Nasal IgA wanes 9 months after hospitalisation with COVID-19 and is not induced by subsequent vaccination.

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Summary

Background
Most studies of immunity to SARS-CoV-2 focus on circulating antibody, giving limited insights into mucosal defences that prevent viral replication and onward transmission. We studied nasal and plasma antibody responses one year after hospitalisation for COVID-19, including a period when SARS-CoV-2 vaccination was introduced.

Methods
Plasma and nasosorption samples were prospectively collected from 446 adults hospitalised for COVID-19 between February 2020 and March 2021 via the ISARIC4C and PHOSP-COVID consortia. IgA and IgG responses to NP and S of ancestral SARS-CoV-2, Delta and Omicron (BA.1) variants were measured by electrochemiluminescence and compared with plasma neutralisation data.

Findings
Strong and consistent nasal anti-NP and anti-S IgA responses were demonstrated, which remained elevated for nine months. Nasal and plasma anti-S IgG remained elevated for at least 12 months with high plasma neutralising titres against all variants. Of 180 with complete data, 160 were vaccinated between 6 and 12 months; coinciding with rises in nasal and plasma IgA and IgG anti-S titres for all SARS-CoV-2 variants, although the change in nasal IgA was minimal. Samples 12 months after admission showed no association between nasal IgA and plasma IgG responses, indicating that nasal IgA responses are distinct from those in plasma and minimally boosted by vaccination.

Interpretation
The decline in nasal IgA responses 9 months after infection and minimal impact of subsequent vaccination may explain the lack of long-lasting nasal defence against reinfection and the limited effects of vaccination on transmission. These findings highlight the need to develop vaccines that enhance nasal immunity.
Research in context

Evidence before the study
While systemic immunity to SARS-CoV-2 is important in preventing severe disease, mucosal immunity prevents viral replication at the point of entry and reduces onward transmission. We searched PubMed with search terms “mucosal”, “nasal”, “antibody”, “IgA”, “COVID-19”, “SARS-CoV-2”, “convalescent” and “vaccination” for studies published in English before 20th July 2022, identifying three previous studies examining the durability of nasal responses that generally show nasal antibody to persist for 3 to 9 months. However, these studies were small or included individuals with mild COVID-19. One study of 107 care-home residents demonstrated increased salivary IgG (but not IgA) after two doses of mRNA vaccine, and another examined nasal antibody responses after infection and subsequent vaccination in 20 cases, demonstrating rises in both nasal IgA and IgG 7 to 10 days after vaccination.

Added value of this study
Studying 446 people hospitalised for COVID-19, we show durable nasal and plasma IgG responses to ancestral (B.1 lineage) SARS-CoV-2, Delta and Omicron (BA.1) variants up to 12 months after infection. Nasal antibody induced by infection with pre-Omicron variants, bind Omicron virus in vitro better than plasma antibody. Although nasal and plasma IgG responses were enhanced by vaccination, Omicron binding responses did not reach levels equivalent to responses for ancestral SARS-CoV-2. Using paired plasma and nasal samples collected approximately 12 months after infection, we show that nasal IgA declines and shows a minimal response to vaccination whilst plasma antibody responses to S antigen are well maintained and boosted by vaccination.

Implications of all the available evidence
After COVID-19 and subsequent vaccination, Omicron binding plasma and nasal antibody responses are only moderately enhanced, supporting the need for booster vaccinations to maintain immunity against SARS-CoV-2 variants. Notably, there is distinct compartmentalisation between nasal IgA and plasma IgA and IgG responses after vaccination. These findings highlight the need for vaccines that induce robust and durable mucosal immunity.
Introduction

Intramuscular (i.m.) vaccines are remarkably effective in preventing severe COVID-19, their use being associated with declining hospitalisation. However, current vaccines provide only transient protection against respiratory viral replication, onward transmission and continuing emergence of variants. By contrast, respiratory infection with SARS-CoV-2 induces mucosal immune defences that can inhibit viral replication and transmission, though the correlation between nasal and systemic immunity is inexact. To date, there have been few longitudinal studies of nasal antibody durability and those that exist give diverse results – suggesting that nasal antibody may persist for anywhere between 3 and 9 months. There is a clear need for additional studies of mucosal and systemic immunity in those recovered from severe disease.

Although i.m. vaccination transiently reduces transmission, vaccinees with breakthrough infections have peak nasopharyngeal viral loads similar to those in unvaccinated individuals. Some studies have shown that viral loads decline more rapidly in vaccinees, but it is unclear whether this effect is mediated by passive transudation of plasma antibody into the mucosa, or whether vaccination can recall mucosal responses primed by infection (as observed after i.m. influenza vaccination following an intranasal (i.n.) priming). Serum IgA and IgG is mostly monomeric and produced in the bone marrow, whereas nasal IgA is polymeric and can be synthesized locally by mucosal plasma cells. It is polymeric nasal IgA that is critical for efficient neutralisation of virus in the upper respiratory tract, and so passive transudation of plasma antibody into the mucosa is unlikely to provide durable sterilizing immunity. Understanding whether i.m. vaccination after COVID-19 can recall nasal IgA responses is an important step towards developing vaccines which prevent infection and transmission.

During worldwide circulation of SARS-CoV-2, multiple successive variants have evolved, driven by enhancements in transmissibility as well as immune evasion. The Omicron subvariants appear less susceptible to vaccine-induced immunity and show high reinfection rates. It seems that immunity induced by successive infection and vaccination may provide superior protection against Omicron compared with either alone, and vaccination regimes which combine i.n and i.m. administration in mice induce enhanced mucosal protection against SARS-CoV-2 variants. This suggests that priming the nasal mucosa is required to induce effective local antibody responses that might provide enhanced immunity against current and future variants. However, the cross-reactivity of nasal antibody after infection with pre-Omicron virus is unknown.

We here report the results of a large multicentre longitudinal study of nasal and plasma antibody responses approximately a year after COVID-19, aiming to understand the longevity of nasal antibody responses after COVID-19 and the effect of subsequent vaccination. We demonstrate durable nasal and plasma IgG responses to ancestral (B.1 lineage) SARS-CoV-2, Delta and Omicron variant that are enhanced by i.m. vaccination. However, nasal IgA responses did not mirror those in plasma, waned after 9 months and were not substantially boosted by vaccination (Figure S1).

Methodology

Study design and participants

Clinical data, nasosorption and plasma samples were collected from hospitalised cases of COVID-19 within the ISARIC4C and PHOSP-COVID multicentre studies of UK adult patients (figure S2).

Adults hospitalised during the SARS-COV-2 pandemic were recruited into the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) World Health Organization Clinical Characterisation Protocol UK (IRAS260007 and IRAS126600). Written informed consent was obtained from all patients. Ethical approval was given by the South Central–Oxford C Research Ethics Committee in England (reference: 13/SC/0149), Scotland A Research Ethics Committee (20/SS/0028) and World Health Organization Ethics Review Committee (RPC571 and RPC572I; 25 April 2013).
After hospital discharge patients >18 years old who had no co-morbidity resulting in a prognosis of less than 6 months, were recruited to the PHOSP-COVID study. Written informed consent was obtained from all patients. Ethical approvals for the PHOSP-COVID study were given by Leeds West Research Ethics Committee (20/YH/0225).

Samples and data were collected on day 1 to 9 of admission and/or at intervals during convalescence (approximately 1 to 14 months after discharge). Disease severity was classified according to the WHO Clinical Progression score.19

See supplementary materials for full methods.

**Procedures and immunoassays**

Nasal samples were collected via nasosorption. Nasal and plasma IgA and IgG responses to Spike (S), Nucleocapsid (NP) and the Receptor-Binding-Domain of Spike (RBD) antigens of ancestral SARS-CoV-2 were measured using MSD (Mesoscale Diagnostics, Rockville, Maryland, USA) V-PLEX COVID-19 Coronavirus Panel 2 Kits. Antibody responses to RBD antigen of Delta and Omicron (BA.1) variants were measured using MSD V-PLEX SARS-CoV-2 panel 22. Nasal samples were diluted 1 in 50 and plasma 1 in 5000 prior to analysis. Nasosorption and plasma samples collected from 25 healthy participants prior to the emergence of SARS-CoV-2 were used as a control group. Plasma neutralisation of ancestral SARS-CoV-2, Delta and Omicron (BA.1) variants was measured using a pseudotype neutralisation assay, as previously described at a dilution of 1 in 50.20 Samples with neutralising activity >90% were titrated to establish the titre resulting in 50% reduction in infectivity (PRNT50).

**Data analysis and Outcomes measured**

Analyses were conducted using the Outbreak Data Analysis Platform (ODAP). Statistical analyses used R version 4.2.0. All tests were two-tailed and statistical significance was defined as a p-value<0.05 after adjustment for false discovery rate. Sample size calculations are detailed in supplementary materials.

Nasal antigen-specific IgA and IgG (AU/mL) was normalised to total isotype (pg/mL) accounting for concentration of sample obtained and matrix effects. Plasma and normalised nasal data were log2 transformed. The data were confirmed to be non-parametrically distributed using quantile Vs quantile plots. To understand the durability of antibody responses, comparisons between timepoints were made using the optimal pooled t-test, which performs well in non-parametric partially paired data.21 To estimate the effect of vaccination on antibody trajectories, a LOESS regression curve was fitted to data from repeated and cross-sectional samples taken from those who were known to be vaccinated. To understand the relationship between compartments, disease severity and age, paired plasma and nasal responses taken from the same individuals were analysed in a correlation matrix measuring the Spearman rank correlation coefficient between variables. The variables in the correlogram were hierarchically clustered using Ward’s minimum variance. To further explore the relationship between nasal IgA and plasma IgA and IgG responses, unsupervised clustering was performed with Ward’s minimum variance and the results were visualised in a heatmap, which was subsequently annotated with age, disease severity and vaccination status to determine factors associated with cluster formation.

Control samples were used to define a nasal antibody threshold. The threshold was equivalent to the geometric mean titre (GMT) + 2 SD of controls and validated against standardized WHO BAU/mL thresholds converted into MSD AU/mL.22

**Results**

A total of 446 adults, hospitalised between February 2020 and March 2021, were recruited and 569 plasma samples were collected, of which 338 represented samples taken from the same individual at sequential timepoints. In addition, 356 nasal samples were collected, of which 143 were taken from
the same individual at sequential timepoints. 174 individuals had paired plasma and nasal samples taken at a given time point. Patient characteristics are shown in Table 1. The 6 and 12 month samples were collected between September 2020 and March 2022, covering the start of the UK vaccination campaign (figure S2).

**Plasma antibody responses are more durable than nasal responses after COVID-19**

Nasal anti-S and anti-NP IgA appeared within 4 weeks after symptom onset but waned after 9 months to levels equivalent to pre-pandemic controls ($p<0.0001$) (figure 1). Anti-S IgG appeared within 14 days of symptom onset ($p<0.0001$) and rose 2181-fold after 9 months ($p<0.0001$) but unlike IgA responses, remained above pre-pandemic controls thereafter ($p<0.0001$) (figure 1 A–B). Both nasal IgA and IgG anti-S titres rose after 10 months, though the median change was only 1.46-fold in the case of IgA ($p=0.011$). Anti-NP IgA and IgG responses remained low after 9 months ($p<0.0001$) (figure 1 E–F).

Pre-pandemic controls allowed a threshold value for nasal antibody to be established, equivalent to the GMT+2SD (figure S3). Applying the same method to plasma samples, we found that the threshold value performed similarly to that of the WHO standards, confirming the validity of this method (figure S4). Using this threshold, we found that the nasal IgA GMT to S and NP fell below threshold after 9 months (Figure S3 A–B) whilst the nasal IgG GMT was durable and remained above threshold for both antigens at 12 months (Figure S3 F–G).

Plasma IgG anti-S and anti-NP responses developed within 14 days of symptom onset and remained elevated at 12 months ($p<0.0001$) (figure 1D and H). Notably, the trajectories of plasma IgA and IgG responses differed to that of nasal IgA. Whilst nasal responses peaked between 6 to 9 months for S and between 3 to 5 months for NP, plasma responses peaked within 4 weeks before waning (figure 1). Notably, plasma anti-NP responses plateaued after 10 months and all individuals were seropositive for both antigens at the final time point, indicating durable plasma responses after COVID-19 (figure 1D, G and H).

Only 2 of 446 individuals showed serological evidence of re-infection (whereby a rise in both anti-NP and anti-S IgG was observed between 103 and 308 days after infection for the first individual and between 238 and 463 days for the second). Furthermore, in 33 individuals where vaccination status was known and from whom samples were taken before and after vaccination anti-S titres rose ($p<0.0001$) whilst anti-NP titres declined ($p=0.00019$), as expected, indicating a low prevalence of re-infection in our cohort (figure S5 A–B). These data therefore demonstrate that nasal and plasma IgG responses are durable after COVID-19, whilst nasal IgA responses last only 9 months.

**Responses during vaccination campaign**

Given the timing of vaccination in most of our cohort (median 20th February 2021) and the timing of the 6 to 9 month visit (median 16th March 2021), we reasoned that the increases in anti-S IgA and IgG seen in both nasal and plasma samples after 9 months were predominantly due to vaccination (figure 1 and S2). Of those with known vaccination status (n=180), 89% of individuals from whom plasma samples were collected and 95% of individuals from whom nasal samples were collected, received their first SARS-CoV-2 vaccination during the study. All vaccinations occurred between December 2020 and March 2022. Of these, 64.7% received ChAdOx1 nCoV-19 as the first dose (table 1). Since vaccines contain only S protein, NP responses are not induced by vaccination, and these responses remained low after 9 months (figure 1 E–H).

We confirmed the effect of vaccination by comparing S and NP antibody titres in individuals known to be vaccinated before and after their first vaccination (figure 2). Outliers who had samples taken >500 days after symptom onset were removed from this analysis to avoid modelling with insufficient data. Although the analysis was limited by the small number of nasal samples collected pre-vaccination (n=4), there were clear differences in the nasal IgA and IgG responses to S and NP after vaccination (figure 2 A–B). Although nasal anti-S IgA responses appeared elevated relative to anti-NP responses 100 days after vaccination, the difference in trajectories was small and the 95% CIs
overlapped. By contrast, nasal IgG anti-S responses rose after vaccination and peaked approximately 150 days after vaccination, whilst the anti-NP trajectory declined. There was no overlap between the 95% confidence intervals (CI) of the regression curve for anti-S and anti-NP IgG responses after vaccination indicating distinct trajectories (figure 2B). Notably, the nasal IgG responses mirrored that of plasma IgA and IgG (figure 2 C–D). Thus, in keeping with the threshold analysis (figure S3), changes in nasal IgA titres after vaccination are minor compared to nasal and plasma IgG which are substantially boosted, suggesting that vaccination cannot fully recall mucosal antibody responses.

Responses to Delta and Omicron (BA.1) variants

All participants were admitted to hospital prior to the emergence of Omicron variant and 71.1% (n=317) were admitted before 10th May 2021 when Delta variant became the dominant strain in the UK.4,12 However, nasal IgA and IgG responses binding both Delta and Omicron RBD were present within 28 days of symptom onset and remained elevated for at least 9 months (figure 3 A–F). Nasal IgA binding Omicron appeared the most short-lived; the GMT only reached the threshold for positivity between 3 and 9 months (figure S3E). Furthermore, at its peak median titre, Omicron binding nasal IgA was only 10-fold above controls (p<0·0001), compared to nasal IgA binding ancestral SARS-CoV-2 RBD which was 28-fold higher (p<0·0001) (figure 3A and C). Plasma IgG responses to Delta and Omicron also developed within 14 days and were sustained for 12 months (figure 3 G–I).

To understand the degree of cross-reactivity between compartments we compared the ratio of antibody binding RBD of Omicron virus and ancestral SARS-CoV-2 (figure S6). There was no difference in the median ratio between nasal IgA (0-10) and nasal IgG (0-12, p=0-67). However, the nasal IgG ratio was higher than that of plasma IgG (0-09, p=0-020) and the nasal IgA ratio was higher than that of plasma IgA (0-08, p=0-00059). These data indicate that infection with pre-Omicron SARS-CoV-2 can induce nasal and plasma antibody that binds Omicron RBD, and that nasal antibody may have greater cross-binding potential. However, despite this, Omicron-binding nasal IgA is slow to reach positive levels and is transiently maintained.

Responses to Delta and Omicron (BA.1) variant after vaccination

The nasal IgA trajectory did not appear substantially different after vaccination (figure S7A) though a small rise in the Omicron- and Delta-binding nasal IgA GMT was seen between 10 and 12 months, when most individuals with known vaccination status had been vaccinated (figure 3 and S3). However, this difference was small and did not reach the positive threshold. Nasal IgG responses to Omicron and Delta variant rose after vaccination (figure S7B), although Omicron-binding responses did not reach the level of those to Delta and ancestral SARS-CoV-2 despite vaccination.

Plasma IgG responses to Delta and Omicron variants also rose after vaccination (figure 7 C–D). To study the effect of vaccination specifically, we identified 33 individuals from whom pre- and post-vaccination plasma samples were collected; these were taken at a median of 54 days (IQR 25-6–68-8) before the first vaccination dose and 176 days (IQR 113–212) after (figure S5). Vaccination substantially boosted Omicron-binding plasma IgG in these individuals, which rose 8-7-fold (p<0-0001). However, no significant difference in Delta-binding titres was seen, as antibody was boosted in some individuals but declined in others (figure S5D). This pattern may relate to a rapid rise and wane of vaccine-boosted antibody, given that samples were taken a median of 168 days after first vaccination and Delta-binding plasma IgG responses were observed to wane approximately 75 days from first vaccination (figure S7D). The subsequent rise in Delta-binding plasma IgG 150 days after vaccination likely results from individuals receiving their second vaccination dose during the study (table 1). These data suggest that vaccination can boost Omicron- and Delta-binding nasal and plasma IgG but enhancement of Delta responses may be short-lived after one vaccine dose. Meanwhile, Omicron- and Delta-binding nasal IgA responses are not significantly affected by vaccination.

Plasma neutralising antibody to SARS-CoV-2 variants.
Plasma neutralising titres against ancestral, Delta and Omicron variants of SARS-CoV-2 remained substantially elevated compared with controls between 3 and 12 months (figure S8). However, neutralising titres against Omicron were generally lower: at 10 to 12 months, 76.2% had neutralising antibody against Omicron, compared to 92.5% against ancestral SARS-CoV-2. Neutralising titres against all three variants were boosted during the vaccination campaign ($p<0.0001$) indicating that i.m. vaccination after COVID-19 can enhance neutralising antibody levels to homologous and heterologous variants.

As expected, neutralising antibody titres correlated with plasma RBD ($R=0.82$, $p<0.0001$) and S IgG ($R=0.81$, $p<0.0001$) (figure S9). Notably, plasma neutralising antibody correlated with nasal anti-RBD IgG ($R =0.59$, $p<0.0001$) and anti-S IgG ($R=0.56$, $p<0.0001$) but not nasal IgA (anti-RBD $R =0.1$, $p=0.39$). This finding, alongside the boosting of nasal IgG after vaccination indicate that nasal IgG responses reflect that of plasma, whilst the nasal IgA response is distinct and compartmentalised (figure 2 and S9).

**Discordance between plasma and nasal antibody responses.**

To characterise the relationship between compartments, paired nasal and plasma samples from 175 individuals were examined. Samples were divided into those taken at approximately 6 months (3–9 months) and 12 months (>10–12 months) after infection (figure 4). At 6 months nasal anti-S IgA responses correlated strongly with nasal anti-NP IgA responses ($R=0.71$, $p<0.0001$) but showed a weaker association with nasal anti-S IgG ($R =0.57$, $p<0.0001$) and plasma anti-S IgA responses ($R =0.50$, $p<0.0001$) (figure 4A). There was no association between nasal IgA responses and plasma IgG response to either S ($p=0.38$) or NP ($p=0.56$). Nasal anti-NP IgA did not correlate with either nasal or plasma anti-NP IgG and correlated weakly with plasma anti-NP IgA ($R=0.40$, $p=0.0021$). Nasal IgG responses correlated with plasma IgG responses to the corresponding antigen, (anti-S $R=0.47$, $p<0.0001$) and the association between nasal and plasma anti-NP IgG was marginally stronger ($R=0.51$, $p<0.0001$) (figure 4B). Age and disease severity showed no association with nasal responses at both time points.

We considered the role of vaccination in driving the compartmentalisation between nasal IgA and plasma responses. At 6 months, 27 of 31 individuals with known vaccination status had received their first vaccination and 10 had received both doses. The median time from first vaccination was 81 days (IQR 20–105). Meanwhile at 12 months, 58 of 63 individuals with known vaccination status had received both vaccinations and the median time from second vaccination was 171 days (IQR 103–246). Thus, we reasoned that the increased compartmentalisation between these time points may result from vaccination; whereby plasma responses are enhanced but nasal IgA is minimally affected.

To explore the relationship between nasal IgA and plasma antibody responses after first vaccination, we performed hierarchical clustering of anti-S/RBD responses from paired samples collected at 6 months (median 81 days after vaccination). Compartmentalisation of nasal IgA from plasma responses was observed with 4 distinct clusters forming (figure 4C). The first cluster exhibited patients with robust nasal IgA and plasma responses. Patients with the weakest plasma IgA and IgG responses were present in cluster 2 whilst patients with the weakest nasal IgA responses were in cluster 4. Although not statistically significant, there was a tendency towards more recent vaccination in cluster 1 compared with cluster 4 (figure S10A). The date of vaccination was not available for any members of cluster 2. Cluster 1 also contained a higher proportion of individuals receiving BNT162b2 vaccination (44%) compared with cluster 4 (27%), although the difference in proportions did not reach statistical significance due to the number of participants with complete vaccination data (figure S10B). There was no association between disease severity or age and cluster membership (figure 4C). Thus, we concluded that the clusters resulted from transient boosting of nasal IgA responses after recent vaccination, with divergence between the nasal IgA and plasma responses with increasing time from vaccination. Given the insubstantial and transient effect of vaccination on nasal IgA responses...
relative to plasma responses, we suggest that i.m. vaccination after COVID-19 is unable to adequately recall mucosal responses.

Discussion
We demonstrate durable nasal and plasma IgG responses to ancestral (B.1 lineage), Delta and Omicron variants of SARS-CoV-2 in 446 adults hospitalised with COVID-19, who were infected with pre-Omicron virus and the majority of whom were subsequently vaccinated. However, we found that nasal virus-specific IgA levels fell back to pre-COVID levels after 9 months and Omicron-binding nasal responses were particularly short-lived. Our results reveal that nasal IgA responses are compartmentalised from systemic responses after vaccination, which boosted nasal and plasma IgG but not nasal IgA.

The durability of nasal antibody responses has hitherto been unclear. Whilst a Dutch study of healthcare workers found that nasal antibody lasted 9 months after mild infection, others demonstrated rapid waning after 3 months. Neither study examined a large cohort of hospitalised patients, and our findings confirm that COVID-19 can induce durable mucosal immunity. We also found that disease severity and age did not impact the longevity of the nasal responses in keeping with a recent study of 26 unvaccinated individuals.

By calibrating nasal antibody levels with pre-COVID samples, we demonstrate that on average, nasal IgA responses disappear after 9 months and Omicron-binding IgA is particularly short-lived. Nasal IgA is the most abundant mucosal antibody and provides an important first-line defence against respiratory infection. The importance of nasal IgA in mediating immunity to SARS-CoV-2 is highlighted by a recent study where nasal IgA but not IgG correlates with nasal neutralisation after COVID-19. The short-lived nasal IgA response demonstrated here may explain the high rates of infection with Omicron variant, despite vaccination, and are in-keeping with real-world data reported in preprint, showing that infection with pre-Omicron virus has minimal influence on the risk of Omicron infection at 15 months.

Whilst we found that i.m. vaccination can boost nasal IgG, it had limited effects on IgA, in keeping with a previous study of salivary antibody in 107 care home residents. We demonstrated correlations between nasal IgG, plasma IgG and plasma neutralisation, whilst nasal IgA responses were compartmentalised, suggesting that the rise in nasal IgG after vaccination could derive from plasma. Notably, we demonstrate that those exhibiting more robust nasal IgA responses had been recently vaccinated and a higher proportion had been vaccinated with BNT162b2 vaccine. Although this analysis was limited by small sample size, our findings suggest that vaccination only transiently boosts nasal IgA, and the type of vaccination received may influence the strength of response. mRNA vaccines tend to induce stronger circulating antibody responses than those using adenoviral vectors, and this may also apply to nasal responses. Taken together, these findings suggest that i.m. vaccination after COVID-19 cannot recall mucosal responses.

The concept of independent mucosal and systemic immunity is supported by recent studies showing that SARS-CoV-2 naïve individuals (whose mucosa have not been primed) do not produce nasal antibody after i.m. vaccination, highlighting that an independent response must occur at mucosal sites. Moreover, previous work has demonstrated that transudation of plasma antibody makes minimal contribution to total antibody concentrations in the mucosa, even in cases of paraproteinaemia where plasma concentrations are extremely high. This would explain why i.m. vaccination has had only transient effects on transmission, since the enhancement of nasal IgG that we observe, while measurable, is unlikely to have a considerable effect on mucosal susceptibility to infection. Future vaccines will need to substantially boost nasal IgA if they are to fully prevent infection and transmission. To date, intranasal and aerosolized vaccines have shown the most promise in doing so. It is therefore essential to prioritise development of mucosal vaccines which can provide better protection against respiratory infections.
Study limitations

Although 338 individuals had samples taken from more than 1 time point after hospital discharge, given the circumstances and scale of this study we were not able to collect longitudinal samples from each participant. However, given that most individuals follow similar antibody kinetics, where longitudinal samples were missing, data were compared to cross-sectional samples taken from individuals in the acute and early convalescent phase of illness.

We did not have vaccination data for all cases, preventing direct comparison of pre- and post-vaccination nasal antibody titres. However, we demonstrated differences in nasal anti-S and anti-NP responses during the period of vaccination, enabling inferences to be drawn. Notably we estimated a peak of nasal anti-S IgG titres 150 days after vaccination which is considerably slower than peak circulating antibody responses after vaccination (28–42 days).\(^3^1\) Future studies using longitudinal data collected at fixed intervals before and after vaccination will better capture the peak of nasal antibody titres after i.m. vaccination.

Conclusions

This is the first study to demonstrate durable but compartmentalised nasal IgA and plasma antibody responses to SARS-CoV-2 after infection and subsequent vaccination. We show enhancement of nasal and plasma IgG responses to ancestral SARS-CoV-2, Delta and Omicron variants after vaccination. However, nasal IgA responses, especially those to Omicron, are more short-lived and are not substantially affected by vaccination. Our results explain the lack of long-term sterilising immunity after previous infection and/or vaccination and highlight the need for mucosal vaccines that target nasal IgA responses. By enhancing nasal antibody responses, mucosal vaccines might prevent infection and transmission more effectively, enabling greater control of the pandemic and limiting the emergence of variants.
Table 1.

| Demographics (n=446)                        | Missing data |
|--------------------------------------------|--------------|
| Age at admission, years                    | 59 (51–67)   | 28 (6.3)     |
| Sex at birth                               |              |              |
| Female                                     | 164 (39·1)   | 27 (6.0)     |
| Male                                       | 255 (60·9)   |              |
| Ethnicity                                  |              |              |
| White                                      | 259 (82·0)   | 130 (29.0)   |
| South Asian                                | 22 (6·9)     |              |
| Black                                      | 20 (6·3)     |              |
| Mixed                                      | 5 (1·6)      |              |
| Other                                      | 10 (3·2)     |              |

| Clinical characteristics (n=446)            | Missing data |
|--------------------------------------------|--------------|
| Disease severity                           |              |
| WHO Class 3-4                              | 60 (14·6)    | 34 (7.6)     |
| WHO Class 5                                | 193 (46·8)   |              |
| WHO Class 6                                | 101 (24·5)   |              |
| WHO Class 7-9                              | 48(11·6)     |              |
| WHO Class 10                               | 10 (2·4)     |              |
| BMI ≥ 30                                   | 164 (62·1)   | 183 (40.9)   |
| Co-morbidities                             |              |
| None                                       | 66 (20·8)    | 129 (28.8)   |
| 1                                          | 69 (21·8)    |              |
| ≥2                                         | 183 (57·4)   |              |
| First vaccination received during the study |              |
| Yes                                        | 160 (89·9)   | 266 (59·4)   |
| No                                         | 20 (11·1)    |              |
| Second vaccination received during the study|              |
| Yes                                        | 114 (65·5)   | 272 (60·7)   |
| No                                         | 60 (34·5)    |              |
| Type of first vaccination                   |              |
| Oxford/ AstraZeneca (ChAdOx1 nCoV-19)      | 101 (64·7)   | 290 (64·7)   |
| Pfizer/Bio-N-Tec (BNT162b2)                | 55 (35·3)    |              |
| Moderna                                    | 0            |              |
| Type of second vaccination                  |              |
| Oxford/ AstraZeneca (ChAdOx1 nCoV-19)      | 65 (58·0)    | 334 (74·6)   |
| Pfizer/Bio-N-Tec (BNT162b2)                | 46 (40·1)    |              |
| Moderna                                    | 1 (0·9)      |              |
Table 1. Summary of clinical and demographic data. Data are n (%) or median (IQR). Percentages were calculated after exclusion of missing data. Disease severity is classified according to the WHO Clinical Progression score: 3–4=no continuous supplemental oxygen needed; 5=continuous supplemental oxygen only; 6=continuous or bi-level positive airway pressure ventilation or high-flow nasal oxygen; 7–9=invasive mechanical ventilation or other organ support; and 10=did not survive. BMI=body-mass index.

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Ethical Approvals

Identified patients hospitalised during the SARS-COV-2 pandemic were recruited into the International Severe Acute Respiratory and Emerging Infection Consortium World Health Organization Clinical Characterisation Protocol UK (IRAS260007 and IRAS126600). Written informed consent was obtained from all patients. Ethical approval was given by the South Central–Oxford C Research Ethics Committee in England (reference: 13/SC/0149), Scotland A Research Ethics Committee (reference: 20/SS/0028) and World Health Organization Ethics Review Committee (RPC571 and RPC572l; 25 April 2013).

Following hospital discharge patients were recruited to the PHOSP-COVID study for which written consent was obtained and ethical approval given by Leeds West Research Ethics Committee (Ref: 20/YH/0225).

Data sharing

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The ISARIC4C protocol, data sharing and publication policy are available at https://isaric4c.net. ISARIC4C’s Independent Data and Material Access Committee welcomes applications for access to data and materials (https://isaric4c.net).

The PHOSP-COVID protocol, consent form, definition and derivation of clinical characteristics and outcomes, training materials, regulatory documents, information about requests for data access, and other relevant study materials are available online: https://phosp.org/resource/. Access to these materials can be granted by contacting phosp@leicester.ac.uk and Phospcontracts@leicester.ac.uk.

All data used in this study is available within ODAP. Data access criteria and information about how to request access is available online: https://phosp.org/resource/. If criteria are met and a request is made, access can be gained by signing the eDRIS user agreement.

PPI dissemination

Patient and public involvement have been integral to the PHOSP-COVID study and consortium since conception. The PHOSP PPI group is co-chaired by NOCRI (Kate Holmes) and Asthma and Lung UK (Krisnah Poinasamy) with representation of over 10 relevant charities. Members of the ‘Long-COVID Facebook support group’ are closely involved and a Leicester BRC PPI group consisting of people with lived experience of a hospital admission for COVID-19. Patients and public are embedded within the PHOSP-COVID infrastructure including our working groups, core management group, and executive and steering groups. Patients were involved in the development of the clinical research study including the overarching aims, choice of outcomes, consent processes and the structure of the study visits. Patients review all patient facing material. We have recently completed a joint patient and clinician research priority questions exercise hosted by advisors from the James Lind Alliance to ensure co-ownership of the direction of PHOSP-COVID research.

ISARIC4C has a public facing website and twitter account (@CCPUKstudy). We are engaging with print and internet press, television, radio, news, and documentary programme makers. We will explore distribution of findings with Asthma and Lung UK and take advice from NIHR Involve and GenerationR Alliance Young People’s Advisory Groups.

Author Contributions
**Felicity Liew** has made substantial contributions to this work including: recruitment of participants, acquisition of clinical samples and data, as well as analysis and interpretation of data. They have co-written this manuscript, including all drafting and revisions. They approve the final version to be published and agree to accountability for all aspects of this work.

**Shubha Talwar** has made substantial contributions to acquisition of nasal and plasma antibody data underlying this study. They have reviewed and approved the data underlying this study and supported drafting and revisions of this work. They approve the final version to be published and agree to accountability for all aspects of this work.

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Declaration of interests
Felicity Liew has no conflicts of interest. Shubha Talwar has no conflicts of interest. Andy Cross has no conflicts of interest. Brian J. Willett has no conflicts of interest. Sam Scott has no conflicts of interest. Nicola Logan has no conflicts of interest. Matthew K. Siggins has no conflicts of interest. Dawid Swieboda has no conflicts of interest. Jasmin K. Sidhu has no conflicts of interest. Claudia Efstathiou has no conflicts of interest. Shona C. Moore has no conflicts of interest. Christopher Davis has no conflicts of interest. Clara King has no conflicts of interest. A.A. Roger Thompson is supported by a British Heart Foundation (BHF) Intermediate Clinical Fellowship FS/18/13/33281. He receives speaker fees and support to attend meetings from Janssen Pharmaceuticals. Sarah L. Rowland-Jones receives support from UKRI for the PHOSP-Covid study. She has grants from UKRI, GCRF, Rosetrees Trust, BHIVA, EDCTP, Globvac. She is on the data safety monitoring board for Bexero trial in HIV+ adults in Kenya. Ewen Harrison has no conflicts of interest. Annemarie B. Docherty has no conflicts of interest. Jennifer K. Quim has no conflicts of interest. James D. Chalmers is the deputy chief editor of ERS. He receives consulting fees from AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Insmed, Janssen, Novartis, Pfizer and Zambon. He has grants from AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Gilead Sciences, Grifols, Novartis and Insmed. Ling-Pei Ho has no conflicts of interest. Alexander Horsley is Deputy chair of NIHR Translational Research Collaboration (unpaid role). He is currently supported by UK Research and Innovation. NIHR and NIHR Manchester BRC. Betty Raman receives support from BHF Oxford Centre of Research Excellence, NIHR Oxford BRC and MRC. She receives honoraria from Axcella therapeutics. Krisnah Poinasamy has no conflicts of interest. Susanna J. Dunachie is supported by an NIHR Global Research Professorship (NIHR300791). She is a member of the PITCH Consortium which has
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Figure 1.

(A) Nasal Spike IgA

(B) Nasal Spike IgG

(C) Plasma Spike IgA

(D) Plasma Spike IgG

(E) Nasal NP IgA

(F) Nasal NP IgG

(G) Plasma NP IgA

(H) Plasma NP IgG

NP IgA/total IgA

NP IgG/total IgG

Time from symptom onset

Spike IgA/total IgA

Spike IgG/total IgG

Time from symptom onset

Control

0-14 days

2-4 weeks

3-5 months

6-9 months

10-12 months

>12 months

NP IgA

Plasma NP IgA

NP IgG

Plasma NP IgG

Figure 1.
Figure 2.
Figure 3.
Figure 4.

A

Nasal S IgA
Nasal NP IgA
Nasal S IgG
Plasma S IgA
Plasma S IgG
Nasal NP IgG
Plasma NP IgG
Severity
Age

6 months

B

Nasal S IgA
Nasal NP IgA
Nasal S IgG
Plasma S IgA
Plasma S IgG
Nasal NP IgG
Plasma NP IgG
Severity
Age

12 months

C

Nasal and plasma antibody at 6 months

Spike RBD
Spike RBD
Spike RBD
Nasal IgA Plasma IgA Plasma IgG

Response
Plasma
Nasal

vaccination
Yes
No
Not known
age
<30
30-39
40-49
50-59
60-69
70-79
80+
severity
WHO – class 3-4
WHO – class 5
WHO – class 6
WHO – class 7-9
Figure legends

**Figure 1.** Nasal IgA (A), nasal IgG (B), plasma IgA (C) and plasma IgG (D) responses to S from ancestral SARS-CoV-2, 12 months after symptom onset and compared to pre-pandemic control samples (grey). Nasal IgA (E), nasal IgG (F), plasma IgA (G) and plasma IgG (H) responses to NP of ancestral SARS-CoV-2, 12 months after symptom onset and compared to pre-pandemic control samples. The blue and red lines indicate the trajectory of median titres across each timepoint. The horizontal dashed line indicates the WHO threshold for a seropositive titre. * = p<0·05, ** = p<0·01, *** = p<0·001, **** = p<0·0001.

**Figure 2.** Trajectory of nasal IgA (A), nasal IgG (B), plasma IgA (C) and plasma IgG (F) anti-S and anti-NP responses before and after first vaccination. Trajectories have been modelled using a LOESS regression curve and 95% confidence intervals are shown in grey. The vertical dashed line indicates the time of first vaccination.

**Figure 3.** Nasal IgA (A–C), nasal IgG (D–G) and plasma IgG (G–I) responses to RBD of ancestral SARS-CoV-2, Delta, and Omicron (BA.1) variants 12 months after symptom onset compared to pre-pandemic control samples (grey). Nasal antibody titres have been normalised to total isotype content of sample. The blue and red lines indicate the trajectory of median titres across each timepoint. The horizontal dashed line indicates the WHO threshold for a seropositive titre. * = p<0·05, ** = p<0·01, *** = p<0·001, **** = p<0·0001.

**Figure 4.** Correlogram of nasal and plasma IgA and IgG responses to S and NP, disease severity and age at 6 months, when 27 of 31 individuals with known vaccination status had received their first vaccination (A) and 12 months, when 58 of 63 individuals with known vaccination status had received both vaccinations (B). All statistically significant correlations are denoted with *. The variables were hierarchically clustered. Heatmap (C) of nasal IgA, plasma IgA and plasma IgG responses to S and RBD at 6–9 months. Rows are annotated with vaccination status, age and disease severity according to the WHO clinical progression score: 3–4 = no continuous supplemental oxygen needed; 5 = continuous supplemental oxygen only; 6 = continuous/bi-level positive airway pressure ventilation or high-flow nasal oxygen; 7–9 = invasive mechanical ventilation or other organ support.
446 patients hospitalised with COVID-19 (Feb 2020 to March 2021)

**Plasma anti-S IgG response:** Durable and boosted after vaccination

**Nasal anti-S IgG response:** Mirrors plasma IgG and boosted after vaccination

**Nasal anti-S IgA response:** distinct, wanes after 9 months and not boosted by vaccination

89% receive 1st vaccination (Dec 2020 to March 2022)
Figure S2.

- **Recruitment to ISARIC4C**
- **Recruitment to PHOSP-COVID**

**6 to 9 month visit:**
- Median: 16th March 2021
- (September 2020 to August 2021)

**>12 month visit:**
- Median: 11th July 2021
- (March 2021 to March 2022)

**Participants vaccinated**

**Median date of vaccination:**
- 20th February 2021
- (December 2020 to March 2022)

**February 2020 to March 2021**

**Prior to emergence of Omicron variant (November 2021)**
Figure S3.
Figure S4.

A

Plasma S IgG (ancestral SARS-CoV-2)

B

Plasma Spike IgG

C

Plasma NP IgG (ancestral SARS-CoV-2)

D

Plasma NP IgG
Figure S5.

**Plasma anti-S IgG (ancestral SARS-CoV-2)**

**Plasma anti-NP IgG (ancestral SARS-CoV-2)**

**Plasma anti-RBD IgG (Omicron BA.1)**

**Plasma anti-RBD IgG (Delta)**

The figures show the distribution of antibodies before and after vaccination for different viral strains. The data is presented in a scatter plot format with box plots, indicating significant differences indicated by asterisks (** for p < 0.001 and *** for p < 0.0001).
Figure S6

Binding titre ratio

Kruskal-Wallis, p = 0.00023

Omicron RBD/ Ancestral RBD ratio

Nasal IgA, Nasal IgG, Plasma IgA, Plasma IgG

Immunoglobulin
**Figure S7.**

A. Nasal IgA (vaccinated)

B. Nasal IgG (vaccinated)

C. Plasma IgA (vaccinated)

D. Plasma IgG (vaccinated)
Figure S8.

A. Ancestral SARS-CoV-2

B. Delta

C. Omicron (BA.1)
Figure S9.
Figure 10. (A) Time from vaccination

(B) Vaccination type

Vaccination type
- Pfizer/Bio-N-Tec
- Oxford/AstraZeneca

| Time from vaccination (days) | 0 | 50 | 100 | 150 | 200 |
|-----------------------------|---|----|-----|-----|-----|
| 1 Month Cluster             | 0%| 10%| 20% | 30% | 40% |
| 3 Month Cluster             | 50%| 60%| 70% | 80% | 90% |
| 6 Month Cluster             | 100%| 100%| 100%| 100%| 100%|

Vaccination type distribution:
- Pfizer/Bio-N-Tec: 44% (n = 9)
- Oxford/AstraZeneca: 56% (n = 11)
Supplementary figure legends

Figures S1. Graphical abstract. Plasma and nasal samples collected at serial intervals from 446 adults hospitalised for COVID-19. Plasma and nasal IgG responses are durable and boosted by vaccination. Nasal IgA responses are compartmentalised from plasma IgG responses and are minimally affected by vaccination. This image was created with BioRender.com

Figure S2. Schematic of study design. Clinical data, plasma and/or nasal samples were obtained during hospital admission and/or 1 to 3 visits during convalescence. The 6 to 9 month visit coincided with the start of the UK vaccination campaign. Vaccination dates are shown as median (range) for individuals where vaccination status was known. Dates in which all study participants attended their 6 to 9 month and >12 month visit are shown in median (range).

Figure S3. Nasal IgA (A-B) and Nasal IgG (F-G) geometric mean titre (GMT) to S and NP from ancestral SARS-CoV-2. Nasal IgA (C-E) and IgG (G-I) GMT to RBD of ancestral SARS-CoV-2, Delta and Omicron BA.1 variant are also shown. The horizontal dotted line indicates the threshold titre derived from GMT+2SD of pre-pandemic samples.

Figure S4. Geometric mean titre (GMT) of plasma anti-S IgG (A) relative to a threshold defined by GMT+2SD of pre-pandemic samples (horizontal dotted line). This has been compared to the WHO threshold titre for seropositivity (horizontal dashed line) (B). The same comparison is shown for anti-NP plasma IgG responses (C–D).

Figure S5. Paired plasma IgG responses to S (A), NP (B) and RBD of Omicron BA.1 (C) and Delta (D) variant, taken before and after vaccination.

Figure S6. Ratio of binding titre to RBD of Omicron BA.1 variant and ancestral SARS-CoV-2 across nasal and plasma compartments.

Figure S7. Trajectory of Nasal IgA (A), nasal IgG (B), plasma IgA (C) and plasma IgG (D) responses to RBD of Omicron (BA.1) and Delta variant before and after vaccination. Responses to NP and RBD of ancestral SARS-CoV-2 are also shown for comparison. Trajectories have been modelled using a LOESS regression curve and 95% confidence intervals are shown in grey. The vertical dashed line indicates the time of vaccination.

Figure S8. Plasma neutralising titres between 3 and 12 months after infection. Neutralisation of ancestral SARS-CoV-2 (A), Delta variant (B) and Omicron variant (C) are shown. The red line indicates the trajectory of the median titre across each time bin. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.

Figure S9. Correlation between plasma neutralising titre and plasma IgG (A–B) and IgA (C–D) binding titre to RBD and S. The correlation between plasma neutralising titre and nasal IgG (E–F) and IgA (G–H) binding titre to RBD and S. A regression line has been fit to the data for which the 95% confidence intervals are shown in grey. R=Spearman-rank correlation coefficient. PRNT<sub>50</sub> = serum dilution resulting in >50% reduction in infectivity.

Figure S10. Time from vaccination (A) in cluster 1, 3 and 4 derived from unsupervised, hierarchical clustering analysis of nasal IgA, plasma IgA and plasma IgG anti-S and anti-RBD responses at 6 months from symptom onset. Date of vaccination was not available for any individual in cluster 2. Proportion of individuals vaccinated with either Pfizer/Bio-N-Tec (BNT162b2) or Oxford/ AstraZeneca (ChAdOx1 nCoV-19) vaccine (B) in each cluster.