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RESEARCH ARTICLE

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Ancestral SARS-CoV-2 and Omicron BA.5-specific neutralizing antibody and T-cell responses after Omicron bivalent booster vaccination in previously infected and infection-naive individuals

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Abstract

Coronavirus disease-2019 (COVID-19) bivalent ancestral/Omicron messenger RNA (mRNA) booster vaccinations became available to boost and expand the immunity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron infections. In a prospective cohort study including 59 healthcare workers, we assessed SARS-CoV-2 ancestral and Omicron BA.5-specific neutralizing antibody and T-cell responses in previously infected and infection-naive individuals. Also, we assessed the effect of an ancestral/Omicron BA.1 bivalent mRNA booster vaccination on these immune responses. 10 months after previous monovalent mRNA vaccinations, ancestral SARS-CoV-2 S1-specific T-cell and anti-RBD IgG responses remained detectable in most individuals and a previous SARS-CoV-2 infection was associated with increased T-cell responses. T-cell responses, anti-RBD IgG, and Omicron BA.5 neutralization activity increased after receiving an ancestral/Omicron BA.1 bivalent booster mRNA vaccination. An Omicron BA.5 infection in addition to bivalent vaccination, led to a higher ratio of Omicron BA.5 to ancestral strain neutralization activity compared to no bivalent vaccination and no recent SARS-CoV-2 infection. In conclusion, SARS-CoV-2 T-cell and antibody responses persist for up to 10 months after a monovalent booster mRNA vaccination. An ancestral/Omicron BA.1 bivalent booster mRNA vaccination increases these immune responses and also induces Omicron BA.5 cross-neutralization antibody activity. Finally, our data indicate that hybrid immunity is associated with improved preservation of T-cell immunity.

KEYWORDS

antibodies, COVID-19, Omicron, SARS-CoV-2, T-cells, vaccination

1 | INTRODUCTION

Shortly after the first detection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron (B.1.1.529) variant in November 2021, Omicron became the global dominant variant that sustains the ongoing coronavirus disease 2019 (COVID-19) pandemic.[1,2] Omicron infections are mainly restricted to the upper respiratory tract and are thus generally associated with mild disease.[3,4] However, Omicron infections may still result in severe disease in immunocompromized patients or those with pre-existing comorbidities, which substantially contributes to hospitalization rates and general disease burden.[5,6]

Several observational studies reported a high incidence of Omicron vaccine-breakthrough infections and reinfections.[7,8] These findings can be explained by the more than 30 substitutions in the spike protein, which make Omicron highly transmissible and very efficient at immune evasion.[9,10] Moreover, these characteristics improved even further in each Omicron subvariant, including the previously dominant BA.1, BA.2, BA.4, and BA.5,[1,10] and the most recent dominant subvariants XBB and XBB1.5.[2,11]

The continuing emergence of new Omicron subvariants and concerns about waning immunity has led to the development of bivalent booster vaccines. From September 2022, these bivalent vaccines, containing spike-encoding mRNA of both the ancestral strain and Omicron BA.1, were first administered. However, a limited number of studies explored the effects of these bivalent vaccinations and latest Omicron variant infections on both neutralizing antibody (nAb) and T-cell responses. Therefore, we performed a prospective cohort study aimed to investigate the impact of an ancestral/Omicron BA.1 bivalent booster vaccination, a recent Omicron BA.5 infection, or a combination of these on ancestral and Omicron BA.5 specific T-cell and nAbs responses.

2 | METHODS

2.1 | Study design

The study population consisted of 59 healthcare workers (HCWs) (28.8% male, age 54 (interquartile range [IQR] 41.5–58) years) who had received their primary COVID-19 vaccinations and were part of our ongoing prospective cohort as described previously.[12] Whole blood was collected at two timepoints: September/October 2022 (T1) and December 2022 (T2). In between these timepoints the majority of HCWs received an ancestral/Omicron BA.1 bivalent booster vaccination. Blood samples were collected in heparin tubes via venipuncture and were processed as described previously.[13] This study received approval from the Medical Research Ethical Committee United (protocol number R20.030) and was performed according to the Declaration of Helsinki as revised in 2013.

2.2 | SARS-CoV-2 S1 IFN-γ ELISpot

Interferon-gamma (IFN-γ) T-cell responses after stimulation with spike protein subunit 1 (S1) and nucleocapsid (N) peptides were assessed using the T-SPOT.COVID (Oxford Immunotec) kit as described previously.[13]

2.3 | SARS-CoV-2 Omicron BA.5 IFN-γ ELIspot

An in-house-developed ELISpot was applied to detect IFN-γ T-cell responses against Omicron BA.5 spike peptides. On Day 1, polyvinylidene fluoride membranes precoated with a monoclonal anti-IFN-γ antibody (mAb 1-D1K; Mabtech) were washed thrice with phosphate-buffered saline (PBS) and were conditioned with AIM-V (AIM-V® + AlbuMAX® (BSA); Gibco) for 30 min at room temperature (RT). The following stimulations were separately added, each in a volume of 50 µL per well: AIM-V medium as negative control, anti-CD3 (1:1000, mAb CD3-2; Mabtech) as positive control, Omicron BA.5 mutation peptides, and corresponding ancestral strain peptides (PepTivator® SARS-CoV-2 Prot_S B.1.1.529/BA.5; Miltenyi Biotec). These peptide pools consisted of 15-mer peptides with 11 amino acids overlap and were added to a final 0.66 µg/mL concentration. An amount of 2.5×10^5 PBMCs in 100 µL AIM-V was added to each well, whereafter the microtiter plate was incubated for 16–20 h at 37°C with 5% CO_2 in a humidified atmosphere. On Day 2, the PBMCs were washed off the plate with PBS, and 100 µL alkaline phosphatase-conjugated antibody (1:200, 7-B6-1-ALP; Mabtech) was added and incubated for two hours at RT. Subsequently, the microtiter plate was washed with PBS, and 100 µL substrate (BCIP-NBT-plus; Mabtech) was added and incubated at RT for 7–12 min, whereafter the reaction was stopped with demineralized water.

2.4 | ELISpot image processing and spot quantification

For the spot quantification, we used the method previously described.[14] In short, images of the ELISpot membranes were made, and an intensity threshold of 95 was applied instead of the previously described threshold of 75 to enhance spot detection sensitivity. The number of spots in the negative control was subtracted from the number of spots in peptide-stimulated conditions per individual sample. Samples were excluded if the positive control resulted in less than 20 spots.

2.5 | SARS-CoV-2 anti-RBD IgG quantitative ELISA

The anti-RBD (ancestral strain) IgG serum concentrations were determined using a quantitative enzyme-linked immunosorbent assay (ELISA) (Beijing Wantai Biological Pharmacy Enterprise) as described previously.[13]

2.6 | SARS-CoV-2 ancestral and Omicron BA.5 sVNT

Surrogate virus neutralization tests (sVNT) were performed to assess the neutralizing activity of serum anti-receptor-binding domain (RBD) antibodies. The neutralizing activity against the ancestral strain RBD was determined using the kit (Genscript Biotech) and protocol as previously described.[15] A second sVNT kit (ACROBiosystems) was used to determine the neutralizing activity of serum antibodies against the Omicron BA.5 RBD. The sVNT was performed according to the manufacturer's guidelines using a fully automatic ETI-MAX (Diasorin) system. Serum samples, as well as the positive and negative control, were diluted 1:9 with a dilution buffer. These dilutions were added 1:1 to RBD-horseradish peroxidase (HRP-RBD) in a pre-coated well, whereafter, this was incubated for 1 h at 37°C. After washing the wells with a washing buffer, a substrate solution was added and incubated for 20 min at 37°C. Lastly, a stop solution was added, and the absorbance was measured at 450 nm and 620 nm. The neutralizing activity was calculated as the percentage of inhibition using the following formula: Inhibition (%) = (1 – (OD450–OD620 nm)) × 100.

2.7 | Statistical analyses

All data obtained in this study were expressed as median with IQR, and statistical analyses were performed using GraphPad Prism v9 (GraphPad Software). The Wilcoxon signed-rank test was applied to compare paired datasets, and the Mann–Whitney *U* test was applied to compare two independent data sets. The Kruskal–Wallis test with Dunn's multiple comparison test was performed to compare three or more independent groups. All statistical tests were performed at a two-tailed α-level of 0.05.

3 | RESULTS

3.1 | SARS-CoV-2-specific T-cell and antibody responses in prior-infected and infection-naive individuals 10 months after previous monovalent mRNA vaccinations

First, we investigated whether a prior SARS-CoV-2 infection in addition to primary and booster vaccinations results in prolonged increased SARS-CoV-2-specific T-cell and antibody responses. Therefore, we determined these immune responses in HCWs who tested SARS-CoV-2 PCR positive more than 3 months ago (previous) or within 3 months (recent) and in HCWs who never tested SARS-CoV-2 positive (naive) until the time of blood collection. All HCWs had received mRNA or viral vector COVID-19 primary vaccinations, and HCWs received no ($n = 4$), one ($n = 40$), or two ($n = 7$) booster mRNA vaccinations. The last vaccination was received at median 307 (IQR 301–314.5) days before the first blood collection in this study. Here, we observed a higher spike S1-specific T-cell responses in previously infected HCWs than in infection-naive HCWs ($p = 0.0351$), whereas responses were comparable between the recently infected and infection-naive HCWs (Figure 1A). Nucleocapsid protein (N)-specific T-cell responses were only observed in prior infected HCWs since immunological responses against the N protein are not elicited by mRNA vaccines (Figure 1B).

For the humoral immunity component, anti-SARS-CoV-2-RBD IgG (of ancestral virus) was detectable in all HCWs, and the serum concentrations were comparable between all groups (Figure 1C). Also, we investigated the neutralizing activity of serum antibodies against Omicron BA.5 spike-RBD (Figure 1D). According to the manufacturer's cut-off value of ≥20% inhibition, 83.3% of previously infected, 76.9% of recently infected, and 38.5% of infection-naive

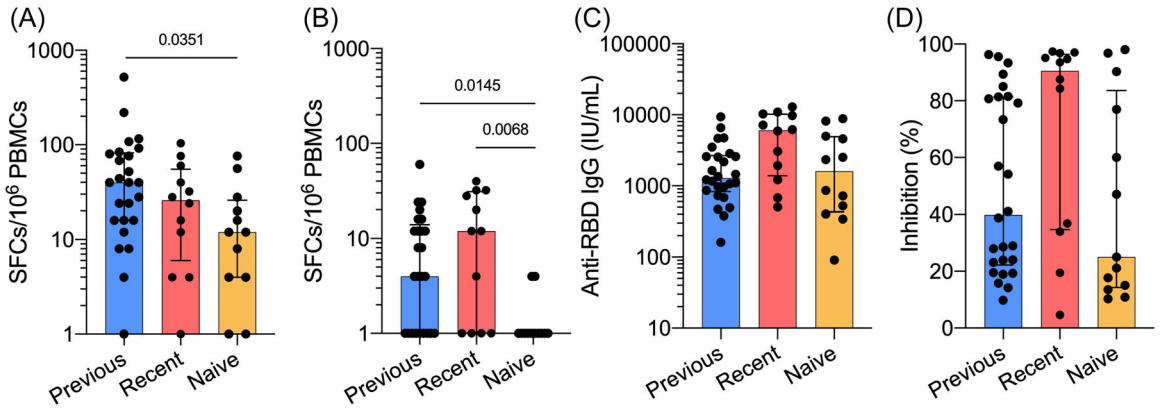

FIGURE 1 SARS-CoV-2-specific immune responses in vaccinated HCWs 10 months after previous vaccinations. Individual data points represent previously infected ($n = 26$; 26.9% male, age 52 (IQR 44–58) years), recently infected ($n = 12$; 41.7% male, age 48 (IQR 38–58) years), or infection-naive ($n = 13$; 23.1% male, age 54 (IQR 42–57) years) HCWs. Differences in age and gender were not statistically significant between these groups. T-cell responses against SARS-CoV-2 (A) spike S1 and (B) nucleocapsid protein. (C) Total serum anti-SARS-CoV-2 RBD (ancestral strain) IgG concentrations. (D) Serum antibody neutralizing activity against Omicron BA.5 spike RBD presented as percentage inhibition. Data are represented as median with IQR and were assessed by a Kruskal–Wallis test with Dunn's post hoc analysis. HCWs, healthcare workers; IQR, interquartile range; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

HCWs were considered positive for the presence of Omicron BA.5 nAbs. We observed considerable intragroup variations, but no significant differences between the groups.

3.2 | The effect of a bivalent ancestral/Omicron BA.1 COVID-19 booster mRNA vaccination on SARS-CoV-2-specific immune responses

After pooling the previously infected, recently infected, and infection-naive HCWs, 18 HCWs received a COVID-19 bivalent booster mRNA vaccination, that is, Comirnaty Original/Omicron BA.1 ($n = 13$) or Spikevax bivalent Original/Omicron BA.1 ($n = 5$), in between T1 and T2. Accordingly, we determined the effect of a bivalent booster vaccination, which contains spike-encoding mRNA of both the SARS-CoV-2 ancestral strain and Omicron BA.1, on T-cell and antibody responses. We observed a considerable increase in S1-specific T-cell responses ($p = 0.0004$), anti-RBD (ancestral) IgG antibodies ($p = 0.0090$), and Omicron BA.5 serum neutralization activity ($p = 0.0023$) after bivalent booster vaccination (Figure 2A–C). Furthermore, we also assessed the immune responses of 8 HCWs who were not vaccinated in between the two timepoints with an interval of 70 (69.5–75.5) days. These HCWs showed comparable immune responses at both timepoints (Figure 2D–F).

3.3 | Ratio of Omicron BA.5 to ancestral SARS-CoV-2 strain-specific immunological responses after Omicron BA.5 infection and ancestral/Omicron BA.1 bivalent vaccination

We determined the effect of a recent Omicron BA.5 infection, an ancestral/Omicron BA.1 bivalent booster vaccination, and both on Omicron BA.5 and ancestral SARS-CoV-2 specific immunity. For this purpose, we stimulated PBMCs with solely Omicron BA.5 mutation peptides and the corresponding ancestral strain spike peptides. Also, we performed sVNT assays using either Omicron BA.5 or ancestral SARS-CoV-2 pseudovirus particles.

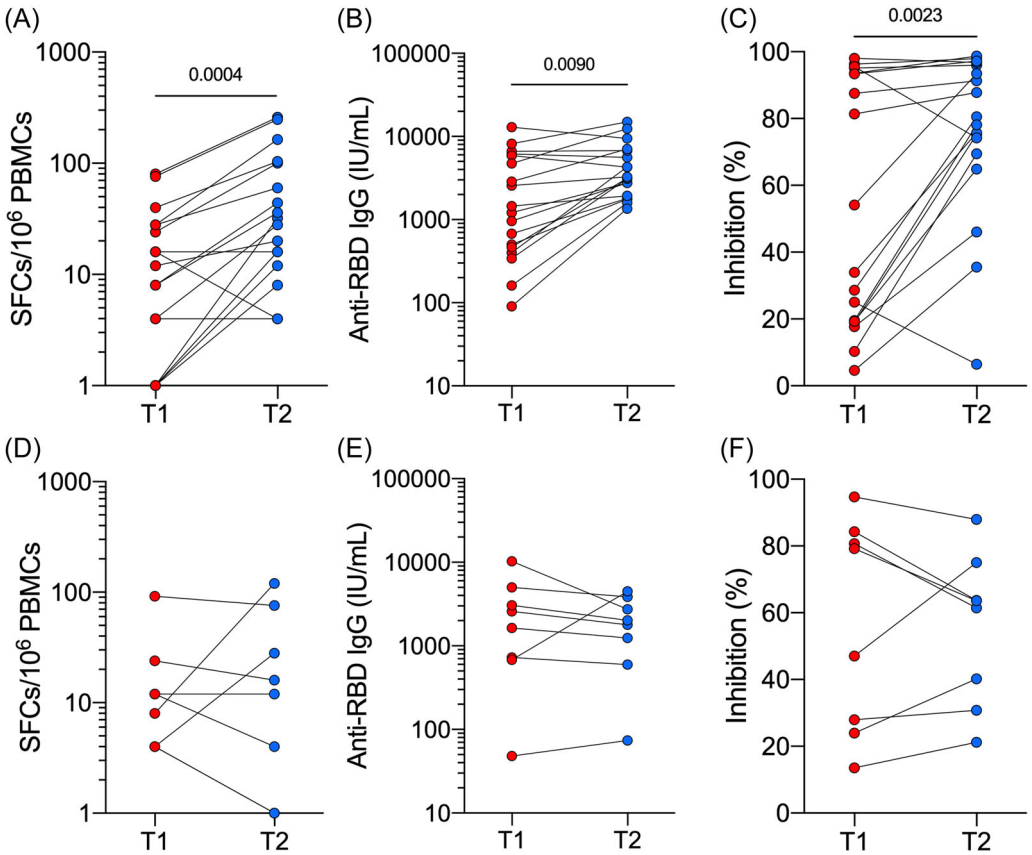

FIGURE 2 SARS-CoV-2-specific immune responses before and after bivalent booster vaccination. (A–C) Blood was collected from 18 HCWs (33.3% male, age 55 (IQR 45.5–58) years) at 11 (IQR 5–31) days before (T1) and 57 (IQR 38–65) days after (T2) bivalent booster vaccination with 70 (IQR 69–71) days in between the two timepoints. (D–F) Immune responses of 8 HCWs (12.5% male, age 41.5 (IQR 33–44.5) years) who received no bivalent booster vaccination were also assessed at similar timepoints with an interval of 70 (IQR 69.5–75.5) days. (A, D) Spike S1-specific T-cell responses. (B, E) Anti-RBD (ancestral) IgG concentrations. (C, F) Omicron BA.5 serum neutralization activity. HCWs were excluded if a previous (booster) vaccination was received within 3 months before T1 or if the HCW tested SARS-CoV-2 RT-qPCR positive in between T1 and T2. Data are represented as median with IQR and were assessed by a Wilcoxon signed-rank test. HCWs, healthcare workers; IQR, interquartile range; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

At T1, the T-cell responses against these specific peptides were overall low, and the Omicron BA.5/ancestral ratios were comparable between the two groups (Figures 3A and S2A). The neutralization activity was not significantly different between the two groups at T1 (Figure 3B). However, 92% of recently infected HCWs versus 57% of nonrecently infected HCWs exhibited higher (i.e., a ratio of >1) neutralization activity against Omicron BA.5 compared with the ancestral strain.

At T2, T-cell responses were also overall low and only the HCWs who were both recently infected with Omicron BA.5 and received a bivalent booster vaccination had 2.2-fold higher T-cell responses against Omicron BA.5 than against ancestral strain (Figures 3C and S2C). Strikingly, this HCW group also demonstrated a 2.4-fold higher neutralizing activity against Omicron BA.5 compared to ancestral strain, which was higher ($p = 0.0214$) than in HCWs who had not received a bivalent vaccination and were not recently infected with Omicron BA.5 (Figure 3D). In contrast, the latter group exhibited a 1.1-fold higher neutralization activity against Omicron BA.5 compared to ancestral strain. Of note, age was not significantly different between the first and latter group.

For comparison, we also assessed the Omicron BA.5/ancestral neutralization ratios in pre-Omicron BA.5 serum samples which were obtained before and after monovalent booster vaccination in 2021.[12] Accordingly, we observed that there was an overall higher neutralizing activity of the ancestral strain than of BA.5 (Figure S2).

4 | DISCUSSION

This study showed that approximately 10 months after receiving the last monovalent (booster) mRNA vaccination, ancestral SARS-CoV-RBD IgG antibodies were present, and a previous SARS-CoV-2

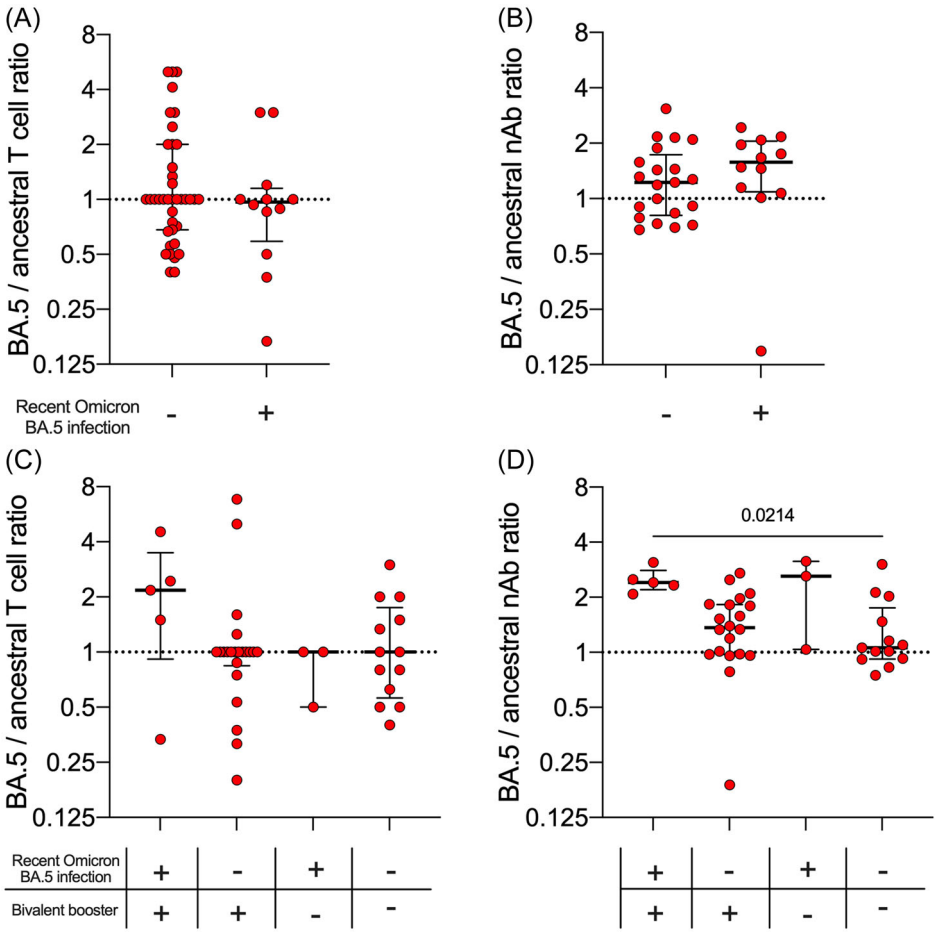

FIGURE 3 SARS-CoV-2 Omicron BA.5/ancestral specific T-cell response and antibody neutralization ratios. Individual data points comprise the ratio of Omicron BA.5 to ancestral (A, C) T-cell responses or (B, D) serum neutralization activity. Recent Omicron BA.5 infection and bivalent booster vaccination are presented as positive (+) or negative (-). (A and B) At T1, HCWs were divided into two groups based on recent (i.e., <3 months) Omicron BA.5 infection ($n = 39$, 25.6% male, age 54 (IQR 43–58) years; $n = 12$, 41.7% male, age 48 (IQR 38–58) years). Differences in age and gender were not statistically significant between these groups. (C and D) At T2, HCWs were divided into four groups based on recent Omicron BA.5 infection and receiving a bivalent booster vaccination (from left to right: $n = 5$, 20% male, age 54 (IQR 33.5–57) years; $n = 22$, 36.4% male, age 56 (IQR 52–61) years; $n = 3$, 33.3% male, age 58 (IQR 48–60) years; $n = 13$, 15.4% male, age 40 (IQR 33–48) years). Age was only significantly different between -/+ and -/-. Absolute values that were used to calculate these ratios are presented in Figure S1A–D. Data are represented as median with IQR and were assessed by an (A and B) Mann–Whitney *U* test or (C and D) Kruskal–Wallis test with Dunn's post hoc analysis. HCWs, healthcare workers; IQR, interquartile range; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

infection in addition to vaccination led to higher T-cell responses against SARS-CoV-2 S1. Subsequently, an ancestral/Omicron BA.1 bivalent booster vaccination significantly increased these T-cell and antibody responses against SARS-CoV-2, including serum antibody neutralization activity against Omicron BA.5. A recent Omicron BA.5 infection together with ancestral/Omicron BA.1 bivalent booster increased the ratio of Omicron BA.5 to ancestral strain neutralization activity compared to no recent infection and no bivalent vaccination.

nAbs are considered the first line of defense within adaptive immunity against viruses as these bind external viral epitopes and thereby prevent infection of host cells. After infection both the nAbs and T-cells contribute to limiting viral replication and preventing disease progression.[16] In our previous study, we similarly assessed anti-RDB IgG antibody and S1-specific T-cell responses up to 7 months post (booster) vaccinations and observed higher anti-RBD IgG concentrations in individuals with a previous or a recent infection in comparison to infection-naive individuals.[12] In the period from 7 to 10 months postvaccination, the infection-induced higher anti-RBD IgG levels seem to normalize towards infection-naive levels. In this current study, a recent Omicron BA.5 infection was not associated with statistically significant higher anti-RBD concentrations, although the recently infected HCWs had 3-fold higher anti-RBD IgG median concentrations than infection-naive HCWs. Nevertheless, all HCWs had detectable anti-RBD IgG antibodies, which is in line with other studies showing waning but yet sustained anti-RBD antibodies levels up to 9 months after mRNA vaccinations.[17–19]

Protection against reinfection is considered to be reduced against the Omicron variant in comparison to previous SARS-CoV-2 variants.[20] The proportion of HCWs who were considered positive for the presence of Omicron BA.5 nABs was increased in both prior-infected groups, and inhibition activity was substantially higher in the recently Omicron BA.5 infected individuals. In addition, a recent Omicron BA.5 infection in combination with a bivalent vaccination led to an increased ratio of Omicron BA.5 to ancestral strain neutralization activity. Moreover, Omicron BA.5 partially escapes nAbs induced by Omicron BA.1 vaccination, indicating that vaccine-induced Omicron BA.1-specific nAbs might not optimally neutralize Omicron BA.5.[21,22] Nevertheless, we observed significantly higher neutralization activity against Omicron BA.5 after Omicron BA.1 bivalent vaccination in comparison to no bivalent booster vaccination.

Infection plus vaccination, termed hybrid immunity, seemed to induce higher T-cell responses against spike S1 than vaccination alone. Although the underlying mechanisms of hybrid immunity is not well understood, it is known that a combination of infection and vaccination induces more polyfunctional spike-specific T-cells than infection or vaccination alone.[23–25] In addition, a SARS-CoV-2 infection also induces T-cell responses against non-spike proteins such as the nucleocapsid protein, allowing for a broader and more protective T-cell response against the virus.[26] Notably, T-cell responses were similar between the recently Omicron BA.5 infected and infection-naive HCWs, which is potentially explained by the mild disease following Omicron BA.5 infection since mild COVID-19 elicits weak T-cell responses.[27,28]

T-cell epitopes in the spike protein remain largely preserved accross SARS-CoV-2 variants, including Omicron BA.5.[29–32] This possibly explains why we observed weak T-cell responses against the Omicron BA.5 peptide pool that solely consists of mutation-containing peptides. However, broader T-cell responses are likely to be cross-reactive against different variants, as T-cells may prevent severe COVID-19 even in the absence of effective nAbs.[30]

Although this study is one of the first to investigate both SARS-CoV-2 ancestral and Omicron BA.5 specific humoral and cellular immune responses after ancestral/Omicron BA.1 bivalent booster vaccination, there are some limitations to consider. First, some subgroups were limited in size, because only a small proportion of HCWs were recently infected during the study period (i.e., Figure 3C–D). Second, we assessed immune responses against Omicron BA.5, while the BA.5 sublineage BQ.1 and the BA.2 sublineage XBB.1.5 became the global dominant variants as of December 2022 and March 2023, respectively.[2,33] However, Omicron bivalent BA.4/BA.5 vaccinations have shown to increase neutralizing antibodies against BQ.1 and XBB.1.5 compared to original monovalent vaccinations.[34] This might be due to the considerable number of epitopes that remain conserved among Omicron subvariants, which makes studying Omicron responses in general still informative.

In conclusion, SARS-CoV-2 specific nAb and T-cell responses persist for up to at least 10 months after monovalent booster mRNA vaccinations, and hybrid immunity is associated with improved preservation of T-cell immunity. An ancestral/Omicron BA.1 bivalent booster mRNA vaccination induces nAb and T-cell responses against the ancestral strain and cross-protective neutralization activity against Omicron BA.5. Future studies must elucidate how nAb and T-cell responses induced by prior-infection and bivalent vaccinations wane over time and how protective these are against new emerging subvariants.

AUTHOR CONTRIBUTIONS

Willem A. Mak: Conceptualization, data curation, validation, visualization, formal analysis, writing—original draft, writing—review & editing. **Wendy Visser**: Investigation, validation, visualization, formal analysis, writing—review & editing. **Marijke van der Vliet**: Project administration, writing—review & editing. **Hilde Y. Markus**: Investigation, writing—review & editing. **Johannes G. M. Koeleman**: Conceptualization, supervision, writing—review & editing. **David S. Y. Ong**: Conceptualization, validation, visualization, supervision, writing—review & editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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