

SARS-CoV-2 antibody responses in children exhibit higher FcR engagement and avidity than in adults

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The article by Cohen et al examines differences in antibody responses to SARS-CoV-2 infection across children and adults following primary infection and prior to the availability of vaccination. The authors follow their cohort longitudinally post infection to define differences in dynamics and function across the cohorts. The strength of this article rests in the large cohort of children, the longitudinal nature of the sampling, and the lack of confounding from vaccination. The manuscript largely confirms what has been published previously regarding pediatric immune responses to SARS-CoV-2. Overall the manuscript and figures would benefit from significant editing to make it easier for the reader to follow the story. The inclusion of so many data values with SD and p values within the text made it very difficult and dense to read. The figures have varying font sizes, and the inclusion/size/placement of the keys for symbols/abbreviations wasn't consistent or easy for reading/interpreting purposes. Legends would benefit from editing particularly paying attention to abbreviations and what lines/bars mean, etc.

Figure 2

Did authors repeat PCR testing? Modeling makes it look like there are waves where antibody titers go up. This could be from individual variation in antibody response or repeat infections causing increases in titers.

Figure 2 – no indication of what the bars and lines mean. Also inconsistent use across individual plots with some cohorts having lines with bars going in both direction and others without.

-This was a problem across figures.

Figure 5 -

It is unclear to why the authors state the adults and children are distinct here when they appear to heavily overlap in Fig 5C. This data isn't particularly convincing.

The multi-factor analysis included in the last section appears to be really the only PC that separates the adults and the children.

--This figure was very difficult to interpret, particularly the top where there was no key for the meaning of lines/weight of lines etc which were only contained in the figure legend.

The article would benefit from discussing how these results differ or confirm other similar publications

1) durable response over a longer period than studied by the current authors with some showing higher responses compared to adults.

Renk et al. Nat Communications 2023

Rybikina et al. JEM 2023

Joshi et al. iScience 2023 (cited by authors)

2) Stronger avidity inversely correlated with age.

Yang et al Jama Network Open 2021

3) Fc activity similar or increased in children compared to adults

Gelderloos et al Clinical and Translational immunology 2024

Minor Comments

IFR is an unnecessary and confusing abbreviation

Cohort description

First sentence of cohort description is run on and confusing.

Sentence describing adults is also confusing (starting line 125)

Figures - use of C and A when there appears to be plenty of space to spell out child and adult seems unnecessary. Also -C and -A was not intuitive. Consider an alternative way to convey.

Figure callout for supplement figure 3 incorrectly labeled on line 192

Supplementary figure 3 legend cut off

Reviewer #2

(Remarks to the Author)

Major Comments to the Author:

1. This study aimed to show the differences in humoral immune responses comparing children and adults following first SARS-CoV-2 infection, since we are past that moment in time (2019 – 2020), please comment in the discussion on what your findings mean for subsequent and likely seasonal SARS-CoV-2 infection and how these data may translate to potentially future coronavirus pandemics.
2. Interestingly, there are no neutralizing antibody responses detailed in this study. I feel this is a huge gap as neutralization is a huge correlate of protection. Understanding how neutralizing antibody responses develop in children vs. adults after first SARS-CoV-2 infection could be revealing.

Minor Comments to the Author:

1. You found that uninfected children have high acute IgM levels and attribute that to high baseline or cross-reactivity. If one of the main conclusions of the manuscript is that SARS-CoV-2 infected children make de novo antibody responses, please discuss what you mean by “high” cross-reactivity in the acute IgM responses.
2. You state that for children “lower magnitude of responses highlights that vaccination is particularly important for maintained serological protection in children”. I have two problems with this statement: (1) We do not understand the threshold of serological protection so your studies cannot allude to that. (2) As the pandemic/endemic has progressed, it is clear that adults (especially older adults) need vaccination to maintain high levels of antibodies.
3. It's interesting that there is little cross-reactive call response in the children, given the ages start at 9 – by which time, they have likely seen 1 or 2 other coronaviruses. Could this result be a function of the use of solely OC43 S protein and not accounting for the other 3 endemic coronaviruses (HKU1, 229, and NL63)?
4. You do not need EMPEM to look at mapping epitopes as suggested in the discussion, this could be done using serum depletions, antibody competition immunoassays (such as ELISA or biolayer interferometry), or simply testing responses to RBD, S1-NTD, or S2 subunit proteins. This analysis would be beneficial to see in this study.
5. If the ADCC function of the antibodies in children was higher and that correlates, per literature, to protection, I again bring you to back your statement: “lower magnitude of responses highlights that vaccination is particularly important for maintained serological protection in children”. There seems to be a disconnect between this finding and that statement.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

My concerns have been addressed

Reviewer #2

(Remarks to the Author)

Thank you for making significant changes made to the manuscript in response to the reviewers' comments. I especially am happy that neutralizing data is included.

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Reviewer 1:

R1.1: The article by Cohen et al examines differences in antibody responses to SARS-CoV-2 infection across children and adults following primary infection and prior to the availability of vaccination. The authors follow their cohort longitudinally post infection to define differences in dynamics and function across the cohorts. The strength of this article rests in the large cohort of children, the longitudinal nature of the sampling, and the lack of confounding from vaccination. The manuscript largely confirms what has been published previously regarding pediatric immune responses to SARS-CoV-2.

Thank you for the positive endorsement of this work. In addition to exploring previously studied paediatric immune responses in our cohort, our study further explains the drivers for the antibody qualitative differences between children and adults by finding high OC43 Spike, and S2 IgG in adults compared to children, that are major contributors to differences in children and adults by multiple factor analysis.

R1.2: Overall the manuscript and figures would benefit from significant editing to make it easier for the reader to follow the story. The inclusion of so many data values with SD and p values within the text made it very difficult and dense to read. The figures have varying font sizes, and the inclusion/size/placement of the keys for symbols/abbreviations wasn't consistent or easy for reading/interpreting purposes. Legends would benefit from editing particularly paying attention to abbreviations and what lines/bars mean, etc.

Thank you for the feedback to make our manuscript easier to follow for the reader. In the main text, we have removed a number of data values and all SD values from the results section to enable smoother reading of the text. We have cleaned up the figures to ensure graphs are more consistent in size and used the same key in the top right corner throughout figures (excluding the key with uninfected children and adults where not used in Supplementary Figure 1, 3, and 7). We have further edited legends throughout for readability.

R1.3: Figure 2. Did authors repeat PCR testing? Modeling makes it look like there are waves where antibody titers go up. This could be from individual variation in antibody response or repeat infections causing increases in titers.

We believe that any waves in antibody titres are likely due to individual variation. While all included participants were RT-PCR positive for SARS-CoV-2 infection at recruitment, participants carried out regular rapid antigen tests (RAT), as was necessary in Hong Kong for strict contact tracing, and to enter workplaces and restaurants. Participants self-reported any reinfection by RAT at follow-up and no reinfection cases were included in our cohort. Therefore, waves are more likely attributable to individual variation rather than reinfection.

R1.4: Figure 2. no indication of what the bars and lines mean. Also inconsistent use across individual plots with some cohorts having lines with bars going in both direction and others without. This was a problem across figures.

Apologies for the confusion. We have added to figure legends that bars represent mean and SD throughout. The lack of a visible lower bar is due to the lower SD being negative in these incidences, which cannot be plotted on logarithmic axes. We have added this information to legends where appropriate.

"Individual data points are shown, with lines and error bars showing mean and standard deviations. Lower error bars are not shown if at 0 or below."

R1.5: Figure 5 - It is unclear to why the authors state the adults and children are distinct here when they appear to heavily overlap in Fig 5C. This data isn't particularly convincing.

The multi-factor analysis included in the last section appears to be really the only PC that separates the adults and the children. This figure was very difficult to interpret,

particularly the top where there was no key for the meaning of lines/weight of lines etc which were only contained in the figure legend.

We apologise for our oversight for the lack of legend key in Figure 5A, we have now added a comprehensive legend. For Figure 5C, we have confirmed that, although adults and children overlap, there is a significant difference as tested with an Analysis of Similarities test (ANOSIM). We have added these statistics to all our PCAs and MFAs graphs. The MFA is a supervised analysis that does take groups into account when plotting the points, which is why the separation is clearer. We used MFA to emphasize what variables were driving the differences between adults and children (avidity for OC43-IgG and N-IgG, N-IgM, OC43-IgM).

R1.6: The article would benefit from discussing how these results differ or confirm other similar publications

1) durable response over a longer period than studied by the current authors with some showing higher responses compared to adults.

Renk et al. Nat Communications 2023

Rybkina et al. JEM 2023

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3) Fc activity similar or increased in children compared to adults

Gelderloos et al Clinical and Translational Immunology 2024

Thank you for suggesting these important references to improve our discussion. We have added the following:

Discussion line 339: *"While our results confirm the lower antibody magnitude in children than adults found previously [14, 15], other studies contrastingly find higher IgG in children than adults, which may be explained by using different inclusion criteria measuring only seropositive children, or comparing later time points [16, 39]."*

Discussion line 347: *"Higher S-IgG avidity in children, driven in part by RBD-IgG avidity, suggests higher quality de novo response, also shown by other work finding high RBD-specific avidity (by lower dissociation rates) in children [45]."*

Discussion line 359: *"Others have found no difference between children and adults for either ADCP or NK cell activation when assessing responses to S1 subunit [46], suggesting these differences are driven by S2."*

Regarding the Rybkina JEM paper, this paper compares T cells and cytokine responses in MIS-C and acute COVID-19 and does not compare children to adults and therefore we have not included it as our study does not include any MIS-C patients and is beyond the scope of our study.

Minor Comments

R1.7: IFR is an unnecessary and confusing abbreviation

Thank you for the comment, we have now removed this.

Cohort description

R1.8: First sentence of cohort description is run on and confusing. Sentence describing adults is also confusing (starting line 125)

We agree and have broken this sentence down:

Results line 118:

"Blood samples were obtained from opportunistic sampling of volunteers with RT-PCR confirmed SARS-CoV-2 primary infection, who were previously infection and vaccination-naïve. Participants were recruited between March 2020 and December 2020 and prior to the introduction of COVID-19 vaccines. Children (0.25-17 years) and adults (18-71 years) were

recruited from hospitals based on RT-PCR positivity, lack of admission to ICU and by WHO severity score <2 (mild) [34].”

R1.9: Figures - use of C and A when there appears to be plenty of space to spell out child and adult seems unnecessary. Also -C and -A was not intuitive. Consider an alternative way to convey.

We have removed the use of C, A, - C and – A on the x-axis, and is included in the legend panel per figure as Children, Adults, -ve Children, -ve Adults. However, including per figure panel, “Child and Adults” made figures overly cluttered and instead we have increased the size of the keys on each figure and described colours in legends.

R1.10: Figure callout for supplement figure 3 incorrectly labeled on line 192

Thanks for highlighting this issue, we’ve corrected figure numbering to supplementary figure 4 and 5 due to inclusion of a new supplementary figure 2.

R1.11: Supplementary figure 3 legend cut off

Thanks for highlighting, we’ve adjusted supplementary figure 5 legend to be on an additional page.

Reviewer #2 (Remarks to the Author):

Major Comments to the Author:

R2.1. This study aimed to show the differences in humoral immune responses comparing children and adults following first SARS-CoV-2 infection, since we are past that moment in time (2019 – 2020), please comment in the discussion on what your findings mean for subsequent and likely seasonal SARS-CoV-2 infection and how these data may translate to potentially future coronavirus pandemics.

Thank you for the comment. We have added to our discussion:

Line 306: “This is promising for children experiencing their first exposures of current and future SARS-CoV-2 variants, and in the event of potential future coronavirus pandemics, where children may similarly generate de novo immune responses with higher avidity and FcR functions than adults.”

And edited the final paragraph, line 372:

“This study confirms that children generate high quality but low IgG magnitude immune responses to SARS-CoV-2 mild and asymptomatic infections. Further study is needed to understand the protective thresholds and mechanisms in children. However, the ability for children to generate higher quality antibody responses than adults is promising in the event of future endemic SARS-CoV-2 infections or coronavirus pandemics in this age group.”

R2.2. Interestingly, there are no neutralizing antibody responses detailed in this study. I feel this is a huge gap as neutralization is a huge correlate of protection. Understanding how neutralizing antibody responses develop in children vs. adults after first SARS-CoV-2 infection could be revealing.

We have added neutralising data from a subset of study participants to Figure 2. We find no differences between children and adults at convalescent time points.

We have added to results line 165: *“While neutralising antibodies at convalescent (>14 days) time points were equivalent between children and adults (Figure 2A)”*

Minor Comments to the Author:

R2.2.1. You found that uninfected children have high acute IgM levels and attribute that to high baseline or cross-reactivity. If one of the main conclusions of the manuscript is

that SARS-CoV-2 infected children make de novo antibody responses, please discuss what you mean by “high” cross-reactivity in the acute IgM responses.

We have tried to clarify this point better. We proposed that high IgM in uninfected children and acute infected time points may be from a high cross-reactive baseline response OR from a non-specific, innate-like response. A cross-reactive baseline would also correspond to higher levels of IgG avidity and class-switched antibodies to the protein as a recall response e.g. OC43 Spike. As we find a high IgM but lower class switching and avidity to OC43, we believe that this indicates the second proposed hypothesis: non-specific innate like response, whereby children have natural IgM antibodies that can react against SARS-CoV-2 in the absence of infection, which are then seen at acute time points. Higher natural IgM that can broadly react has been studied in children before (doi: 10.3389/fimmu.2021.690534, 10.1016/S2352-4642(20)30135-8).

We have added these references and edited the results and discussion to clarify that the high IgM at acute and uninfected children, and lower OC43 class switching and avidity indicate a *de novo* and not cross-reactive memory response.

Results, line 152: *“Furthermore, uninfected children’s IgM were equivalent to those in the infected children group indicating either a high cross-reactive baseline, or an immature and non-specific response.”*

Discussion, line 326: *“Children are known to have greater innate-like B cells expressing natural IgM than adults [40, 41]. This suggests that adults have a class-switched cross-reactive memory antibody response to OC43, while children’s is lower avidity and more innate-like from fewer exposures and lower OC43 immune memory.”*

R2.2.2. You state that for children “lower magnitude of responses highlights that vaccination is particularly important for maintained serological protection in children”. I have two problems with this statement: (1) We do not understand the threshold of serological protection so your studies cannot allude to that. (2) As the pandemic/endemic has progressed, it is clear that adults (especially older adults) need vaccination to maintain high levels of antibodies.

We agree with the reviewer and have removed this statement from the text.

R2.2.3. It’s interesting that there is little cross-reactive call response in the children, given the ages start at 9 – by which time, they have likely seen 1 or 2 other coronaviruses. Could this result be a function of the use of solely OC43 S protein and not accounting for the other 3 endemic coronaviruses (HKU1, 229, and NL63)?

The ages of children in fact start at 3 months, with an average of 9.4 years of age and an interquartile region of 5-13 years, rather than starting from 9 years of age. We have now highlighted this in the text to ensure it is clear for the reader. Line 122: *“Children (0.25-17 years) and adults (18-71 years) were recruited from hospitals based on RT-PCR positivity...”*

We see that OC43 IgG, like SARS-CoV-2 also significantly correlates with age (in supplementary figure 3), specifically in children (Figure 5B). Therefore we think that the little cross-reactivity is indeed from fewer common cold coronaviruses in children, also seen by lower avidity in children than adults. From work by others, OC43 seems to account for the highest antibody levels out of the 4 endemic coronaviruses in infected and uninfected children (Dowell et al., <https://doi.org/10.1038/s41590-021-01089-8>), hence we don’t believe other common cold coronaviruses will show higher levels of recall response.

Antibody responses to HKU1, 229, and NL63 were not tested in our study, and OC43 used as a closely related common beta-coronavirus.

To clarify, we have added to Discussion line 32: *“While antibodies to other CCCoV HKU1, 229E and NL63 were not tested in our study, OC43 was used as a closely related common*

beta-coronavirus, and accounts for the highest antibody levels out of the 4 endemic CCCoV [39].”

R2.2.4. You do not need EMPEM to look at mapping epitopes as suggested in the discussion, this could be done using serum depletions, antibody competition immunoassays (such as ELISA or biolayer interferometry), or simply testing responses to RBD, S1-NTD, or S2 subunit proteins. This analysis would be beneficial to see in this study.

We thank the reviewer for pointing this out, and to address this issue of antibody domain specificity where prior cross-reactive responses may skew immunodominance, we have conducted a series of antibody binding assays for infected adults' and children's samples.

New experimental data includes IgG and avidity data against SARS-CoV-2 Wuh1 derived RBD (catalogue 40592-V08H, Sino Biological), S1-NTD (catalogue 40591-V49H, Sino Biological) and S2 (catalogue 40590-V08H1, Sino Biological) subunits as relevant detectors to determine immunodominance with age.

We find lower S2 IgG and avidity responses in children than adults, yet similar RBD-specific IgG in children, confirming our previous results that children are able to make a more focussed and specific SARS-CoV-2 response, whilst prior memory in adults leads to a larger S2-IgG increase.

We have added results:

Line 170: *“The difference in SARS-CoV-2 response was driven by significant differences ($p < 0.0001$) S2 domain IgG (Figure 2A), while Receptor Binding Domain (RBD) and S1-N-Terminal Domain (NTD) IgG was equivalent (Supplementary Figure 2).”*

And discussion:

Line 333: *“We found adults had higher S2 and S2 avidity responses compared to children, driving their higher total response, while RBD directed response and neutralisation was equivalent in children supporting the greater de novo response in children.”*

R2.2.5. If the ADCC function of the antibodies in children was higher and that correlates, per literature, to protection, I again bring you to back your statement: “lower magnitude of responses highlights that vaccination is particularly important for maintained serological protection in children”. There seems to be a disconnect between this finding and that statement.

Thanks for highlighting this discrepancy. Based on the reviewer's previous comment (R2.2.2), we have removed the statement regarding the importance of vaccination in children as a conclusion in this manuscript. And instead conclude with:

Discussion:

Line 362

“Ultimately, the ability for children to generate higher quality antibody responses than adults is promising in the event of future endemic SARS-CoV-2 infections or coronavirus pandemics in this age group.”