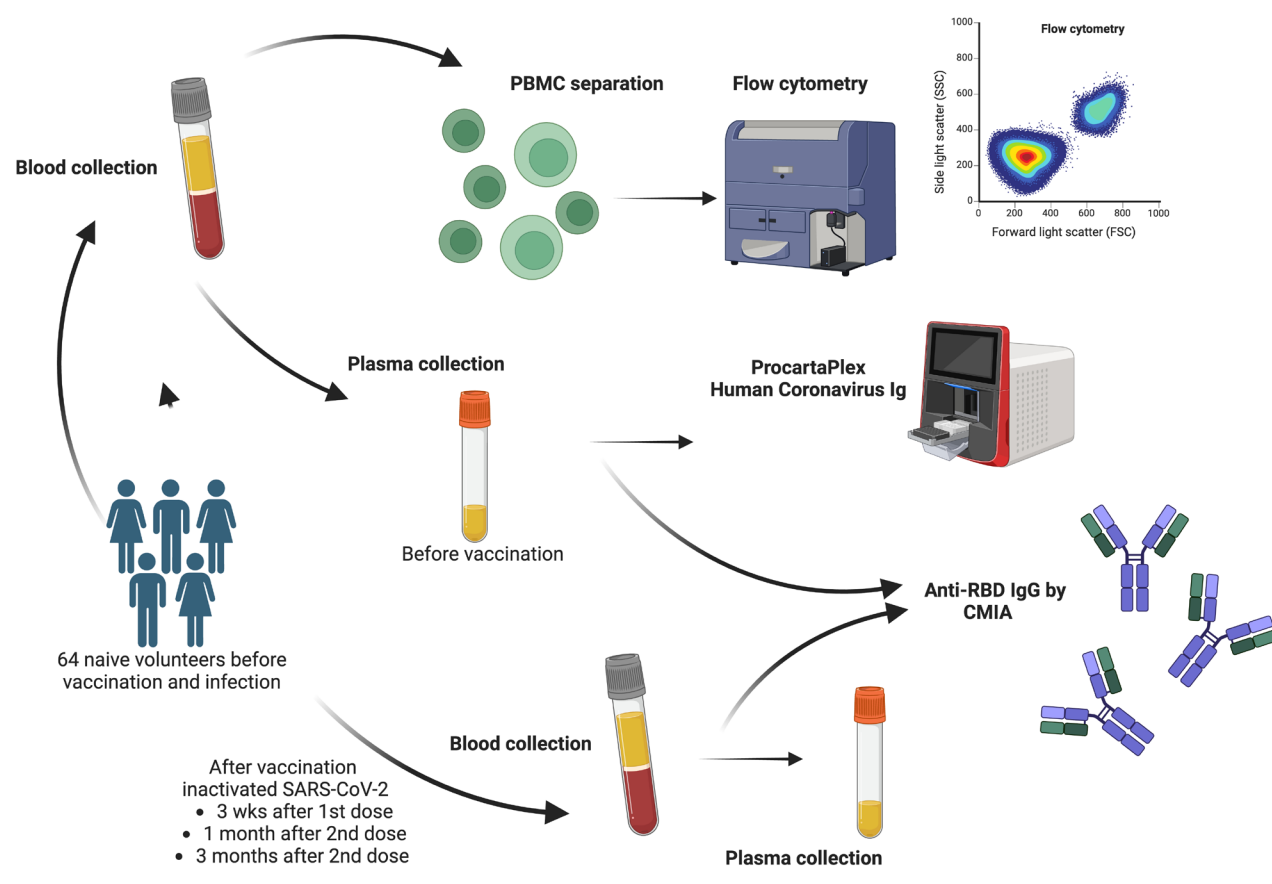


Impact of pre-existing immunity on humoral and cellular responses to CoronaVac in SARS-CoV-2 variants: A focus on common human Coronaviruses

Nawamin Pinpathomrat,¹ Bunya Seeyankem,¹ Ratchanon Sophonmanee,¹ Jomkwan Ongarj,¹ Smonrapat Surasombatpattana²

Abstract

Impact of Pre-existing Immunity on Humoral and Cellular Responses to CoronaVac in SARS-CoV-2 Variants: A Focus on Common Human Coronaviruses



Background: The global COVID-19 pandemic, caused by SARS-CoV-2, has highlighted the importance of understanding immune responses elicited by vaccines.

Objectives: This study evaluated antibody and T cell responses to the inactivated CoronaVac vaccine, as well as the role of pre-existing immunity to common human coronaviruses (HCoVs) in shaping vaccine-induced immunity.

Methods: We enrolled 64 participants (17 males and 47 females) and measured IgG levels against HCoVs before and after vaccination. T cell responses were analysed by stimulating peripheral blood mononuclear cells (PBMCs) with wild-type, Delta, and Omicron spike peptides.

Results: We found pre-existing antibodies against HCoV-229E, HCoV-HKU1, HCoV-NL63, and HCoV-OC43 were present before vaccination. Notably, a positive correlation was observed between pre-existing antibodies to HCoV-229E and HCoV-HKU1 and anti-RBD IgG levels post-vaccination. Pre-existing CD4⁺ T cell responses were observed for the wild-type strain before vaccination, with a significant reduction in IFN- γ secretion after Delta re-stimulation and partial restoration after Omicron re-stimulation. IL-4 production by CD4⁺ T cells was significantly reduced upon re-stimulation with Delta and Omicron compared to wild-type. CD8⁺ T cells again showed a reduction of IL-4 production after Delta re-stimulation compared to the original strain.

Conclusion: This work demonstrate that CoronaVac induces robust humoral and cellular immune responses, though variant-specific responses vary. Pre-existing immunity to certain HCoVs may influence vaccine-induced antibody responses, underscoring the importance of monitoring immunity to emerging SARS-CoV-2 variants and informing future vaccine design.

Key words: HCoV, SARS-CoV-2, Variant, pre-existing immunity, T cell, antibody and vaccination

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Affiliations:

¹ Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

² Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

Corresponding author:

Nawamin Pinpathomrat
E-mail: nawamin.p@psu.ac.th

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread around the world, causing a wide range of symptoms from mild, moderate to severe illnesses. Some patients might have respiratory failure and death.¹ This outbreak affected not only humans but also global economic losses. Since the beginning of the epidemic, scientists have worked hard to understand every mechanism of the virus and developing vaccines to prevent epidemic crises, but many aspects are still not revealed. Moreover, current vaccines are unable to prevent infection from all known and future SARS-CoV-2 strains.^{2,3} As a reason, the development of a dream vaccine would include not only SARS-CoV-2 and its variations of concern, but also future variants of concern and other coronaviruses that might cause human coronavirus illness in the future.⁴

Before the COVID-19 pandemic, human coronaviruses (HCoVs) of six different kinds have been identified comprising both highly pathogenic groups (SARS-CoV-1 and MERS-CoV) that resulted in severe respiratory pneumonia and four human common cold coronaviruses (HCoVs) have spread worldwide: HCoV-HKU1, HCoV-OC43, HCoV-229E, and HCoV-NL63. It frequently causes moderate upper respiratory tract illness and contributes to approximately 30% of the human common cold.^{5,6} As a result, previous infections may provide cross-protection against SARS-CoV-2 infection. However, studies that the specific antibody for HCoV activated after SARS-CoV-2 infection continues to have conflicting results. According to some findings, common CoV immunity that targets the receptor binding domain (RBD) associated with viral infection does not appear to impact the rapid functional evolution of SARS-CoV-2 immunity.⁷ Although some researchers have lately discovered a rise in the particular HCoV-OC43 antibody.⁸ Furthermore, the longitudinal study discovered an increase in both HCoV-HKU1 and HCoV-OC43 antibodies.^{9,10} However, it is unclear whether pre-existing antibodies against HCoVs generated by natural infection are associated with SARS-CoV-2 protection.

Here, the purpose of this research was to identify a correlation between pre-existing immunity of antibody and T cell responses against SARS-CoV-2 as well as other human pathogenic coronaviruses. Antibody responses after receiving completed (CoronaVac) vaccines in healthy individuals were also included. We found pre-exposure to HCoV-229E and HCoV-HKU1 positively correlated with enhanced SARS-CoV-2 S1S2 IgG after completed CoronaVac vaccination. Pre-existing T cell responses were relatively conserved across the strains. However, IL-4 producing T cells were affected after the mutation of spike antigens. This result provides better understanding of serological responses of coronavirus species across one another which would benefit in vaccine design.

Material and Method

Study population

The individuals for this study were provided written informed consent and approval from the Human Research Ethics Committee were acquired (REC.64-194-4-1). In this experiment was carried out in according to good clinical research practices. The blood of all participants in this study were collected during April 2021 to November 2022. In total 64 individuals ranging in age from 21 to 67 years who received a homologous CoronaVac (Sinovac) vaccine. The vaccine interval was 3-4 weeks between the 1st and the 2nd dose. The study enrollment was completed before the first COVID-19 case reported in our hospital.

Collection of blood samples

Blood samples were collected just before vaccination and then one month after the 1st dose of vaccination. After completed vaccination, participants were also followed up and sampled blood at one and three months. Five mL of the blood samples were taken and transferred in a clotted blood tube. The serum is then collected through the process. The tubes of clotted blood were centrifuged at 363 ×g for 10 min. Following centrifugation, the serum was immediately harvested, and stored serum at -80°C until used.

Separation of Peripheral Blood Mononuclear Cells (PBMCs)

To collect human PBMCs, 10 mL of blood was divided split into two heparinized tubes. The blood samples were diluted with RPMI and placed into a 50 mL tube containing Lymphoprep (STEMCELL Technologies, BC, Canada). PBMCs were separated and collected as described previously.¹¹ The samples were aliquoted and transferred into liquid nitrogen to await the subsequent investigation.

Flow Cytometry Analysis

Peripheral blood mononuclear cells (PBMCs) were thawed and adjusted to 2–3 × 10⁶ PBMCs/mL in R10 medium. The cells were treated with 10 U/mL of benzonase for 2 hours at 37°C with 5% CO₂ and resuspended in R10 medium. 1 × 10⁶ PBMCs per well were seeded into a 96-well plate. The cells were washed with R10 and centrifuged at 470 × g for 5 minutes at 22°C. Cells were stimulated using wild-type S1-peptide pools (ProImmune, Oxford, UK), consisting of 15-mers overlapping by 10 amino acids. Delta and Omicron spike peptide pools (PepTivator®, Miltenyi Biotec) were also used for re-stimulation. Peptide pools were diluted to a final concentration of 2 µg/mL in R10, supplemented with anti-human CD28 and CD49d antibodies. The cells were cultured at 37°C in 5% CO₂ for 18 hours. GolgiPlug (BD Biosciences, NJ, USA) was added after 2 hours of stimulation to block cytokine secretion. After incubation, plates were centrifuged, and cells were washed with phosphate-buffered saline (PBS). Cells were stained for viability using LIVE/DEAD Fixable Aqua Dead Cell Stain for 10 minutes. Surface staining was performed by incubating cells for 30 minutes

with fluorochrome-conjugated antibodies diluted in FACS buffer (1% bovine serum albumin in PBS). Following surface staining, cells were fixed and permeabilized using CytoFix/CytoPerm solution (BD Biosciences, NJ, USA) as per the manufacturer's instructions. Intracellular staining was performed using fluorochrome-conjugated antibodies. The cells were resuspended in FACS buffer and analysed using a CytoFLEX S flow cytometer (Beckman Coulter, CA, USA). Data were processed using FlowJo software (FlowJo, OR, USA) as per the gating strategy.

Anti-S RBD antibodies against SARS-CoV-2 quantification

In human serum, the level of anti-S RBD IgG of the S1 subunit spike protein of SARS-CoV-2 was evaluated and quantified. The measurement was performed utilizing the ARCHITECT I System (Abbott, Abbott Park, Illinois, USA) chemiluminescent microparticle immunoassay (CMIA) (SARS-CoV-2 IgG II Quant, Abbott Ireland, Sligo, Ireland) with a range of measurements reported from 6.8 Abbott Arbitrary Unit (AU/mL) to 80,000.0 AU/mL (up to 40,000 AU/mL with onboard 1:2 dilution). Qualities were determined as those greater than 50 AU/mL. According to the World Health Organization (WHO) International Standard (NIBSC Code 20–136), the Binding Antibody Unit (BAU/mL) measurement utilized consistent with the most recent notice to be used with the SARS-CoV-2 IgG II Quant test.

Multiplex Bead Based Immunoassay

ProcartaPlex Human Coronavirus Ig Total 11-Plex Panel (Invitrogen™, Thermo Fisher Scientific, Massachusetts, USA) is an assay that is based on the Luminex xMAP™ fluorescent bead-based technology (Luminex Corporation., 12,212 Technology Blvd, Austin, TX, 78727, USA). It is designed to identify antibodies directed against four separate SARS-CoV-2 targets induce the spike trimer (S_{trimer}), S1 subunit, receptor binding domain (RBD), and N protein, as well as antibodies directed against S1 proteins of SARS-CoV-1, MERS-CoV, and all four human common cold coronaviruses including HCoV-NL63, HCoV-HKU1, HCoV-229E, and HCoV-OC43. Each set of beads was color-coded and coated with a unique target. Signal-coupled secondary anti-human IgG antibodies were used to identify specific antibodies bound to these targets. To differentiate the beads, distinct light-emitting diodes (LEDs) were utilized. High, medium, and low positive controls were used as standards for quantitative detection in each run. All controls and samples were tested in duplication and the findings were reported as mean fluorescence intensity (MFI) values. All controls and samples were tested in duplicates and the findings were presented as mean fluorescence intensity (MFI) values. Signals were measured using MAGPIX® System (Luminex Corporation, 12,212 Technology Blvd, Austin, TX 78727, USA).

Data Analysis

GraphPad Prism 9 software (GraphPad Software Inc., San Diego, CA, USA) was used to perform statistical analyses. The data of demographic characteristics were used Mann-Whitney test. Nonparametric Kruskal-Wallis with the Dunn's multiple comparisons test was performed to determine the level of IgG antibody before and after vaccination. Pearson's formulation with multiple correlation testing correction was used to calculate the correlation coefficients between pre-existing HCoV antibodies and the post-vaccination antibody response and values of $p \leq 0.05$ were considered statistically significant. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$, ns non significance.

Results

Demographics characteristic

64 CoronaVac vaccinated participants were male ($n = 17$) and female ($n = 47$). Table 1 provides demographic characteristics of study participants with age of male with mean and SD (38.00, 13.15) did not differ significantly from those of female (39.40, 11.17). The mean participant weight and height were difference between the male [(74.44, 14.11) and (172.4, 10.07)] and female group [(57.45, 11.04) and (156.3, 5.256)], respectively.

Table 1. Demographic characteristics of study population.

Characteristics	Post vaccination (CoronaVac) N = 64		p-value
	Male N = 17	Female N = 47	
Age, year; mean (SD)	38.00 (13.15)	39.40 (11.17)	0.5137
Weight	74.44 (14.11)	57.45 (11.04)	< 0.0001
High	172.4 (10.07)	156.3 (5.256)	< 0.0001

Antibody responses before and after completed vaccination of CoronaVac

To explore antibody responses, we measured the level of anti-RBD IgG antibody in participants before and after booster doses of CoronaVac (Sinovac). Anti-RBD IgG levels significantly increased one month after the first dose and one month and three months after the second doses compared with before vaccination (Figure 1a). Pre-existing IgG levels against Corona virus species were measured and presented as final concentrations (U/mL) (Figure 1b). immunoglobulin levels against HCoV-229E, HCoV-HKU-1, HCoV-NL63 and HCoV-OC43 were detectable since before vaccination. However, SARS-CoV-1 and SARS-CoV-2 antibodies were undetectable before vaccination.

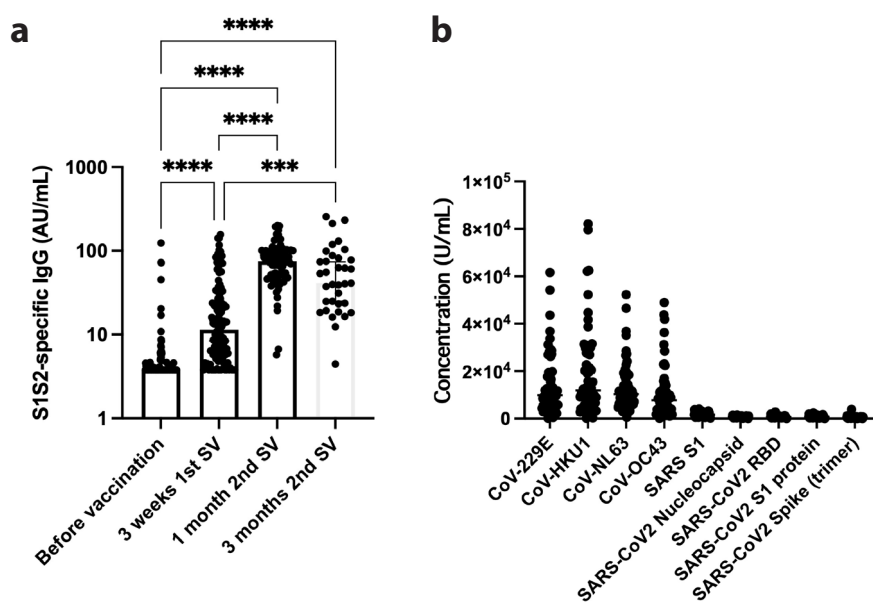


Figure 1. The concentrations of IgG and Multiplex antibodies against coronaviruses. (a) The Abbott anti S-RBD IgG levels of SARS-CoV-2 before and after vaccination as active unit per mL (AU/mL). (b) IgG antibodies against previously identified coronaviruses before vaccination as unit per mL (U/mL). Each symbol represents one participant presenting as median with 95%CI ($n = 64$ volunteers). Statistical significance was determined using Kruskal-Wallis test, with Dunn's multiple comparisons test between different timepoints. $***p \leq 0.001$ and $****p \leq 0.0001$.

Correlation analysis of Multiplex antibody and the level of anti-IgG antibody detection

The final concentrations (U/ml) and Median Fluorescence Intensity (MFI) values of pre-existing antibodies against previously identified coronaviruses (Anti-CoV) were generated from the Multiplex serological assay for anti HCoV level (Figure 2a and 2b). There was a trend of higher anti-RBD IgG level post vaccination when pre-existing

antibodies against previously identified coronaviruses were presented. The Pearson's correlation values for each pair of IgG levels after vaccination and multiplex serology output were showed in Figure 2c-h. We obtained the weakly positive correlation in IgG level post vaccination versus HCoV-229E Ig total with Pearson's correlation coefficient ($r = 0.3478$, $p = 0.0049$) and HCoV-HKU-1 Ig total ($r = 0.2741$, $p = 0.0284$). On the other hand, our results

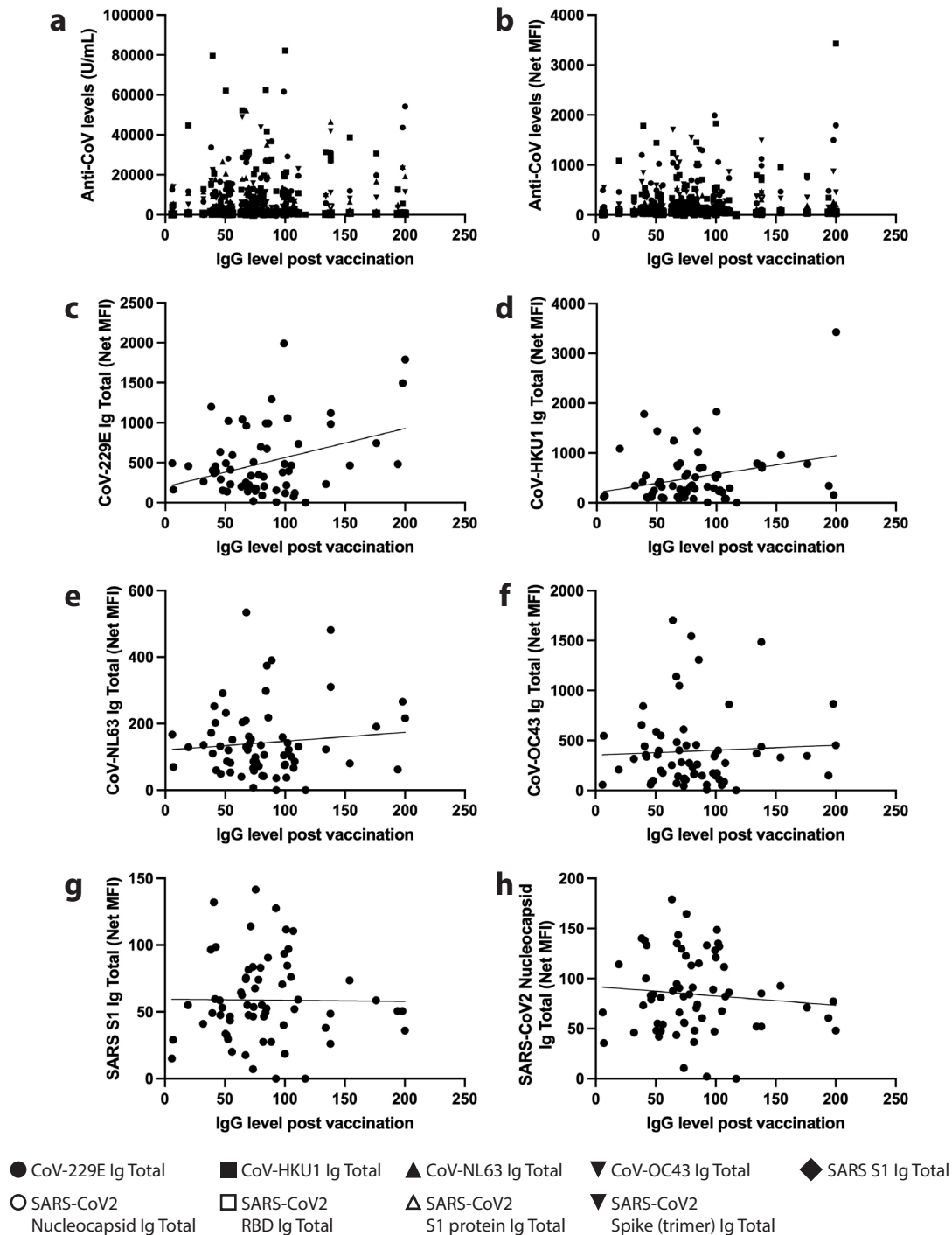


Figure 2. Correlation between pre-existing IgG levels and IgG post CoronaVac vaccination. Pre-existing antibodies against previously identified coronaviruses (Anti-CoV) and RBD-specific IgG after completed CoronaVac vaccination presented as (a) final concentrations U/mL and (b) Median Fluorescence Intensity (MFI). Spearman's correlation coefficient of IgG level at 1 month post CoronaVac primary series and pre-existing IgG against Coronavirus species which are human coronaviruses (HCoVs) (c) 229E, (d) HKU-1, (e) NL-63, (f) OC-43, (g) SARS-CoV S1, and (h) SARS-CoV-2 nucleocapsid.

Table 2. Correlation analysis between HCoV-229E and IgG level post vaccination.

	IgG level post vaccination vs. CoV-229E Ig Total	IgG level post vaccination vs. CoV-HKU1 Ig Total	IgG level post vaccination vs. CoV-NL63 Ig Total	IgG level post vaccination vs. CoV-OC43 Ig Total	IgG level post vaccination vs. SARS S1 Ig Total	IgG level post vaccination vs. SARS-CoV2 Nucleocapsid Ig Total
Pearson r						
r	0.3478	0.2741	0.1021	0.05388	-0.01055	-0.09916
95% confidence interval	0.1116 to 0.5469	0.03029 to 0.4871	-0.1474 to 0.3394	-0.1945 to 0.2958	-0.2557 to 0.2359	-0.3368 to 0.1503
R squared	0.1210	0.07511	0.01042	0.002903	0.0001113	0.009833
P value						
P (two-tailed)	0.0049	0.0284	0.4222	0.6724	0.9340	0.4356
P value summary	**	*	ns	ns	ns	ns
Significant? (alpha = 0.05)	Yes	Yes	No	No	No	No
Number of XY pairs	64	64	64	64	64	64

Spearman's correlation coefficient (r), * $p \leq 0.0332$, ** $p \leq 0.0021$, and ns = non significance.

showed that there was no correlation in HCoV-NL63, HCoV-OC43, SARS-CoV-S1 and SARS-CoV-2 nucleocapsid as shown in **Table 2** and **Figure 2e-h**. Therefore, our results provided the better understanding of pre-existing immunity of HCoV-229E and HCoV-HKU-1 compared with anti-RBD-IgG levels after CoronaVac vaccination.

Pre-existing CD4⁺ T cell responses against different strains of SARS-CoV-2

As far as T cell responses are concerned, PBMCs were collected and analysed to observed antigen specific T cell immunity against Wildtype (WT), Delta and Omicron strains of SARS-CoV-2. Cytokine producing CD4⁺ T cells against WT strain were observed since before vaccination.

IFN- γ secretion was significantly decreased when re-stimulated with the Delta spike protein (**Figure 3a**, $p = 0.0056$). The IFN- γ response was restored after Omicron spike re-stimulation when compared to the WT response. Wild type S1-specific IL4 secreting CD4⁺ T cells were detected before vaccination. Interestingly, these IL-4 responses were significantly declined and almost undetectable after re-stimulated with Delta and Omicron strains compared to the original strain (**Figure 3d**, $p < 0.0001$ and $p = 0.0040$ respectively). TNF- α and IL-2 production of CD4⁺ T cells were comparable across different strains of SARS-CoV-2 (**Figure 3b and c**). However, IL-2⁺ producing CD4⁺ T cells were reduced after Omicron re-stimulation compared to Delta strain, but the difference was not statistically significant (**Figure 3c**, $p = 0.0553$).

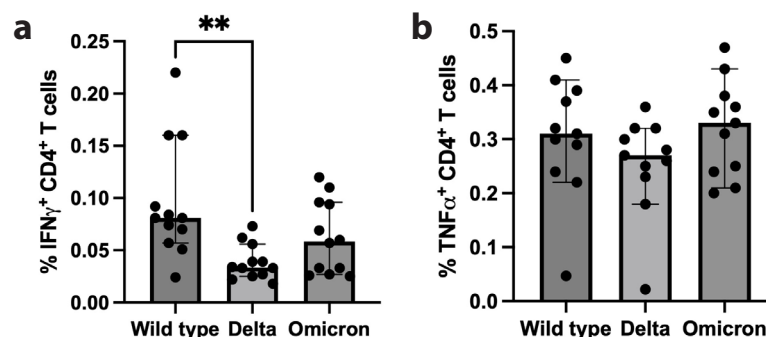


Figure 3. Cytokine responses of CD4⁺ T cells against different SARS-CoV-2 strains before vaccination. (a) Percentages of IFN- γ ⁺ CD4⁺ T cells and **(b)** TNF- α ⁺ CD4⁺ T cells. **(c)** Percentages of IL2⁺ CD4⁺ T cells and **(d)** IL4⁺ CD4⁺ T cells. Each spot represents median with a 95%CI. Statistical significance was assessed based on the Kruskal-Wallis test, followed by Dunn's multiple comparisons test.

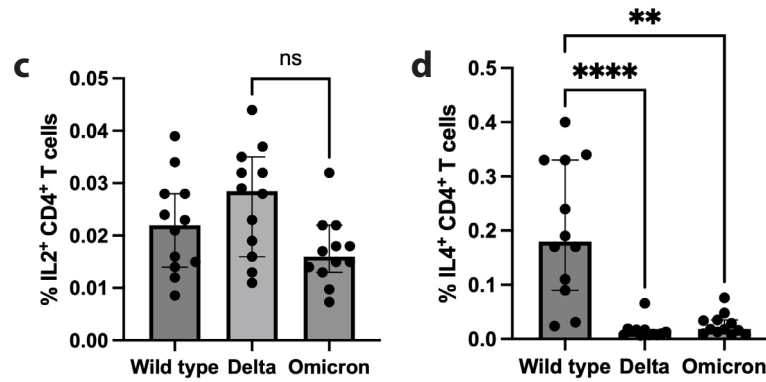


Figure 3. (Continued)

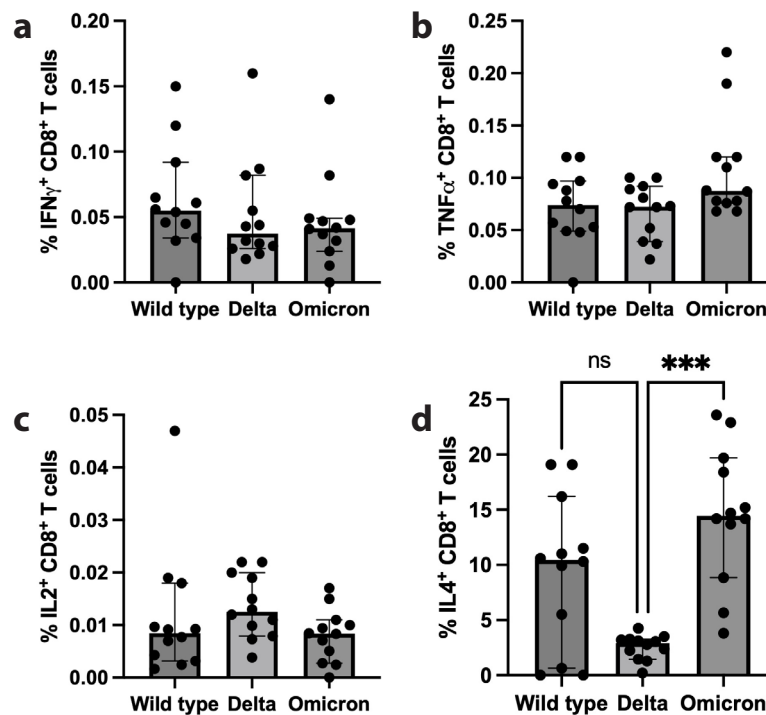


Figure 4. Cytokine responses of CD8⁺ T cells against different SARS-CoV-2 strains before vaccination. (a) Percentages of IFN-γ⁺ CD8⁺ T cells and (b) TNF-α⁺ CD8⁺ T cells. (c) Percentages of IL-2⁺ CD8⁺ T cells and (d) IL-4⁺ CD8⁺ T cells. Each spot represents median with a 95%CI. Statistical significance was assessed based on the Kruskal–Wallis test, followed by Dunn's multiple comparisons test.

Pre-existing CD8⁺ T cell responses against different strains of SARS-CoV-2

Cytotoxic lymphocytes are responsible for killing virally infected cells. It is shown to also be the case of SARS-CoV-2. Cytokine producing CD8⁺ T cells were obtained before vaccination against all three strains tested in this study. IFN-γ, TNF-α and IL-2 production were comparable among different strains (Figure 4a-c). Interestingly, more than one in ten of CD8⁺ T cells was IL-4 producing cells. The IL-4 responses were comparable when re-stimulated with WT and Omicron peptides. However, these responses were significantly decreased after Delta strain re-stimulation compared to the Omicron strain (Figure 4d, $p = 0.002$) but the difference was not significant when compared to the WT strain ($p = 0.0776$).

Discussion

This study aimed to explore pre-existing antibody and T cell responses against various human coronaviruses and evaluate immunogenicity before and after receiving the CoronaVac. Our results provide valuable insights into the humoral and cellular immune responses elicited by the vaccine and how pre-existing immunity may influence vaccine efficacy.

The antibody response to CoronaVac, measured by anti-RBD IgG levels, significantly increased following vaccination. As expected, IgG levels were undetectable before vaccination for SARS-CoV-1 and SARS-CoV-2, as our samples were collected before the pandemic. After the first and second doses of the CoronaVac vaccine,

a significant rise in anti-RBD IgG levels was observed, peaking at one month after the second dose. This result is consistent with many previous published works on CoronaVac immunogenicity.¹²⁻¹⁵ This rise demonstrates the ability of the vaccine to effectively elicit humoral immunity against SARS-CoV-2. Pre-existing antibodies against other HCoVs (HCoV-229E, HCoV-HKU1, HCoV-NL63, and HCoV-OC43) were present in participants before vaccination, which suggests prior exposure to these endemic coronaviruses. This could be because the participants in this study were mostly health care workers who were likely to be exposed to these HCoVs. A few studies have reported prevalences of HCoVs detected using RT-PCR^{16,17} but there was no report on seroprevalence against these common coronaviruses before COVID-19 pandemic.

Correlation analyses revealed a weak positive relationship between pre-existing antibodies to HCoV-229E and HCoV-HKU1 and the anti-RBD IgG response post-vaccination. Specifically, there was a modest correlation for HCoV-229E ($r = 0.3478$, $p = 0.0049$) and HCoV-HKU1 ($r = 0.2741$, $p = 0.0284$), indicating that participants with higher pre-existing immunity to these common cold coronaviruses may experience a more robust antibody response to SARS-CoV-2 vaccination. However, no significant correlation was found between pre-existing immunity to HCoV-NL63, HCoV-OC43, SARS-CoV-1 and SARS-CoV-2, and the post-vaccination anti-RBD IgG levels. This suggests that pre-existing immunity to certain HCoVs may not universally predict enhanced vaccine-induced immune responses, potentially due to differences in antigenic relatedness among these viruses.¹⁸ Our finding consistent with Asamoah-Boaheng et al reporting that higher HCoV-229E and also HCoV-NL63 anti-spike IgG antibodies were associated with increased SARS-CoV-2 IgG antibodies.¹⁹ However, this study was carried out during the COVID-19 pandemic which could affect the seroprevalence of HCoVs. In children population, Li et al observed positive correlations between the cross-reactive antibodies against SARS-CoV-2 with the S-specific IgG antibodies against each of the HCoVs, especially with HCoV-OC43.²⁰ This could explain from different of HCoV exposure in different population and age groups.

Our study demonstrated that pre-existing CD4⁺ T cell responses were present against the wild-type (WT) strain of SARS-CoV-2 before vaccination. Following re-stimulation, we observed a significant reduction in IFN- γ production when cells were re-exposed to the Delta variant compared to WT ($p = 0.0056$). Interestingly, the IFN- γ response was restored when re-stimulated with the Omicron strain, highlighting the variant-specific nature of T cell responses. This restoration of IFN- γ production after Omicron exposure may reflect a broader immune recognition of this variant's spike protein.^{15,21} IL-4-producing CD4⁺ T cells, specific to the WT strain, were detected before vaccination, but these responses were significantly diminished upon re-stimulation with Delta and Omicron ($p < 0.0001$ and $p = 0.0040$, respectively). These findings suggest that

the CoronaVac-induced CD4⁺ T cell response may be less effective against variants, particularly in eliciting Th2 responses.

CD8⁺ T cells play a critical role in cytotoxic responses against SARS-CoV-2-infected cells.²² In our study, CD8⁺ T cells produced comparable levels of IFN- γ , TNF- α , and IL-2 across the WT, Delta, and Omicron strains, indicating that cytotoxic T cell responses were relatively conserved across different variants. The cross recognition of T cells against different SARS-CoV-2 variants has been widely reported.^{23,24} Notably, a subset of CD8⁺ T cells produced IL-4, with responses to WT and Omicron remaining similar. However, IL-4 production was significantly lower after Delta stimulation compared to Omicron ($p = 0.002$). This suggests that Delta-specific CD8⁺ T cell responses may differ in their cytokine profiles compared to Omicron, which may impact the effectiveness of cellular immunity against different variants.

The findings from this study have several important implications. The weak correlation between pre-existing immunity to common cold coronaviruses and post-vaccination antibody responses suggests that prior exposure to these viruses might provide a limited cross-reactive boost to SARS-CoV-2 vaccination, though this effect appears to be strain-specific. Our T cell analyses suggest that while CoronaVac induces a robust T cell response against the original WT strain, these responses may be less effective against the Delta variant, particularly in terms of Th1 and Th2 cytokine production. The partial restoration of IFN- γ responses against Omicron is encouraging, but the diminished IL-4 responses highlight potential challenges in achieving durable and broad T cell immunity against emerging variants.

This study has a few limitations. Firstly, the demographic characteristics of the study cohort, comprising 17 male and 47 female participants, did not show significant differences in age distribution between the sexes. However, male participants had higher mean weight and height compared to females. These baseline characteristics are essential in ensuring that the immune responses observed are not influenced by demographic differences. Secondly, the participants recruited in this study were mainly healthcare workers. The pre-existing immunity of this population may hard to apply to other normal populations as the exposures of HCoVs are different. Extending this study to other populations would be beneficial to understand more on negative/positive effects of pre-existing immunity to host immune responses. Lastly, even though both antibody and T cell responses were analysed in this study, it would be comprehensively supporting the findings, if some functional assays were included such as neutralization or killing assay to show the functioning effect of the pre-existing immunity. However, the limited numbers of cells and funding did not allow the assays to be conducted. Further research should also explore the role of pre-existing immunity in shaping vaccine responses to SARS-CoV-2, with a focus on elucidating the mechanisms underlying cross-reactivity between endemic coronaviruses and SARS-CoV-2.

Overall, these findings underscore the need for ongoing monitoring of immune responses to SARS-CoV-2 variants, particularly in populations receiving inactivated virus vaccines like CoronaVac. Future vaccine strategies may benefit from incorporating variant-specific components to enhance both humoral and cellular immunity against newer strains.

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Author contributions

- P.S., R.S., J.O., S.S., and B.S. performed the experiment.
- N.P. designed the experiment and interpreted the data.
- B.S., and N.P. wrote the manuscript.
- Before the paper was submitted, all authors had access to the data and authorized it.

Competing Interests

The authors declare no competing financial or non-financial interests.

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