Primary Omicron infection elicits weak antibody response but robust cellular immunity in children

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Abstract

The Omicron variant of SARS-CoV-2 is now globally dominant but despite high prevalence little is known regarding the immune response in children. We determined the antibody and cellular immune response following Omicron infection in children aged 6-14 years and related this to prior SARS-CoV-2 infection and vaccination status. Primary Omicron infection elicited a weak antibody response and only 53% of children developed detectable neutralising antibodies. In contrast, children with secondary Omicron infection following prior infection with a pre-Omicron variant developed 24-fold higher antibody titres and neutralisation of Omicron. Vaccination elicited the highest levels of antibody response and was also strongly immunogenic following prior natural infection with Omicron. Cellular responses against Omicron were robust and broadly equivalent in all study groups. These data reveal that primary Omicron infection elicits a weak humoral immune response in children and may presage a clinical profile of recurrent infection as seen with antecedent seasonal coronaviruses. Vaccination may represent the most effective approach to control infection whilst cellular immunity should offer strong clinical protection.

Introduction

The emergence of the SARS-CoV-2 Omicron variant (B.1.1.529) marked a major shift in the course of the COVID-19 pandemic. The acquisition of multiple mutations, primarily within the spike receptor binding domain (RBD), enhanced Omicron infectivity and led to substantial evasion of antibody responses elicited from prior infection or vaccination [1-3]. As such, the variant demonstrated remarkable capacity to outcompete pre-Omicron SARS-CoV-2 variants and Omicron and its subvariants now dominate infections globally.

Omicron infection rates have been high in all age groups and have been particularly notable in children [4]. Indeed, in one study SARS-CoV-2 seroprevalence in this age group rose from 40% to 82% following emergence of the Omicron variant [5]. However, despite this epidemiology there is very little information to date on the nature of the immune response elicited by Omicron infection in children. Such studies are vital as infection with pre-Omicron variants elicits a reduced level of protection against Omicron [1] and recurrent infections with Omicron and the subvariants BA.4 and BA.5 are now a major concern. Initial studies in adults show that booster vaccination or prior pre-Omicron infection elicits the generation of robust Omicron-specific neutralising antibodies following Omicron infection. However, primary Omicron infection in unvaccinated people generates only low level and Omicron-specific humoral immunity with poor cross-variant protection [6].

Profound differences are observed in the age-dependency of the immune response against SARS-CoV-2. Infections are generally mild or asymptomatic in children and potential determinants of this may include increased expression of IFN-response genes within the mucosal epithelium and enhanced innate immune responses [7, 8]. Despite this, children have been observed to develop robust systemic adaptive immune responses against pre-Omicron variants [9] with increased clonality compared to adults [8], although it is not known if similar

findings will be seen following infection with Omicron and related variants. Furthermore, COVID-19 vaccination is now offered to children in many countries but most vaccines utilize a Wuhan spike immunogen which provides lower protection against Omicron compared to previous SARS-CoV-2 variants. Of note, this relative loss of efficacy is somewhat more marked in children aged 5-11 years, although the basis for this is unclear [10].

Here we studied antibody and cellular immunity in a cohort of 43 children following a recent Omicron infection and related this to prior pre-Omicron SARS-CoV-2 infection and vaccination status. Immune profiles were compared to 32 vaccinated children as well as six children with post-vaccine breakthrough infection and 5 with a previous history of primary Omicron infection. Primary Omicron infection elicited weak and poorly neutralising antibodies whilst hybrid immunity or vaccination elicited robust Omicron-specific antibody responses although cellular immunity was equivalent in all cases. Importantly vaccination following primary Omicron restored robust antibody responses to all variants. These findings indicate the potential for susceptibility to recurrent Omicron infection in unvaccinated children.

RESULTS

Antibodies generated following childhood infection with a pre-Omicron SARS-CoV-2 variant bind poorly to Omicron spike protein

Previous studies have shown that children develop strong spike-specific antibody binding and neutralisation following natural infection with pre-Omicron SARS-CoV-2 variants. In initial studies we were interested to assess the ability of these sera to bind and neutralise the Omicron variant. Studies in adults have shown that antibody binding to Omicron is markedly suppressed in this setting due to the large number of critical mutations in the Receptor Binding Domain (RBD) of the spike protein [11, 12].

Antibody binding to Wuhan and Omicron spike and RBD regions was assessed in stored sera from 54 children aged 5-15 years who had been infected with SARS-CoV-2 prior to the emergence of Omicron. These findings were compared to 30 adult donors and both children and adults were unvaccinated. Relative antibody binding to both the Spike and RBD domains of Omicron was dramatically reduced in both children and adults when compared to Wuhan. Indeed, relative binding fell by 85% and 88% respectively in children and only 41% demonstrated a positive RBD response (**Figure 1A&B**). These data show that antibody recognition of Omicron in sera following pre-Omicron infection is markedly and comparably reduced in both children and adults.

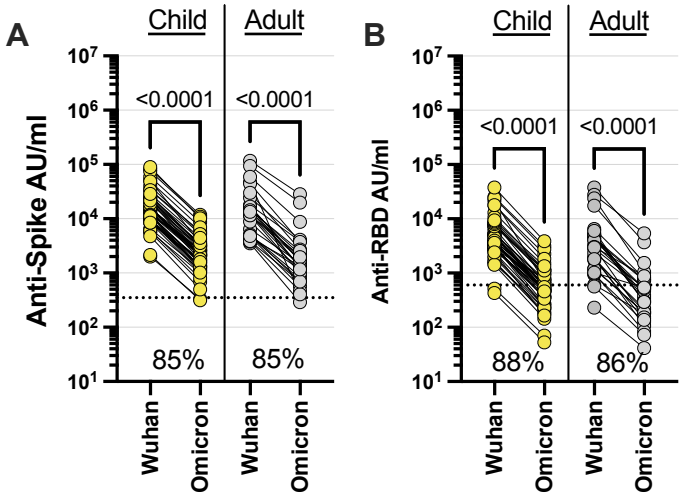

Figure 1. **Marked reduction in Omicron-specific antibody binding in sera from children with pre-Omicron natural infection**

Antibody binding to Wuhan or Omicron spike (A) or RBD-domain (B) in seropositive children (n=54, aged 5-14 years) and adults (n=30; aged >18 years), infected and sampled prior to the emergence of Omicron. Lines join individual donors. Inset percentage indicates the average reduction in binding (AU/ml) to Omicron protein compared to Wuhan. Kruskal-Wallis test with Dunn's multiple comparisons test. Dotted lines indicate seropositive cut-offs as determined for Wuhan-specific response.

Primary Omicron infection elicits low antibody titre and poor neutralisation in children

43 unvaccinated children who had a positive SARS-CoV-2 test during the Omicron wave were recruited for study (**Supplementary Figure 1A**). This comprised 29 donors from the sKIDs study of children aged 6-14 years [13] and 14 children aged 11-14 years in the Born in Bradford study [14]. Demographics are provided in **Supplementary Table 1**.

Initial studies determined antibody responses following primary or secondary infection. Primary infection referred to cases where Omicron was the first SARS-CoV-2 exposure whilst secondary infection was defined as Omicron infection in children with prior infection with a pre-Omicron SARS-CoV-2 variant. Pre-infection serological samples were available from 15 children (**Supplementary Figure 1B**) of whom 4 were seronegative and 11 seropositive for prior SARS-CoV-2 infection.

Primary Omicron infection elicited low level antibody responses against Omicron and pre-Omicron variants in 3 donors (**Figure 2A&B**). Antibody titres were somewhat higher in 1 child, most particularly against the more recently prevalent Delta variant, and it is likely that this donor had a recent unconfirmed Delta infection prior to Omicron infection.

In contrast, secondary Omicron infection elicited a strong antibody response against all pre-Omicron variants with an 8- and 10-fold rise in titre against the Wuhan and Omicron RBD domain respectively. However, the relative reduction of Omicron-specific titres compared to previous variants was maintained despite infection with Omicron. As such, Omicron-specific spike responses were 85% lower compared to the Wuhan titres prior to Omicron infection and remained suppressed by 70% thereafter. (**Figure 2C&D**).

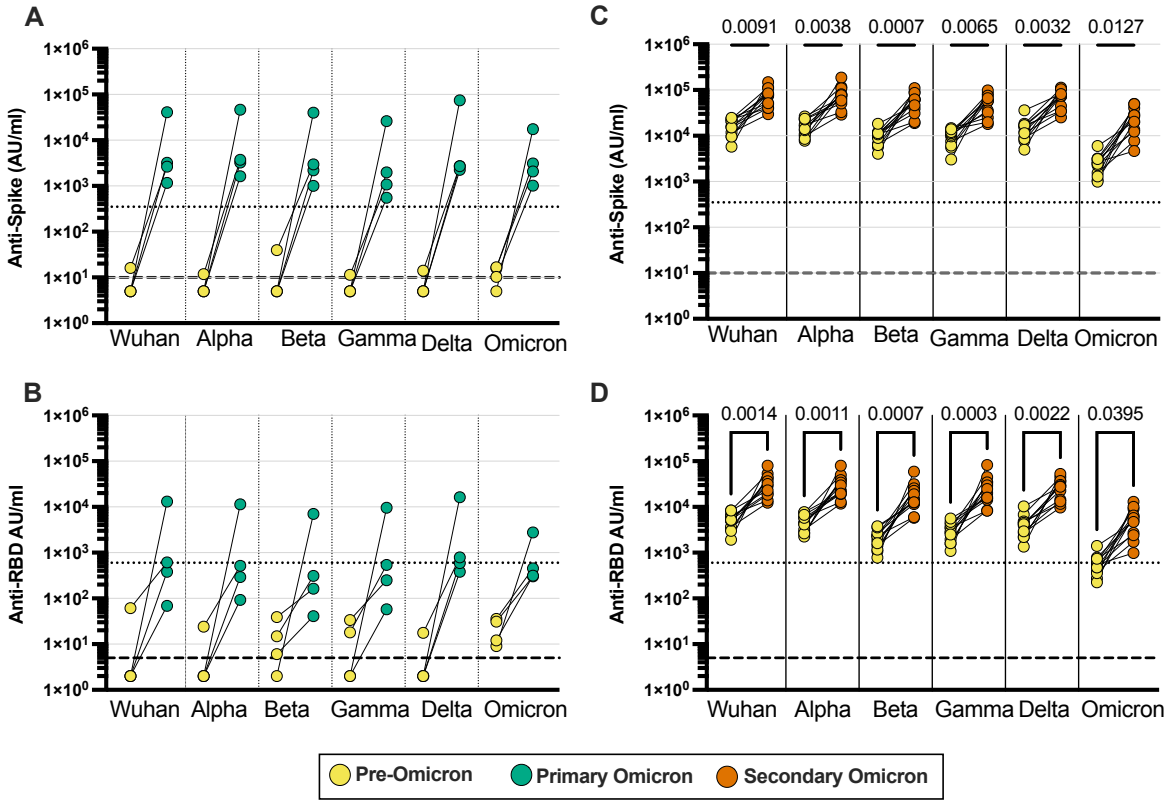

Figure 2. Profile of SARS-CoV-2-specific antibody response following primary or secondary Omicron infection

SARS-CoV-2-specific antibody responses were determined in longitudinal plasma samples from children with Omicron infection.

Spike (A) and RBD-specific (B) antibody binding against viral variants in samples from children (n=4) who were seronegative prior to Omicron infection (blue) and following Omicron infection (yellow).
Spike (C) and RBD-specific (D) antibody binding against viral variants in samples from children (n=11) who were seropositive prior to Omicron infection (blue) and following Omicron infection (orange).
Dotted lines indicate sero-positive cut-offs as determined for Wuhan-specific antibody response; dashed lines indicate below limit of detection. Friedman Test with Dunn's multiple comparisons test.

These data were then used to determine serological responses following primary or secondary infection in the total cross-sectional cohort of 43 children. Bimodal Wuhan-specific antibody titres revealed 20 donors with primary and 23 children with secondary Omicron infection (**Supplementary Figure 2**).

Primary Omicron infection elicited broadly equivalent antibody responses against Omicron and pre-Omicron variants but these were of low titre. Indeed, titres against pre-Omicron variants were only 22% of those seen in a historical cohort of donors with pre-Omicron infection (n=54) whilst Omicron-specific responses were comparable. In contrast, secondary Omicron infection strongly boosted antibody responses against all viral variants (**Figure 3A&B**). Nucleocapsid-specific responses were also lower after primary Omicron infection compared to secondary infection ($p<0.0001$; Kruskal-Wallis test with Dunn's multiple comparisons test) although comparable to historical pre-Omicron infection (**Figure 3C**).

Functional humoral immunity was determined using a pseudovirus-based neutralisation assay following primary (n=17) or secondary (n=9) Omicron infection. Neutralisation after primary infection was weak and only 53% of children had a measurable neutralisation titre despite recent infection. In contrast, neutralisation was robust following secondary infection with all donors developing measurable neutralisation (**Figure 3D**).

These data show that primary Omicron infection in children elicits a poor antibody response against both Omicron and pre-Omicron variants. In contrast, prior infection with a pre-

Omicron variant primes the immune system to develop robust humoral immunity to all viral variants following a secondary infection.

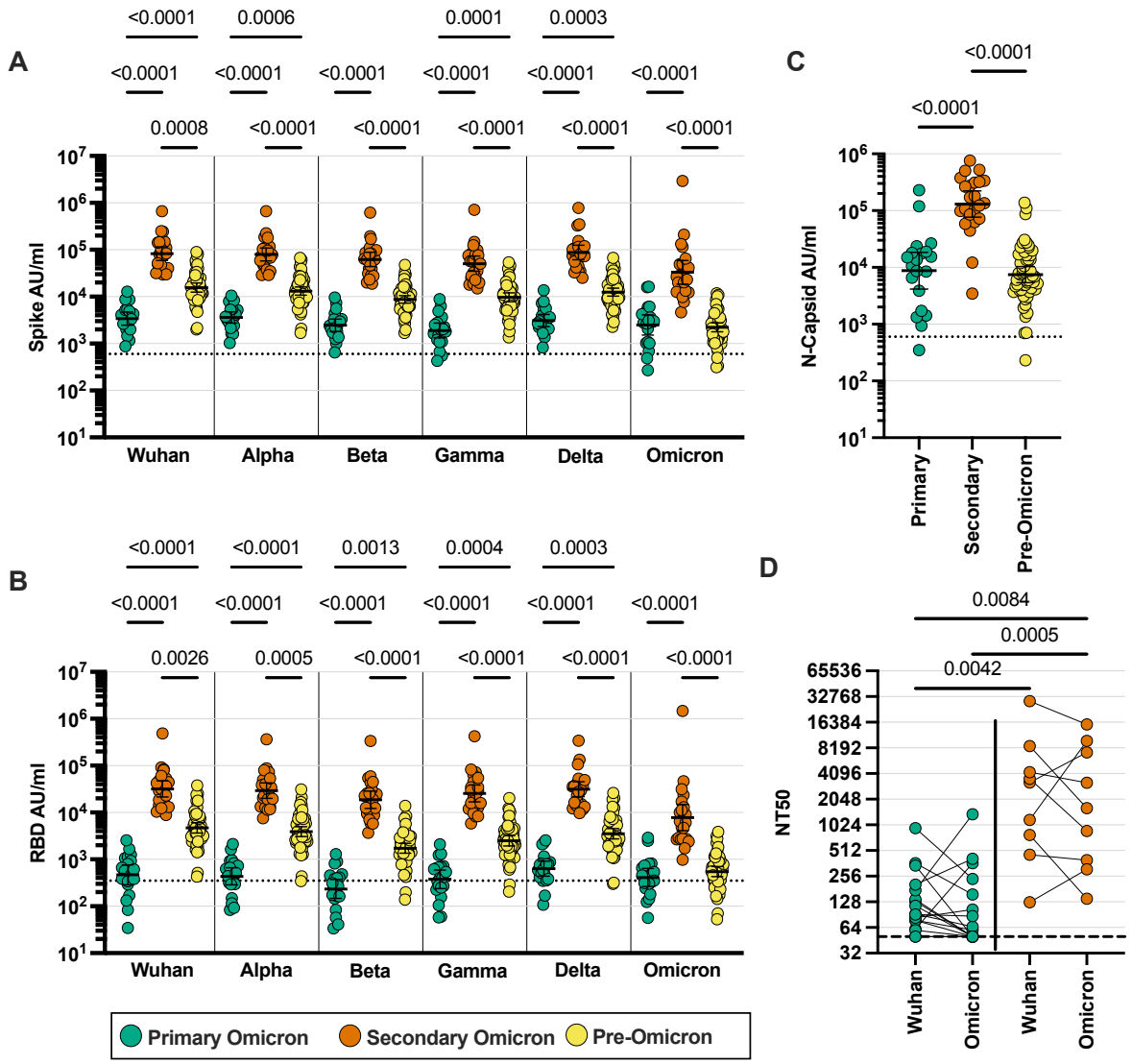

Figure 3. Primary Omicron infection elicits low antibody titre against Omicron and pre-Omicron variants

Children (n=43, aged 6-14 years) known to be infected with the Omicron variant were assessed on the MSD-platform for antibodies specific to Spike protein (A) and RBD-domain (B) from variants as indicated. The Omicron infected children were divided based on those above (Orange, n=23) and those below (Yellow, n=20) the Wuhan specific antibody geo. mean titre. These are compared to antibody levels from children infected and sampled prior to Omicron (blue, n=54, aged 5-14 years). Friedman Test with Dunn's multiple comparisons test. C). Antibody levels specific for Wuhan sequence nucleocapsid. Bars indicate geo. Mean±95% confidence interval. Dotted likes indicate cut-offs defined on Wuhan sequence. D) Neutralisation of pseudo-virus baring Wuhan or

Omicron sequence spike protein (RBD-Hi n=9, RBD-Lo n=17). Dashed lines indicate limit of detection. Kruskal-Wallis test with Dunn's multiple comparisons test.

Vaccination induces strong antibody titres with neutralisation against Omicron

In order to contrast humoral immunity following natural infection with that against vaccination we next recruited 32 children aged 6-14 years who had been vaccinated with BNT162b2 (Pfizer-BioNTech, Cominarty) of which 10 had received two doses and 22 had received one dose. Prior infection status was not established.

Dual vaccination elicited strong antibody responses against the Spike and RBD domain of all SARS-CoV-2 variants. Some variation was evident within children who had received one dose and likely reflect prior infection status. Spike and RBD-specific responses against Omicron were reduced by 74% and 79% respectively in comparison to Wuhan (**Figure 4A&B**) and are likely to reflect the Wuhan spike domain within BNT162b2. This difference was retained irrespective of whether a one or two vaccine doses had been received.

Notably, spike-specific antibody levels against Wuhan were 7.3 and 19-fold higher after single or double vaccination than following pre-Omicron natural infection whilst Omicron-specific responses were 14 and 31-fold higher respectively.

Analysis of 15 vaccinated donors showed that all were able to neutralise Wuhan whilst 14 had measurable titres against Omicron (**Figure 4C**). This neutralization titre against Wuhan was 1.7-fold higher than after secondary Omicron infection but 50% lower against Omicron. In contrast, values were 60 and 14-fold higher respectively in vaccinated donors than after primary Omicron infection.

As such these data indicate that BNT162b2 vaccination generates substantially stronger Omicron-specific neutralisation than primary Omicron infection.

We were also interested to assess the extent to which an Omicron breakthrough infection following vaccination might enhance Omicron-specific antibody responses, as seen following secondary Omicron infection. As such an additional 6 vaccinated children with Omicron breakthrough infection following vaccination were recruited. Omicron-specific antibody responses were not enhanced after infection and remained 71% lower compared to Wuhan (**Figure 4D-F**). Indeed, antibody responses remained lower in these children than seen in vaccinees without breakthrough infection. Whilst pre-breakthrough samples were not available these data suggest limited enhancement of Omicron-specific antibody responses following breakthrough Omicron infection after BNT162b2 vaccination.

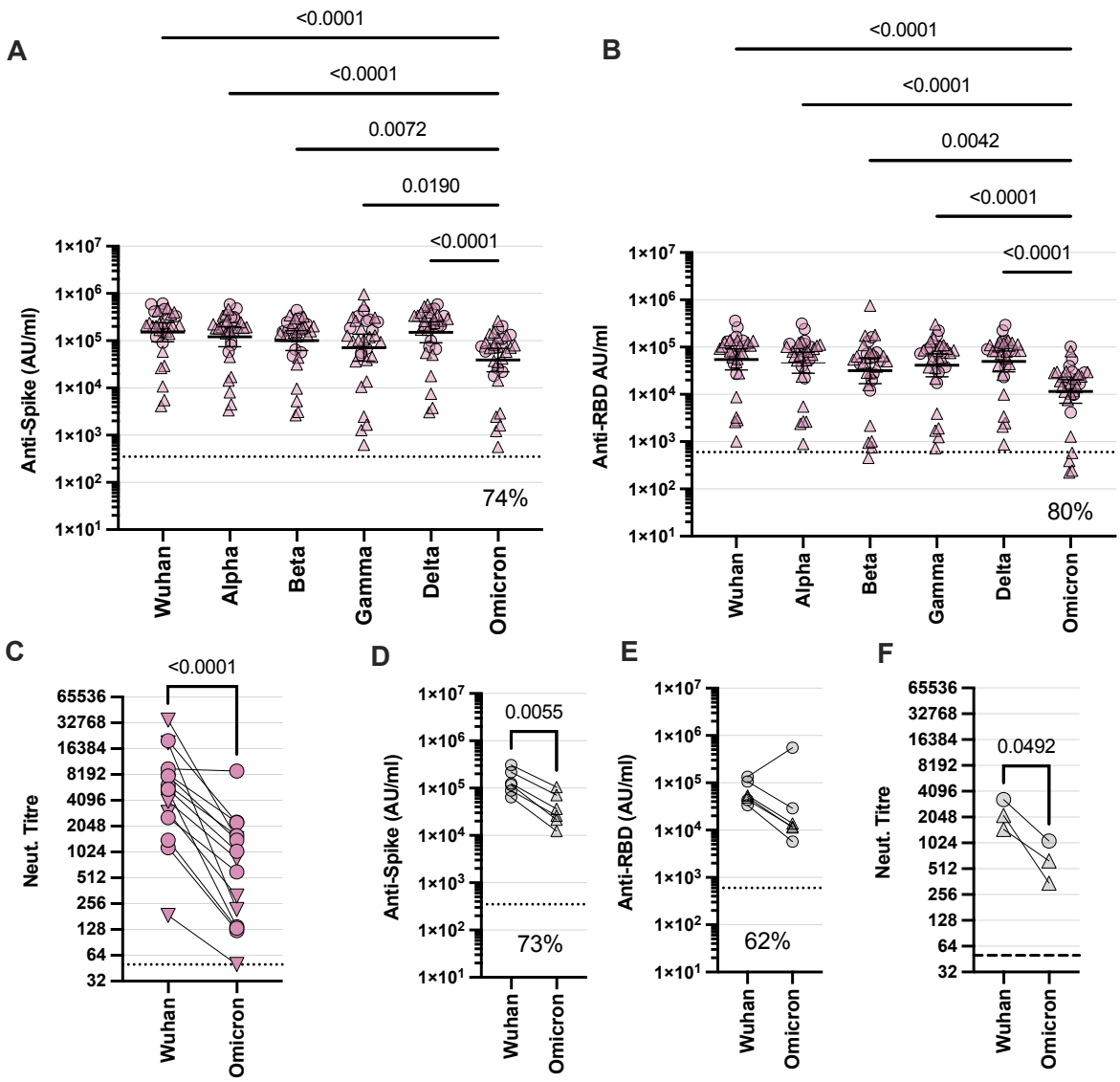

Figure 4. Vaccination induces robust Omicron-specific antibody responses

Vaccinated children were aged 6-14 years and included 22 donors who had received one dose (triangle) and 10 who had received two doses (circle).

Antibody levels to spike (A) and RBD-domain (B) from viral variants following vaccination. Inset percentage indicates the relative level of Omicron titre in respect to Wuhan titre. Bars indicate geo. Mean ±95% CI. Dotted line indicates cut-off as defined by Wuhan sequence. Friedman Test with Dunn's multiple comparisons test.

C) Pseudo-virus neutralisation titres against virus bearing Wuhan or Omicron sequence spike protein. Dotted line indicates lower limit of the assay. Two-tailed paired t-test.

D-E) Wuhan and Omicron sequence spike (D) and RBD (E) titre in six children with Omicron breakthrough infection (two after a single vaccine dose (triangle) and four after two doses (circle). Dotted line indicates cut-off as defined by Wuhan sequence. Two-tailed ratio paired t-test.

F) Pseudo-virus neutralisation titres in three children with vaccine breakthrough infection against virus bearing Wuhan or Omicron sequence spike protein. Dotted line indicates limit of the assay. Two-tailed ratio paired t-test.

COVID-19 vaccination markedly boosts antibody responses in children following prior primary Omicron infection

As primary Omicron infection was found to induce low antibody responses we next assessed if subsequent vaccination could act to boost humoral immunity. As such samples were obtained from five children with low antibody responses following primary Omicron infection who had subsequently received one dose of BNT192b2.

Notably, spike-specific antibody titres against both Omicron and pre-Omicron were markedly enhanced following vaccination revealing a powerful effect of hybrid immunity. Importantly relative Omicron-specific titre was maintained despite use of the Wuhan spike immunogen (**Figure 5A&B**).

These data demonstrate that primary Omicron infection primes the humoral immune response to respond strongly to vaccination and also to retain relative equivalent recognition against Omicron. The paediatric antibody response towards Omicron is summarised in **Supplementary Table 2**.

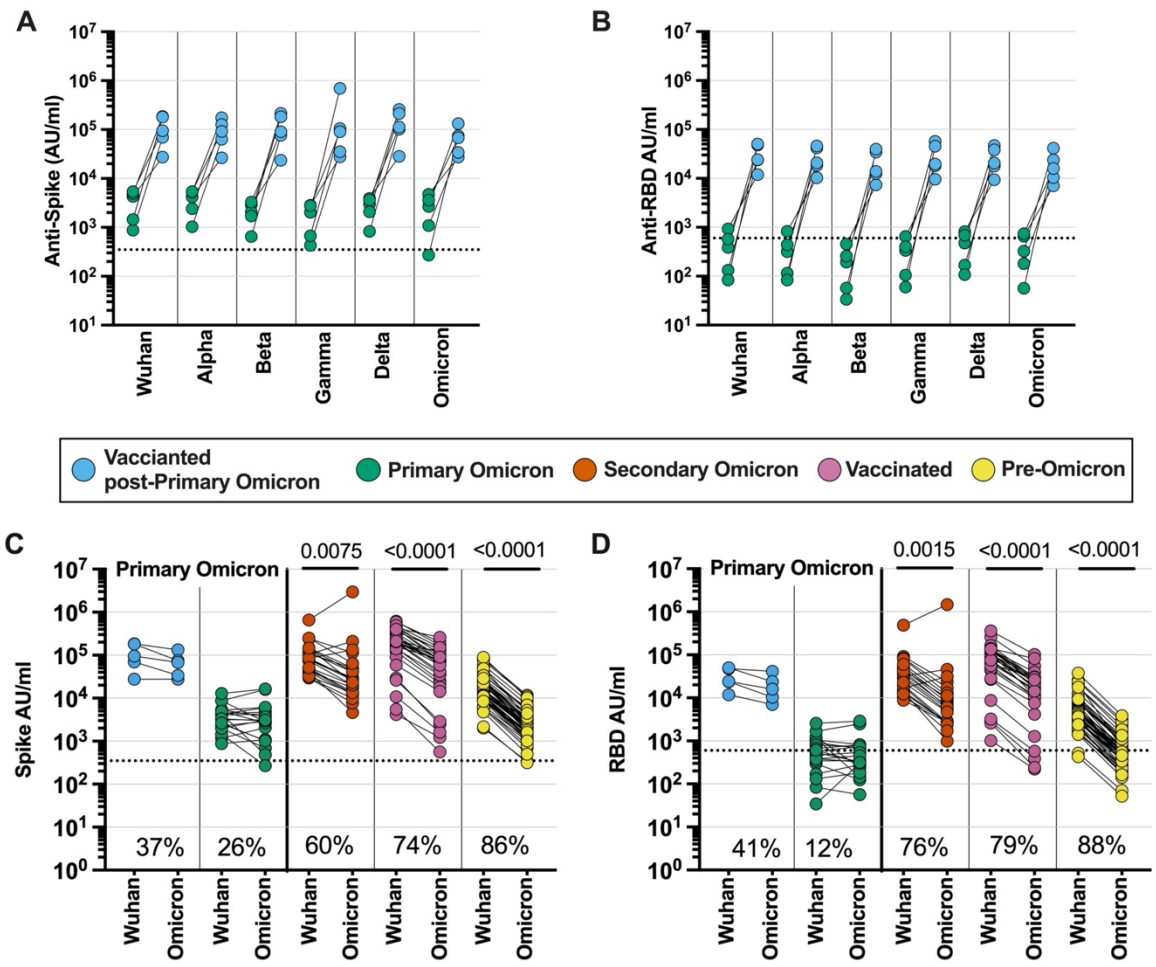

Figure 5. Vaccination enhances the immune response after primary Omicron infection. Five children who experienced a primary Omicron infection prior to vaccination and following one dose of BNT192b2. Antibody levels to spike (A) and RBD-domain (B) from viral variants before (green) and after vaccination (blue). C-D) Comparison of Wuhan and Omicron Spike (C) and RBD (D) titres from children vaccinated after a primary Omicron infection (blue), Primary Omicron infection (green), Secondary infection with Omicron (orange), vaccination (pink) or natural infection by a pre-omicron variant (yellow). Inset percentage indicates the reduction in titre of Omicron in comparison to Wuhan. Dotted line indicates positive cut-off as defined for Wuhan. Two-tailed paired Wilcoxon test.

Cellular responses are comparable in all seropositive children

SARS-CoV-2-specific T cell responses play an important role in the control of infection but have not yet been assessed in children with Omicron infection. Importantly, 94% (16/17) of donors developed spike-specific cellular responses after primary Omicron infection (**Figure 6A**) and comparable responses were present in all children following secondary Omicron infection (**Figure 6B**). Furthermore, cellular immunity was broadly equivalent against peptide pools from either the Wuhan or Omicron spike domain.

Omicron-specific cellular responses were also assessed in stored samples from 5 children who had been infected with SARS-CoV-2 prior to the emergence of Omicron. Again, there was no significant difference in the magnitude of the Wuhan or Omicron-specific response (**Figure 6C**). As such, the T cell response against Omicron is well maintained in children and comparable following natural infection with any SARS-CoV-2 variant.

Analysis of vaccinated children showed cellular responses in 92% (17/19) donors with responses of similar magnitude to those seen following natural infection (**Figure 6D**).

These data show that a cellular response is induced following Omicron primary infection, despite poor antibody responses. The response is of similar magnitude as seen with previous variants showing that the cellular response in children is not affected by the mutations present in the Omicron sequence spike.

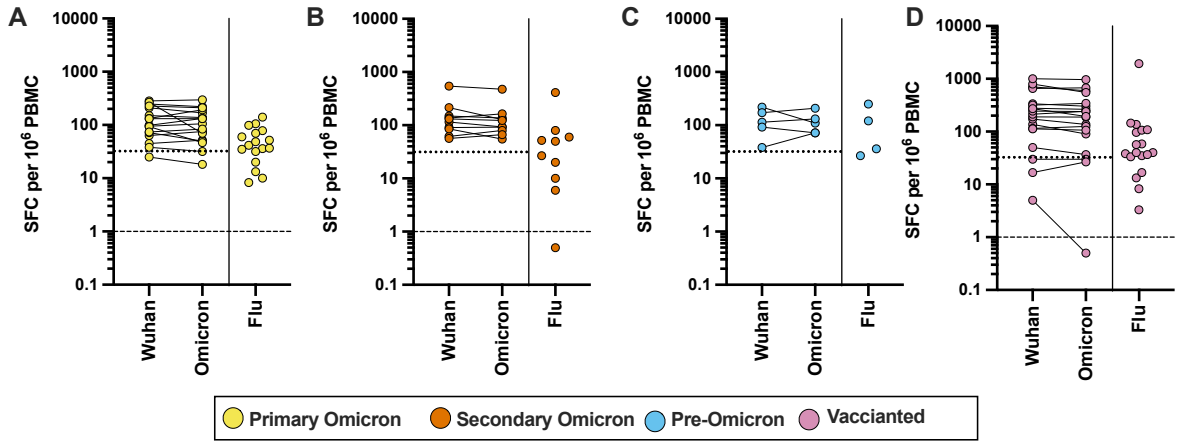

Figure 6. T cell responses are induced and maintained to Omicron sequence spike in children.

IFNγ ELISpots were used to assess the response to Wuhan and Omicron sequence peptide pools, in addition peptides from Influenza (Flu) were included as a relevant control. A) Children characterised as a secondary Omicron infection (n=8) two-tailed paired t-test. B) Children with primary Omicron infection (n =16) two-tailed paired Wilcoxon test. C) Children sampled prior to the Omicron wave (n=5) two-tailed paired t-test. D) Vaccinated children without known Omicron infection (n= 13) two-tailed paired Wilcoxon test.

Discussion

Omicron and related subvariants now dominate the COVID-19 pandemic and have a high infection rate across all age groups, including children. Despite this, little is known regarding the nature of the immune response to infection in this age group. Here we report a number of characteristic features of primary or secondary Omicron infection in children that have potential implications for the trajectory of the pandemic in this age group.

We initially assessed the relative loss of antibody binding to Omicron in serum samples from children infected with pre-Omicron viral variants. This revealed an 86% reduction in binding to the Omicron spike protein and is broadly comparable to the profile seen in adults. As such we did not find any differential capacity in the ability of the pre-Omicron serological response to cross react with the Omicron variant.

We were particularly interested to characterise the immune response following primary Omicron infection in children. A striking feature was the low magnitude of the antibody response and associated neutralisation activity against Omicron and pre-Omicron lineages. Indeed, nearly half of children failed to demonstrate neutralisation activity against Omicron despite recent primary infection. These data reveal that primary Omicron infection elicits poor humoral immunity and augurs badly for future protection against reinfection. The magnitude of the adaptive immune response to SARS-CoV-2 is related to clinical severity and the somewhat milder nature of Omicron infection may therefore by one determinant of this observation [15].

Four additional endemic alpha- and beta-coronavirus (HCoV) circulate widely in the population and have evolved a pattern of recurrent and generally mild infection in children. Infections typically elicit modest antibody responses that are unable to prevent recurrent infections [16-19]. These repeat infections progressively drive maturation of the antibody

response which typically achieve adult levels towards the onset of puberty [17-19]. The Omicron variant itself displays preferential viral replication within the upper rather than lower respiratory tract which may underpin a somewhat less severe clinical profile compared to pre-Omicron variant infection [20-23], and may also relate to the muted humoral response [24]. The extent to which Omicron may mimic the immune response to current HCoV is unclear but could be an important predictor of potential clinical features of Omicron infection in children. Of note, antibody responses against both Omicron and pre-Omicron variants were broadly comparable following a primary Omicron infection and neutralisation activity was also equivalent. This indicates that, whilst infection with a pre-Omicron virus deviates the humoral immune response away from Omicron, the reverse pattern is not observed. In addition, although spike-specific antibody responses after Omicron were weaker than those seen after a pre-Omicron variant infection, the magnitude of the nucleocapsid-specific response was comparable and may indicate a more equivalent recognition of the whole viral proteome, although waning of nucleocapsid-specific antibody responses in the pre-Omicron variant samples make interpretation of this difficult [9].

In contrast, secondary Omicron infection following initial exposure a pre-Omicron variant elicited a strong antibody boost against all variants and whilst relative recognition of pre-Omicron and Omicron variants was maintained despite the Omicron boost. This is likely to reflect the impact of immune priming against pre-Omicron spike and indicates the potential challenge of deviating humoral immunity towards new viral variants due to 'antigenic seniority' [25, 26]. The magnitude of the humoral response, however, was impressive with robust neutralisation of all variants. This indicates that children whose immune system has been primed by pre-Omicron variants develop robust protective immunity against subsequent Omicron infection, at least in the short term.

The prospective nature of the study cohort allowed analysis of the impact of vaccination on adaptive immunity and identified a number of important observations. Firstly, vaccination elicited very strong antibody responses which were higher than following natural secondary Omicron infection. These findings indicate strong immunogenicity of SARS-CoV-2 vaccines in children despite incomplete clinical protection [27].

Importantly vaccination following primary Omicron was found to markedly increase the antibody response to all variants including Omicron and is encouraging for potential future protection. In addition, the relative profile of antibody response against Omicron and pre-Omicron variants that was seen after primary infection was preserved despite the Wuhan spike vaccine immunogen. This is additional evidence for the strong influence of the initial antigen exposure in priming the specificity of subsequent challenge with antigenic variants which is likely to be an important determinant of the longer-term profile of coronavirus-specific humoral immunity in children.

Cellular immunity against SARS-CoV-2 is an important mediator of protection against severe disease. Here we observed strong T cell responses following primary or secondary Omicron exposure. Moreover, cellular responses against both pre-Omicron and Omicron were broadly comparable, reflecting the ability of cellular immune recognition to tolerate viral variants that elicit confirmational changes that evade humoral recognition [28]. T cell responses against endemic HCoV are also robust and somewhat higher in younger people [29] suggesting a differential age-dependent priming of immunity against coronaviruses.

Limitations of the study include absence of samples from vaccinees prior to breakthrough infection and absence of donors with recurrent Omicron infection which will become important given recent emergence of the BA.4 and BA.5 variants.

Our findings, summarised in **Figure 7**, have a number of potential implications for the future course of the COVID-19 pandemic within children. The most striking observation was that primary Omicron infection induces a modest antibody response with poor neutralisation activity. As such, children may be at risk of repeated infection although the underlying cell immunity should continue to protect against severe disease. As such the Omicron variants of SARS-CoV-2 may have evolved to represent a fifth seasonal coronavirus infection within the paediatric population although the potential emergence of future SARS-CoV-2 viral variants must be monitored closely. COVID-19 vaccines are strongly immunogenic in children but the profile and specificity of this immune response is markedly influenced by prior infection with a pre-Omicron or Omicron variant. The potential utility of recurrent vaccination in the face of high prevalence of baseline immunity and relatively modest clinical burden will be a subject of ongoing debate.

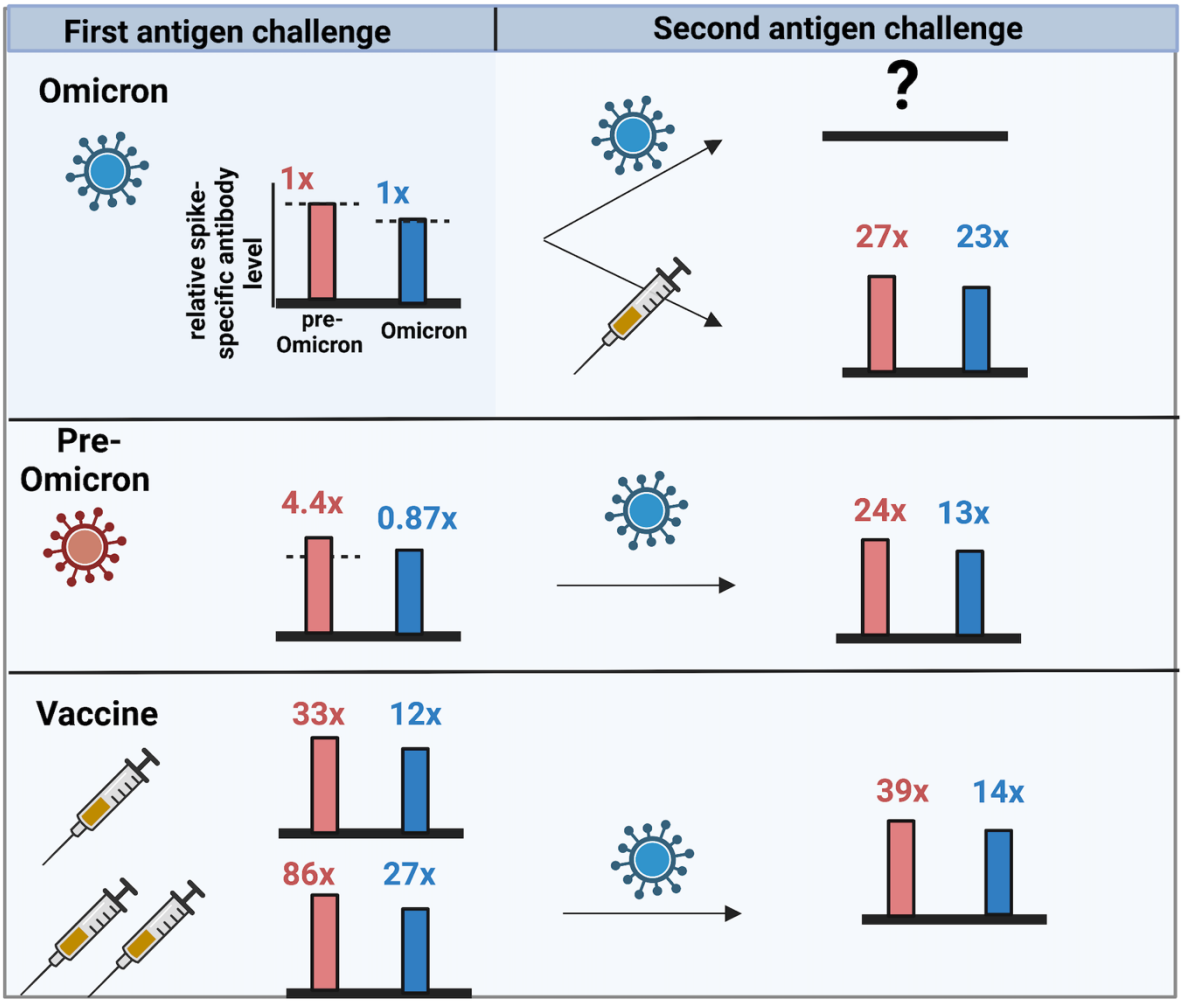

Figure 7
Schematic representation of the relative magnitude of antibody responses against spike protein from Wuhan (pre-Omicron) and Omicron in children following natural infection or vaccination. Values are show relative to the magnitude of response to primary Omicron infection and related to the first or second immune challenge to antigen from infection or vaccination.

Acknowledgments

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Data availability

All data generated or analysed during this study are included in this published article (and its supplementary information files).

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Methods

Sample collection

The SARS-CoV-2 surveillance in primary schools (sKIDs) study was initiated by the United Kingdom Health Security Agency (UKHSA) after schools reopened following the easing of national lockdown in June 2020. Subsequent to the Omicron wave (December-January 2022) a further prospective collection recruited children aged 6-14 years to assess the immunological response to Omicron infection in the paediatric population. Samples were taken 23 March 2022 - 14 April 2022. Additional follow-up samples were obtained 31 June 2022 - 06 July 2022. The protocol for sKIDs is available online (https://www.gov.uk/guidance/covid-19-paediatric-surveillance).

Further plasma samples were obtained from a prospective longitudinal collection conducted by the Born in Bradford study [14].

Samples taken from children prior to the Omicron wave were obtained as part of earlier rounds of the sKIDs or Born in Bradford studies. Between 23 November 2020 – August 2022.

Omicron infection was determined through linkage with the national SARS-CoV-2 testing database (SGSS) held by UKHSA and the NIMS database (which is used to record all COVID-19 vaccinations for individuals in England), was used to obtain records of COVID-19 vaccination and vaccine manufacturer for each dose. These data were accessed in May 2022.

No statistical methods were used to predetermine sample sizes. Researchers were blinded to the status of donors before ELISpot and serological assessment.

Ethical review for the sKIDs study was provided by the PHE Research Ethics and Governance Group (PHE R&D REGG ref. no. NR0209). Ethical Review of The Born in Bradford study was provided by the National Health Service Health Research Authority Yorkshire and the Humber (Bradford Leeds) Research Ethics Committee; REC reference: 16/YH/0320. Children and

parents or guardians were provided with age-appropriate information sheets prior to enrolment. Written informed consent was obtained from all from parents or guardians of all participants.

PBMC and Plasma Preparation

Lithium Heparin blood tubes were processed within 24hrs of collection. Briefly tubes were spun at 300g for 10mins prior to removal of plasma which was then spun at 800g for 10mins and stored as aliquots at -80°C. Remaining blood was diluted with RPMI and PBMC isolated on a Sepmate ficol density gradient (Stemcell), cells were washed with RPMI and rested in RPMI+10% batch tested FBS for a minimum of 4 hours prior to cellular assays.

Serological analysis of SARS-CoV-2-specific immune response

Quantitative IgG antibody titres were measured using Mesoscale Diagnostics multiplex assays; Coronavirus Panel 22 and 24, as previously describe [9] following the manufacturer instructions. Briefly, samples were diluted 1:5000 and added wells of the 96 well plate alongside reference standards and controls. After incubation, plates were washed and anti-IgG-Sulfo tagged detection antibody added. Plates were washed and were immediately read using a MESO TM QuickPlex SQ 120 system. Data was generated by Methodological Mind software and analysed with MSD Discovery Workbench (v4.0) software. Data are presented as arbitrary units (AU)/ml determined relative to the standard curve generated. Cut-offs were previously defined against pre-pandemic adult and paediatric samples [9].

Pseudotype-based neutralisation assays

Constructs and 293-ACE2 cells were previously described [9, 30]. The assay was performed as previously described [9, 30], briefly neutralising activity in each sample was measured against pseudo-virus displaying SARS-CoV-2 spike, either Wuhan sequence or B.1.1.529:BA.2 sequence, by a serial dilution approach. Each sample was serially diluted in triplicate from

1:50 to 1:36450 in complete DMEM prior to incubation with approximately $1x10^6$ CPS (counts per second) per well of HIV (SARS-CoV-2) pseudotypes, incubated for 1 hour, and plated onto 239-ACE2 target cells. After 48-72 hours, luciferase activity was quantified by the addition of Steadylite Plus chemiluminescence substrate and analysis on a Perkin Elmer EnSight multimode plate reader (Perkin Elmer, Beaconsfield, UK). Antibody titre was then estimated by interpolating the point at which infectivity had been reduced to 50% of the value for the no serum control samples.

IFN-γ ELISpot

T cell responses were measured using a IFN-γ ELISpot Pro kit (Mabtech) as previously described. [9] Pepmixes pool containing 15-mer peptides overlapping by 10aa from either SARS-CoV-2 spike S1 or S2 domains from the Wuhan or Omicron (B1.1.529; BA.1) variant were purchased from JPT technologies.

Briefly, fresh PBMC were rested overnight prior to assay and $0.25\text{-}0.3x10^6$ PBMC were added in duplicate per well containing either pep-mix, anti-CD3 (positive) or DMSO (negative) control. Samples were incubated for 16-18hrs. Plates were developed following the manufacturer's instructions and read using an AID plate reader (AID).

Data visualisation and statistics

Data was visualised and statistical tests, including normality tests, performed as indicated using GraphPad Prism v9 software. Only results found to be significant ($p<0.05$) are displayed.

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