SHORT COMMUNICATION

T-cell responses to SARS-CoV-2 Omicron spike epitopes with mutations after the third booster dose of an inactivated vaccine

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Abstract

The rapidly spreading severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron variant contains more than 30 mutations that mediate escape from antibody responses elicited by prior infection or current vaccines. Fortunately, T-cell responses are highly conserved in most individuals, but the impacts of mutations are not clear. Here, we showed that the T-cell responses of individuals who underwent booster vaccination with CoronaVac were largely protective against the SARS-CoV-2 Omicron spike protein. To specifically estimate the impact of Omicron mutations on vaccinated participants, 16 peptides derived from the spike protein of the ancestral virus or Omicron strain with mutations were used to stimulate peripheral blood mononuclear cells (PBMCs) from the volunteers. Compared with the administration of two doses of vaccine, booster vaccination substantially enhanced T-cell activation in response to both the ancestral and Omicron epitopes, although the enhancement was slightly weakened by the Omicron mutations. Then, the peptides derived from these spike proteins were used separately to stimulate PBMCs. Interestingly, compared with the ancestral peptides, only the peptides with the G339D or N440K mutation were detected to significantly destabilize the T-cell response. Although more participants need to be evaluated to confirm this conclusion, our study nonetheless estimates the impacts of mutations on T-cell responses to the SARS-CoV-2 Omicron variant.

KEYWORDS
mutations, Omicron, SARS-CoV-2, T-cell responses, vaccine

1 | INTRODUCTION

The emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant of concern Omicron (B.1.1.529), which has more than 30 mutations in the spike protein, has raised an alarm related to the control of coronavirus disease 2019.[1–5] Recent data have shown that the induction of a neutralizing antibody response against the Omicron variant by either vaccines or infection is drastically reduced,[5–12] while T-cell responses are largely preserved.[5,13–16] The T-cell response to the Omicron variant is based on dozens of peptide epitopes; several groups have performed computational analyses and found that even for the Omicron strain with more than 30 mutations, ~80% of the antigenic peptides recognized by T cells are identical to those of original ancestral strain.[14,17–19] As most of the peptides in the overlapping peptide pools previously used to stimulate immune cells are the same as those of the ancestral strain, the impact of mutations on T cells might be underestimated.[13–16]

There are three important reasons to interrogate whether the mutant sites cause viral escape from T cells. First, if some mutations lead to epitope escape from T cells, the observation that T-cell responses are largely preserved could be the result of the limited accumulation of mutations. After enrichment with more mutations, new variants may completely escape the immune system of vaccinated individuals. In this case, vaccines focusing on the mutations of variants are needed. The second reason is that if some mutations allow the virus to escape the T-cell response, a distinct proportion of individuals with certain Human leukocyte antigen (HLA) types that tend to recognize viral peptides with mutations may be more susceptible to the impact of the Omicron variant. In fact, reduced T-cell reactivity was recently reported in ~21% of the participants.[20] Third, identification of the mutations that can reduce T-cell responses induced by vaccines may provide clues to estimate the spread and virulence of emerging Omicron variants, such as the newly emerged variant Deltamicron.[21] Thus, clarifying the effects of Omicron mutations on the T-cell response is important for both addressing immunological questions and controlling the epidemic.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

This study and some of the relevant experiments were approved by the Beijing Youan Hospital Research Ethics Committee (No. 2021-031 and 2021-079), and written informed consent was obtained from each participant in accordance with the Declaration of Helsinki. The clinical samples were collected for research use only. The methods used conformed to approved guidelines and regulations.

2.2 | Study design

This study was a single-center, open-label, randomized controlled clinical trial initiated at Beijing Youan Hospital, China, in April 2021, among health workers. Participants received two doses of CoronaVac with a 28-day interval approximately 6 months before the study and then voluntarily received a third dose of the same vaccine (CoronaVac). Peripheral blood mononuclear cells (PBMCs) were collected from 20 vaccinated individuals (14 males and 6 females, 10 aged >40 years old and 10 aged <40 years old) 6 months after the second vaccine dose but before the third dose (week 0) and 2 (Week 2) and 4 weeks (Week 4) after the third (booster) dose of the inactivated vaccine.

2.3 | Peptides and stimulations

Peptides spanning the SARS-CoV-2 spike protein sequences of the ancestral strain and Omicron variant[1,22–24] were synthesized for use in antigen-specific T-cell assays. All peptides were reconstituted in dimethyl sulfoxide at a concentration of 1 mg/ml. Cells were stimulated for approximately 24 h. For the experiment shown in Figure 1A, all the peptides were used at a final concentration of 0.2 µg/ml each. For the work shown in Figure 1B–E, 16 peptides were mixed and used at a final concentration of 10 µg/ml each. For the work shown in Figure 2, one peptide was used at a final concentration of 10 µg/ml. Peptide sequence details are shown in Supporting Information: Table S1.

2.4 | Ex vivo interferon-γ enzyme-linked immunospot assays

Enzyme-linked immunospot (ELISpot) assays were performed with PBMCs collected after the administration of two or three vaccine doses as previously described.[22] The assays were performed using Human IFN-γ ELISpotPRO (ALP) plates (Mabtech 3420-2AST-10) following the manufacturer's instructions. In brief, after five washes with phosphate-buffered saline (PBS) and blocking with cell culture medium for 30 min, approximately 2×10^5 PBMCs were added to each well. After an hour, mixed peptides or one peptide (Supporting Information: Table S1) were added. PBS was added to the negative control wells. The cells were incubated for 24 h at 37°C with 5% CO_2. The supernatants were carefully collected. The plates were then washed four times with PBS, followed by the addition of an anti-IFN-γ detection antibody (7-B6-ALP) in PBS containing 0.5% fetal bovine serum (FBS) to each well. After a 2-h incubation, the plates were washed again and detected with BCIP/NBT-plus, which was included in the kit.

ELISpot plates were counted with an automated ELISpot counter (AID) using the same suggested system settings for all plates. The mean response of the unstimulated (negative control) wells was subtracted. The results were corrected according to the number of cells added and are expressed as a spot-forming unit (SFU)/10^6 PBMCs.
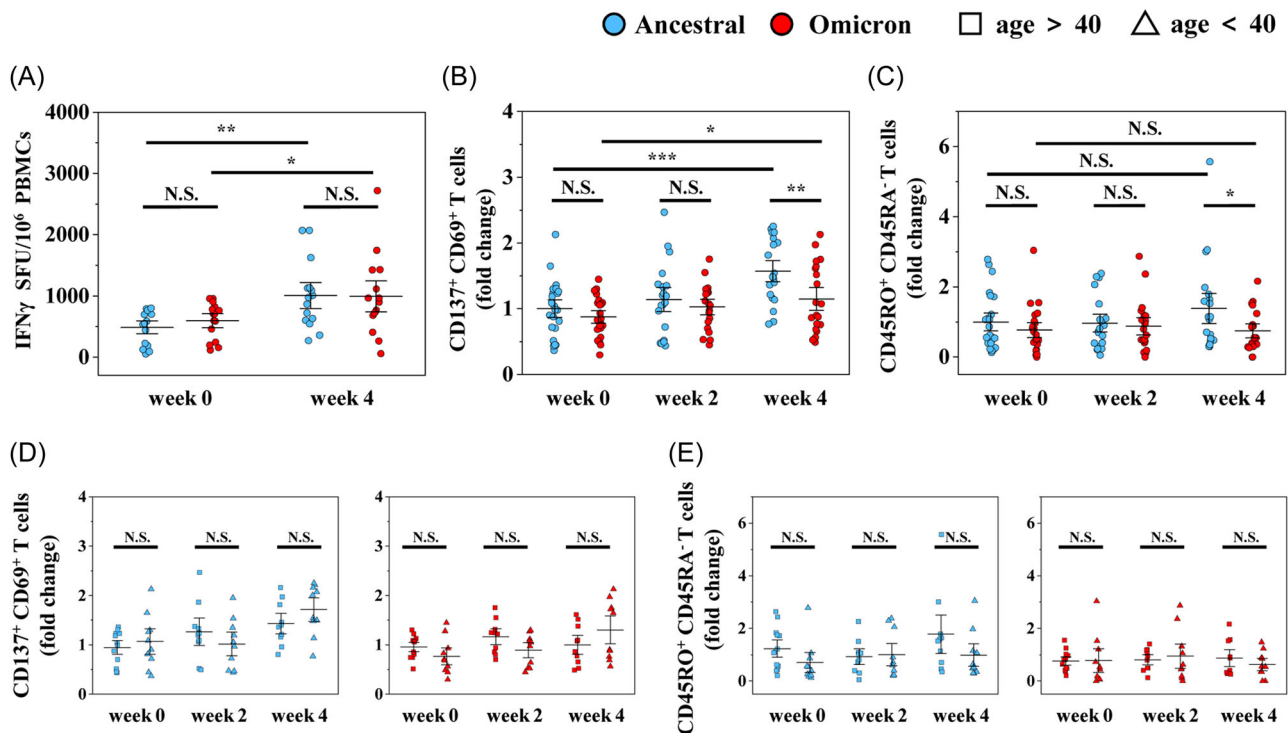
FIGURE 1 Vaccine-induced memory and T-cell responses to the ancestral virus and Omicron variant. Each dot represents a single participant. The notation *Week 0* denotes the time 6 months after when the participants were vaccinated with the second dose but before receiving the third booster dose. The notations *Week 2* and *Week 4* denote the length of time after the third booster dose in the same group of participants (2 and 4 weeks, respectively). (A) Grouped scatter and box plot of the comparative IFN-γ ELISpot spot-forming units (SFUs) per 10^6 peripheral blood mononuclear cells (PBMCs) from individuals given two or three vaccine doses; the PBMCs were stimulated with peptides spanning the spike protein of the ancestral virus (blue) or Omicron variant (red). (B,C) Grouped scatter and box plot of the fold changes in activated T cells ($CD69^+CD137^+$) or activated memory T cells ($CD69^+CD137^+CD45RO^+CD45RA^-$) after stimulation of PBMCs with peptides derived from the spike protein of the ancestral virus or Omicron variant with one or several mutations at three timepoints. Fold changes were calculated relative to the average percentage of activated T cells stimulated by the ancestral peptides at the *Week 0* timepoint. (D,E) Comparison of two groups of participants according to the age of 40. The horizontal lines and error bars represent the mean value ± 1.5 SE in (A–E); $N = 15$ for (A), $N = 20$ for (B,C), and $N = 10$ for (D,E). (B,C) Significance was analyzed by a paired parametric t-test. Significance was analyzed by an unpaired two-sample parametric t-test with *Welch's* correlation in (A,D,E). ELISpot, enzyme-linked immunosorbent spot; IFN-γ, interferon γ; NS, not significant. ***$p < 0.0005$, **$p < 0.01$, *$p < 0.05$.

2.5 | Activation-induced marker assays

Freshly isolated PBMCs ($1–2 \times 10^6$) were thawed quickly and resuspended in 500 μl of Roswell Park Memorial Institute 1640 medium in 24-well U-bottom plates and supplemented with 40 U/ml interleukin-2 (IL-2). The PBMCs were stimulated with each of the 16 peptides spanning the SARS-CoV-2 spike protein sequence of the ancestral strain or Omicron variant at a concentration of 10 μg/ml. After a 24-h incubation at 37°C with 5% CO_2, the cells were collected by centrifugation (450*g*, 5 min).

Cells were stained with fluorophore-labeled antibodies (APC anti-human CD14, 325608; BioLegend; APC anti-human CD16, 302012; BioLegend; APC anti-human CD19, 302212; BioLegend; PE anti-human CD3, 300308; BioLegend; Brilliant Violet 421™ anti-human CD137, 309820; BioLegend; Brilliant Violet 605™ anti-human CD69, 310938; BioLegend; APC/Cy7 anti-human CD45RA, 304128, FITC anti-human CD45RO, 304242). In brief, cells were resuspended in PBS with 2% FBS at a density of 1×10^7 cells/ml. The cells were blocked

with Human TruStain FcX™ for 10 min and then incubated with antibodies for 30 min on ice in the dark. For every 100 μl of cell solution, 5 μl of TruStain FcX™ or antibody was used. Activated cells were sorted on a CytoFlex cytometer (Beckman).

2.6 | Multiplex cytokine profiling

The cytokine responses in the culture supernatants of PBMCs stimulated as described above were measured. Supernatants were subpackage and stored at −80°C until use. Cytokine responses were analyzed using the LEGENDplex™ HU Th Cytokine Panel (12-plex) w/VbP V02 Kit according to the manufacturer's instructions.

Briefly, supernatants were mixed with beads coated with capture antibodies specific for IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IFN-γ, tumor necrosis factor α (TNF-α), IL-17a, IL-17F, or IL-22 and then incubated in a 96-well V-bottom plate with shaking at 650 rpm for 3 h. After centrifugation, the beads were washed and incubated with

(A)

○ Ancestral ● Omicron
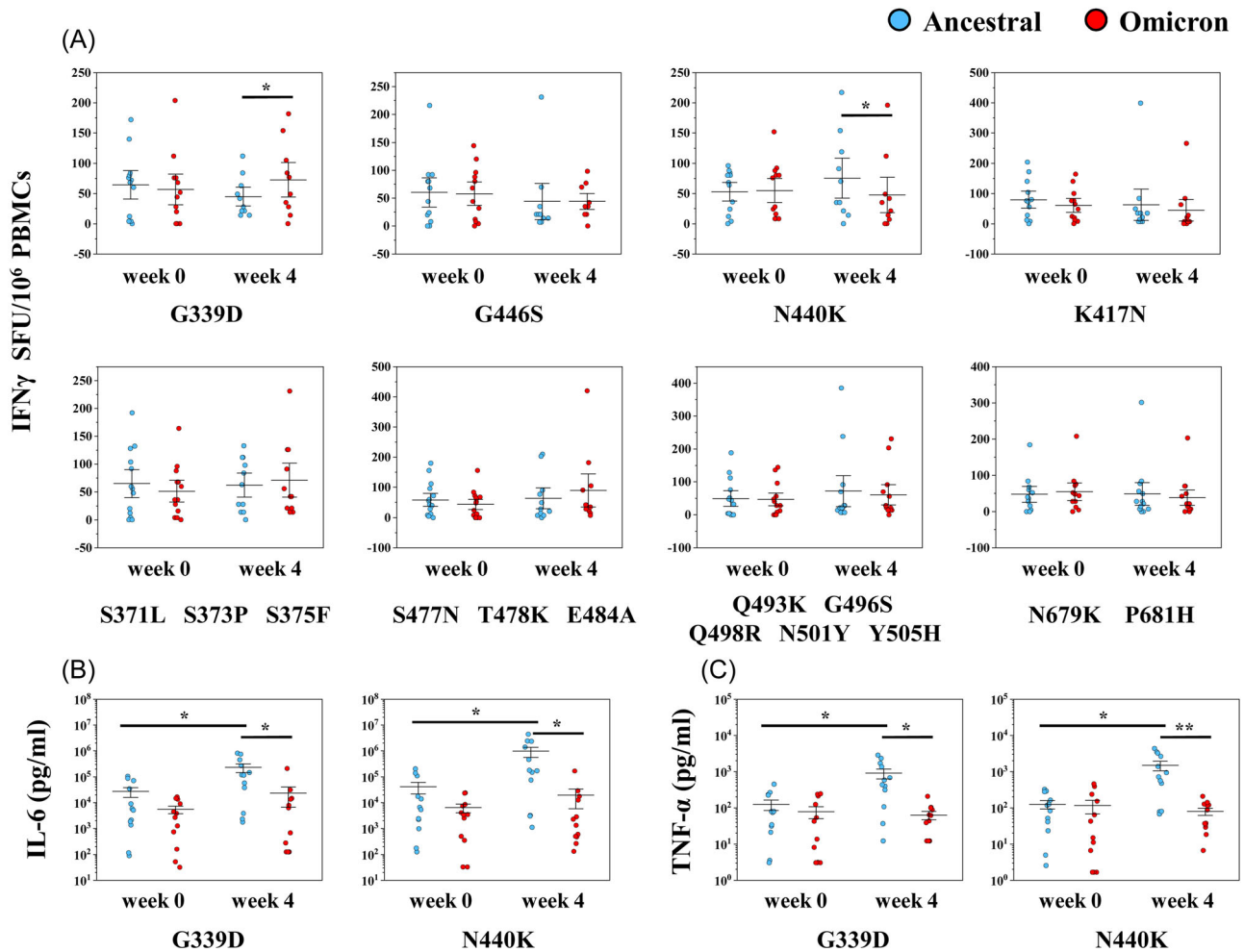
FIGURE 2 Impacts of mutations on T-cell responses. Each dot represents a single participant. The meanings of *Week 0* and *Week 4* are described in the legend of Figure 1. (A) Grouped scatter and box plot of the comparative IFN-γ SFUs per 10^6 PBMCs from individuals given two or three vaccine doses; the PBMCs were stimulated with one peptide derived from the spike protein of the ancestral virus (blue) or Omicron variant (red) with one or several mutations. The mutated site in each peptide is shown below the figure. (B, C) Concentrations of IL-6 and TNF-α in the supernatant of PBMCs stimulated with G339D or N440K mutant peptides at two timepoints (*Week 0* and *Week 4*). The horizontal lines and error bars represent the mean values ± 1.5 SE in (A) and mean values ± SE in (B,C); $N = 14–16$. Significance was analyzed by a paired parametric *t*-test. IFN-γ, interferon γ; IL-6, interleukin-6; NS, not significant; PBMC, peripheral blood mononuclear cell; SFU, spot-forming unit; TNF-α, tumor necrosis factor α. **$p < 0.01$, *$p < 0.05$.

biotinylated detection antibodies for 1.5 h, followed by a final incubation with streptavidin-PE for 45 min without washing. All incubation procedures were performed at 27°C. The beads were analyzed by flow cytometry using a CytoFlex cytometer (Beckman). Analysis was performed using the online analysis software LEGEN-Dplex, which distinguishes between the 12 different analytes on the basis of bead size and internal dye.

2.7 | Data analysis and statistics

Flow cytometry data were analyzed using CytExpert (2.3.0.84). ELISpot plates were counted using an ELISpot Reader (AID) using the same suggested system settings for all plates. The concentrations of cytokines were analyzed with the online analysis software LEGENDplex. Statistical

analyses were performed using Prism version 6 (GraphPad). Significance was analyzed by a paired parametric *t-test* or an unpaired two-sample parametric *t-test* with Welch's correlation.

3 | RESULTS

We collected PBMCs from 20 healthy vaccinated individuals (14 males and 6 females, 10 aged >40 years old and 10 aged <40 years old) 6 months after their second vaccine dose but before the third dose (Week 0) and 2 (Week 2) and 4 weeks (Week 4) after the third (booster) dose of the inactivated CoronaVac vaccine. To briefly assess the total (CD4+ and CD8+) effector T-cell response, we performed an ELISpot assay to detect IFN-γ-secreting cells following stimulation with pooled peptides

(Supporting Information: Table S1) spanning the full length of the wild-type or Omicron spike protein. Consistent with previous reports,[13-16] the T-cell responses in vaccinated participants could cross-recognize the SARS-CoV-2 Omicron spike protein, and the responses were enhanced by the booster dose. The number of SFUs significantly increased from *Week 0* to *Week 4* in both the ancestral strain peptide ($p = 0.0038$)- and Omicron peptide ($p = 0.0448$)-stimulated groups, while no significant differences were detected by intragroup comparison at either of the two timepoints (Figure 1A).

To specifically estimate the impact of the Omicron mutations on vaccinated participants, cells were stimulated in parallel with 16 peptides derived from the spike protein of the ancestral strain and 16 peptides derived from that of the Omicron variant with the same sequence but also including one or several mutations (Supporting Information: Table S1). Activation-induced marker (AIM) assays were used to evaluate spike-specific T-cell responses via the evaluation of the upregulation of CD69 and CD137 (4-1BB).[25] For activated T cells (CD69$^+$CD137$^+$), the percentage of memory cells (CD45RO$^+$CD45RA$^-$) was further analyzed. The fold change was calculated relative to the average percentage of activated T cells stimulated by ancestral peptides at the *Week 0* timepoint.

The overall magnitude of the SARS-CoV-2 spike-specific T-cell response showed an increase after the third vaccine dose (Figure 1B), while no significant differences were detected by intragroup comparisons at the *Week 0* and *Week 2* timepoints. Four weeks after the third vaccine dose, the percentage of activated T cells in stimulated PBMCs was significantly greater than that in the same participants measured before receiving the booster dose, as analyzed by a paired *t*-test. The results were consistent between the ancestral strain peptide ($p = 0.0008$)- and Omicron peptide ($p = 0.0106$)-stimulated groups (Figure 1B). At the *Week 4* timepoint following booster dose administration, the percentage of activated cells stimulated by the peptides was significantly reduced by the Omicron mutations ($p = 0.0015$, paired *t*-test). Changes in the percentage of activated memory T cells were seldom observed. No significant differences in the percentages of activated memory T cells were detected through intergroup comparisons of SARS-CoV-2 spike-specific T-cell responses at different timepoints. During *Week 0* and *Week 2*, the percentage of activated memory T cells was not significantly different between the Omicron- and ancestral strain peptide-stimulated groups (Figure 1C). Nevertheless, at the *Week 4* timepoint, a statistically significant decrease was measured in the Omicron-stimulated group compared with the ancestral strain peptide-stimulated group ($p = 0.0356$, paired *t*-test). When we divided the participants by the age of 40 and compared the groups, the differences for both activated T cells and activated memory T cells remained nonsignificant (Figure 1D,E). Overall, the frequency of AIM$^+$ spike-specific T cells was clearly enhanced for both the Omicron and ancestral strains after the third vaccine dose, but the Omicron mutations did weaken this enhancement.

To further investigate which mutation in the Omicron spike protein evades the T-cell response to SARS-CoV-2 epitopes, eight peptides with 17 mutations were used to separately stimulate PBMCs collected at *Week 0* and *Week 4*. Based on sample availability, PBMCs from 14 or 16 participants were used in the ELISpot assay to detect IFN-γ-secreting cells (Figure 2A). Interestingly, compared with the ancestral peptides, only the peptides with the G339D or N440K mutation were found to significantly change the number of IFN-γ SFUs (Figure 2A). The finding that most mutations did not change T-cell reactivity was consistent with the calculation that HLA binding was well conserved for the majority of the epitopes with mutations.[14] Surprisingly, even for the peptides with three or five mutations, no significant difference in the number of SFUs was observed by paired comparison with the ancestral peptides. Notably, the number of SFUs stimulated by only a single peptide was very small, generally less than 100 per 10^6 PBMCs. T-cell responses to a single type of peptide are quite weak in most individuals, which may be the reason that significant differences were seldom detected. The small number of SFUs was suspected to be a consequence of HLA restriction. Interestingly, the G339D mutation increased the number of SFUs, while the N440K mutation decreased this number (Figure 2A).

To further assess the impacts of the G339D and N440K mutations on T-cell responses, cytokines in the supernatants of the wells assessed in the ELISpot assay were detected. From *Week 0* to *Week 4*, the concentrations of IL-6 ($p = 0.0435$ for G339D, $p = 0.0428$ for N440K, Figure 2B) and TNF-α ($p = 0.0297$ for G339D, $p = 0.0135$ for N440K, Figure 2C) significantly increased in the ancestral peptide-stimulated groups, which again indicated the enhancement in the T-cell response induced by the booster vaccination. At the *Week 4* timepoint, by paired comparison, both the G339D and N440K mutations significantly reduced the concentrations of IL-6 ($p = 0.033$ for G339D, $p = 0.041$ for N440K, Figure 2B) and TNF-α ($p = 0.0109$ for G339D, $p = 0.0079$ for N440K, Figure 2C). No statistically significant differences were detected by comparing the IL-6 and TNF-α concentrations stimulated by the Omicron peptides at the *Week 0* and *Week 4* timepoints. Overall, the G339D and N440K mutations in the spike protein of the Omicron variant destabilize T-cell responses.

4 | DISCUSSION

Encouragingly, as the vast majority of T cell epitopes are fully conserved, ancestral SARS-CoV-2-specific T cells are expected to cross-recognize the Omicron variant, which has been shown by our data and several recent works.[13-16] Previous studies reported that a third vaccine dose showed a satisfactory safety profile and induced a stronger immune response to SARS-CoV-2 and variants of concern.[26-29] Our study provides further evidence that after two doses of an inactivated vaccine, a third booster vaccination substantially enhanced T-cell responses to the spike proteins of both the ancestral strain and the Omicron variant, although the enhancement was slightly weakened by Omicron mutations, especially G339D and N440K.

The prevalence of the mutation G339D is the highest among those mutations in the receptor-binding domain region, which is

96.6% with a χ^2 test value of 0.115.[30] The single mutations G339D and N440K allow escape from a subset of neutralizing antibodies,[7] which indicates that G339D and N440K may also change the binding affinity between T-cell epitopes and the major histocompatibility complex and consequently increase the transmissibility and infectivity of the Omicron variant. We computationally assessed the predicted binding affinity of ancestral and Omicron peptides used in ELISpot assays for 12 Chinese common HLA alleles[31] using NetMHCIIpan-4.0.[32] The predicted binding affinity and ELISpot results were not consistent. More powerful tools, such as cryo-electron microscopy-based structure analysis, may be needed to further understand the impacts of mutations on the binding affinity of T cell epitopes.

Our research focused on Chinese individuals vaccinated with CoronaVac. As T-cell responses are HLA restricted, the effects of booster vaccination and mutations in the spike protein need to be further investigated in people from different countries treated with different vaccine formulations. To elaborate on the effects of mutations on T-cell responses, further investigation of the phenotype and function of responding cells should also be performed. Moreover, additional assays, such as single-cell sequencing, intracellular cytokine staining following peptide stimulation, and multimer staining, could be performed to understand SARS-CoV-2-specific immune responses.[33]

AUTHOR CONTRIBUTIONS

Yongzheng Li and Bin Su designed the study, wrote the manuscript, and supervised the whole study. Yongzheng Li, Xiuwen Wang, and Junyan Jin performed the activation-induced marker assay and enzyme-linked immunosorbent spot assay. Yongzheng Li, Xiuwen Wang, and Junyan Jin did data analysis. Bin Su and Zhenglai Ma collected the peripheral blood mononuclear cells. Xin Zhang and Yongzheng Li took part in the discussion and wrote the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Most data needed to evaluate the conclusions in the paper are presented in the paper. The materials described in the study are commercially available. Data and materials are available from the corresponding authors upon request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.