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BRIEF REPORT

Variant-Specific IgA Protects Against Omicron Infection

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Background. The emergence of rapidly evolving severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants, coupled with waning vaccine-induced immunity, has contributed to the rise of vaccine breakthrough infections. It is crucial to understand how vaccine-induced protection is mediated.

Methods. We examined 2 prospective cohorts of mRNA vaccinated and boosted individuals during the Omicron wave of infection in Singapore.

Results. We found that individuals who remain uninfected over the follow-up period had a higher variant-specific IgA, but not IgG, antibody response at 1 month after booster vaccination, compared with individuals who became infected.

Conclusions. We conclude that IgA may have a potential contributory role in protection against Omicron infection.

Clinical Trials Registration. NCT05142319.

Keywords. SARS-CoV-2; COVID-19; S protein; antibodies; IgA; Omicron; mRNA vaccine.

To develop an effective vaccine, it is important to identify the immune correlates of protection. The current coronavirus disease 2019 (COVID-19) vaccines have been instrumental in curbing severe infections [1]. However, vaccine breakthrough infections do occur. Analyses from clinical trials of the current COVID-19 vaccines [2, 3] and vaccine breakthrough cohort studies [4, 5] have shown support for spike-specific antibody level as a correlate of protection following a 2-dose primary vaccination. However, emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants, including the Omicron variants, are rapidly evolving with multiple mutations within and beyond the receptor-binding domain. They are better at escaping vaccine-induced immunity [6], spurring booster vaccination programs. Shortly after the emergence of the Omicron variant in November 2021, Kuhlmann reported vaccine breakthrough infections with the Omicron variant despite a mRNA vaccine booster dose [7]. While robust humoral and cellular responses were detected, these samples were collected 2–4 days following symptoms onset. It is possible that the immune responses detected were boosted following exposure. Here, we investigated the potential contributory role of spike-specific antibody in protection against infection in 2 prospective cohorts of mRNA-vaccinated-and-boosted individuals during the Omicron wave of infection in Singapore.

METHODS

Ethics Statement and Study Population

The prospective cohort study included 2 cohorts (Supplementary Table 1): (1) 55 individuals from the SCOPE study and (2) 93 individuals from the PRIBIVAC Phase A study. A total of 55 individuals, aged 60–76 years, from the SCOPE [8] study was recruited into this study. The SCOPE [8] study includes individuals between 22 and 82 years old, who received 2 doses of Pfizer/BioNTech BNT162b2 mRNA primary vaccination 21 days apart in March-April 2021. At the time of this study, older individuals (>60 years old) were identified as a priority group for booster vaccination in Singapore, and 55 individuals aged >60 years old were randomly selected from the SCOPE study and recruited into this study (September-October 2021). All 55 individuals were

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subsequently boosted with 1 dose of BNT162b2 mRNA vaccine. The infection status of the individuals was followed for 8 months post booster. Information on infection status was acquired through self-reporting of positive Antigen Rapid Test (ART) and/or polymerase chain reaction (PCR) results throughout the study period and infection was assessed by the presence of antibodies against SARS-CoV-2 nucleocapsid (N) protein (via Elecsys anti-N assays performed at all the blood collection time points). None of the 55 individuals received a second booster vaccination the follow-up period. The study design and protocol were assessed by the ethics committee, the National Healthcare Group Domain Specific Review Board and approved under institutional review board study number 2012/00917.

All 93 participants from the PRIBIVAC Phase A study [9], a randomized, subject-blinded study to study the immunogenicity and safety of a monovalent Pfizer/BioNTech BNT162b2 or Moderna mRNA-1273 vaccine (ClinicalTrials.gov identifier: NCT05142319) were recruited into this study, except for individuals with a known history of SARS-CoV-1 or SARS-CoV-2 infection, recipients of SARS-CoV-2 monoclonal antibody therapy, and a history of being immunocompromised (eg, requiring immunosuppressant medication, undergoing chemotherapy, diagnosed with leukemia, etc.). There were no other inclusion/exclusion criteria. All 93 individuals, aged 21–84 years, had received a primary vaccination of 2 doses of mRNA vaccine (either Pfizer/BioNTech BNT162b2 or Moderna mRNA-1273) before study enrolment and were boosted with either BNT162b2 or mRNA-1273 vaccine in this study. The infection status of the individuals was followed for 12 months post booster. Information on infection status was acquired through self-reporting of positive ART and/or PCR results throughout the study period and infection was assessed by the presence of antibodies against SARS-CoV-2 nucleocapsid (N) protein (via Elecsys anti-N assays performed at all the blood collection time points). None of the 93 individuals received a second booster vaccination during the follow-up period.

Sample Collection

Blood samples were collected in Cell Preparation Tubes (CPT; Greiner). For the SCOPE study, blood samples were collected before booster vaccination (day 0), and 1 month, 4 months, and 8 months after booster vaccination, and plasma samples were used for immunological analysis. For the PRIBIVAC Phase A study, blood samples were collected before booster vaccination (day 0), and 1 month, 6 months, and 12 months after booster vaccination and plasma samples were used for immunological analysis.

Spike Protein Flow Cytometry-Based Assay for Spike-Specific Antibody Detection

The spike protein flow cytometry-based assay was performed as previously described [10, 11]. HEK293T cells expressing full-length spike protein of either the Wuhan wild-type (WT), Omicron BA.1 (BA1), or Omicron XBB (XBB) variants [12], were seeded at 1.5×10^5 cells per well in 96-well V-bottom plates (ThermoFisher Scientific). Cells were incubated with human plasma samples (diluted 1:100 in 10% fetal bovine serum; HyClone) for 30 minutes at 4°C, followed by a secondary 30 minutes incubation at 4°C with a double stain, comprising Alexa Fluor 647-conjugated anti-human IgG (1:500 dilution; ThermoFisher Scientific) and propidium iodide (1:2500 dilution; Sigma-Aldrich). Cells were acquired using a BD Biosciences LSR4 laser cytometer (Becton-Dickinson) and analyzed using FlowJo (Tree Star, BD Biosciences). The assay was performed as 2 independent experiments.

Detection of SARS-CoV-2 Anti-N Antibody

SARS-CoV-2 anti-N antibody were qualitatively measured using the Elecsys anti-SARS-CoV-2 chemiluminescent immunoassay (Roche) following the manufacturer's instructions. Elecsys anti-N results were interpreted as follows: cutoff index <1.0, negative; cutoff index ≥ 1.0, positive.

RESULTS

The first prospective cohort (SCOPE) included 55 individuals (median age 69 years; interquartile range [IQR], 63.5–72 years), who were first vaccinated with 2 doses of the Pfizer/BioNTech BNT162b2 mRNA vaccine in March-April 2021 and randomly selected from individuals >60 years old from the parent SCOPE [8]. This is because, at the time of this study (September-October 2021), individuals >60 years old were identified as a priority group for booster vaccination in Singapore (Supplementary Table 1). The 55 individuals were subsequently vaccinated with 1 dose of BNT162b2 mRNA vaccine. No individuals were infected prior to booster vaccination. By 8 months after booster vaccination, 26 individuals were infected while 29 remained uninfected. Infection status was assessed by presence of anti-N antibodies. Both groups were age matched and sex-matched. We analyzed the plasma antibody response at 1 month after booster vaccination and found no significant difference in immunoglobulin G (IgG; median binding 49.94% and 50.08% in individuals with no infection and individuals with infection, respectively) and IgA (median binding 34.19% and 33.06% in individuals with no infection and individuals with infection, respectively) responses against WT spike between the 2 groups of individuals (Figure 1). Analysis of IgA responses (median binding 20.32% and 14.2% in individuals with no infection and individuals with infection, respectively), but not IgG (median binding 31.67% and 31.43% in individuals with no infection and individuals with infection, respectively), against Omicron BA1 spike revealed lower levels of BA1-specific binding (Figure 1) in individuals who eventually became infected. We observed no difference

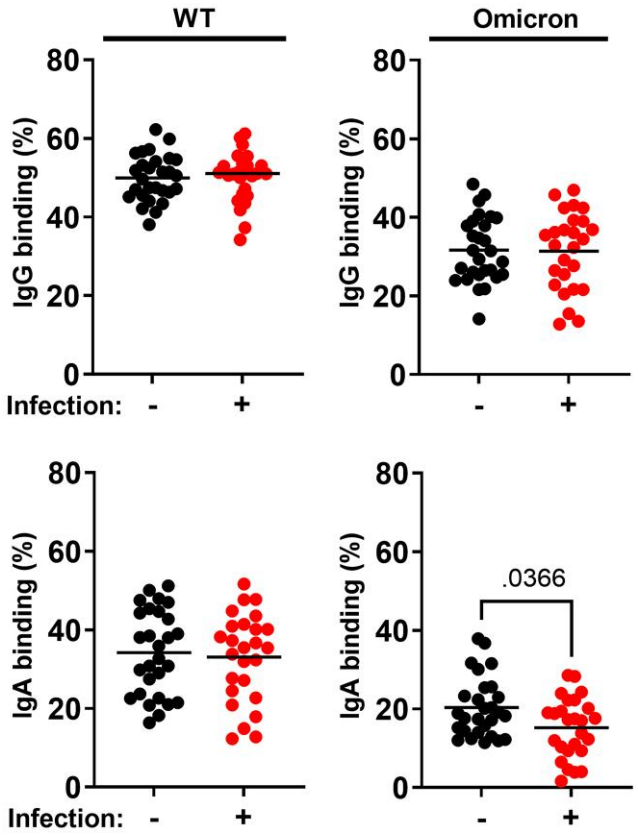

Figure 1. Specific responses against wild-type (WT) and Omicron BA1 spike at 1 month after booster vaccination in the SCOPE cohort (n = 55). No individuals were infected prior to booster vaccination. By 8 months after booster vaccination, 26 individuals were infected (indicated by +) while 29 individuals remained uninfected (indicated by −). Plasma immunoglobulin G (IgG) and IgA against WT and Omicron BA1 spike responses at 1 month after booster vaccination were analyzed by the spike protein flow cytometry-based assay. Bar represents the median value. Comparisons between groups were performed using Mann-Whitney test. Only significant comparison is indicated in the figure, as marked by the bracket and the numerical P-value of the comparison.

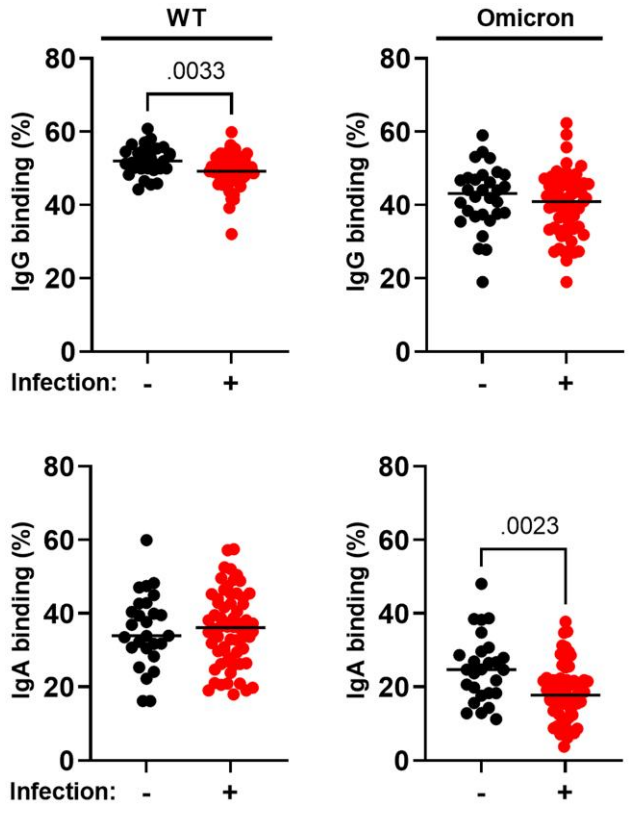

Figure 2. Specific responses against WT and Omicron BA1 spike at 1 month after booster vaccination in the PRIBVAC Phase A cohort (n = 93). No individuals were infected prior to booster vaccination. By 12 months after booster vaccination, 66 individuals were infected (indicated by +) while 27 individuals remained uninfected (indicated by −). Plasma immunoglobulin G (IgG) and IgA against WT and Omicron BA1 spike responses before booster vaccination were analyzed by the spike protein flow cytometry-based assay. Bar represents the median value. Comparisons between groups were performed using Mann-Whitney test. Only significant comparison is indicated in the figure, as marked by the bracket and the numerical P-value of the comparison.

in IgG and IgA response against both WT spike (median IgG binding 25.29% and 26.13% and median IgA binding 7.97% and 9.45% in individuals with no infection and individuals with infection, respectively) and Omicron BA1 spike (median IgG binding 2.05% and 2.92% and median IgA binding 1.43% and 2.52% in individuals with no infection and individuals with infection, respectively) between the 2 groups before booster vaccination (Supplementary Figure 1).

As the booster vaccination program was rolled out to other age groups, we recruited a second prospective cohort (PRIBIVAC Phase A) [9] of 93 individuals (median age 58 years; IQR, 32–66 years) who received a primary vaccination of 2 doses of BNT162b2 mRNA vaccine at least 6 months before study enrolment and were boosted with either BNT162b2 or mRNA-1273 vaccine (October-November 2021). All individuals were not infected prior to booster vaccination. The individuals were then followed for 12 months following booster vaccination. A total of 66 became infected while 27 individuals remained uninfected. Infection status was assessed by presence of anti-N antibodies. Both groups were age matched and gender matched. In this second prospective cohort, we also found significantly higher IgA responses against Omicron BA1 spike in individuals who remained uninfected (median binding 26.05%) compared with individuals who eventually became infected (median binding 18.6%) (Figure 2). IgG responses against WT spike were also significantly higher in individuals who remained uninfected (median binding 53.42% and 49.29% in individuals with no infection and individuals with infection, respectively). We did not observe any difference in IgG and IgA response against both WT spike (median IgG binding 12.19% and 10.19% and median IgA binding 2.95% and 2.81% in individuals with no infection and individuals with infection, respectively) and Omicron BA1 spike (median IgA binding 4.09% and 4.25% and median IgA binding 1.23% and 1.45% in individuals with no infection and individuals

with infection, respectively) between the 2 groups before booster vaccination (Supplementary Figure 2).

DISCUSSION

In this study, we examined the potential contributory role of antibodies in protection against infection. Information on infection status was acquired through self-reporting of positive ART and/or PCR results throughout the study period and infection was assessed by the presence of antibodies against the N protein. Most studies examining antibody responses have focused on IgG responses. Low IgG responses have been found to correlate with disease severity in vaccinated individuals and higher IgG responses may protect against severe infection [13].

While IgG responses are often studied, IgA responses are less studied. Our study shows that higher variant-specific IgA antibody was found in individuals who remained uninfected over a follow-up period of at least 8 months following booster vaccination, compared with individuals who became infected. This highlights the potential contributory role of IgA in protection against infection during the Omicron infection wave. Due to the Singapore vaccination strategies to prioritize older individuals at the time of this study, the median age of the cohorts in this study are high (69 and 58 years for SCOPE and PRIBIVAC Phase A, respectively). More studies are needed to determine if similar findings are observed in a younger cohort.

The importance of IgA responses was highlighted in both prospective studies described here. An earlier study (77 individuals; median age 47 years) [14] found that individuals with breakthrough infections within 3 months of primary vaccination had lower vaccine-induced serum IgA, but no difference in serum IgG, at 2–4 weeks after primary vaccination. Spike-specific IgA was also found to negatively correlate with the length of breakthrough infections following primary vaccination [15]. The feasibility of variant-specific IgA as a correlate of protection against infection warrants further investigation. While the association between higher variant-specific IgA and reduced infection risk may represent a true protective effect, it is also possible that it may be a surrogate marker. Whether the protection against infection is directly due to the higher level of variant-specific IgA remains to be determined. While our study found lower variant-specific IgA responses at 1 month after booster vaccination in individuals who eventually became infected at later time points, we also noted the considerable overlap in the antibody responses between the groups. There are likely other factors involved. However, in both cohorts, the 2 groups (with and without an eventual infection) were age-matched and sex-matched. We hypothesize that, in addition to binding antibody, Fc-effector antibody functions may play a role.

Taken together, IgA has a potential contributory role in protection against infection. Low variant-specific plasma IgA at 28 days after booster vaccination may indicate higher risk of infection at up to 8 months after booster vaccination. This has clinical implications in informing public health decisions on vaccination strategies against COVID-19. Individuals, especially older individuals, with low variant-specific plasma IgA may need another vaccination dose for better protection against emerging SARS-CoV-2 variants. This may also serve as a valuable tool in the development of effective and long-lasting vaccines. Vaccines that can induce a robust variant-specific IgA may offer better protection.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Author contributions. Y. S. G. conceptualized the study, designed and performed the experiments, analyzed data, and wrote the manuscript. S. W. F., P. X. H., C. Y. L., W. B., S. N. M. S., E. Z. X. N., and R. T. C. L. performed the experiments and analyzed data. X. Y. P., S. R., P. Y. C., S. W. X. O., T. H. L., C. L., J. T., S. P., L. J. S., D. L. S. O., J. S., E. S. L., and NCID Study Group supervised and coordinated cohort recruitment and sample collection. COVID-19 Cohort Study Group processed samples. S. M. S., C. I. W., Y. S. L., D. C. L., B. E. Y., L. F. P. N., and L. R. conceptualized the study. All authors reviewed and approved the final version of the manuscript.

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Potential conflicts of interest. A patent application for the spike protein flow cytometry-based assay has been filed (Singapore patent No. 10202009679P: A Method of Detecting Antibodies and Related Products) by Y. S. G., L. R., and L. F. P. N. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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