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Nasal mucosal IgA levels against SARS-CoV-2 and seasonal coronaviruses are low in children but boosted by reinfection

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SUMMARY

Repeated coronavirus infections in childhood drive progressive maturation of systemic immune responses into adulthood. Analyses of immune responses in children have focused primarily upon systemic assessment but the importance of mucosal immunity is increasingly recognised. We studied virus-specific antibody responses in contemporaneous nasal swabs and blood samples from 99 children (4–15 years) and 28 adults (22–56 years), all of whom had prior SARS-CoV-2 infection. Whilst mucosal IgA titres against Influenza and Respiratory Syncytial virus were comparable between children and adults, those against all coronaviruses, including SARS-CoV-2, were lower in children. Mucosal IgA antibodies demonstrated comparable relative neutralisation capacity in both groups and retained activity against recent omicron variants such as XBB.1 which are highly evasive of IgG neutralisation. SARS-CoV-2 reinfection preferentially enhanced mucosal IgA responses whilst the impact of vaccination was more modest. Nasal IgA levels against coronaviruses thus display a pattern of incremental response to reinfection which likely determines the natural history of reinfection. This highlights the particular significance of developing mucosal vaccines against coronaviruses in children.

Introduction

The mucosal barrier of the upper respiratory tract provides the first line of defence against respiratory pathogens. The local immune system comprises a range of specialized cells including mucosal-associated B cells which produce predominantly IgA antibodies which are actively secreted as dimers across the epithelium.[1] These mucosal responses can neutralise or eliminate viruses prior to breaching of the mucosal barrier and establishment of infection. Additionally, the mucosal IgA response may temper the spread of respiratory infections through reduced viral shedding.[2,3]

Mucosal IgA responses have previously been associated with protection from Influenza[4] and Respiratory Syncytial virus (RSV)[3,5] infection, and more recently with protection from SARS-CoV-2 Omicron infection.[6,7] However, intramuscular COVID-19 vaccination induces modest mucosal responses in adults[1,8,9] although these may be boosted by hybrid immunity,[8] and this may contribute to the incomplete protection from infection offered by vaccination.

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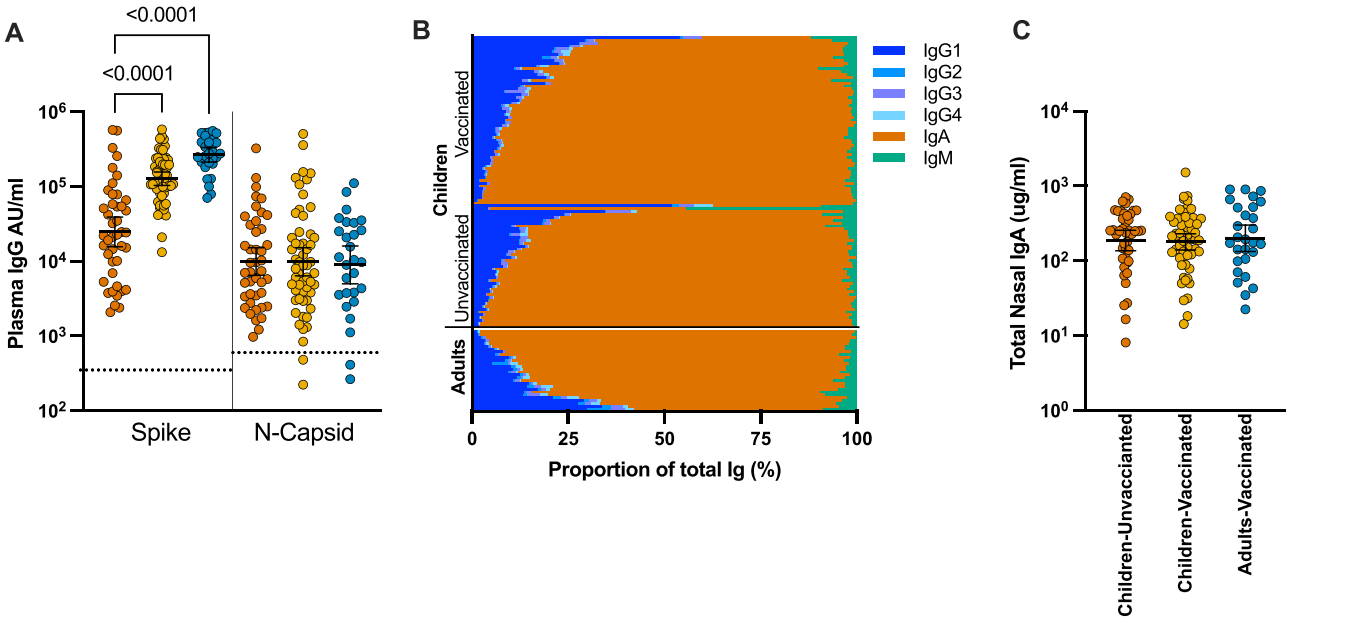


Fig. 1. Nasal immunoglobulin isotypes are dominated by IgA and comparable between children and adults A) SARS-CoV-2 spike and nucleocapsid-specific plasma IgG levels in the study cohort; unvaccinated (n = 43, orange) and vaccinated (n = 56, yellow) children and vaccinated adults (n = 28, blue). Dotted lines indicate previously defined sensitivity and donors below the nucleocapsid cut-off were included in the study as they had prior confirmed SARS-CoV-2 infection. IgG cut-offs were previously defined against pre-pandemic adult and paediatric samples.[38] Bars indicate geometric mean ± 95% CI. Kruskal-Wallis test with Dunn's correction for multiple comparisons. B) Immunoglobulin isotypes as a proportion of total immunoglobulin in nasal samples from children (n = 99) and adults (n = 28). C) Total IgA concentration in nasal samples. Bars indicate median ± 95%CI.

In contrast to systemic IgG responses, total secretory IgA levels show enhanced maturation in children and reach adult levels at approximately 1 year of age.[10] Whilst systemic seasonal coronavirus (HCoV)-specific IgG responses develop slowly during childhood, through repeated reinfection,[11–13] little is known about the development of the mucosal response to HCoV. Furthermore, how this pattern may compare to mucosal immunity against the newly emergent SARS-CoV-2, and other respiratory pathogens, is not known.

Children generally experience mild or asymptomatic SARS-CoV-2 infection and this is likely to be underpinned by differences in the underlying immune response across the life course.[14] Hitherto, studies in children have primarily focused upon the systemic immune response to SARS-CoV-2. Investigation of the mucosal immune response following infection has identified enhanced innate activation in children[15] whilst rapid IgA secretion is correlated inversely with severity of infection.[16] Although the majority of children have now been exposed to SARS-CoV-2,[17] the protective impact of mucosal immunity against future protection, including those from viral variants, is not clear. Additionally, no data exists regarding the mucosal immune response in children following SARS-CoV-2 vaccination which, given current low rates of vaccine uptake in this age group, is relevant for driving optimal vaccine design.

Here we measured virus-specific antibody levels and SARS-CoV-2 neutralisation activity in mucosal and plasma samples from 99 children and 28 adults who had previously been infected with SARS-CoV-2. Differential age and tissue-specific patterns of immunity are observed which have implications for understanding protective immunity against SARS-CoV-2 and HCoV.

Results

Children and adults have comparable levels of total immunoglobulin and relative isotypes within the nasal mucosa

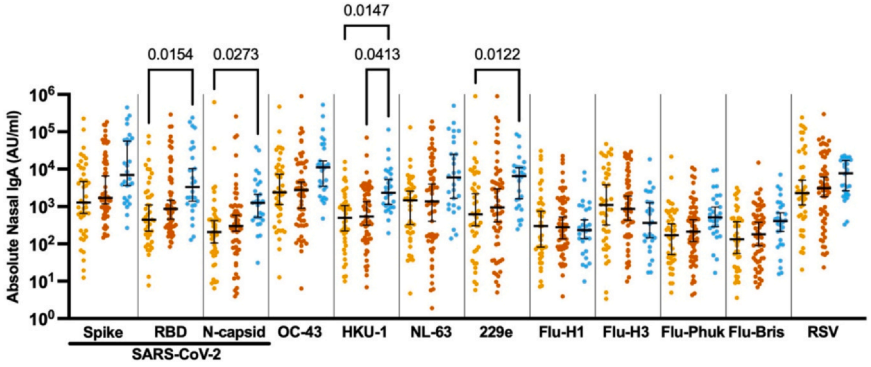
Matched nasal swab and blood samples were collected prospectively from 99 children and 28 adults with clinical and/or serological evidence of prior SARS-CoV-2 infection (Fig. 1A). Due to high rates of population seroprevalence it was not possible to recruit sufficient infection-naïve donors for comparative analysis. Children had a median age 10 years (range 4–14 years) and 43 were unvaccinated whilst 56 had received at least one COVID-19 BNT162b2 vaccine (1 dose, n = 14; 2 doses, n = 39 and 3 doses, n = 3). Adults had median age of 35 years (range 22–56) and all had received COVID-19 vaccinations (2 doses, n = 2; 3 doses, n = 22; and 4 doses, n = 4).

We initially determined the concentration of IgG1–4, IgA and IgM immunoglobulin isotype in each nasal swab sample. Children and adults had similar median total immunoglobulin levels although a range of values were seen in each cohort and likely represent variation in the quality of nasal sampling. IgA comprised the main immunoglobulin isotype in both groups (children: median 90% [28–98%] vs adults: 84% [49–98%]) (Fig. 1B-C).

Mucosal IgA responses against coronaviruses are reduced in children compared with adults

The level of IgA antibody specific for the spike, receptor binding domain (RBD) and nucleocapsid proteins from SARS-CoV-2 was next determined in blood (systemic) and nasal swab (mucosal) samples using the Mesoscale Diagnostics (MSD) system. Antibody binding against a range of additional respiratory pathogens was also determined including the additional endemic beta (OC43 and HKU-1) and alpha (NL-63 and 229e) coronaviruses (HCoV); 2 subtypes of Influenza A and Influenza B; and respiratory syncytial virus (RSV). To account for differences in the quality of nasal swab collection, virus-specific antibody titres were normalised to the total IgA concentration from individual samples and this correction factor was applied to the values measured against each viral protein. Uncorrected raw values are available in Extended Fig. 1 and describe a similar profile.

Marked differences were observed in relative nasal and systemic virus-specific IgA titres between children and adults. In particular, a striking pattern was a reduced level of antibody binding to coronaviruses in the nasal mucosa of children (Fig. 2A, Table 1). This was observed for all coronaviruses where median nasal beta-HCoV



Extended Fig. 1. Absolute Mucosal coronavirus-specific IgA responses. A) Mucosal IgA responses specific for SARS-CoV-2 Spike, RBD, and Nucleocapsid (N-capsid); spike protein from the four seasonal human coronaviruses; Haemagglutinin from 4 Influenza subtypes; and RSV pre-fusion F protein were measured in 99 children (SARS-CoV-2 unvaccinated, orange (n = 43); vaccinated, yellow (n = 56), and vaccinated adults, blue (n = 28). Results are shown as AU/ml as measured, without normalisation to total IgA concentration. Bars indicate Median ± 95% CI.

(OC-43 and HKU-1) and alpha-HCoV (NL-63 and 229e)-specific titres were 3.3 and 4.3 times, and 6.9 and 6.5 times, higher respectively in adults. Nasal antibody titres against SARS-CoV-2 nucleocapsid protein were also 4.2 times higher in adults. SARS-CoV-2 vaccination increased SARS-CoV-2 spike-specific nasal responses in children by 4.1 times such that these were then only 1.7 times lower than in adults. However, vaccination did not influence relative binding against other HCoV. In contrast to this pattern seen against coronaviruses, nasal antibody responses against Influenza and RSV antigens were comparable between adults and children.

Systemic titres of IgA antibodies against respiratory pathogens were broadly uniform in children and adults although binding against Influenza Hong Kong/2014/H3 was 6.8 times lower in adults which may again reflect differential vaccine or infection history (Fig. 2B, Table 2). Levels of SARS-CoV-2 spike-specific IgA were markedly increased by COVID-19 vaccination status, being 5.4 times lower in unvaccinated children compared to adults ($p = 0.041$) but comparable following vaccination.

Relative correlation between mucosal and systemic IgA responses against each pathogen was then assessed in 99 children (43 seropositive-unvaccinated and 56 hybrid-vaccinated). Positive correlation was seen only for SARS-CoV-2 (R_2 0.3466, p0.0004) (Extended Fig. 2) and here subgroup analysis showed that this was itself driven by vaccination (hybrid immune children; R_2 0.35, p0.0075; unvaccinated; $R_2$0.19, p0.23). In contrast, IgA responses to beta-coronaviruses and RSV were correlated in adults (SARS-CoV-2; R_2 0.74, $p < 0.0001$, OC43; R_2 0.36, p0.061, HKU-1; R_2 0.40, p0.024, RSV; R_2 0.56, p0.0021) although those against alpha-coronaviruses were not. A sub-group of children did appear to show greater correlation between systemic and mucosal IgA and these may represent progressive maturation of the response into adulthood although no association was seen with age.

These data show that children and adults have comparable IgA antibody levels against respiratory viruses in blood but responses against coronaviruses are lower within the nasal mucosa of children. As such, an age-related difference is observed in the balance of the nasal and systemic IgA response to coronaviruses.

SARS-CoV-2 spike-specific mucosal IgA show limited cross-recognition of seasonal coronavirus

SARS-CoV-2 infection in children can promote 'back-boosting' of systemic IgG responses (selective enhancement of antibody responses to related viruses) against the beta-coronaviruses OC43 and HKU-1 through cross-reactive antibodies directed predominantly towards the spike S2 domain.[18] Given that mucosal IgA responses against SARS-CoV-2 and OC43 were comparable across age groups (Table 1) we next investigated if such cross-reactive responses also exist in the nasal IgA repertoire.

To test this, swab samples from children and adults were pre-absorbed against plate-bound trimeric SARS-CoV-2 spike and then assessed for residual binding to coronavirus spike proteins. As expected, this markedly reduced binding to SARS-CoV-2 whilst only a small reduction in binding to HCoV spike proteins was seen, indicating limited cross reactivity in the mucosal spike-specific IgA response (Fig. 3).

Overall, these results show that the mucosal IgA antibody response to SARS-CoV-2 infection is not substantially comprised from boosting of cross-reactive responses to HCoV but reflects the generation of a *de novo* SARS-CoV-2-specific response in all age groups.

Spike-specific IgA within nasal mucosa of children and adults demonstrates equivalent functional capacity to neutralise SARS-CoV-2

The HCoV-specific B cell repertoire in children has been reported to be broader than that seen in adults[19] and may potentially allow wider recognition of SARS-CoV-2 variants.[20] As such we next assessed the functional capacity of mucosal IgA to inhibit binding of spike proteins from a range of different SARS-CoV-2 variants to ACE2. Samples were selected from donors with comparable total spike and RBD-specific IgA concentrations in order to minimize variation in nasal sampling quality (Fig. 4A).

Median inhibition of ancestral Wu-Hu-1 spike binding was two times higher in adults than children and may reflect its usage in vaccine regimens, although vaccination in children did not improve functional activity. Inhibition of all other variants was similar across the cohorts (Fig. 4B).

These data show that mucosal IgA antibodies have comparable levels of functional neutralisation capacity against SARS-CoV-2 viruses in children and adults.

SARS-CoV-2 reinfection elicits greater enhancement of mucosal IgA responses against spike compared to nucleocapsid protein

Systemic IgG responses against coronaviruses are enhanced by repeated infections and we therefore assessed if mucosal responses against SARS-CoV-2 were similarly increased by reinfection. 13 children with reinfection were identified by a > 2-fold rise in nucleocapsid-specific antibody titre compared to paired plasma samples taken 6 months previously. In addition, 7 children had received a primary vaccination during this period.[21]

As expected, reinfection or vaccination increased systemic spike-specific IgG titres which rose by 4.6 times (75533 vs 16352 AU/ml; $p = 0.0087$) or 6.7 times (109657 vs 16352 AU/ml; $p = 0.0076$)

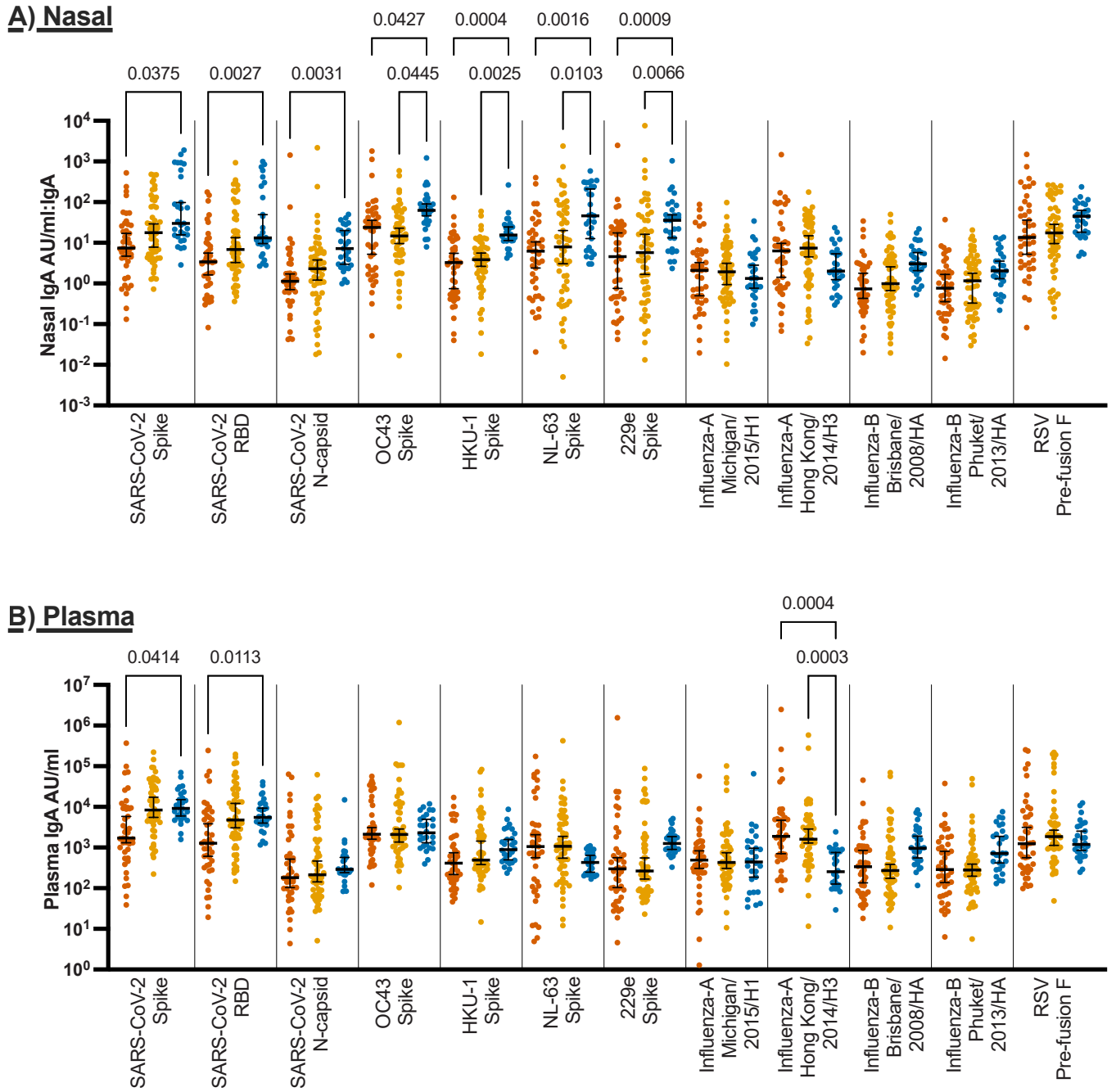


Fig. 2. Mucosal coronavirus-specific IgA responses are lower in children. A) Mucosal IgA responses specific for SARS-CoV-2 Spike, RBD, and Nucleocapsid (N-capsid); spike protein from the four seasonal human coronaviruses; Haemagglutinin from 4 Influenza subtypes; and RSV pre-fusion F protein were measured in 99 children (SARS-CoV-2 unvaccinated, orange (n = 43); vaccinated, yellow (n = 56), and vaccinated adults, blue (n = 28). Results are normalised and expressed as a ratio to total IgA. Bars indicate Median ± 95% CI. B) Systemic IgA responses to the same antigens defined in plasma samples. Results are given as arbitrary units per ml. Bars indicate geometric mean ± 95%CI. Kruskal-Wallis test with Dunn's correction for multiple comparisons.

respectively whilst systemic IgA increments were more muted at 2.3-times and 2.2-times respectively (p = NS). An 8.3 times increase in nucleocapsid-specific IgG was seen following reinfection only (40833 vs 4944 AU/ml) (Fig. 5).

Analysis of nasal IgA responses revealed a 5.6-times increment against spike (28.5 vs 5.1 AU:IgA/ml) following reinfection but it was noteworthy that no increase in nucleocapsid-specific response was observed.

Children who received vaccination showed no increase in nasal spike-specific IgA response but the limited cohort size limited statistical analysis (7.5 vs 5.1 AU:IgA/ml).

These data show that SARS-CoV-2 reinfection in children selectively enhances nasal IgA responses against spike compared to nucleocapsid protein.

Discussion

SARS-CoV-2 is now an endemic virus and infection rates remain high despite widespread deployment of vaccines that provide strong protection against severe disease. As such, there is increasing interest in defining the tissue-specific profile of immunity and how this varies across the life course. Here we demonstrate that levels of

Table 1
Comparison of respiratory virus specific mucosal IgA responses in children and adults. Median AU/ml:IgA (IQR).

	Child-Unvacc	Child-Vaccinated	Adult-Vaccinated	Child-Unvacc vs Child-Vacc	Child-Unvacc vs Adult-Vacc	Child-Vacc vs Adult-Vacc
	Subgroup Comparison vs SARS-CoV-2 spike*			**Comparison across cohorts****		
SARS-CoV-2 Spike	7.41 (3.48–27.03)	17.72 (4.53–79.25)	30.19 (14.74–520.4)	> 0.9999	**0.0375**	> 0.9999
SARS-CoV-2 RBD	3.40 (0.91–9.31)	6.84 (2.47–38.72)	13.30(8.66–188.0)	0.7122	**0.0027**	> 0.9999
	0.0186	0.0706	0.1972			
SARS-CoV-2 N-Capsid	1.13 (0.60–3.01)	2.30 (0.57–5.90)	7.27 (2.65–20.57)	> 0.9999	**0.0031**	0.1261
	< 0.0001	**< 0.0001**	**0.0003**			
OC43 Spike	23.92(2.13–52.85)	14.64 (5.02–41.70)	63.21 (35.36–108.6)	> 0.9999	**0.0427**	**0.0445**
	> 0.9999	> 0.9999	> 0.9999			
HKU-1 Spike	3.28 (0.52–10.97)	3.87 (1.48–7.56)	15.51 (11.18–25.92)	> 0.9999	**0.0004**	**0.0025**
	0.0014	**< 0.0001**	0.1069			
NL-63 Spike	4.60 (0.47–22.05)	5.78 (0.60–41.87)	35.66 (10.07–73.87)	> 0.9999	**0.0009**	**0.0066**
	0.0206	**< 0.0001**	> 0.9999			
229e Spike	6.27 (0.78–17.55)	7.91 (0.75–60.83)	45.94 (7.04–241.4)	> 0.9999	**0.0016**	**0.0103**
	> 0.9999	**0.0020**	> 0.9999			
Influenza A H1	2.09 (0.46–5.76)	1.95 (0.48–7.05)	1.33 (0.60–4.33)	> 0.9999	> 0.9999	> 0.9999
	< 0.0001	**< 0.0001**	**< 0.0001**			
Influenza A H3	6.34 (0.87–22.03)	7.44 (1.59–26.28)	2.02 (1.01–5.72)	> 0.9999	> 0.9999	0.7710
	> 0.9999	**0.0060**	**< 0.0001**			
Influenza B Brisbane	0.76 (0.19–1.91)	1.16 (0.24–3.39)	2.03 (1.05–4.92)	> 0.9999	> 0.9999	> 0.9999
	< 0.0001	**< 0.0001**	**< 0.0001**			
Influenza B Phuket	0.74 (0.32–2.59)	0.98 (0.32–3.84)	3.04 (1.87–6.39)	> 0.9999	0.2701	> 0.9999
	< 0.0001	**< 0.0001**	**< 0.0001**			
RSV Pre-F	13.54 (3.19–68.67)	17.56 (4.63–66.48)	44.62 (16.68–80.43)	> 0.9999	0.9798	0.9369
	> 0.9999	> 0.9999	> 0.9999			

* Friedman test with Dunn's correction for multiple comparisons
** Kruskal-Wallis test with Dunn's correction for multiple comparisons

coronavirus-specific IgA within the nose are relatively reduced in children but show incremental improvement following reinfection. These observations provide understanding of how the natural history of SARS-CoV-2 infection may evolve in this age group as well as potential insights into future optimal vaccine design.

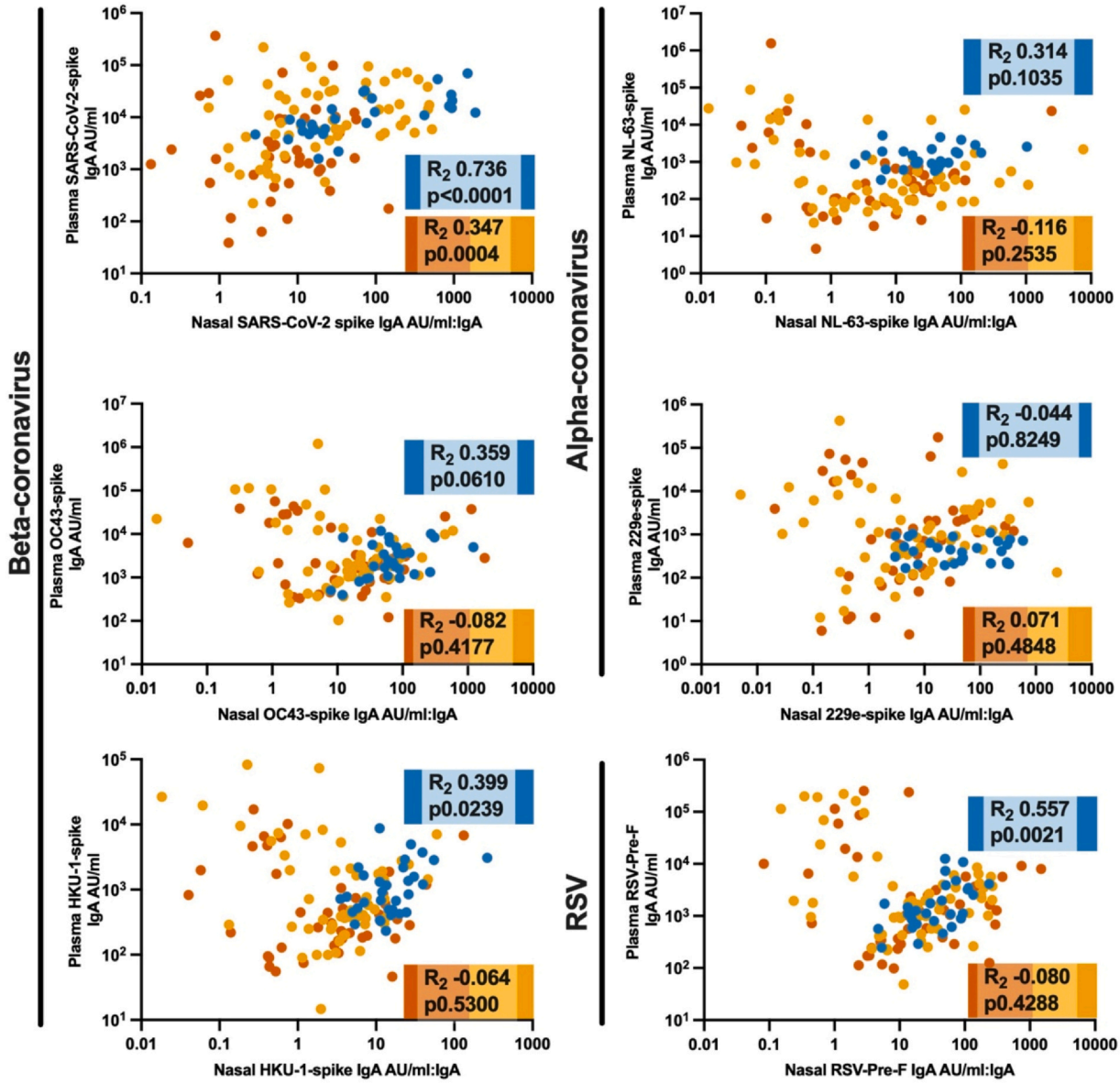
Nasal sampling showed equivalent total levels of immunoglobulin within children and adults and a strong predominance of the IgA isotype in line with previous investigations.[22] Comparison of IgA antibody levels against a range of respiratory viruses within the nose and plasma revealed that nasal antibody levels against RSV and Influenza were equivalent in children and adults. This is reassuring as RSV is a major cause of respiratory infections in young children and an infection wave was elicited following lifting of lockdown restrictions[23] and raised concerns of an 'immunity debt'.[24]

In contrast, within children, the IgA titres against coronaviruses were markedly reduced within the nose. This was seen across all coronavirus subtypes, including SARS-CoV-2 and the four additional endemic coronaviruses. This pattern was also seen both for spike and nucleocapsid-specific antibody responses. As such, this profile of relative suppression of nasal IgA responses appears to be a feature of coronavirus infection.

Table 2
Comparison of respiratory virus specific systemic IgA responses in children and adults Median AU/ml (IQR).

	Child-Unvacc	Child-Vaccinated	Adult-Vaccinated	Child-Unvacc Child-Vacc	Child-Unvacc Adult-Vacc	Child-Vacc Adult-Vacc
	Subgroup Comparison vs spike*			**Comparison across cohorts****		
SARS-CoV-2 Spike	1706 (780.1–9420)	8327 (4237–30829)	9168 (5494–19746)	0.0608	**0.0414**	> 0.9999
SARS-CoV-2 RBD	1277 (359.6–6059)	4779 (1707–19972)	5558 (3807–11965)	0.0535	**0.0113**	> 0.9999
	0.3192	0.1514	> 0.9999			
SARS-CoV-2 N-Capsid	181.0 (79.10–1566)	212.8 (82.36–1520)	290.7 (231.5–616.1)	> 0.9999	> 0.9999	> 0.9999
	< 0.0001	**< 0.0001**	**< 0.0001**			
OC43 Spike	2122 (909.7–11007)	2090 (1047–11395)	2303 (1103–5469)	> 0.9999	> 0.9999	> 0.9999
	> 0.9999	0.0764	0.1160			
HKU-1 Spike	416 (176.6–1197)	490.5 (290.1–2357)	888.0 (460.3–2064)	> 0.9999	> 0.9999	> 0.9999
	< 0.0001	**< 0.0001**	**< 0.0001**			
NL-63 Spike	300.8 (85.06–925.5)	265.9 (86.11–1460)	1266 (859.3–1916)	> 0.9999	0.0679	0.0876
	< 0.0001	**< 0.0001**	**< 0.0001**			
229e Spike	1056 (113.6–3523)	1077 (247–1077)	429.1 (217.9–714.4)	> 0.9999	> 0.9999	0.3932
	0.0110	**< 0.0001**	**< 0.0001**			
Influenza A H1	492.5 (241.7–1250)	429.3 (187.3–1136)	443.9 (101.2–1290)	> 0.9999	> 0.9999	> 0.9999
	< 0.0001	**< 0.0001**	**< 0.0001**			
Influenza A H3	1882 (598.3–5089)	1601 (541.9–4545)	254.8 (120.2–790.8)	> 0.9999	**0.0004**	**0.0003**
	> 0.9999	**< 0.0001**	**< 0.0001**			
Influenza B Brisbane	286.1 (77.13–1201)	279.8 (103.7–575.9)	718.7 (311.5–2436)	> 0.9999	> 0.9999	0.2880
	< 0.0001	**< 0.0001**	**< 0.0001**			
Influenza B Phuket	338.3 (80.81–1077)	272.1 (114.5–838.8)	966.3 (476.3–2629)	> 0.9999	0.3270	0.1715
	< 0.0001	**< 0.0001**	**< 0.0001**			
RSV Pre-F	1250(319.3–6503)	1868 (913.7–5273)	1192 (721.1–3407)	> 0.9999	> 0.9999	> 0.9999
	> 0.9999	**0.0016**	**0.0002**			

* Friedman test with Dunn's correction for multiple comparisons
** Kruskal-Wallis test with Dunn's correction for multiple comparisons



Extended Fig. 2. Differential correlation of Mucosal and Systemic IgA responses in children and adults. Mucosal and systemic IgA antibody responses in 99 children (43 unvaccinated; orange and 56 vaccinated; yellow) and 28 adults (blue) specific for SARS-CoV-2-Spike, and the four seasonal human coronavirus spike protein, and RSV pre-fusion F protein were correlated. Two-way Spearman's correlation was used.

In order to determine if there was also variation in the functional capacity of coronavirus-specific nasal IgA responses across the life course we also determined neutralisation capacity.[25–27] Here, equivalent functional responses were seen between children and adult samples. Neutralisation of viral variants was reduced compared to the ancestral spike protein although there was no further reduction for recent subvariants such as BQ.1.1 and XBB.1, a pattern which differs somewhat from the pattern seen in serological assessment of adults.[28,29] Mucosal IgA is secreted as dimers or multimers and studies in influenza have shown that this increases avidity and enhances neutralisation.[30,31] Whether such an effect also acts to retain relative affinity against SARS-CoV-2 viral variants which largely evade neutralisation by IgG is unclear but, if so, may act to limit future risk of reinfection.

Taken together, these quantitative and qualitative assessments indicate that the overall protective level of virus-specific nasal antibodies against coronaviruses is reduced within children. The reasons why nasal IgA-specific immune responses against this viral family are reduced in children is unknown but suggests that an early interaction between coronaviruses and the immune system act to subvert the generation of tissue resident IgA plasma cells within nasal mucosa. These findings are in contrast with our previous observation of enhanced systemic SARS-CoV-2 spike IgG responses in children.[18,32,33] Recent data has shown that primary SARS-CoV-2 infection also generates relatively low levels of plasma cells within bone marrow.[34]

Given this selective reduction of nasal antibody responses against coronaviruses, we were also keen to assess whether there was potential cross-reactivity of IgA responses between the different viruses.[18] However, adsorption of nasal samples with spike protein from SARS-CoV-2 showed only modest levels of reduction in antibody binding to spike proteins of other coronaviruses, indicating that the serological immune response to SARS-CoV-2 spike is generated *de novo* and does not significantly comprise boosting of cross-reactive responses from other viruses. A modest degree of cross-reactivity was apparent, most notable against the alpha-coronavirus

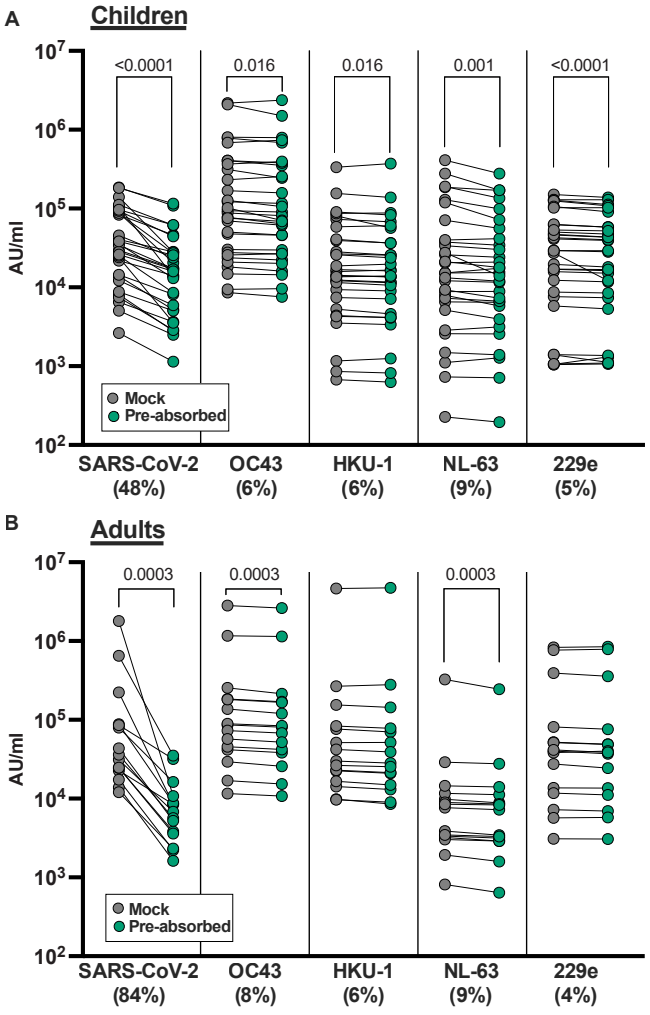


Fig. 3. Mucosal SARS-CoV-2-specific IgA shows limited cross-reactivity against seasonal coronavirus. Cross-reactive spike-specific mucosal IgA responses were determined in samples from 31 children and 15 adults. Antibodies were preabsorbed on plate bound trimeric SARS-CoV-2 spike or mock treated. Differences in binding to spike proteins from SARS-CoV-2, beta-coronaviruses (OC43 and HKU-1) and alpha-coronaviruses (NL-63 and 229e) were determined. A,B) Spike specific-IgA levels (AU/ml) in absorbed and mock treated samples from children (A) and adults (B). Percentages indicate median reduction in titre. Paired two-tailed Wilcoxon test with Holm-Šídák correction for multiple comparisons.

NL-63, and was broadly comparable between children and adults despite prior observations of greater pre-existing neutralising IgG in children.[35]

Given the low levels of nasal-specific antibodies again SARS-CoV-2 in children we were also interested to assess how viral reinfection might impact on tissue-specific antibody responses and were able to study this in children although adult samples were not available. Nasal IgA levels against spike increased 5.6 fold, compared to a 2.3 fold increase within blood, and suggest a potential relative increase in nasal mucosa which may contribute to the protective effect of natural infection.[7] Vaccination enhanced strong systemic immunity, which likely underpins its clinical efficacy in children[36,37] and enhancement of mucosal responses was also apparent.[6,8,9,38] Of note, nasal IgA responses against nucleocapsid were not increased following reinfection. It is established that children make preferential immune responses against spike compared to nucleocapsid[18] although the reasons for this are not clear and will be important to address in future studies.

Recent studies indicated higher mucosal spike-specific IgA was associated with protection from subsequent Omicron infection in adults.[6,7] As such, these observations reveal that SARS-CoV-2 infection in children generates low levels of protective nasal IgA antibodies but these increase incrementally following reinfection. This would be consistent with a pattern for the development of systemic immunity towards seasonal coronaviruses whereby repeated infections progressively drive maturation of the antibody response to reach adult levels towards the onset of puberty.[11–13] Until then, however, children will likely be more susceptible to SARS-CoV-2 reinfection than adults. In addition, the finding that nasal IgA nucleocapsid-specific levels against SARS-CoV-2, a recently emergent coronavirus, are also lower in childhood suggests that relative nasal IgA deficit also has a direct age-related effect.

A limitation of this study was the single collection of swab samples and removal of national testing in the community after March 2022; as such, we were unable to assess protection or directly compare durability or changes in mucosal antibody responses following infection and/or vaccination. Future studies to address relative levels of nasal and systemic spike-specific antibodies in vaccinated infection-naive donors would also be of interest. In addition, whilst the primary interest was in paediatric immunity, the study included 28 adults. Finally, whilst the study was performed over 1 year following the end of lockdown restrictions it is possible that suppression of respiratory virus infections during this period may have influenced the profile of virus-specific immunity at the sampling timepoint.

In conclusion, these data reveal a unique pattern of coronavirus immunity in which nasal virus-specific antibodies are relatively suppressed within children but increase following reinfection. Such a profile may indicate evolution of an immune relationship which facilitates repeated infections with coronaviruses, most particularly in childhood, a pattern which is compatible with epidemiological observations. Whilst children are generally spared from the severe clinical effects of infection, greater understanding of the role of mucosal responses to SARS-CoV-2 and its importance for transmission and future protection is important. The findings also have implications for potential vaccine design to protect children against

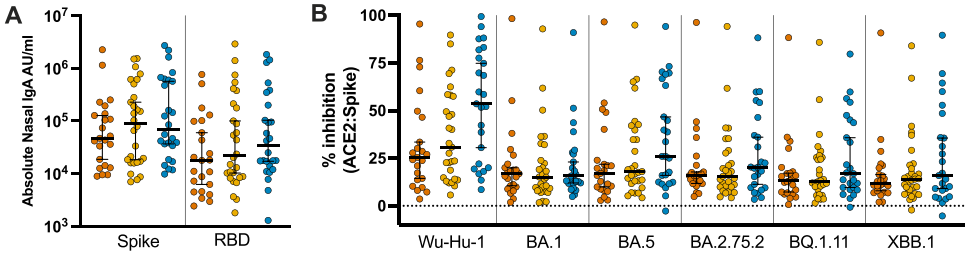


Fig. 4. Nasal IgA-mediated neutralisation of SARS-CoV-2 variants is equivalent in children and adults The functional ability of mucosal antibodies to inhibit spikes from SARS-CoV-2 variants binding to ACE2 was determined in children (23 unvaccinated; orange, 28 vaccinated; yellow) and adults (26 vaccinated; blue). Donor samples were selected on the basis of comparable concentrations of nasal spike specific IgA. A) Baseline spike- and RBD-specific mucosal IgA levels in samples tested. B) Relative inhibition of binding of spike protein from SARS-CoV-2 variants to ACE2. Bars show median ± 95% CI.

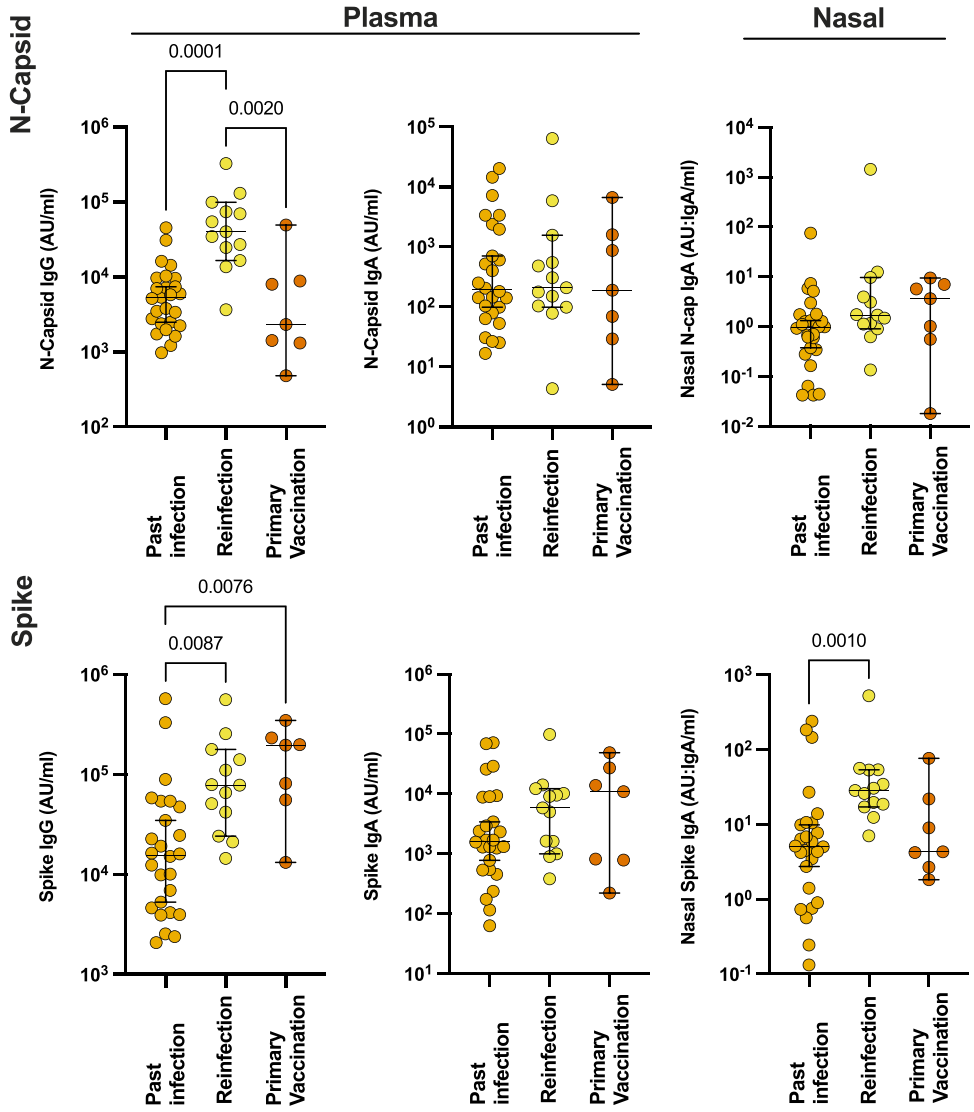


Fig. 5. SARS-CoV-2 reinfection boosts nasal IgA responses against spike but not nucleocapsid protein. Systemic IgG and IgA, and nasal IgA, responses specific for Spike or Nucleocapsid (N-capsid) in children with no reinfection ('past infection'; n = 26) or following reinfection (n = 13) or vaccination (n = 7) within the preceding 6 months. Mucosal IgA results are normalised and expressed as a ratio to total IgA. Bars indicate Median ± 95% CI. Kruskal-Wallis test with Dunn's correction for multiple comparisons.

coronavirus infection. As second-generation nasal vaccines are developed, vaccination of children may be warranted to boost mucosal immunity, thereby reducing the burden of repeated natural infections and potentially reducing transmission and community spread.

Methods

Sample collection

Paediatric samples were collected as part of the final round of the United Kingdom Health Security Agency (UK-HSA) SARS-CoV-2 surveillance in primary schools (sKIDs) a cross sectional study initiated in June 2020 (https://www.gov.uk/guidance/covid-19-paediatric-surveillance). Ethical review for the sKIDs study was provided by the PHE Research Ethics and Governance Group (PHE R&D REGG ref. no. NR0209). 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.

5–10 ml of Lithium Heparin blood and matched nasal swabs (MWE, UK) were taken by trained staff between 9th-16th December 2022. Matched plasma samples from children were available from a prior round of collection between 27th June-4th July 2022. No statistical methods were used to predetermine sample sizes. Vaccination status was accessed in January 2023 from the NIMS database, a record of all COVID-19 vaccinations in England. All children received BNT162b2 mRNA Pfizer-BionTech COVID-19 vaccine.

Blood and nasal swab samples were also obtained from healthy adult donors between November 2022-Febuary 2023 as part of an ongoing study of Coronavirus Immune responses. Ethical permission was provided by the North West–Preston Research Ethics Committee, United Kingdom (20/NW/0240) and all participants gave written informed consent. Blood was collected by a trained phlebotomist whilst nasal swabs were self-administered following instruction.

Plasma and swab preparation

Lithium Heparin blood tubes were processed within 24hrs of collection. Tubes were spun at 300 g for 10 mins prior to removal of plasma which was then spun at 800 g for 10 mins and stored as aliquots at −80 °C.

Nasal swabs were transported in Sterilin polypropylene 30 ml Universal tubes (Fisher Scientific), swabs were removed to a 15 ml polypropylene centrifuge tube (Sarstedt). The transport tube was rinsed with 500ul of Diluent 100 (Mesoscale Diagnostics) and added to the swab. Swabs were eluted with agitation for 3hrs at room temperature or overnight at 4 °C. Liquid was removed to a Costar-Spin-X 0.22 µm spin column (Sigma-Aldrich). The swab was inverted and spun at 400 g for 10 min to elute absorbed volume and added to the column. Columns were spun at 10000 g for 5 min, in some cases when the sample was not fully eluted a further spin or transfer to a new column was necessary. Eluted nasal sample was aliquoted and stored at −80 °C.

Swab dilution calculation

To define the dilution used to interpret results for swab samples 10 nasal swab samples were taken under laboratory conditions from healthy adults. Swabs were weighed before and after use using a fine balance capable of microgram measurements. The difference was calculated, 1 ug was equated to 1ul. Median sample was 10ul (range; 4–18), nasal sample were thus assigned as a 1:50 dilution.

Serological analysis

Quantitative IgG and IgA antibody titres were measured using Mesoscale Diagnostics (MSD) multiplex assays; Respiratory Panel 2 and Coronavirus Panel 7 following the manufacturer instructions. Briefly, plasma samples were diluted 1:5000 for IgG or 1:500 for IgA, nasal samples were neat (assumed dilution of 1:50) additional 1:10 dilutions were used for samples above the detection limit. Samples were added to wells of the 96 well MSD plate alongside reference standards and controls. After incubation, plates were washed and anti-IgG or IgA-Sulfo tagged detection antibody added. After incubation, plates were washed and 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 an 8-point standard curve generated on each MSD plate with standards provided by MSD. IgG cut-offs were previously defined against pre-pandemic adult and paediatric samples.[18] Wuhan-Hu-1 Spike-specific antibody titres were used to define seropositivity in unvaccinated children. Nucleocapsid titres, in the absence of reported SARS-CoV-2 test results, were used to define infection-naïve vaccinated donors.

Characterization of total nasal immunoglobulin

The proportion and level of IgG1–4, IgA and IgM were determined in nasal swab samples using a 6-plex Legendplex Immunoglobulin Isotyping Panel kit (Biolegend), all reagents were supplied. Briefly, samples were diluted 1:300–1:1000 in assay buffer and mixed with pre-mixed microbeads coated with isotype specific antibodies, known concertation standards were also included in duplicate. Bound antibody was then detected with biotin conjugated secondary antibodies and streptavidin-PE. Samples were run on a LSR-II flow cytometer (BD bioscience). The eight-point standard curve allowed quantification using the cloud-based Legendplex analysis software suit (Biolegend).

Cross-reactive absorption assay

Nasal samples were diluted 1:4 (children) or 1:5 (adults) in Diluent 100 (MSD), 120ul of sample was then added to either a blank 96 well plate or a Coronavirus Panel 7 plate, containing bound SARS-CoV-2 trimeric spike, both plates were blocked (Blocker A, MSD) and washed prior to sample addition. Plates were incubated with shaking for 2hrs, a second round of absorption was then repeated. 45ul of absorbed or mock sample were plated in duplicate onto a Coronavirus Panel 2 plate (MSD) and analysed as with other MSD assays. A cut off 30% reduction in SARS-CoV-2 spike titre in comparison to the Mock samples was used.

Spike-ACE2 Receptor inhibition assay

Inhibition of trimeric Spike binding of ACE-2 by nasal samples was measured using an MSD V-PLEX COVID-19 ACE2 Neutralization Kit (SARS-CoV-2 Plate 32) following manufacturer's instructions. Briefly, samples were added in duplicate to the plate coated with trimeric spike from SARS-CoV-2 variants. After pre-incubation, Sulfo-tagged Human ACE-2 protein was added to the plate and incubated for 1 h. Plates were washed and read immediately using a MESO ™ QuickPlex SQ 120 system. Data was generated by Methodological Mind software and analysed with MSD Discovery Workbench (v4.0) software. Presented data were expressed as a percentage of maximal binding in reference to mock treated wells.

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.

Declaration of Competing Interest

The authors declare no competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2023.08.013.

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