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Short communication

Immune responses associated with protection from SARS-CoV-2 infection

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ABSTRACT

Conflicting results have emerged regarding immune correlates of protection against SARS-CoV-2. In this secondary analysis of a trial of immediate versus delayed bivalent mRNA booster vaccination, we studied a range of humoral and cellular immune responses 28 days after vaccination and analyzed their association with subsequent symptomatic and asymptomatic SARS-CoV-2 infection. We found neutralizing antibodies and other plasma antibody titers correlated with prevention of SARS-CoV-2 infection. In a multivariate model, only neutralizing antibodies remained associated with protection from infection ($p = 0.021$). There was no significant correlation observed between B and T cell responses to Spike and protection. Our findings add to evidence of antibody correlates of prevention of SARS-CoV-2 infection.

Trial registration: ANZCTR 12622000411741.

1. Introduction

Understanding immune protection from SARS-CoV-2 is important in the generation of improved vaccines and therapies. Neutralizing antibodies (Nab) have consistently been identified as a strong correlate of protection from symptomatic and severe COVID-19 [1,2]. Other responses including spike binding antibodies, mucosal and cellular immunity have also been implicated as correlates of protection, but with some variation across studies [3–5]. Ongoing assessment of correlates of protection for COVID-19 are important as circulating variants and immune histories grow more complex.

We previously conducted a randomized controlled trial of the Moderna bivalent mRNA vaccine in healthy Australian adults with intensive monitoring for antibody responses and symptomatic and asymptomatic SARS-CoV-2 infection ($n = 48$) [6]. Of these 7 were infected prior to blood samples being taken for immunological analysis. Of the remaining 41 participants, we analyzed both antibody, B cell and T cell immunity in 30 participants [7]. The trial occurred during high level community transmission of SARS-CoV-2 and 11 of the 30 participants acquired symptomatic or asymptomatic SARS-CoV-2 infection after vaccination (timeline shown in Fig. 1A). The serial samples, wide immunologic monitoring and high proportions of defined infections allowed us to analyze immune correlates of protection from symptomatic and asymptomatic SARS-CoV-2 infection.

2. Methods

2.1. Clinical trial

We studied in detail 30 participants of a trial of the Moderna bivalent (Ancestral/BA.1) mRNA vaccine that has previously been reported for both cellular and humoral immunity for the relationship between humoral immune responses and infection [6,7] (Fig. 1). We also studied an additional 11 participants where we had humoral immunity data only (total 41 participants) for the relationship between humoral immune responses and infection [6] (Supplementary Fig. 1). The study was approved by ethics committees at the Royal Melbourne Hospital (study no. 2021/272) and the University of Melbourne (approval nos. 13,793

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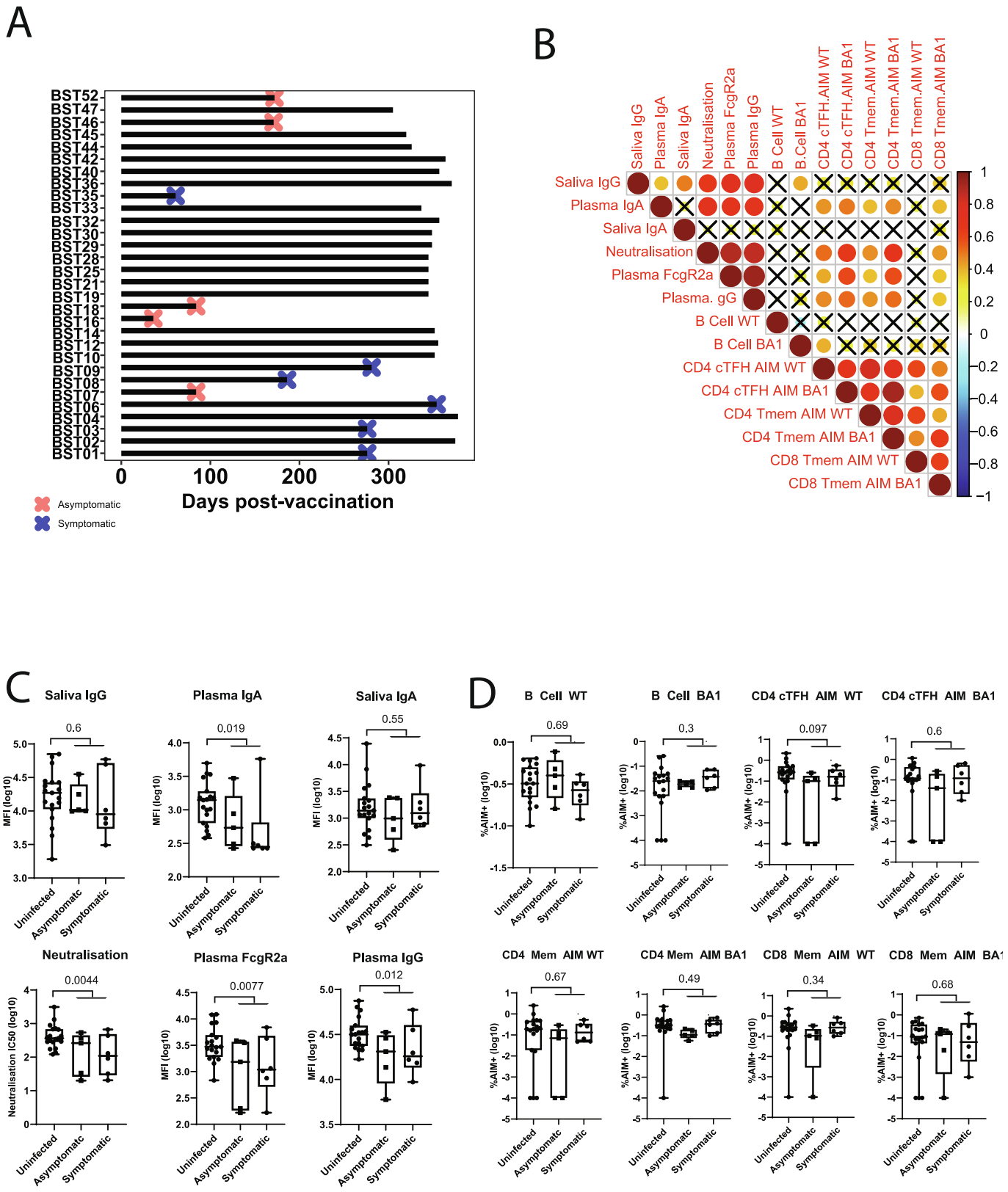

Fig. 1. Analysis of humoral and cellular correlates of symptomatic and asymptomatic SARS-CoV-2 infection in 30 vaccinated adults.
(A) Representation of the 30 subjects and the timing of either asymptomatic (rise in N and S antibodies) or symptomatic (symptoms plus PCR or rapid antigen test positive) SARS-CoV-2 infection after bivalent mRNA vaccination. (B) Correlation of the humoral and cellular immune responses measured 28 days after vaccination. Serological responses were measured against the circulating Omicron XBB.1.5 variant and cellular responses measured against either ancestral (WT) or Omicron BA.1 variants in the vaccine. Correlations (Spearman's, two-tailed) that did not reach significance of $P < 0.05$ were displayed as crosses. Size of the circle represents the statistical significance, while the colour represents the Spearman correlation coefficient value. (C) Levels of humoral and (D) cellular immune responses in subjects without infection, with symptomatic infection and with asymptomatic infection.

and 23,497). Written informed consent was obtained from all participants prior to enrolment in the study. This study was registered with the Australian New Zealand Clinical Trials Registry (anzctr.org.au, no. 12622000411741). The mean age of the 30 participants was 44 years, 15 subjects were female and 15 male. All had previously received 2–3 COVID-19 vaccine doses encoding the ancestral spike. SARS-CoV-2 was diagnosed as previously defined [6] as either symptomatic infection with a positive PCR or rapid antigen test, or asymptomatic infection with who had a rise in both N antibodies by ELISA and XBB.1.5 neutralization titres (>4-fold increase over the previous sampling time point).

2.2. Immune responses

Blood and saliva taken 28 days after vaccination were studied for this report. Details of the immune responses measured have been published previously [6,7]. In brief, serum neutralization titres were measured by a live virus assay to the Omicron XBB.1.5 strain that was circulating at the time and other antibody responses were measured by a bead-based multiplex array to XBB.1.5 spike [6]. Spike specific IgG, IgA and Fc-functional antibodies (those binding dimeric FcγRIIa) were measured by using a bead-based multiplex assay in serum and saliva. B cell responses were measured on stored PBMC by flow cytometry using labelled spike probes to WT and BA.1 variants [7]. T cell responses were measured by an activation induced marker assay measuring the upregulation of activation markers on gated memory CD4 T cells, circulating CD4 Tfh cells or memory CD8 T cells following overnight stimulation with spike peptide pool derived from either the ancestral strain or the BA.1 strain [7].

2.3. Analysis

The serological and T cell responses were analyzed using *t*-tests to compare the uninfected versus infected groups and the asymptomatic versus symptomatic groups. To predict infection status, logistic regression was performed with serological and B and T cell responses as the predictor variables. First, univariate regression models were used to evaluate the individual significance of each serological and T cell response in relation to infection status. Subsequently, a multivariate regression model was employed to assess the collective relationship between the predictor variables (serological and B and T cell responses) and infection status. Throughout the analysis, we reported the regression coefficients, which quantify the magnitude of the relationship between each predictor and infection status, along with the associated *p*-values to determine the statistical significance of these predictors. The best multivariate model was selected using both forward and backward stepwise selection methods, aiming to minimize the Akaike Information Criterion (AIC). Statistical significance was defined as p-values <0.05. All models were fitted using the *glmm* and *stepAIC* libraries in R v4.4.0.

3. Results

We analyzed a range of humoral and cellular immune responses 28 days after mRNA vaccination in 30 adults for an association between these immune responses and SARS-CoV-2 infection (symptomatic or asymptomatic, as defined above in methods) over the subsequent year after vaccination (Fig. 1A). We first correlated each immune response with other responses (Fig. 1B). We found, not surprisingly, that different measures of serum spike-specific antibody responses (Spike-specific-IgG, -IgA, and FcγRIIa binding antibodies) and tended to correlate well with each other. Spike-specific salivary IgA only correlated with salivary IgG (Spearman $r = 0.49$, $p = 0.0056$), whereas serum IgA correlated with other humoral responses. T cell responses correlated with other T cell responses, but less so with humoral or B cell responses, except for CD4 T helper cell responses correlating with neutralizing antibody responses.

When we analyzed the univariate relationship between immune responses and infection (Fig. 1C), we found that serum neutralizing antibody responses to XBB.1.5, a circulating variant at the time of the study, were lower in individuals who later acquired an infection (symptomatic or asymptomatic) compared with individuals who remained uninfected ($p = 0.0044$ – geometric mean of 120.98 vs 408.22 in infected vs uninfected, respectively). Similarly, we found spike-specific plasma IgG responses and plasma antibodies that cross-linked FcγRIIa were associated with protection from acquiring infection ($p = 0.013$ and $p = 0.0077$). As noted above, these 3 humoral responses correlated well with each other (Fig. 1B). We noted that there were lower plasma antibody responses in individuals with symptomatic SARS-CoV-2 infection compared to asymptomatic individuals (Fig. 1C), although this difference was not significant (Supplementary Table 1). Given that we had serological data for the entire cohort of 41 subjects, we repeated analyses of serological immunity with the full set of participants and the results were similar (Supplementary Fig. 1).

In contrast to the humoral immune responses, there were no significant differences (univariate analysis) in spike-specific B and T cell responses between uninfected and infected participants (Fig. 1D). We studied responses to both components of the bivalent vaccine used (ancestral and BA.1 strains) but still found no correlations with protection.

Given the large number of immune responses studied and that some of the participants were vaccinated 3 months later (ie, immediate vs delayed vaccination) and that some of these responses naturally correlated with each other, we also performed a multivariate logistic regression to determine if these associations were influenced by potential confounders and to find the strongest predictors of protection from infection. Using multivariate logistic regression, we identified neutralizing antibodies to be the best predictor of protection, and once these were included in the model, no additional responses improved the model (Table 1). We also repeated this analysis with the serological data from 41 subjects and found the same results (Supplementary Table 2).

4. Discussion

Neutralizing antibodies have emerged as a correlate of protection from both mild-moderate and severe SARS-CoV-2 infection, but less is known about protection from asymptomatic infection. Here we confirm that neutralizing antibodies correlate with protection from any SARS-CoV-2 infection. Other antibody parameters, spike-specific serum IgG

Table 1
Predictors of any SARS-CoV-2 infection in 30 subjects with humoral and cellular immunity.

Univariate logistic regression of predicting any covid infection			Multivariate best model (with forward and backward model selection)	
Variable	Odds Ratio	*p*-value*	Odds ratio	p-value
Age	0.98	0.46	–	–
Study arm	0.62	0.56	–	–
Gender (M)	0.22	0.055	–	–
Saliva IgG	0.58	0.59	–	–
Saliva IgA	0.56	0.53	–	–
Plasma IgG	0.0066	0.0093	–	–
Plasma IgA	0.073	0.014	–	–
Neutralisation	0.07	0.0037	0.07	0.0037
Plasma FcgR2a	0.081	0.0068	–	–
B cell WT	0.49	0.68	–	–
B Cell BA1	1.76	0.27	–	–
CD4 cTFH WT	0.54	0.093	–	–
CD4 Memory WT	0.8	0.67	–	–
CD4 cTFH BA1	0.85	0.58	–	–
CD4 Memory BA1	0.75	0.48	–	–
CD8 Memory WT	0.73	0.33	–	–
CD8 Memory BA1	0.92	0.8	–	–

* Note that the p-values presented in this table differ slightly from the *p*-values obtained from t-tests depicted in Fig. 1C, as these are the result of a likelihood ratio test applied to a logistic regression model rather than a *t*-test.

and spike antibodies capable of engaging the FcγRIIa receptor also correlated with protection from infection. Serum antibodies were non-significantly higher in asymptomatic infections than symptomatic infections (Fig. 1C). Although larger studies are needed to assess this finding, it would be consistent with a higher level of antibodies being protective against symptomatic disease. Like our previous work showing that a gradient exists where lower levels of Nab are required to prevent severe infection than mild-moderate infection [8], we speculate that even higher titers of neutralizing antibodies could be required to prevent asymptomatic infection.

One might ask if there is any value in preventing asymptomatic infection since such infections do not cause disease by definition and these infections may serve to boost immunity. The role of asymptomatic infections in maintaining immunity to other pathogens has been documented [9]. However, asymptomatic infections contribute to the spread of COVID-19 globally (albeit less than symptomatic infections [10]) and chest CT abnormalities and longer-term sequelae following asymptomatic infection have been described [11]. More accurately defining the correlates of protection from asymptomatic infection could further studies on the immune correlates of protection.

Despite some prior evidence that T cells could play a role in prevention of SARS-CoV-2 infection [12–14], we were unable to find a robust association here. It may be that the T cell assays used are less sensitive and/or that much larger numbers of subjects would be required to demonstrate such an association. Our previous work has shown that early activation of T cells after onset of infection may help control virus levels [15]. This work is consistent with T cells may play a more important role in controlling severity of infection rather than preventing infection altogether.

Our study was limited by the small number of subjects and infections documented and larger studies are warranted. None-the-less, our finding that antibody parameters are correlated with protection from SARS-CoV-2 infection even in this small study adds to the weight and consistency of evidence that neutralizing antibodies are a robust correlate of protection. Our findings on antibody parameters associated with protection from asymptomatic infection suggest further studies are warranted in this area.

CRediT authorship contribution statement

Arnold Reynaldi: Writing – review & editing, Formal analysis. **Wen Shi Lee:** Writing – review & editing, Data curation. **Kevin J. Selva:** Writing – review & editing, Investigation, Data curation. **Jennifer Audsley:** Writing – review & editing, Data curation. **Mai-Chi Trieu:** Writing – review & editing, Investigation, Data curation. **Amy W. Chung:** Writing – review & editing, Investigation, Data curation. **Deborah Cromer:** Writing – review & editing, Formal analysis. **Hyon-Xhi Tan:** Writing – review & editing, Data curation. **Adam K. Wheatley:** Writing – review & editing, Data curation. **Jennifer A. Juno:** Writing – review & editing, Supervision, Data curation. **David S. Khoury:** Writing – review & editing, Supervision, Formal analysis. **Miles P. Davenport:** Writing – review & editing, Supervision. **Stephen J. Kent:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stephen Kent reports financial support was provided by The University of Melbourne. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors contribution

All authors attest they meet the ICMJE criteria for authorship. SJK designed the study, KJS, MCT, JAJ, WSL, and HXT performed experiments, AR, DC, DSK and MPD analyzed and interpreted data, AKW, JAJ, AWC, DSK, and MPD provided supervision. SJK wrote the manuscript. All authors critically revised the manuscript and approved the final version.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2026.128579.

Data availability

Data will be made available on request.

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