

BRIEF REPORT

Seroprevalence and T-cell response in 32 children 10 months after COVID-19

Protective cellular and humoral immune responses to SARS-CoV-2 have been found to persist 1 year after recovery from COVID-19 in adults.¹ Less is known about the kinetics of SARS-CoV-2 antibodies and T-cell memory response in children. We determined IgG antibodies against spike protein (anti-S), nucleocapsid protein (anti-N) and IFN- γ T-cell response 10 months after PCR-confirmed COVID-19 in 32 children aged 1–18 years.

All patients, <19 years of age, with a PCR-confirmed SARS-CoV-2 infection May–July 2020 in Region Västmanland, Sweden, were invited to participate in the study. After written informed consent, blood samples were collected 10 months (± 7 days) after the positive PCR test. A short questionnaire was completed at the time of blood sampling. For qualitative detection of anti-N IgG, a chemiluminescent microparticle immunoassay (CMIA) was used (SARS-CoV-2 IgG, Abbott). An index of the absorbance of the specimen and calibrator ≥ 1.1 was reported as positive. For semi-quantitative detection of anti-S IgG, a CMIA was used (SARS-CoV-2 IgG II Quant assay, Abbott). A signal/cut-off ratio ≥ 14 BAU (binding antibody unit)/ml was reported as positive. The T-cell responses to peptide pools from spike protein (S1), spike (S1 and S2), nucleoprotein, membrane protein and open-reading frame proteins (SNMO) were assessed using an enzyme-linked immunospot assay (ELISpot Confirm kit: SARS-CoV-2 (T-cells and INF- γ), Mabtech, Sweden) following manufacturers' instruction, using freshly prepared peripheral blood monocytes. A positive control (anti-CD3 mAb) and a negative control well was set up for each individual. A positive T-cell response (towards either S1 or SNMO) was defined as a well with >7 spots after subtraction of the number of spots in the negative control well and with at least two times the number of spots in the negative control well. T-cell analysis was performed by abclabs, Solna, Sweden, whereas serology was performed at the Department of Microbiology, Region Västmanland, Sweden. The study was approved by the Swedish Ethical Review Board (Dnr 2020–06283).

Thirty-two out of 52 eligible children were included in the study. The median age was 13.5 years (range 1.5–18 years). 19/32 (62%)

participants were female. The most commonly reported symptoms were fever (23/32), fatigue (13/32), cough (12/32) and anosmia/ageusia (12/32). No child was hospitalised due to COVID-19. No re-infections were encountered during follow-up (10 months ± 7 days). The presence of anti-N IgG, anti-S IgG (including binding antibody unit [BAU] levels) and T-cell response towards SARS-CoV-2 antigens is presented by age-group in Table S1. Anti-S IgG was detected in 25/32 (78%) of the children and anti-N IgG in 2/32 (6%). A T-cell response towards SARS-CoV-2 S1 was demonstrated in 17/32 (53%) and towards the combined SMNO-peptide pool in 20/32 (63%), with a concordance rate of 78%. All but two children with a T-cell response (to either the S1 peptide pool or the SMNO-peptide pool) were positive for anti-S IgG (Figure 1).

In this study, 78% of the children had positive anti-S IgG 10 months after SARS-CoV-2 infection, but only 2/32 were positive for anti-N IgG. A T-cell response was detected in two-thirds of the children and almost completely overlapped the presence of anti-S IgG.

It has been shown that SARS-CoV-2 infected children predominantly develop antibodies towards S1, and that the level of anti-S IgG correlates with neutralising activity.^{2,3} Neutralising antibodies (nAbs) persist 8 months after COVID-19 in children, whereas antibodies directed at a combined target of spike and nucleocapsid proteins are largely undetectable after 7–9 months.² This is in line with the almost nonexistence of positive anti-N IgG in this cohort of PCR-confirmed mild cases of COVID-19 and favours anti-S IgG assays in children when investigating humoral immunity months after presumed infection. However, the discrepancy between anti-S and anti-N could also be related to low sensitivity of the anti-N assay in late convalescent sera.

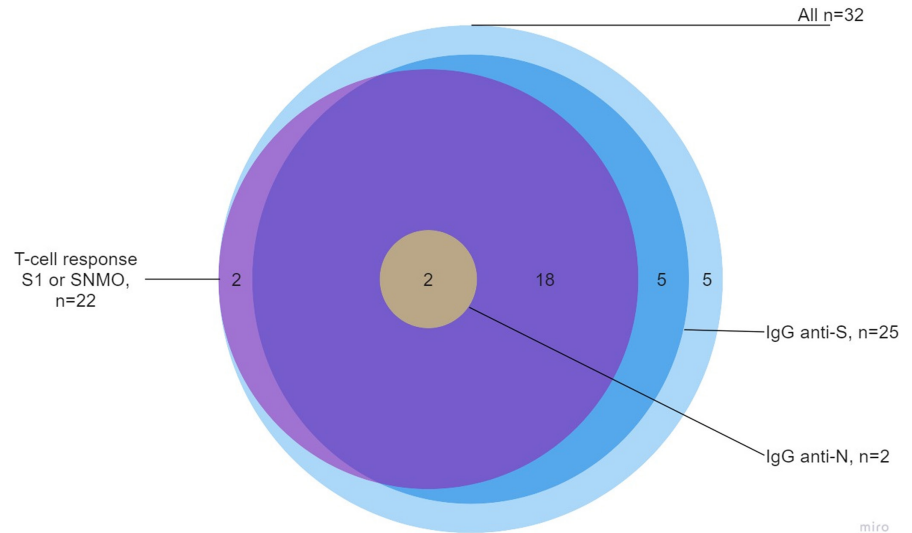
The nature of long-term T-cell response has, to our knowledge, not previously been presented after PCR-confirmed SARS-CoV-2 infection in children. Robust T-cell response to SARS-CoV has been found to persist 17 years after the SARS outbreak in 2003, which suggested that even though antibody levels to SARS-CoV-2 are waning with time, T-cell immunity may still persist. In adults, the frequency

Abbreviations: anti-N IgG, immunoglobulin G directed at SARS-CoV-2 nucleocapsid protein; anti-S IgG, immunoglobulin G directed at SARS-CoV-2 spike protein; BAU, binding antibody unit; CMIA, chemiluminescent microparticle immunoassay; COVID-19, coronavirus disease 2019; ELISpot, enzyme-linked immunospot; IgG, immunoglobulin G; INF, interferon; nAb, neutralising antibody; number, *n*; ORF, open-reading frame; PCR, polymerase chain reaction; SARS-CoV, severe acute respiratory syndrome coronavirus; SNMO, spike nucleocapsid membrane open-reading frame.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. *Acta Paediatrica* published by John Wiley & Sons Ltd on behalf of Foundation Acta Paediatrica.

FIGURE 1 Overlapping immune responses in a cohort of 32 children with PCR-confirmed COVID-19. anti-N, directed against SARS-CoV-2 nucleocapsid protein; anti-S, directed against SARS-CoV-2 spike protein; IgG, immunoglobulin G; S1, spike protein domain 1; SNMO, spike nucleocapsid membrane open-reading frame



of T-cell response to SARS-CoV-2 decreased six months posthospital discharge but then remained stable at 79% at 12 months.¹ A Swedish study on young adults found that 67% of IgG positive individuals demonstrated a positive T-cell response up to 6–7 months after presumed infection.⁴ Available data indicate that children to a lesser extent than adults demonstrate T-cell response towards SARS-CoV-2 early (weeks) after infection (83% vs. 100%).⁵ As previously suggested, this difference could be related to age and/or disease severity.⁴ We found that 68% of children demonstrated T-cell response to SARS-CoV-2 antigen at ten months, indicating long-lasting cellular immune response.

Limitations of this study include the small number of participants, the lack of a control group and the absence of an assay to determine nAbs.

To summarise, this study indicates a persistent immunity of at least ten months after natural infection for most children and suggests that a long-lasting T-cell response may be present in the majority of children after mild infection.

ACKNOWLEDGEMENT

The authors would like to thank the paediatric nurses at the Department of Paediatrics, Hospital of Västmanland, Västerås.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

David Moritz¹
Anders Krifors^{2,3,4}
Elisabeth Freyhult⁵
Emeli Månsson^{2,3,6} 

¹Department of Paediatrics, Uppsala University, Uppsala, Sweden

²Department of Infectious Diseases, Västmanlands Hospital, Västerås, Sweden

³Centre of Clinical Research Region Västmanland, Uppsala University, Uppsala, Sweden

⁴Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

⁵Department of Microbiology, Västmanlands Hospital, Västerås, Sweden

⁶School of Medical Sciences, Örebro University, Örebro, Sweden

Correspondence

Emeli Månsson, Department of Infectious Diseases, Västmanlands Hospital Västerås, 721 89 Västerås, Sweden.
Email: emeli.mansson@regionvastmanland.se

ORCID

Emeli Månsson  <https://orcid.org/0000-0003-2867-1044>

REFERENCES

- Feng C, Shi J, Fan Q, et al. Protective humoral and cellular immune responses to SARS-CoV-2 persist up to 1 year after recovery. *Nat Commun.* 2021;12(1):4984.
- Bonfante F, Costenaro P, Cantarutti A, et al. Mild SARS-CoV-2 infections and neutralizing antibody titers. *Pediatrics.* 2021;148(3):e2021052173.
- Weisberg SP, Connors TJ, Zhu Y, et al. Distinct antibody responses to SARS-CoV-2 in children and adults across the COVID-19 clinical spectrum. *Nat Immunol.* 2021;22(1):25-31.
- Bjorkander S, Du L, Zuo F, et al. SARS-CoV-2-specific B- and T-cell immunity in a population-based study of young Swedish adults. *J Allergy Clin Immunol.* 2022;149(1):65-75.e8.
- Kaaijk P, Pimentel VO, Emmelot ME, et al. Children and adults with mild COVID-19 symptoms develop memory T cell immunity to SARS-CoV-2. *medRxiv.* 2021. doi:10.1101/2021.09.10.21263333

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.