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Phenotypic and functional changes of T cell subsets after CoronaVac vaccination

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Abstract

Background: The pandemic coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, is a significant global public health concern [1], with global impacts on social-, economic- and health-inequalities [2]. Sev-

1. Introduction

The pandemic coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, is a significant global public health concern [1], with global impacts on social-, economic- and health-inequalities [2].
eral protective vaccines as well as preventive and therapeutic approaches have been developed to prevent serious disease and death. The World Health Organization (WHO) Emergency Use Authorization (EUA), has approved seven vaccines for use (https://extranet.who.int/pqweb/sites/default/files/documents/Status_COVID_VAX_23Dec2021.pdf). CoronaVac (Sinovac Life Sciences, Beijing, China), an inactivated whole virus, has been tested and validated for immunogenicity and safety in phase II and III clinical trials [3,4]. Following a two-dose vaccination regimen, neutralizing antibody (NAb) titres were high against the Wuhan strain (wild-type) but substantially decreased against variants of concern (VOC) such as alpha, beta, and delta variants [5]. Although CoronaVac elicited serum antibody levels in most vaccinated individuals, levels were lower than those in serum of convalescent COVID-19-infected individuals [5]. Previous studies have mainly focused on humoral immune responses of CoronaVac vaccination, with few studies on cellular responses [4–6]. However, a report from Chile showed a significant increase in spike proteinspecific interferon gamma (IFN-gamma)-producing helper T cells after full CoronaVac vaccination, suggesting that the vaccine also induces cellular immune responses [6]. Much less is known if CoronaVac vaccine elicits long-term T cell responses and hence provides prolonged protection. This study was a longitudinal surveillance study of RBD-specific IgG levels, NAb levels, T cell subsets, and activation, and memory B cells of a CoronaVac-vaccinated population of 356 individuals.

2. Methods

2.1. Study design and participants

This randomised study was conducted between May 2021 to January 2022, by the Department of Microbiology, Faculty of Medicine, Khon Kaen University under the approval of the Khon Kaen University Ethics Committee for Human Research (KKUEC, approval numbers HE641266 and HE641338). During this study period the Wuhan strain was the prevalent virus in circulation, which peaked in March 2020 and then was largely contained until December 2020. In April 2021, the alpha variant was the dominant strain in circulation in Thailand, followed by the Delta variant in June 2021 [7]. All 356 volunteers gave informed consent before commencing the study. Eligibility criteria for participating in the study were healthcare staff within Khon Kaen province aged > 18 years with no history of seizures, acute febrile illness, pregnancy, HIV infection and COVID-19 infection. Participant demographics are shown in Table 1 and no participant reported

2.2. Peripheral blood sample process

Whole blood samples were collected in clot gel tubes, centrifuged at 500 × g for 10 min to collect serum and stored at –20 °C until use. Heparinized blood samples were used for isolation of peripheral blood mononuclear cells (PBMCs) by gradient centrifugation using SepMate (STEMCELL Technologies, USA) for immediate use, or with Isospeed (Robbins Scientific, USA) for samples stored at –80 °C until use.

2.3. Serum anti-SARS-CoV-2 receptor binding domain (RBD) IgG measurements

SARS-CoV-2 WT RBD-specific IgG (anti-RBD) levels were quantified by ELISA (Abbott Laboratories, USA) as previously described [8]. The lower limits of sensitivities were 50 AU/mL using a value of 0.143 for Binding Antibody Unit (BAU) calculations.

2.4. Microneutralization (MN) assay

The cytopathic effect (CPE) based-MN assay was performed as previously described [9]. Briefly, two strains of SARS-CoV-2, the Wuhan strain (hCoV-19/TH/MUMT-3/2020) and the Delta variant (hCoV-19/TH/MUMT-53/2021), isolated and propagated in Vero cells, were used. Antibody titres were determined from the reciprocal of the highest serum dilution that protected at least 50% of cells from virus infection. For these experiments, randomized SARS-CoV-2 RBD-positive serum from 70 participants were tested for neutralization of the Wuhan strain and 30 of these samples were also tested for neutralization of the Delta variant.

2.5. T cell activation assay

Fresh heparinized peripheral whole blood (150 μL) was stained with anti-human CD3-APC-H7, anti-human CD4-PerCP, anti-human CD8-FITC and anti-CD69-PE (BD Pharmingen, USA) at room
temperature for 15 min in the dark. Red cells were lysed with 1x BD FACS lysis buffer (BD Biosciences, USA) for 10 min in the dark. After centrifugation at 450 \( \times g \) for 10 min, samples were washed once with phosphate-buffered-saline (PBS) before analysis using a BD FACSCantoTM II flow cytometer (BD Bioscience, USA). Activated (CD69+) T cells were determined as shown in Supplementary Figure S1. To account for the possibility of asymptomatic infection at CV0 (and hence elevated Th responder cell numbers), we measured the changes in CD4 T cell numbers from CV0-CV1 or from CV1-CV2. Participants were sub-grouped into non-T helper (Non-Th) responders (115/151 [76.2%]) and Th responders (36/151 [23.8%]) based on the increased numbers of helper lymphocytes between from CV0 to CV1 and/or CV2. An average of a > 1.47 fold (+SD) change in Th numbers (non-responders vs responders) was taken as a vaccine-dependent difference.

2.6. IFN-gamma releasing assay by QuantiFERON

SARS-CoV-2 specific T cell responses were measured by IFN-gamma release using a QuantiFERON SARS-CoV-2 kit (QIAGEN Science Inc., USA), following the manufacturer’s instruction. Briefly, 1 mL of heparinized blood was added to the Quantiferon tube containing: pooled peptides from spike-peptides; mixed nucleoprotein, membrane protein and open reading frame protein (NMO) peptide pools of SARS-CoV-2; mitogen (positive control) or no additions (negative control). After mixing, tubes were incubated at 37 \( ^\circ C \) for 24 h prior to centrifugation at 500 \( \times g \) for 10 min. Supernatants were collected for IFN-gamma measurements by enzyme-linked immunosorbent assay (ELISA) using a DS2 instrument (QIAGEN Science Inc., USA).

2.7. T cell subset identification and intracellular cytokine staining

Isolated PBMCs were stimulated with 100 ng/mL phorbol-12-myristate-13-acetate (PMA) (Sigma, USA) and 1 \( \mu \)g/mL ionomycin (calcium salt, Sigma, USA), incubated with 3 \( \mu \)g/mL GolgiStop (BD Biosciences, USA) at 37 \( ^\circ C \) for 1.5 h, and then stained with the following antibodies: anti-human CD3-APCeFluor 780 (Clone: OKT3); anti-human CD4-eFluor 450 (Clone: RPA-T4) and anti-human CD45-PE-Cyanine7 (Clone: HI30). Intracellular staining was sequentially performed after surface staining as previously described [10]. Antibody panels were anti-human IFN gamma-PE (Clone: 4S.B3); anti-human IL-4-Alexa Fluor 488 (Clone: BD4-8); anti-human IL-17A-APC (Clone: eBio64DEC17) and anti-human Foxp3-PertCP-Cyanine5-5 (Clone: PCH101). Two distinct sets of samples; 6 from an unvaccinated group and 7 from the vaccinated group (CV2) were analyzed using a BD FACSCantoTM II (BD Bioscience, USA) and data were analyzed using FlowJo version 10 (Three Star, USA) (Supplementary Figure S2).

2.8. B cell responses

For detection of specific SARS-CoV-2B cell responses, RBD-specific IgG was measured in stimulated and non-stimulated cells, as described previously [11]. Briefly, 5 \times 10^8 PBMCs were incubated with 1 \( \mu \)g/mL R848 and 10 ng/mL IL-12 for 72 h. After incubation, a 96-well plate coated with anti-human IgG (clone: M91/145) was prepared, washed with PBS and then R10 media (Gibco, USA) was added for 30 min at room temperature. Stimulated cell suspensions were added into the washed plate and incubated for 18 to...
24 h. RBD-specific IgG was detected by adding RBD-WASP and visualized by anti-WASP-ALP (Mabtech, Sweden).

2.9. Enzyme linked immunospot (ELISpot)

ELISpot for T cells specific to the SARS-CoV-2 spike (S) protein plus combined N, M protein and open reading frame protein (NMO) peptide pools, were performed according to the manufacturer’s protocol (Mabtech, Sweden). Briefly, 96-well plates were coated with anti-IFN-gamma (clone: 1-D1K), washed with PBS and incubated with AIM-V media (Gibco, USA) for 30 min at room temperature. After removing the media, peptides (2 μg/mL) were added to the well followed by freshly-isolated PBMCs (2.5 × 10^5 cells/well) and incubated at 37 °C, with 5% CO2 for 24 h. The IFN-gamma producing T cells were detected by adding 7-B6-ALP detection antibody (Mabtech) and incubated at room temperature for 2 h. Spots were visualized by adding BCIP/NBT-plus substrate and helper T central memory (TCM) cells were counted and calculated to be spot forming unit per million (SFU/million) by CTL immuneSpot S6 Universal Analyzers.

2.10. Statistical analysis

Samples were randomly selected for the above assays. Qualitative data are presented as numbers with percentages, and statistical significances were analyzed using the Chi-square test. Semi-quantitative data are presented as geometrical mean and geometrical standard deviation (SD). Continuous parameters were tested for normalization, and data with a normal distribution presented as mean ± SD, while non-normally distributed data are presented as median and interquartile range (IQR). Differences between independent variables were determined using an unpaired t test. Data from paired samples were analyzed using Wilcoxon matched-pairs signed-rank test or one-way ANOVA (Friedman test) following Dunn’s multiple comparisons test in case of multi-group comparison. Correlation of two parameters was analyzed using linear regression from log10 transformed data. Statistically significant differences were determined at P < 0.05. All analyses were performed using GraphPad Prism 8 (version 8.4.3) software.

3. Results

3.1. Patient description

Three hundred and fifty-six participants were enrolled in this study. Peripheral whole blood samples were collected 1–2 days before receiving CoronaVac, considered as baseline data (CV0), then the participants returned for blood collection 3–4 weeks after receiving the first dose (CV1, n = 356 [100%]), and 3–4 weeks after receiving the second dose (CV2, n = 335 [94.1%]) (Fig. 1A). The demographics of participants are shown in Table 1. The median age was 28.0 years (IQR 22.0–41.0) for CV0 and CV1, and 28.5 years (IQR 22.0–41.0) for CV2 participants. Approximately, two-thirds of participants were female (68.3% for CV0 and CV1, and 68.7% for CV2). Underlying conditions were reported in 86 cases (24.2%) from CV0 and CV1, and 79 cases (23.6%) from CV2 with allergy and/or asthma the most prevalent, followed by hypertension and diabetes mellitus. Around 50% participants (198 cases [55.6%] of CV0 and CV1, and 183 cases [54.6%] of CV2) presented with mild side effects of vaccination (Table 1), such as headache, fatigue and drowsiness (119 cases [33.4%] of CV0 and CV1, and 110 cases [32.8%] of CV2). Furthermore, we found that one-third of the participants at CV0 and CV1 took dietary supplements (128 cases [36.0%], and 122 cases [36.4%] of CV2), the most common being vitamin C (94 cases [26.4%] of CV0 and CV1, and 90 cases [26.9%] of CV2).

3.2. Antibody responses

The ELISA results showed that 82.6% of participants developed serum anti-RBD IgG (above the lower sensitivity level of 50 AU/mL) 3–4 weeks after CV1 (P = 0.0001 compared to CV0). Antibody levels further increased in every participant 3–4 weeks after CV2 (P = 0.0001 compared to either CV0 and CV1). However, these levels significantly declined (P < 0.0001) 3 months after CV2 (Fig. 1B). Median numbers of anti-RBD IgG secreting B cells in PBMCs at CV2 + 3 mo with CoronaVac vaccination were 10/10^6 PBMCs (IQR = 2–64 SFU/million PBMCs) (Fig. 1C). However, we found no significant differences in antibody titres in participants who reported different underlying conditions (Supplementary Figure S3). We found a significant neutralizing effect (≥ 1:10) against wild-type virus in 87.1% of the samples, while 66.7% of the CV2 serum samples neutralized the Delta variant (Fig. 1D). However, IC50 titre values of CV2 against the Delta variant (B.1.167.2) were significantly lower (P < 0.0001, n = 30) than for the wild-type strain but were higher than those of CV0 (P < 0.0001) (Fig. 1D). Furthermore, the levels of anti-RBD IgG were highly correlated with the IC50 titres that neutralized the wild-type virus (R^2 = 0.6835 and P < 0.0001), and also for the B.1.167.2 variant, albeit with lower correlation (R^2 = 0.3797 and P = 0.0004) (Supplementary Figure S4). These data show that full dose vaccination with CoronaVac elicits NAb responses in > 85% of the vaccinated population, and these antibodies are particularly protective against the wild-type strain.

3.3. T cell responses

The total numbers of peripheral blood lymphocytes and CD3+CD8+ T cells (cytotoxic cells) did not change between the CV0, CV1, and CV2 timepoints (Fig. 2A and 2B). However, the number of peripheral CD3+CD4+ T lymphocytes (helper cells) significantly increased between the CV0 and two later timepoints (CV1: P = 0.0013 and CV2: 0.0120, Fig. 2C). Furthermore, the Th-responder group showed a significant increase in helper T cells between CV0 to CV1 and CV2, respectively (P < 0.0001); however, there was no change in the non-Th-responder group (Fig. 2D). Moreover, activation of helper T cells during vaccination was observed. Our results show that Th-responder participants had significantly increased CD3+CD4+CD69+ (activated helper) T cells at CV1 compared to CV0 (P = 0.0251 compared to CV0), but then decreased to baseline by CV2 (CV1 vs CV2; P = 0.0029) (Fig. 2E). There were correlations between numbers of activated helper T cells and total numbers of peripheral blood helper T cells, which suggests that increases in total number of T cells is another marker of increased numbers of activated T cells. (Supplementary Figure S5). We also show that at CV2, participants developed specific IFN-gamma+ T cells against RBD (34/42, 81%) and S1S2 antigens (35/42, 83%) measured as IFN-gamma secretion after antigen stimulation and this activity was not significantly different 3 months after CV2 (Fig. 2F). Activation of helper T cells from the non-Th responder cells was also observed at CV1 (P = 0.0251 compared to CV0), but then decreased to baseline by CV2 (CV1 vs CV2; P = 0.0029) (Fig. 2E). There were correlations between numbers of activated helper T cells and total numbers of peripheral blood helper T cells, which suggests that increases in total number of T cells is another marker of increased numbers of activated T cells. (Supplementary Figure S5). We also show that at CV2, participants developed specific IFN-gamma+ T cells against RBD (34/42, 81%) and S1S2 antigens (35/42, 83%) measured as IFN-gamma secretion after antigen stimulation and this activity was not significantly different 3 months after CV2 (Fig. 2F). Furthermore, absolute numbers of helper T central memory (TCM) cells in blood samples from participants at 3 months after CV2 were significantly lower than the numbers in samples at CV2 (Fig. 2G). The number of specific T cells against S and NMO pooled antigens at CV2 + 3 mo is shown in Fig. 2H. However, numbers of T cells were not significantly correlated with antibody responses (Supplementary Figure S6) and there was no correlation between levels of anti-RBD IgG levels and number of IFN-gamma+ T cells (Supplementary Figure S7).
3.4. CoronaVac induced persistent Th2 responses

As shown above (Fig. 2C), numbers of helper T lymphocytes increased after vaccination. We then investigated helper T cell subtypes in the bloodstream by measurements of activated intracellular cytokine levels after stimulation as: T helper 1 (Th1; CD3+/CD4+/IFN-gamma+); T helper 2 (Th2; CD3+/CD4+/IL-4+); and T regulatory (Treg; CD3+/CD4+/FOXP3+) lymphocytes (Supplementary Figure S2). Polyclonal activation of PBMCs from participants after CoronaVac vaccination at CV2 revealed higher numbers of Th1 (P = 0.0357), Th2 (P = 0.0339), and Treg (P = 0.0355) compared to unvaccinated participants at CV0 (Fig. 3A). Additionally, the ratio of Th1:Th2 decreased in the CV2 group (Fig. 3B).

Pooled S peptides only stimulated low numbers of IFN-gamma producing Th1 cells which were significantly lower than after activation with NMO peptides (P = 0.0049) (Fig. 3C). These observations correlated with our observations using the ELISpot assay (Fig. 2H) in which stimulation with NMO peptides induced higher IFN-gamma production than with S peptides. There were no differences in IL-4 expressing Th2 cells stimulated by either set of pooled peptides and FOXP3 expression by Treg was again not statistically-significant between S or NMO peptides (Fig. 3C). The ratio of Th1 and Th2 cells after cytokine staining was < 1.0, reflecting that the responses shifted to a Th2 bias (Supplementary Figure S8).

Finally, we collected PBMCs from six participants who received two doses of CoronaVac and then received a booster dose with BNT162b2 (Pfizer-BioNTech). Results show a similar pattern of Th1 and Th2 responses after the booster dose with BNT162b2, whereas a participant with a full dose vaccination followed by BNT162b2 had a higher proportion of Th1 and Th2 cells (Supplementary Figure S9). Taken together, these results show that vaccination with CoronaVac increased both the number and activation status of circulating helper T lymphocytes in most of the participants.

4. Discussion

CoronaVac, an inactivated whole virus vaccine, has been used to vaccinate healthcare workers in several developing countries, including Thailand. Although, complete vaccination can lead to robust antibody responses [3,4], long-term T cell responses have not been extensively studied. Antibody levels are only one form of immunity and both T cell responses and development of mem-
We observed a successful induction of IgG antibodies against RBD of the S protein of the SAR-CoV-2 after full-dose vaccination with CoronaVac. The median concentration of anti-RBD IgG was 1081 AU/mL (IQR = 700–1807 AU/mL), similar to previous studies in both the quality and quantity of anti-RBD IgG levels after two doses of vaccination [6,14–16]. Antibody levels can be further enhanced by a third dose of the same vaccine[17]. Three months after the second dose of vaccination, antibody levels in our study declined to 404 AU/mL (IQR = 250–630 AU/mL), as has been previously reported [14,16]. The induced IgG could effectively neutralize the wild-type virus strain, but was less effective in protection against the B.1.167.2 (Delta) variant, as seen by significantly lower NAb (Fig. 1D). This decline has also been reported for other vaccines, and there is a need to design new vaccines to effectively protect against new variants that arise, primarily because of spike-protein mutations [18]. The B.1.167.2 strain might escape from specific IgG antibodies generated against S protein of the wild type SAR-CoV2 due to lower epitope recognition by activated B cells. The presence of peripheral memory B cell three months after the second dose of vaccine has been shown in our study, but at lower levels compared to those reported previously [16]. This inconsistency may be explained by differences in T cell responses after vaccination. Our data show that the magnitude of antibody production did not correlate with increased numbers or activation of T cell responses. Therefore, the responses in our population may be mainly via B cell activation and less dependent on T cell activation.

T cell responses are important in combating SAR-CoV2 infection and an effective vaccine should elicit both Th1 and Th2 responses whilst inhibiting Treg responses. Although our study shows that CoronaVac activated T cells in ~25% of participants, these T cells were predominantly Th2/Th1. This could be because inactivated virus particles, which do not have their infectivity, are mostly ingested by antigen presenting cells (APCs), a typical phenomenon of vaccinated vaccines. Antigen presenting cells present the antigen through MHC-II, a process that skews towards Th2-type T cell responses. On the other hand, Tregs, that are unfavorable for effective vaccination, were increased by CoronaVac, suggesting a potential tolerance to the
vaccine. This is consistent with a previous study utilizing inactivated virus, which showed that Tregs contribute to poor response to vaccination [20]. Moreover, cytotoxic T cells, that clear virally infected cells, were unchanged upon vaccination, suggesting poor activation of cell-mediated responses that is important for infected cell clearance. Our investigation may help explain why this inactivated vaccine produces a lower and shorter duration of protection against SARS-CoV-2 virus, compared to other vaccine types [21]. However, the ideal vaccine should include Th1 activation for long-lasting immunity similar to that of vector-based or mRNA vaccines. The booster dose (Supplementary Figure S8) showed a Th2 bias confirming the Th2 priming function of CoronaVac.

Our study has several limitations. First, our study did not test the participants whether they have previous COVID-19 infection. However, the incidence in our study area was extremely low during the study period, infer that the responses were not affected by natural infections. Second, sample collection was completed three months after full vaccination because Thai national policy provides a third dose of vaccine, that is outside our study. Third, using CD69 as a marker may not represent vaccine-induced specific T cells. Even though immune responses towards this vaccine might be limited, several reports have shown reduction of disease mortality rate [22]. A third booster dose should be considered for effective protection [23]. This study confirmed the effectiveness of CoronaVac in terms of antibody response in both SARS-CoV-2 specific IgG levels and protective activity and provides new insights, and information in persisting Th2 subset responses after CoronaVac vaccination.

Data sharing
Individual participant data will be made available when the study is complete, on reasonable requests made to the corresponding author; data can be shared through secure online platforms after proposals are approved.

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CRediT authorship contribution statement
Wisitsak Phoksawat: Conceptualization, Investigation, Methodology, Validation, Writing – original draft. Arnone Nithichanon: Conceptualization, Investigation, Methodology, Validation, Writing – original draft. Hatairat Lerdسام: Investigation, Methodology. Surasakdi WongratanaCheewin: Conceptualization, Funding acquisition, Supervision, Validation, Writing – original draft, Writing – review & editing. Atibordee Meesing: Conceptualization and Investigation. Chonlatip Piptanaboon: Conceptualization, Investigation and Methodology. Sakawrat Kanthawong: Conceptualization, Investigation and Methodology. Chonlatip Piptanaboon: Conceptualization, Investigation and Methodology. Meeya Lulitanond: Conceptualization, Investigation and Methodology. Viraphong Lulitanond: Conceptualization, Validation. Sorujsiri Chareonsudjai: Conceptualization. Pilaipan Puthavathana: Conceptualization, Writing – review & editing. Ludthawun Kamuthachad: Investigation, Methodology. Chatcharin Kamson: Investigation, Methodology. Chavont Koiran: Investigation, Methodology. Kanin Salao: Writing – original draft. Arunya Chonlapan: Investigation, Methodology. Phumjitar Nawnawikhun: Investigation, Methodology. Jarunee Prasertspon: Investigation, Methodology. Hans J. Overgaard: Writing – original draft, Writing – review & editing. Steven W. Edwards: Writing – original draft, Writing – review & editing. Supranee Phanthanawiboon: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Data availability
Data will be made available on request.

Declaration of Competing Interest
The authors 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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Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.10.017.

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