Phenotypic and functional changes of T cell subsets after CoronaVac vaccination

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Summary

Background The pandemic coronavirus disease 2019 (COVID-19) is a major global public health concern and several protective vaccines, or preventive/therapeutic approaches have been developed. Sinovac-CoronaVac, an inactivated whole virus vaccine, can protect against severe COVID-19 disease and hospitalization, but less is known whether it elicits long-term T cell responses and provides prolonged protection.

Methods This is a longitudinal surveillance study of SARS-CoV-2 Receptor binding domain (RBD)-specific IgG levels, neutralizing antibody levels (NAb), T cell subsets and activation, and memory B cells of 335 participants who received two doses of CoronaVac. SARS-CoV-2 RBD-specific IgG levels were measured by enzyme-linked immunosorbent assay (ELISA), while NAb were measured against two strains of SARS-CoV-2, the Wuhan and Delta variants. Activated T cells and subsets were identified by flow cytometry. Memory B and T cells were evaluated by enzyme-linked immune absorbent spot (ELISpot).

Findings Two doses of CoronaVac elicited serum anti-RBD antibody response, elevated B cells with NAb capacity and $CD4^+$ T cell-, but not $CD8^+$ T cell-responses. Among the $CD4^+$ T cells, CoronaVac activated mainly Th2 ($CD4^+$ T) cells. Serum antibody levels significantly declined three months after the second dose.

Interpretation CoronaVac mainly activated B cells but T cells, especially Th1 cells, were poorly activated. Activated T cells were mainly Th2 biased, demonstrating development of effector B cells but not long-lasting memory plasma cells. Taken together, these results suggest that protection with CoronaVac is short-lived and that a third booster dose of vaccine may improve protection.

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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] Several 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 protein-specific 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.

Methods

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 that they smoked.

Participants received two doses of 600 spike unit (SU) of CoronaVac vaccine lot number C202103044 and C202104065 with a one-month interval between doses, at Srinagarind Hospital, Khon Kaen, Thailand. Eligible participants were enrolled for blood collection at four timepoints: baseline, one to two days before the 1st vaccination (CV0); three to four weeks after receiving the 1st vaccination (CV1); three to four weeks after the 2nd vaccination (CV2) and three months after CV2 (CV2 + 3 mo) (Figure 1A). While 356 participants were initially enrolled, 335 remained at the CV2 timepoint. Some volunteers (n=132) also provided blood samples 3 months after CV2. Volunteers were screened for underlying conditions, side effects, and consumption of dietary supplements (e.g. vitamins). A complete blood count (CBC) was also performed.

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 -20ºC 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 Isoprep (Robbins Scientific, USA) for samples stored at -80ºC until use.

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.

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.

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×*g* for 10min, samples were washed once with phosphate-buffered-saline (PBS) before analysis using a BD FACSCanto^{TM} 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.

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°C for 24 h prior to centrifugation at 500×*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).

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 µg/mL ionomycin (calcium salt, Sigma, USA), incubated with 3 µg/mL GolgiStop (BD Biosciences, USA) at 37°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: 8D4-8); anti-human IL-17A-

APC (Clone: eBio64DEC17) and anti-human Foxp3-PerCP-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 FACSCanto™ II (BD Bioscience, USA) and data were analyzed using FlowJo version 10 (Three Star, USA) (Supplementary Figure S2).

B cell Responses

For detection of specific SARS-CoV-2 B cell responses, RBD-specific IgG was measured in stimulated and non-stimulated cells, as described previously.[11] Briefly, $5x10^5$ PBMCs were incubated with 1 μg/mL R848 and 10 ng/mL IL-12 for 72 h. After incubation, a 96-well plate coated with anti-human IgG (clone: MT91/145) 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)

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 ($2{\cdot}5 \times 10^5$ cells/well) and incubated at 37^oC, with 5% CO_2 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.

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 $\log_{10}$ transformed data. Statistically significant differences were determined at $P < 0{\cdot}05$. All analyses were performed using GraphPad Prism 8 (version 8·4·3) software.

Results

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%]) (Figure 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).

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 ($P < 0{\cdot}0001$ compared to CV0). Antibody levels further increased in every participant 3-4 weeks after CV2 ($P < 0{\cdot}0001$ compared to either CV0 and CV1). However, these levels significantly declined ($P < 0{\cdot}0001$) 3 months after CV2 (Figure 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 SFUs/million PBMCs) (Figure 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 (Figure 1D). However, IC_{50} 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) (Figure 1D). Furthermore, the levels of anti-RBD IgG were highly correlated with the IC_{50} 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.

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 (Figures 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, Figure 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 (Figure 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 < 0·0001) and the numbers of activated helper T cells remained elevated at CV2 (Figure 2E). 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) (Figure 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 (Figure 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 (Figure 2G). The number of specific T cells against S and NMO pooled antigens at CV2 + 3 mo is shown in Figure 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).

CoronaVac induced persistent Th2 responses.

As shown above (Figure 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; $CD3^+/CD4^+/IFN\text{-}gamma^+$); T helper 2 (Th2; $CD3^+/CD4^+/IL\text{-}4^+$); 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 ($P = 0{\cdot}0357$), Th2 ($P = 0{\cdot}0339$), and Treg ($P = 0{\cdot}0355$) compared to unvaccinated participants at CV0 (Figure 3A). Additionally, the ratio of Th1:Th2 decreased in the CV2 group (Figure 3B).

Pooled S peptides only stimulated low numbers of IFN-gamma producing Th1 cells which were significantly lower than after activation with NMO peptides ($P = 0{\cdot}0049$) (Figure 3C). These observations correlated with our observations using the ELISpot assay (Figure 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 (Figure 3C). The ratio of Th1 and Th2 cells after cytokine staining was $< 1{\cdot}0$, 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 shown that vaccination with CoronaVac increased both the number and activation status of circulating helper T lymphocytes in most of the participants.

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 memory cells are key to long-term protection. Beside investigation of antibody responses after CoronaVac vaccination, we also measured phenotypic and functional

changes of T cell subsets and development of memory cells. This is the first study reporting evidence of integrated antibody and T cell responses, especially regarding T cell subset functions.

This study enrolled 356 participants who received the first dose of the vaccine, but 21 withdrew from the second dose, citing personal reasons. However, the demographics CV0, CV1 and CV2 were not different, and so this number of withdrawals did not affect data interpretation. We followed the participants up to three months after CV2, and there were no reported incidences of COVID-19 infections. However, we could not definitively demonstrate that they were protected from infection as their exposure to the virus and their behaviors after vaccinations were not determined. In line with other studies,[3,5] we observed that >50% of participants only reported mild side-effects including headaches and other minor discomforts. Serious side effect were not found with this vaccine.[12] In contrast, mRNA- or vector-vaccine vaccination have been reported to cause serious side effects including myocarditis, pericarditis and blood clotting disorders, albeit at very low frequencies.[13] Additionally, there were no differences in the reported side-effects of CoronaVac between doses (Table 1).

We observed a successful induction of IgG antibodies against RBD of the S protein of the SAR-CoV2 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 (IQ=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 (Figure 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 of 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 T helper cells. The presence of SAR-CoV2 protein-specific IFN-gamma-releasing $CD4^+$ T cells, but not $CD8^+$ T cells after full dose vaccination has also been reported.[6] Unlike mRNA and viral vector vaccines, that elicit Th1-skewed T cell immune responses,[19] our study shows that CoronaVac skews immunity towards Th2 responses as indicated by increased ratio of Th2/Th1. This could be because inactivated virus particles, which have lost their infectivity, are mostly ingested by antigen presenting cells (APCs), a typical phenomenon of inactivated 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.

Contributors

Conceptualization: SP, SW, SC, PP, VL, WP and AN; Data collection, data analysis, data interpretation, figures and table: AN, WP, KS, LK, SP, ML and CT; Funding acquisition: SP and SW; Participant recruitment: AM, SC, PM, CP, ML, SA, UY and SK; Investigation and methodology: HL, SP, WP, AN, LK, CK, CT, AC, JP and PN; Project administration: SP; Supervision: SP and SW; Validation: SW, SP, VL, WP and AN; Writing-original draft: SP, KS, AN, SWE, SW, HJO and WP; and Writing-review and editing: SWE, HJO, SW, PP and SP. All authors approved the final version of the published manuscript.

Declaration of interests

The authors declare no conflict of interest.

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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Figure legends

Figure 1. Vaccination with CoronaVac elicited anti-Spike serum antibodies and B cells with neutralizing capacity. Participants (N = 356) were enrolled and peripheral whole blood samples were collected before CoronaVac vaccination at baseline (CV0), at 3 - 4 weeks after the first CoronaVac vaccination (CV1), and 3 - 4 weeks after the second CoronaVac vaccination (CV2), and three months after CV2 (CV2 + 3 mo) (A). Serum samples were quantified for anti-Spike RBD IgG by ELISA, with data shown as an individual dot plots with median and interquartile range: the red dash-line represents a positive cut-off at >50 AU/ml (B). PBMCs from participants were cultured in the presence of RDB protein and IgG-secreting B cells were enumerated from 15 participants using ELISpot: data are compared between unstimulated medium control and RBD activation (C). Serum 50% inhibitory concentration (IC50) neutralization titers of CV2 serum against wild type (n = 70) virus: 30 of these samples were also tested for neutralisation of the B.1.167.2 strain (n = 30) (D). Statistically-significant differences were determined using the Wilcoxon matched-pairs signed rank test: ***, $P < 0·001$, ****, $P < 0·0001$.

Figure 2. Increasing of overall helper T cell responses after Sinovac vaccination. Whole blood samples from participants (CV0, before vaccination; CV1, after the first vaccination; CV2, after the second vaccination) were enumerated and lymphocyte sub populations determined by flow cytometry (A). T lymphocyte subsets were analyzed by flow-cytometry as populations of CD3+/CD8+ (cytotoxic) T cells (B) and CD3+/CD4+ (helper) T cells (C). Number of CD3+/CD4+ (helper) T cells (D), or CD3+/CD4+/ CD69+ (activated helper) T cells from each group are shown (E). IFN-gamma secreting T cells specific to RBD or S1S2 antigens were quantified in CV2 and three months after CV2 (CV2 + 3 mo) (F). Number of total helper T central memory (TCM) cells in peripheral blood were enumerated (G). Activation of IFN-gamma by PBMCs against pooled peptides of Spike (S) and pooled nucleoprotein, membrane protein, and open reading frame proteins (NMO) were measured with ELISpot (H). Data are shown as dot plots with median and interquartile range. Statistical significances of samples were tested by one-way ANOVA (Friedman test) following Dunn's multiple comparisons test or Wilcoxon matched-pairs signed rank test; ns, non-significance, *, $P < 0·05$, **, $P < 0·01$, ***, $P < 0·001$, ****, $P < 0·0001$.

Figure 3. CoronaVac vaccination induced responses to polyclonal activation, S defined pooled peptides and NMO pooled peptides from helper T cell subsets. PBMCs from participants were stained for intracellular expression of IFN-gamma, IL-4 and FOXP3, which are markers for T helper 1 (Th1), T helper 2 (Th2) and T regulatory (Treg) lymphocytes, respectively. Results from polyclonal activation of PBMCs with PMA and

Ionomycin comparing unvaccinated samples at CV0 (black circle) and vaccinated samples at CV2 (red close circle) (A), and ratio of Th1: Th2 (B). Data are plotted as scatter dot plots showing mean levels (bar columns) ±SD. PBMCs were collected at 3 months after CV2 (CV2 + 3 mo, n = 12, red open circle) for stimulation with pooled peptides of Spike protein (S) and pooled peptides of nucleoprotein, membrane protein, and open reading frame protein (NMO), or anti-CD3 as a positive control (C). Data are shown as dot plots with median and interquartile range. Statistical significances between samples were tested with unpaired t test while repeated samples were tested by Wilcoxon matched-pairs signed rank test; ns, non-significance, *, $P < 0{\cdot}05$, **, $P < 0{\cdot}01$.

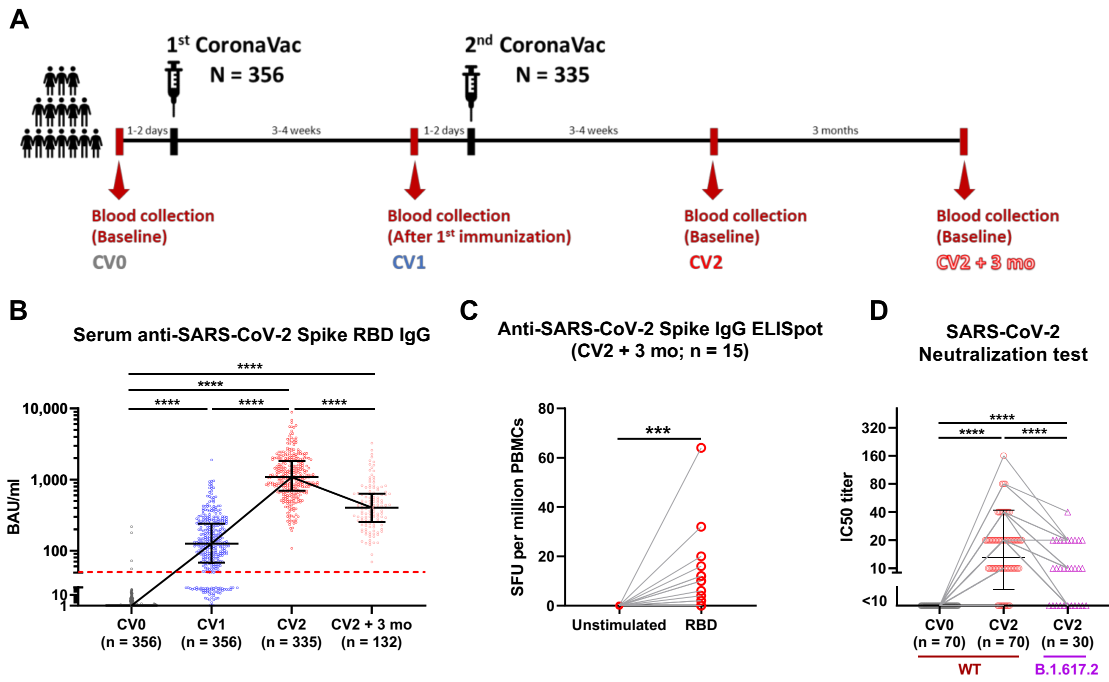

Figure 1

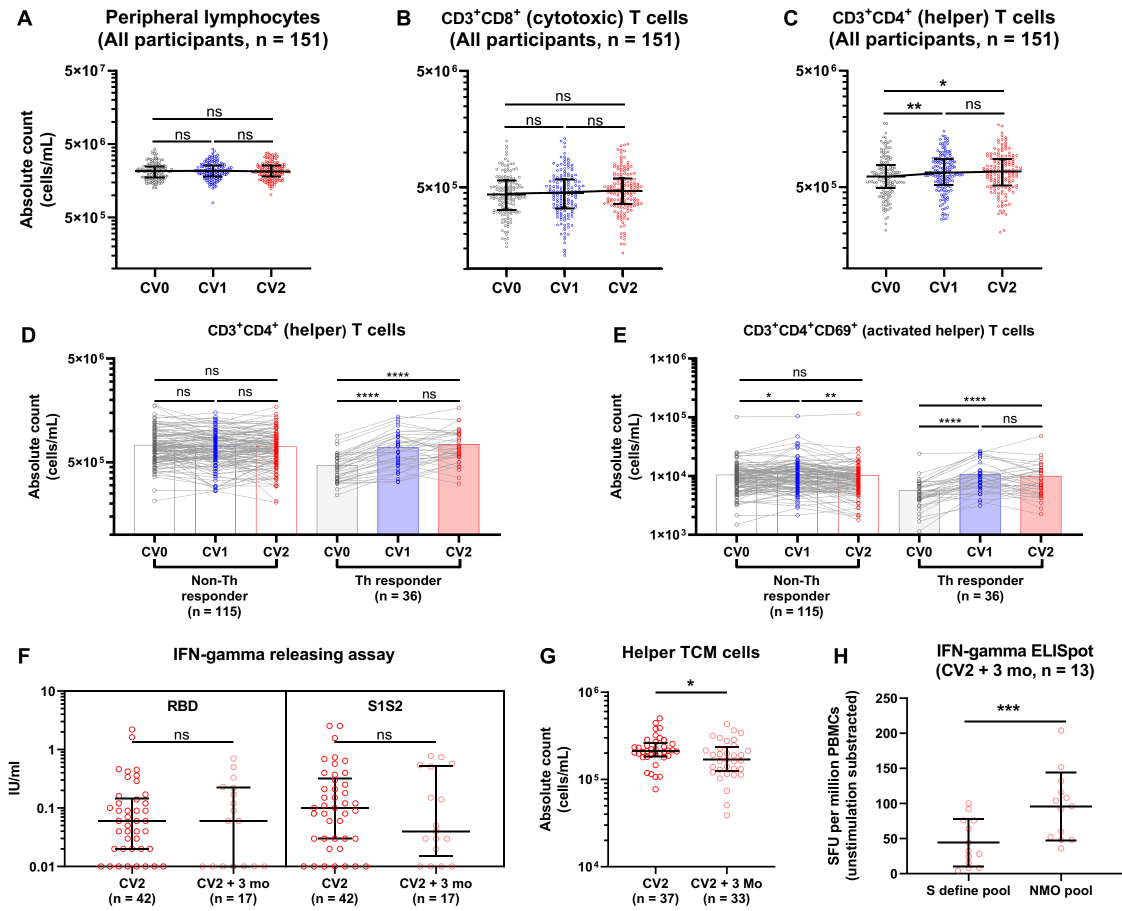

Figure 2

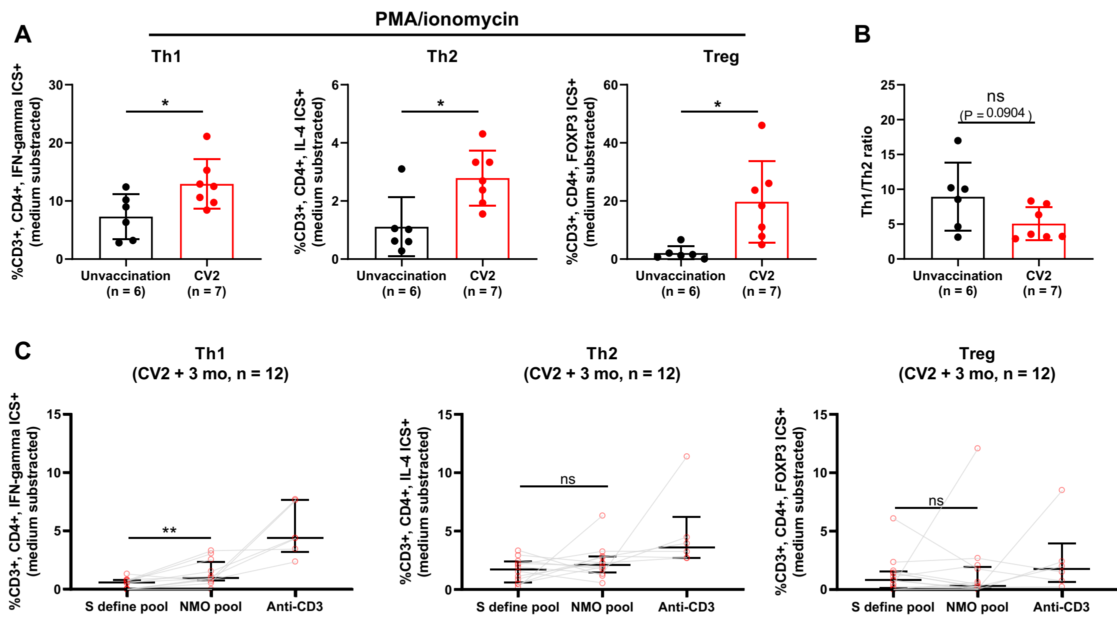

Figure 3

Table 1. Demographics of participants

CV0; before vaccination, CV1; after getting the first CoronaVac, CV2; after getting the second CoronaVac, ns; non-significant.

	CV0 and CV1 (n = 356)	CV2 (n = 335)
Age		
Median (interquartile range)	28·0 (22·0 - 41·0)	28·5 (22·0 - 41·0)
Sex		
Female, n (%)	243 (68·3%)	230 (68·7%)
Male, n (%)	113 (31·7%)	105 (31·3%)
Underlying conditions, n (%)	86 (24·2%)	79 (23·6%)
Allergy and/or asthma	37 (10·4%)	32 (9·6%)
Hypertension	14 (3·9%)	14 (4·2%)
Diabetes mellitus	10 (2·8%)	10 (3·0%)
Hyperlipidemia	7 (2·0%)	7 (2·1%)
Thyroiditis	7 (2·0%)	7 (2·1%)
Systemic lupus erythematosus (SLE)	4 (1·1%)	4 (1·2%)
Hepatitis B virus infection	2 (0·6%)	2 (0·6%)
Others	16 (4·5%)	14 (4·2%)
Side effects after vaccination, n (%)	198 (55·6%)	183 (54·6%)
Headache, fatigue, drowsy	119 (33·4%)	110 (32·8%)
Discomfort	55 (15·4%)	52 (15·5%)
Numb	21 (5·9%)	19 (5·7%)
Diarrhea	11 (3·1%)	10 (3·0%)
Nausea/vomiting	8 (2·2%)	7 (2·1%)
Low grade fever	5 (1·4%)	5 (1·5%)
Others	12 (3·4%)	11 (3·3%)
Supplementation, n (%)	128 (36·0%)	122 (36·4%)
Vitamin C	94 (26·4%)	90 (26·9%)
Vitamin B	30 (8·4%)	28 (8·4%)
Minerals	30 (8·4%)	28 (8·4%)
Cod liver oil	17 (4·8%)	17 (5·1%)
Vitamin D	9 (2·5%)	8 (2·4%)
Multi-vitamin	2 (0·6%)	2 (0·6%)
Vitamin A	1 (0·3%)	1 (0·3%)
Others	16 (4·5%)	15 (4·5%)