Distinct T Cell Functional Profiles In Unvaccinated SARS-CoV-2 Seropositive And Seronegative Children Associated With Human Coronavirus HKU1 Cross-Reactivity

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BACKGROUND

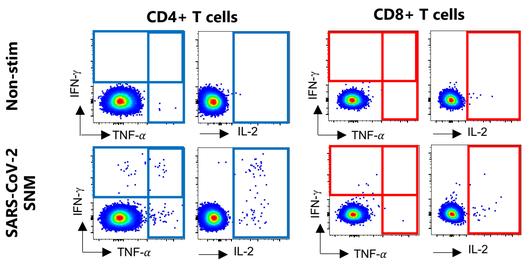
- Children infected with SARS-CoV-2 are more likely to exhibit mild or even asymptomatic disease compared to adults.
- One of the proposed mechanisms for protection from severe COVID-19 in children is cross-reactive immune responses against human coronaviruses, which cause frequent mild childhood infection.
- The immune mechanisms for the differences in disease progression between children and adults is not fully understood and studies assessing SARS-CoV-2 immune responses of paediatric populations in Africa is still scarce.

We aimed to investigate SARS-CoV-2-specific T cell responses and functional profiles in SARS-CoV-2 seropositive and seronegative unvaccinated South African children.

METHODS

- Participants were recruited in Cape Town, South Africa from 1 February 2021- 20 May 2021.
- Blood samples were stimulated with SARS-CoV-2 Spike, Nucleocapsid and Membrane peptide pools for 24 hrs using whole blood assay.
- Plasma samples were used for SARS-CoV-2 indirect ELISA.
- Cytokine expression was quantified using BD Fortessa flow cytometer.
- Data analysis was done using FlowJo v10 and GraphPad Prism v9.

Representative examples of SARS-CoV-2 specific T-cell responses



RESULTS

Demographics of children and adults included in the study

	Children	Convalescent adults
N	71	30
Age, median (IQR)	7 (2.8 - 9)	37.5 (32.3 - 45.3)
Gender, (n, %, female)	24 (34%)	22% (73%)
SARS-CoV-2 vaccination (n,%)	0 (0%)	0 (0%)
SARS-CoV-2 PCR positivity (n,%)	ND	30 (100%)
Days since PCR+ (median, IQR)	N/A	224 (189-239)
Collection date range	1 Feb-20 May 2021	22 Jan-23 Feb 2021

SARS-CoV-2-specific antibody responses in children

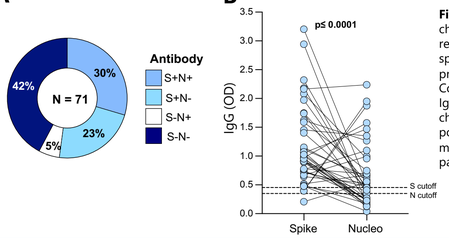


Figure 1: (A) Proportion of children (N=71) with antibody responses against SARS-CoV-2-spike, and/or nucleocapsid protein. **(B)** Magnitude of SARS-CoV-2-spike and nucleocapsid IgG antibodies in seropositive children (n=41). A cut-off for positivity was calculated as mean optical density of pre-pandemic control samples.

To characterise the children serologically, IgG responses against SARS-CoV-2-spike and nucleocapsid antibody was measured, seropositive (light blue) and seronegative (dark blue). Overall, 58% of the children had SARS-CoV-2-specific antibody responses indicative of past infection. 23% (16/71) of seropositive children had undetectable antibody responses against nucleocapsid possibly due to faster waning.

SARS-CoV-2-specific T cell responses and functional profile in seropositive and seronegative children

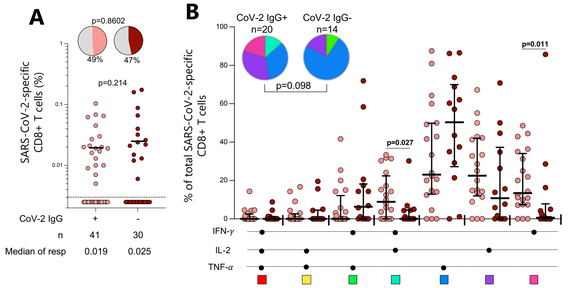


Figure 2: (A) Frequency of SARS-CoV-2-specific CD8+ T cells producing IFN-γ, TNF-α or IL-2. **(B)** The functional profile of SARS-CoV-2-specific CD8+ T cells in SARS-CoV-2 seropositive and seronegative children. Each pie chart slice represents the median contribution of each combination of IFN-γ, TNF-α or IL-2 expression to the total SARS-CoV-2 responses.

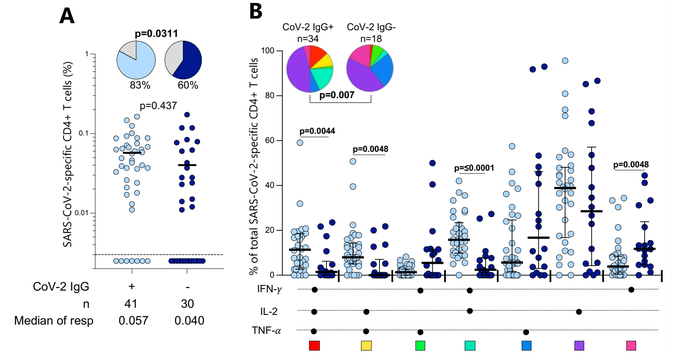


Figure 3: (A) Frequency of SARS-CoV-2-specific CD4+ T cells producing IFN-γ, TNF-α or IL-2. **(B)** The functional profile of SARS-CoV-2-specific CD4+ T cells in SARS-CoV-2 seropositive and seronegative children.

Seropositive children had significant higher proportion of CD4 responders, while no significant differences were observed in the magnitude of SARS-CoV-2-specific CD4+ T cell responses between the groups. However, seropositive children had CD4+ T cells that were polyfunctional, producing different combinations of IFN-γ, TNF-α and IL-2. In contrast, seronegative children were observed to have a monofunctional profile, dominated by IFN-γ producing CD4+ T cells.

No differences were observed in the proportion of responders or magnitude of SARS-CoV-2-specific CD8+ T cell responses between the groups. The functional profile showed seropositive children had significantly higher IFN-γ+ IL-2+ dual producing and IFN-γ monofunctional CD8 T cells compared to seronegative children.

SARS-CoV-2 CD4+ T cell responses cross-reactivity to endemic HCoV-HKU1

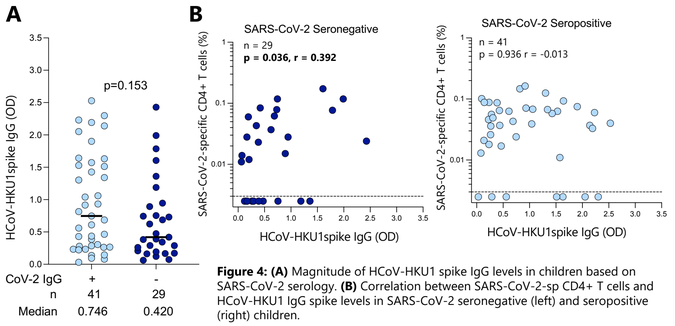


Figure 4: (A) Magnitude of HCoV-HKU1 spike IgG levels in children based on SARS-CoV-2 serology. **(B)** Correlation between SARS-CoV-2-sp CD4+ T cells and HCoV-HKU1 IgG spike levels in SARS-CoV-2 seronegative (left) and seropositive (right) children.

HCoV-HKU1 spike IgG levels were comparable between SARS-CoV-2 seropositive and seronegative children. Furthermore, there was a moderate correlation between the frequency of SARS-CoV-2-specific CD4+ T cells and HCoV-HKU1 spike IgG in seronegative children. No correlation was found in seropositive children.

SARS-CoV-2-specific T cell responses in seropositive children compared to convalescent adults

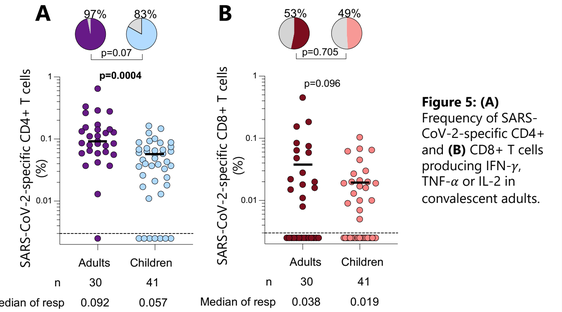


Figure 5: (A) Frequency of SARS-CoV-2-specific CD4+ and **(B)** CD8+ T cells producing IFN-γ, TNF-α or IL-2 in convalescent adults.

A trend towards a higher proportion of CD4 responders was observed in convalescent adults compared to seropositive children. Additionally, convalescent adults had a higher magnitude of SARS-CoV-2-specific CD4+ and CD8+ T cell responses compared to seropositive children.

CONCLUSION

- The majority seronegative children had SARS-CoV-2-specific T cells with a monofunctional T cell profile, despite having no SARS-CoV-2-specific antibodies.
- This, together with the moderate correlation with HCoV-HKU1 may indicate that cross-reactivity to endemic HCoV-HKU1 possibly contributes to the generally positive clinical outcome of COVID-19 in children.
- The increased magnitude of SARS-CoV-2-specific T cells in convalescent adults could be due to age-related T cell differences.

ACKNOWLEDGEMENTS

The authors would like to thank the study participants and their parents/guardians, the clinical team, laboratory research teams, and our funders.

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