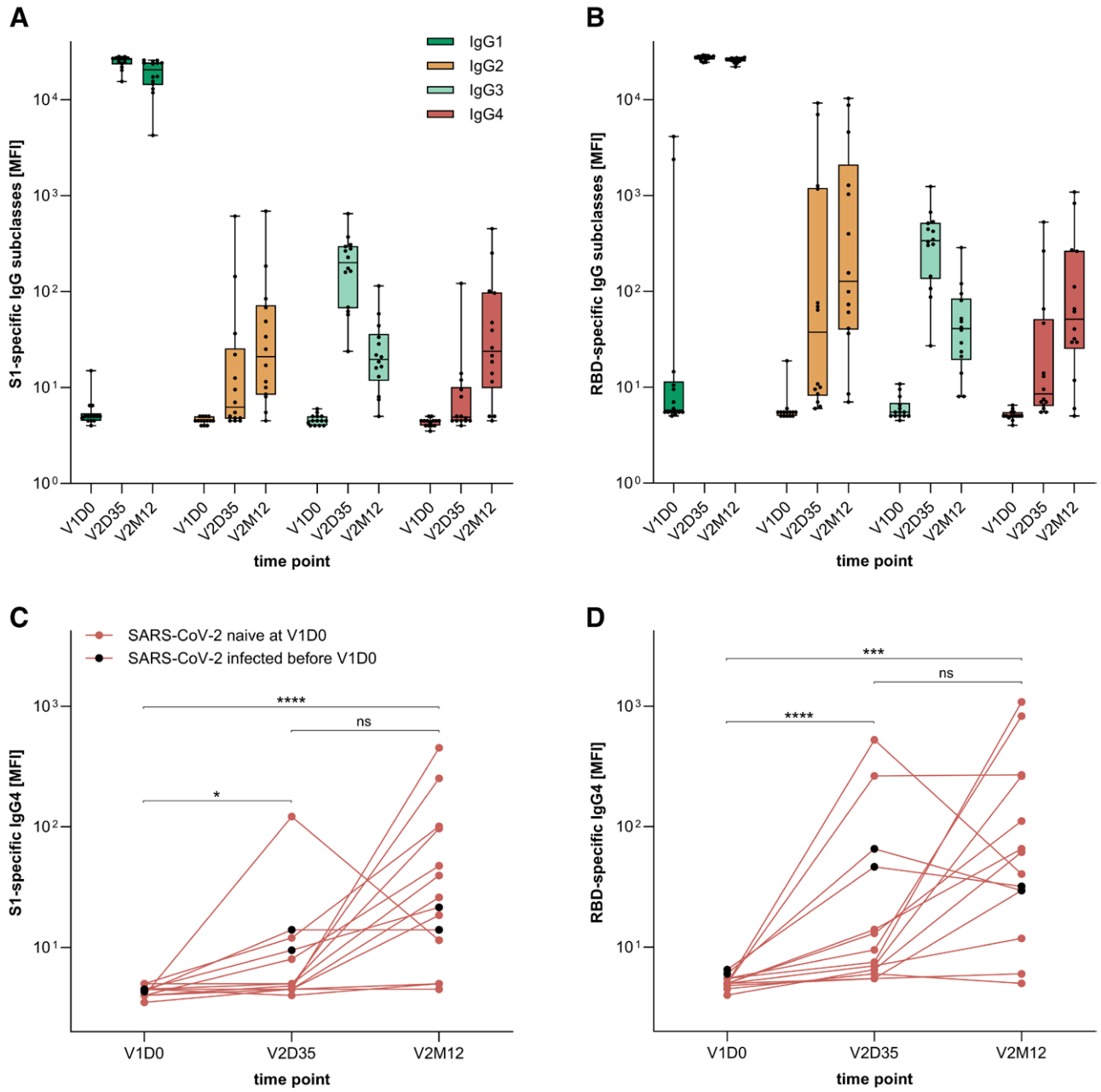

FIGURE 1. Longitudinal IgG subclass composition of spike-specific antibodies following BNT162b2 vaccination in children. A and B: Subclass composition of (A) S1- and (B) RBD-specific IgG subclasses at baseline (V1D0), 4 weeks (V2D35) and 1 year (V2M12) after the second vaccination. Boxplots indicate median, IQR and min-max range. Dots represent individual study participants. C and D: Longitudinal dynamics of (C) S1- and (D) RBD-specific IgG4 induction. Connecting lines indicate data points belonging to the same study participant. All data points are reported as median fluorescent intensity (MFI), measured by the Bio-Plex 200 system. Fourteen children [median age, 8.5 (IQR, 6.4–10.0) years] received 2 doses of the mRNA-BNT162b2 vaccine (10 µg, Corminaty, BioNTech/Pfizer) with a median interval of 27.5 (IQR, 27–28) days; blood was collected on the day of the first dose (V1D0), as well as 5 weeks [V2D35; median, 35.5 (IQR, 30–45) days] and 1 year [V2M12; 350.5 (IQR, 344–364) days] after the second dose. Two individuals had been infected prior to V1D0 (indicated by black circles in C and D), and all previously uninfected children experienced breakthrough SARS-CoV-2-Omicron infection until V2M12. Statistical comparisons between V1D0, V2D35 and V2M12 are only shown for IgG4 subclasses, irrespective of statistical significance. Comparison over time within either the S1 or RBD group was done by the Kruskal-Wallis equality-of-populations rank test, followed by Dunn's test, Bonferroni-adjusted for multiple comparisons (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; and ****$P < 0.0001$). ns indicates not significant.

girls, 8 (57%) boys; for details see Table, Supplemental Digital Content 1, http://links.lww.com/INF/F656] of the well-characterized PRINCE (Prenatal Identification of Children's Health) birth cohort study.[11]

All parents and study participants signed informed consent forms, and the study protocol was approved by the ethics committee of the Hamburg Chamber of Physicians under the license numbers PV3694 and PV7571 (10376-BO-ff). The children were vaccinated with 2 doses of BNT162b2 vaccine (10 µg, Corminaty, BioNTech/Pfizer), with a median interval of 27.5 (IQR, 27–28) days between doses. Blood was collected on the day of the first dose (V1D0), as well as 5 weeks [V2D35; median, 35.5 (IQR, 30–45) days] and 1 year [V2M12; 350.5 (IQR, 344–364) days] after the second dose. None of the participants had more than mild postvaccination reactions. In 2 children with a history of mild COVID-19, evaluation of humoral and cell-mediated immune responses confirmed previous SARS-CoV-2 infection. With the emergence of the Omicron variant, all children became infected with no or only mild symptoms by the time of long-term follow-up (V2M12) and showed respective positive hybrid immunity responses (for more information see Figures, Supplemental Digital Content 2-3, http://links.lww.com/INF/F657; http://links.lww.com/INF/F658).

To measure S1- and RBD-specific IgG subclass levels, plasma samples were diluted 1:200 and analyzed by a bead-based multiplex assay using the MILLIPLEX SARS-CoV-2 Antigen Panel 1 IgG Kit (Merck KGaA, Darmstadt, Germany) following the manufacturer's instructions. Detection antibodies were substituted with PE-conjugated antibodies specific to IgG1-4 (SouthernBiotech, Birmingham, AL), added at a concentration of 0.65 µg/ml in 80 µL/well. The samples were analyzed using a Bio-Plex 200 system. Statistical comparison between time points was performed by the Kruskal-Wallis equality-of-populations rank test, followed by Dunn's test, Bonferroni-adjusted for multiple comparisons, using STATA statistical software (Release 18, StataCorp, College Station, TX). GraphPad Prism 9 (San Diego, CA) was used for visualization.

The children's antibody response 5 weeks after the second BNT162b2 vaccination was dominated by the IgG1 and IgG3 subclasses, which subsequently decreased over time. By contrast, IgG2 and IgG4 levels were relatively low at week 5 after the second vaccination and increased in frequency until the late follow-up for both S1 and RBD (Fig. 1A and B), as also observed in our adult cohort.[10] Specifically, S1- and RBD-specific IgG4 antibody levels increased significantly 1 year after the second vaccination compared to baseline (Fig. 1C and D). As reported by Buhre et al[8] for adults, we observed higher IgG4 levels in infection-naive children at the time of first vaccination compared to the previously infected individuals although the small number of participants hampered statistical calculations.

It remains unclear how the specific subclass kinetics with delayed IgG2 and IgG4 induction by mRNA vaccination, here first described in children, affects long-term immunity. A study by de Jong et al performed immunophenotyping and sequencing of peripheral blood mononuclear cells from young children and adults to investigate sequential IgG subclass switching. Their findings suggest that an accumulation of specific IgG2- and IgG4-expressing memory B cells occurs with age, is associated with increasing levels of somatic hypermutation and might represent a marker for an efficient generation of memory responses.[9] We hope that our observation will stimulate more studies on both similarities and differences of immune responses in adults and children, for example, regarding switching of IgG subclasses and functionality of vaccine-induced antibodies.

IgG4, as the least abundant IgG subclass in humans, has some unique structural and functional features resulting in them being described as "blocking" and "anti-inflammatory" antibodies with an inability to activate antibody-dependent immune effector responses. In addition, IgG4 is the only subclass capable of fragment antigen binding-arm exchange, rendering it bispecific for antigen binding and functionally monovalent, which can either be beneficial or damaging, as recently discussed.[12] Furthermore, varying glycosylation between different IgG molecules can affect antibody functions, such as Fcγ receptor activation and complement activation, and warrants further investigation in pediatric vaccinology.[4] It has been proposed that persistent germinal center responses in lymph nodes, either specific to lipid nanoparticles or spike-antigen, or freely circulating spike-antigen in the priming phase of the immune response after mRNA vaccination, might drive IgG4 class switching.[2,13] One potential explanation is the important role of cluster of differentiation 4 + T follicular helper (TFH) cells in establishing long-term immunity elicited by this new vaccine type.[14] TFH cells are also described as involved in regulating IgG4 class switching and the pathogenesis of IgG4-related diseases.[2,15] Factors deemed important in this process include TFH localization related to the germinal center, its differentiation, functionality and age-related tempering of inflammation, as well as its control by cytokine signals, T cell antigen receptor stimulation and costimulation.[16] Understanding the role and interplay of these regulatory factors will be crucial to design safe and effective vaccines for all age groups in the future.

In summary, we report on increased spike-specific IgG4 levels in children 1 year after BNT162b2 vaccination, such as the effect observed in adults. While our study does not allow to predict effects on population level due to the small cohort size, it gives insight into the longitudinal dynamics of the spike-specific IgG subclass composition in children. IgG4 responses should gain more attention in health and disease, especially in the context of mRNA vaccination. Understanding the unusual mechanism triggering IgG4 production is crucial, as more mRNA vaccines are currently under development and could hit the global market soon.

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