Kinetics of pro- and anti-inflammatory spike-specific T-cell responses in long-term care facility residents after COVID-19 mRNA primary and booster vaccination: A prospective longitudinal study in Japan

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Abstract

Background

The magnitude and durability of cell-mediated immunity in older and severely frail individuals following coronavirus disease 2019 (COVID-19) vaccination remain unclear. A controlled immune response could be the key to preventing severe COVID-19; however, it is uncertain whether vaccination induces an anti-inflammatory cellular immune response. To address these issues, this 48-week-long prospective longitudinal study was conducted. A total of 106 infection-naive participants (57 long-term care facility [LTCF] residents [median age; 89.0 years], 28 outpatients [median age; 72.0 years], and 21 healthcare workers [median age; 51.0 years]) provided peripheral blood mononuclear cell (PBMC) samples for the assessment of spike-specific T-cell responses before primary vaccination, 24 weeks after primary vaccination, and three months after booster vaccination. CD4+ T-cell responses to severe acute respiratory syndrome coronavirus 2 spike protein were examined by measuring interferon (IFN)-γ, tumor necrosis factor (TNF), interleukin (IL)-2, IL-4, IL-6, and IL-10 levels secreted from the spike protein peptide-stimulated PBMCs of participants.

Results

LTCF residents exhibited significantly lower IFN-γ, TNF, IL-2, and IL-6 levels than healthcare workers after the primary vaccination. Booster vaccination increased IL-2 and IL-6 levels in LTCF residents comparable to those in healthcare workers, whereas IFN-γ and TNF levels in LTCF residents remained significantly lower than those in healthcare workers. IL-10 levels were not significantly different from the initial values after primary vaccination but increased significantly after booster vaccination in all subgroups. Multivariate analysis showed that age was negatively associated with IFN-γ, TNF, IL-2, and IL-6 levels but not with IL-10 levels. The levels of pro-inflammatory cytokines, including IFN-γ, TNF, IL-2, and IL-6, were positively correlated with humoral immune responses, whereas IL-10 levels were not.

Conclusions

Older and severely frail individuals may exhibit diminished cell-mediated immune responses following COVID-19 vaccination compared to the general population. A single booster vaccination may not adequately enhance cell-mediated immunity in older and severely frail individuals to a level comparable to that of the general population. Furthermore, booster vaccination may induce not only a pro-inflammatory cellular immune response but also an anti-inflammatory cellular immune response, potentially mitigating detrimental hyperinflammation.

Background

The spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused a global outbreak of coronavirus disease 2019 (COVID-19), leading to a significant number of fatalities. As vaccination rates increase and the Omicron variant replaces earlier strains, a considerable decrease in COVID-19 morbidity and mortality has been observed (1). However, older adults and individuals with multiple comorbidities have higher COVID-19 mortality and morbidity rates than those with influenza (2, 3). Among these individuals, long-term care facility (LTCF) residents are particularly vulnerable to COVID-19 outbreaks given the high infectivity of SARS-CoV-2.

The COVID-19 vaccine remains crucial in safeguarding the older and severely frail populations against severe COVID-19. However, recent evidence suggests that antibody levels following COVID-19 vaccination may decline more rapidly in older adults than in younger or middle-aged individuals (4–13). We previously reported that following COVID-19 mRNA primary vaccination, LTCF residents displayed lower neutralizing activity against the wild-type and Delta variants of SARS-CoV-2 than the general population (13). Although T-cells may maintain their defense capabilities against severe disease (14–21), the magnitude and durability of cell-mediated immunity in older and severely frail individuals, such as LTCF residents, compared to those in the general population remain unclear.

Advanced age is the most significant risk factor for severe COVID-19 (22–25). Nevertheless, SARS-CoV-2 infection in older adults typically causes only asymptomatic or mild symptoms similar to those of the common cold, and few older individuals experience severe illness (26–30). This diversity in immune responses may be attributed to various factors; however, the specific underlying mechanisms remain to be elucidated.

Markedly elevated pro-inflammatory cytokines (including interleukin [IL]-6) are associated with critical and fatal COVID-19, and blocking the inflammatory pathway may prevent disease progression (31, 32). The cytokine storm observed during the initial stages of SARS-CoV-2 infection can precipitate severe disease when the deleterious effects of the immune response outweigh the immediate antiviral benefits (33). High cytokine and chemokine levels during this phase are associated with an increased likelihood of experiencing a severe form of the disease and a higher risk of mortality (31, 34). Interventions targeting the virus demonstrate effectiveness in the early stages, whereas those targeting the immune response show greater efficacy during later stages. Data from randomized trials support the use of glucocorticoids and tocilizumab for severe cases of COVID-19 (35–39), indicating the significance of harmful hyperinflammatory responses during the advanced stages of the disease.

Considering the points mentioned above, eliciting an immune response that controls excessive immune responses may be crucial for preventing the development of severe COVID-19; however, researchers are unsure whether vaccination induces such a response. Moreover, it is unclear whether these
responses are associated with factors such as age, sex, nutritional status, or underlying comorbidities.

To address these issues, we designed a one-year prospective longitudinal study focusing on pro- and anti-inflammatory spike-specific T-cell responses in older and severely frail individuals within the distinctive context of COVID-19 vaccination, wherein individuals were uniformly exposed to the same antigenic stimulus. We examined responses following both primary and booster vaccinations, including not only LTCF residents but also outpatients and healthcare workers. This comprehensive approach enabled comparisons among older adults requiring extended care, those living independently at home, and healthy younger individuals.

Our results will be useful for the development of robust booster strategies as protective measures against the development of severe COVID-19 in older and severely frail individuals, such as LTCF residents.

**Methods**

**Study design and population**

Written informed consent was obtained from all participants or their legal guardians. The study protocol adhered to the Declaration of Helsinki and was approved by the Institutional Review Board of Yamaguchi University Hospital (registration no. 2020 – 214). This prospective longitudinal study was registered in the UMIN Clinical Trials Registry (UMIN Trial ID: UMIN000043558). The detailed protocol for this study is available at https://center6.umin.ac.jp/cgi-open-bin/ctrctr_view.cgi?recptno=R000049712. The other objectives of this study were 1) to evaluate humoral immune responses after COVID-19 vaccination and 2) to investigate the relationship between the microbiomes in the intestinal tract and the immunogenicity and durability of the COVID-19 vaccine. We previously published an interim report on humoral immune responses post COVID-19 vaccination (13). However, obtaining conclusive results regarding the relationship between intestinal microbiomes and the immunogenicity and durability of the COVID-19 vaccine will require additional time.

This study was conducted from March 5, 2021, to July 6, 2022, and included LTCF residents, outpatients, and healthcare workers who had not yet received their first COVID-19 vaccine dose. The LTCFs included four nursing homes and one long-term care hospital in Yamaguchi, Japan, and outpatient clinics included Yamaguchi University Hospital or Hofu Rehabilitation Hospital in Yamaguchi, Japan. All participants were tested for antibodies that target the viral nucleocapsid protein [IgG(N)] to rule out COVID-19 breakthrough infection during the study period (at baseline and 8, 12, 24, and 48 weeks after the first dose). A nucleic acid amplification test for SARS-CoV-2 was performed if any COVID-19-associated symptom or exposure to a SARS-CoV-2-infected person was reported. The eligibility criteria included the absence of SARS-CoV-2 infection, and individuals with positive results were excluded from the final analyses. All participants were asked to provide peripheral blood samples for the assessment of spike-specific T-cell responses before and 24 and 48 weeks after primary vaccination with the BNT162b2 (Pfizer-BioNTech) COVID-19 vaccine (two intramuscular doses of 30 mcg each were given three weeks apart) The endpoint of the study for any participant was defined as 350 days after administration of the first vaccine dose, death, or lack of follow-up.

**Isolation of peripheral blood mononuclear cells (PBMCs)**

PBMCs were isolated from 16 mL of whole blood in BD Vacutainer CPT tubes (BD Biosciences, Franklin Lakes, NJ, USA) or SepMate-50 tubes (STEMCELL Technologies, Vancouver, Canada) according to the manufacturer’s instruction. The PBMCs were frozen in Cellbanker 1plus (ZENOGEN PHARMA, Fukushima, Japan) at ~80 °C overnight and stored in liquid nitrogen until further use.

**T-cell stimulation with synthetic peptides of SARS-CoV-2 spike protein**

T helper type 1 (Th1) and T helper type 2 (Th2) responses to SARS-CoV-2 spike protein were examined by measuring interferon (IFN)-γ, tumor necrosis factor (TNF), IL-2, IL-4, IL-6, and IL-10 secreted from the spike protein peptide-stimulated PBMCs of subjects. Frozen PBMCs were thawed and incubated in RPMI 1640 medium (Merck, Kennebunk, ME, USA) at 1 x 10⁶ / 100 µL/well. A peptide library of 15-mers overlapping by 11 amino acids spanning the full-length spike protein sequence (315 peptides in total, PreMix SARS-CoV-2 Spike Glycoprotein; JPT Peptide Technologies, Berlin, Germany) were contained in the culture medium at 2 µg/mL per peptide, and 100 µL of the peptide-containing medium was added to the PBMC culture (final concentration 1 µg/mL/peptide). After incubation for 18 h, the culture supernatants were harvested after centrifugation of the plate at 1500 rpm for 3 min and stored at −80 °C until use. IFN-γ, IL-2, TNF, IL-4, IL-6, and IL-10 levels in the supernatants were measured with a Human Th1/Th2 Cytokine Cytometric Beads Array Kit II (BD, San Diego, CA, USA) and FACSLyric (BD, San Diego, CA, USA) according to the manufacturer’s instructions. The results were analyzed using FCAP Array software ver. 3.0 (BD, San Diego, CA, USA).

**Serological assays**

In this study, we utilized our previously reported results of serological testing for antibodies against the receptor-binding domain (RBD) of the S1 subunit of the viral spike protein [IgG(S-RBD)], IgG(N), and surrogate virus neutralization against the wild-type virus, Delta (B.1.617.2), and Omicron
variants (B.1.1.529, sublineage BA.1) (13) to investigate the correlation between the spike-specific T-cell responses tested in this study and the humoral immune responses demonstrated in our prior interim report (13).

Statistical analysis

The data were stratified into three groups: healthcare workers, outpatients, and LTCF residents. Values were summarized as median and interquartile range (IQR) for continuous variables and as frequencies (percentage) for categorical variables. Differences in cytokine levels between time points were tested pairwise using the Dunn test for multiple comparisons, whereas between-group differences were tested using Fisher's exact test for categorical variables and the Mann-Whitney test or the Kruskal-Wallis test for numerical variables. All pairwise comparisons after the Kruskal-Wallis test were performed using Dunn's test with the Bonferroni correction for multiple testing. Correlations between variables were calculated using Spearman's rank correlation coefficient ($r_s$). Factors causing variation in the IFN-$\gamma$, TNF, IL-2, IL-6, and IL-10 levels were analyzed using multiple regression analyses (MRAs) by setting each type of cytokine as an objective variable and the following demographic/clinical factors as explanatory variables: age, sex, number of comorbidities, immunosuppressive status, Eastern Cooperative Oncology Group Performance Status (ECOG-PS), serum albumin, estimated glomerular filtration rate (e-GFR), glycated hemoglobin, and booster vaccination type. The optimal regression model was built using repeated stepwise selection from the explanatory variables using a threshold $P$ value of 0.05. The overall goodness of fit of the model was determined using the adjusted coefficient of determination. During the selection process, "age" was included in the model as a control variable to avoid confounding effects on other parameters. The practical significance of each parameter retained in the regression model was interpreted based on its standardized partial regression coefficient, which corresponds to the partial correlation coefficient ($r_{pj}$) and takes values between $-1.0$ and $1.0$. In reference to Cohen's criterion for the effect size of the correlation coefficient (40), we regarded $0.20 \leq |r_{pj}| < 0.3$, $0.30 \leq |r_{pj}| < 0.5$, and $0.5 \leq |r_{pj}|$ as indicative of a "weak," "moderate," and "strong" correlations, respectively. All statistical analyses were performed using StatFlex for Windows Ver. 7 (Artech Inc., Osaka, Japan). Scatter and box-and-whisker plots were generated using JMP Pro 16.1.0 (SAS Institute Inc., Cary, NC, USA).

Results

Study population and serological assays

The final study sample comprised 106 infection-naive participants (57 LTCF residents, 28 outpatients, and 21 healthcare workers). The distribution of demographic characteristics and data on coexisting conditions among participants at baseline are shown in Additional File 1. The cohort in the current study, which examined the cellular immune response, overlapped with that of our previous study, which investigated the humoral immune response (13). However, for this study, we required participants to have undergone a minimum of two assessments of the spike-specific T-cell response from the baseline period for the final analysis. Consequently, the final sample size was lower than that in our previous study. The sample population consisted of 100% Asians, 58.5% of whom were females. The median age of the LTCF residents was 89.0 years, with an IQR of 83.0–93.0 years. Among LTCF residents, 57.9% had an ECOG-PS score of 4 and 29.8% had an ECOG-PS score of 3. The number of participants included in the final analysis who underwent assessment of spike-specific T-cell responses at each period is shown in Additional File 2. One participant refused to complete both vaccination doses and was thus excluded from the final analysis. The remaining participants completed two vaccination doses with the BNT162b2 (Pfizer-BioNtech) COVID-19 vaccine in the primary vaccine series. From 24 to 48 weeks after the primary vaccination, two participants failed to receive booster vaccinations and were excluded from the final analysis at 48 weeks. The remaining participants received booster vaccinations from 24 to 48 weeks after the primary vaccination. Therefore, the assessment at 48 weeks after the first dose took place approximately three months after the booster vaccination, wherein all healthcare workers, 14 of 26 outpatients, and 15 of 50 LTCF residents received the BNT162b2 (Pfizer-BioNtech) COVID-19 vaccine, and 12 of 26 outpatients and 35 of 50 LTCF residents received the mRNA-1273 (Moderna) COVID-19 vaccine. No participants were identified as being infected with COVID-19 during the study period. However, five participants showed positive IgG(N) results during the study period; these participants were considered to have been infected asymptptomatically with SARS-CoV-2 during the study period and excluded from the final analysis.

Spike-specific T-cell response kinetics

Figure 1 shows the kinetics of the spike-specific T-cell responses before and six months after the primary vaccination, as well as three months after the booster (third dose) vaccination. IFN-$\gamma$, IL-2, and IL-6 levels were significantly elevated after the primary vaccination compared to baseline levels in all subgroups. TNF levels were also significantly elevated after the primary vaccination compared to baseline levels in healthcare workers and LTCF residents, but not in outpatients. IFN-$\gamma$ and IL-6 levels were not significantly elevated after booster vaccination compared to levels before booster vaccination in any subgroup. IL-2 and TNF levels did not significantly increase after booster vaccination compared to levels before booster vaccination in healthcare workers or LTCF residents but did increase in outpatients. IL-10 levels were not significantly elevated after primary vaccination compared to those before primary vaccination in any subgroup but were significantly elevated after the booster vaccination compared to baseline and pre-booster vaccination levels in all subgroups. IL-4 levels were not significantly elevated following primary or booster vaccination in any subgroup.

Comparison of the spike-specific T-cell response among subgroups
Figure 2 shows a comparison of spike-specific T-cell responses among the subgroups. LTCF residents exhibited significantly lower IFN-γ, TNF, IL-2, and IL-6 levels than healthcare workers after the primary vaccination. Outpatients had significantly lower TNF, IL-2, and IL-6 levels than healthcare workers but comparable IFN-γ levels after the primary vaccination. After booster vaccination, IL-2 and IL-6 levels in LTCF residents and outpatients, as well as TNF levels in outpatients, were comparable to those in healthcare workers. In contrast, LTCF residents exhibited significantly lower IFN-γ and TNF levels than healthcare workers after the booster vaccination. No significant difference in IL-10 levels following primary vaccination was observed among the subgroups, whereas outpatients exhibited significantly higher IL-10 levels than healthcare workers after booster vaccination.

Correlations between age and IFN-γ, TNF, IL-2, IL-6, and IL-10 levels

Figure 3 shows the correlation between age and IFN-γ, TNF, IL-2, IL-6, and IL-10 levels. Age exhibited negative correlations with IFN-γ, IL-2, and IL-6 levels six months after the primary vaccination ($r_S$: −0.390, −0.319, and −0.228, respectively) and with IFN-γ, TNF, and IL-2 levels three months after the booster vaccination ($r_S$: −0.421, −0.354, and −0.351, respectively). No significant correlation was observed between age and IL-10 level.

MRA of possible factors responsible for the variation in IFN-γ, TNF, IL-2, IL-6, and IL-10 levels

Table 1 shows MRA results. When $|r_p| \geq 0.20$ was set as a practical level of importance in the univariate analysis, age was negatively correlated with IFN-γ, TNF, IL-2, and IL-6 levels six months after primary vaccination and with IFN-γ, TNF, and IL-2 levels three months after booster vaccination. In the multivariate analysis, in addition to the negative association with age, ECOG-PS score and serum albumin levels were positively correlated with TNF levels six months after the primary vaccination. A nearly identical association pattern of these three parameters was also found with IFN-γ levels three months after the booster vaccination. e-GFR showed a weak positive correlation with IL-2 levels three months after booster vaccination. ECOG-PS score and serum albumin levels showed moderate negative correlations with IL-10 levels three months after the booster vaccination.
Table 1
Multiple regression analyses of possible factors underlying IFN-γ, TNF, IL-2, IL-6, and IL-10 level variations

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Multivariate analysis of factors responsible for variation in cytokine levels</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Age</td>
</tr>
<tr>
<td>6 months after primary vaccination</td>
<td>106</td>
<td>-0.321</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>106</td>
<td>-0.205</td>
</tr>
<tr>
<td>TNF</td>
<td>106</td>
<td>-0.320</td>
</tr>
<tr>
<td>IL-2</td>
<td>106</td>
<td>-0.235</td>
</tr>
<tr>
<td>IL-6</td>
<td>106</td>
<td>0.163</td>
</tr>
<tr>
<td>IL-10</td>
<td>93</td>
<td>-0.386</td>
</tr>
<tr>
<td>3 months after booster vaccination</td>
<td>93</td>
<td>-0.329</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>93</td>
<td>-0.318</td>
</tr>
<tr>
<td>TNF</td>
<td>92</td>
<td>-0.091</td>
</tr>
<tr>
<td>IL-2</td>
<td>93</td>
<td>0.135</td>
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<tr>
<td>IL-6</td>
<td>93</td>
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<tr>
<td>IL-10</td>
<td>93</td>
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</table>

The levels of each cytokine were set as objective variables, and the following factors were considered candidate explanatory variables: age, sex, number of comorbidities (Com), immunosuppression, Eastern Cooperative Oncology Group Performance Status Scale (ECOG-PS) score, serum albumin, estimated glomerular filtration rate (e-GFR), glycated hemoglobin (HbA1c), and booster vaccination type. The values presented in the table are r_p values of statistical significance (P < 0.05), except for those in the n (sample size) and R (multiple regression coefficient) columns.

Abbreviations: IFN, interferon; TNF, tumor necrosis factor; IL, interleukin

*1 The dummy variable "sex" was coded as male = 0 and female = 1.

*2 Immunosuppression included receiving steroids, immunosuppressive agents, chemotherapy, or biological therapy.

*3 The dummy variable "booster vaccination type" was coded as BNT162b2 (Pfizer-BioNTech) = 0 and mRNA-1273 (Moderna) = 1.

Additional files

Correlations between cytokine levels and IgG (S-RBD) level and neutralizing activity

Figure 4 shows the correlations of cytokine levels with serum IgG (S-RBD) levels and the neutralizing activity of sera against the wild-type virus and the Delta and Omicron variants. Six months after the primary vaccination, IgG (S-RBD) levels showed a positive correlation with IFN-γ, TNF, IL-2, and IL-6 levels (r_S: 0.394, 0.203, 0.304, and 0.325, respectively). Neutralizing activity against the wild-type virus and the Delta variant showed a positive correlation with IFN-γ, IL-2, and IL-6 levels (r_S: 0.401, 0.338, and 0.342, respectively, for wild-type; r_S: 0.326, 0.261, and 0.277, respectively, for Delta). Neutralizing activity against the Omicron variant showed a negative correlation with IL-6 (r_S: -0.222). Three months after the booster vaccination, IgG (S-RBD) levels showed a positive correlation with IFN-γ, TNF, and IL-2 levels (r_S: 0.315, 0.246, and 0.316, respectively). Neutralizing activity against the wild-type virus and the Delta variant showed a positive correlation with IFN-γ, TNF, and IL-2 levels (r_S: 0.336, 0.286, and 0.229, respectively, for wild-type; r_S: 0.421, 0.361, and 0.299, respectively, for Delta). Neutralizing activity against the Omicron variant showed a positive correlation with IFN-γ (r_S: 0.214). IL-10 levels exhibited no notable correlation with IgG (S-RBD) levels or neutralizing activity six months after the primary vaccination or three months after the booster vaccination.

Correlations between IFN-γ, TNF, IL-2, IL-6, and IL-10 levels

Figure 5 shows pairwise correlations among IFN-γ, TNF, IL-2, IL-6, and IL-10 levels. Six months after the primary vaccination (Fig. 5a), IFN-γ levels were positively correlated with TNF, IL-2, and IL-6 levels (r_S: 0.615, 0.757, and 0.554, respectively); TNF levels with IL-2 and IL-6 levels (r_S: 0.549 and
0.496, respectively); IL-2 levels with IL-6 levels (rS = 0.596); and IL-6 levels with IL-10 levels (rS = 0.216). Three months after primary vaccination (Fig. 5b), IFN-γ levels were positively correlated with TNF, IL-2, and IL-6 levels (rS: 0.837, 0.768, and 0.468, respectively); TNF levels with IL-2 and IL-6 levels (rS: 0.647 and 0.608, respectively); IL-2 levels with IL-6 levels (rS = 0.510); and IL-6 levels with IL-10 levels (rS = 0.283). No significant correlation was found between IL-10 levels and IFN-γ, TNF, or IL-2 levels after the primary or booster vaccination.

Discussion

This prospective longitudinal study, conducted over 48 weeks, aimed to assess the dynamics of pro- and anti-inflammatory spike-specific T-cell responses post COVID-19 mRNA vaccinations among residents of LTCFs. Our study sought to determine if older, severely frail individuals, such as LTCF residents, can develop and sustain cellular immunity over time similar to younger, healthier individuals following COVID-19 vaccination. Additionally, the study aimed to investigate whether vaccination induces an anti-inflammatory cellular immune response. This study is unique in several respects. Firstly, it included LTCF residents with advanced age (median age: 89.0 years, IQR: 83.0–93.0 years) and severe frailty (57.9% had an ECOG-PS score of 4 and 29.8% had an ECOG-PS score of 3). It demonstrated that the cellular immune response of very old and severely frail individuals differs significantly from that of older individuals living independently in the general community. Second, it employed a prospective longitudinal design, tracking the same subjects over a span of one year. Finally, this study assessed cellular immune responses not only following the primary vaccination series but also after booster vaccination, marking a novel approach compared to prior reports.

T-cells play a crucial role in controlling SARS-CoV-2 infection, and there is growing evidence that they may help prevent or limit disease severity (19, 41). We observed a lower magnitude of pro-inflammatory spike-specific T-cell responses in LTCF residents and outpatients than in healthcare workers following primary vaccination, suggesting that the level of cell-mediated immunity following COVID-19 vaccination in older and more vulnerable individuals may be lower than that in healthy younger populations. These findings align with those of previous studies reporting diminished T-cell reactions in older individuals (12, 42–45). Although mortality rates from COVID-19 have decreased to levels comparable to those of influenza in younger individuals, they are still higher in older individuals (2). This may be because of a weak cellular immune response following COVID-19 vaccination in the older population. The mitigation of SARS-CoV-2 infection control measures is progressing in the general healthy population; however, it may still be necessary to continue infection control measures in LTCFs to prevent outbreaks.

Booster vaccinations may not significantly enhance cell-mediated immunity, as evident from the absence of significant increases in IFN-γ, TNF, IL-2, and IL-6 levels in all examined subgroups, except for TNF and IL-2 levels in outpatients following booster vaccination, when compared with the levels before the booster vaccination. This contrasts with the results of a previous study conducted with the same participants (13), where booster vaccination elicited a significant increase in neutralizing activity against the wild-type and Delta variants. Notably, although humoral immunity may wane over time, cell-mediated immunity established via COVID-19 vaccination tends to last for an extended period (14–17). T-cells can confer prolonged immunity to conserved SARS-CoV-2 epitopes, thereby potentially safeguarding against severe illnesses caused by diverse viral variants (14–17). Thus, the role of booster vaccinations may be limited to enhancing cellular immunity. Given that the objective of COVID-19 control has shifted from infection prevention to severe disease prevention, the interval between the primary vaccination series and the booster vaccination could have been longer. However, we were unable to establish this point because we did not assess data from individuals who did not receive the booster. In the absence of booster vaccination, pro-inflammatory spike-specific T-cell responses could have diminished over time, whereas a booster shot might have helped preserve levels similar to those experienced after the primary vaccination.

On the other hand, booster vaccination may partially improve pro-inflammatory spike-specific T-cell responses in older and more vulnerable individuals to a level comparable to that of the general population. After receiving booster vaccinations, LTCF residents and outpatients exhibited similar IL-2 and IL-6 levels to those of healthcare workers. This confirms previous findings and emphasizes the possible value of repeated vaccinations (46–48). However, even with booster shots, LTCF residents showed lower levels of IFN-γ and TNF than healthcare workers. Meanwhile, the levels in outpatients were similar to those in healthcare workers. Therefore, a single booster vaccination may not be sufficient to increase cell-mediated immunity in older, severely frail populations. These findings suggest that the ideal booster intervals differ between older severely frail individuals and younger healthy individuals. Older severely frail individuals may require more frequent booster vaccinations than healthy younger individuals. However, the best interval to administer booster vaccinations is unknown and requires clarification in future studies.

The reduction in COVID-19 mortality and morbidity rates via booster vaccinations (2) could be partly attributed to the induction of a response that regulates detrimental hyperinflammation, which is a significant factor in COVID-19 severity. In the present study, IL-10 levels increased significantly following booster vaccination but not primary vaccination. IL-10 is recognized as a key inhibitor of adaptive T-cell responses (49, 50) and has shown lung-protective activity in various infection models (51–53). In mouse models of influenza virus A pneumonia, IL-10 produced by effector T-cells protects against immune-mediated lung damage (53). In humans, IL-10 is associated with tissue shielding from an aggravated antimicrobial immune response during bacterial and viral infections (54–57). Considering these findings, increased T-cell IL-10 production after booster vaccination could curb hyperinflammation and prevent severe cases of COVID-19. Recent evidence has shown that fully vaccinated individuals display reduced levels of inflammatory markers during the onset and recovery phases of symptomatic COVID-19 compared to their unvaccinated counterparts (58). This suggests that vaccination is associated with decreased inflammation in both the short and long terms. The IL-10 levels following booster vaccination in LTCF residents were not significantly different from those in healthcare workers or outpatients in the present study, suggesting that booster vaccination has a comparable impact on anti-inflammatory immune response in older, more vulnerable individuals and in the general population.
In this study, no significant correlation was found between IL-10 and serum IgG (S-RBD) levels or neutralizing activity, suggesting that IL-10 levels do not negatively affect humoral immune responses. In contrast, higher levels of IFN-γ, IL-2, and IL-6 following primary vaccination were associated with increased serum IgG (S-RBD) levels and neutralizing activity against the wild-type virus and Delta variant, suggesting that these pro-inflammatory spike-specific T-cell responses play an important role in eradicating the virus partially by inducing the production of antibodies by B cells. The booster shot may initiate a balanced immune response involving both pro-inflammatory and anti-inflammatory components, enabling the eradication of the virus and avoiding excessive inflammation.

As the immune system ages, it undergoes senescence that may lead to a diminished response to vaccines (59). These alterations result in impaired immune functions, including limited germinal center responses, reduced numbers of naïve cells, amplified memory cell populations, and increased inflammatory subsets of adaptive immune cells (60–63). The present findings show large inter-individual differences in spike-specific T-cell responses following COVID-19 vaccination and imply that age is associated with a decrease in cellular immunity. Age exhibited negative correlations with levels of pro-inflammatory cytokines, including IFN-γ, TNF, IL-2, and IL-6, following COVID-19 vaccination.

However, it remains unclear whether immune senescence is solely related to chronological age, or whether other factors also play a role. As shown in the correlation chart in Fig. 3, chronological aging alone cannot fully account for this diversity. To investigate the potential factors responsible for the variability in cytokine levels, we used MRA and found that no significant factors other than chronological age were associated with these individual differences except for TNF levels six months after primary vaccinations and IFN-γ, IL-2, and IL-10 levels three months after booster vaccination. It was difficult to identify the significant factors responsible for variability in spike-specific T-cell responses because of the small sample size in this study. Further studies are required to determine the factors associated with the diversity in cellular immunity.

There are some limitations in this study. First, although this study identified spike-specific T-cell responses as a potential indicator of the cellular immune response, it did not provide a clear understanding of how this cellular response translates to actual immune protection against the development of severe disease. Although the presence of neutralizing antibodies is indicative of protection against infection (64, 65), it is unclear whether the extent of cellular immune responses in vitro is associated with protection against severe diseases. Further research is crucial for developing a better understanding of how cellular immune responses may correlate with immune protection, and future studies may include correlation analyses between cellular responses and tangible disease outcomes. Second, the reason for the difference between humoral and cellular immune responses following booster vaccination is currently unknown. Our previous research demonstrated that booster vaccination significantly attenuated individual differences in neutralizing activity against the wild-type virus and the Delta variant (13). However, in this study involving the same participants, considerable individual differences in spike-specific T-cell responses persisted even after booster vaccination. The potential for repeated booster vaccinations to diminish these variations in spike-specific T-cell responses awaits further exploration in subsequent studies. Finally, cellular sources of cytokines were not identified in the present study. Regulatory T-cells (Tregs) play important roles in suppressing inflammation and maintaining immune homeostasis. Future research should investigate the CD4 + T-cell subtypes elicited by vaccination, examining if and how the ratio of Th1, Th2, and Tregs varies over time after repeated vaccinations, and if there are any disparities present in different age groups or populations. Answering these questions could provide a more comprehensive understanding of the CD4 + T-cell response to COVID-19 vaccination and potentially lead to the development of more effective vaccines.

**Conclusions**

Older and more vulnerable individuals may exhibit inferior cell-mediated immunity following COVID-19 vaccination compared to the general population. A single booster vaccination may not adequately enhance cell-mediated immunity in this demographic. Elevated IL-10 levels post-booster vaccination suggest that the booster may trigger both pro-inflammatory and anti-inflammatory cellular immune responses, potentially regulating harmful hyperinflammation. Our results will be useful for the development of robust booster strategies as protective measures against the development of severe COVID-19 in older and severely frail individuals, such as LTCF residents.

**Abbreviations**

IFN  
interferon  
TNF  
tumor necrosis factor  
IL  
interleukin  
IQR  
interquartile range  
ECOG-PS  
Eastern Cooperative Oncology Group Performance Status Scale  
WBC  
white blood cell count
Declarations

Ethics for approval and consent to participate: Written informed consent was obtained from all participants or their legal guardians. The study protocol adhered to the Declaration of Helsinki and was approved by the Institutional Review Board of Yamaguchi University Hospital (Registration No. 2020-214). This prospective longitudinal study was registered in the UMIN Clinical Trials Registry (UMIN Trial ID: UMIN000043558).

Consent for publication: All authors give their consent for publication.

Availability of data and materials: The data are available to approved individuals upon reasonable request to the Yamaguchi University after fulfilling specific requirements.

Competing interests: TK and KD are employees of the Department of Pulmonology and Gerontology, Graduate School of Medicine, Yamaguchi University, Ube, Japan, funded by the Medical Corporation WADOKAI. The other authors declare no conflicts of interest.

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Authors’ contributions: TK and YM conceptualized the study and designed the study protocol. TK, KD, YO, HK, and MK were responsible for the project administration. KD and YO performed isolation of peripheral blood mononuclear cells. YM and YM-K performed spike-specific T-cell response examination. TK and YM curated and validated the data. TK drafted the manuscript. All authors reviewed and edited the manuscript. TK, YM, and YO verified the data. All authors had full access to the data used in this study.

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Figures

Figure 1

Kinetics of spike-specific T-cell responses

CD4+ T-cell responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein, as examined by measuring (a) interferon (IFN)-γ, (b) tumor necrosis factor (TNF), (c) interleukin (IL)-2, (d) IL-6, (e) IL-10, and (f) IL-4 secreted from the spike protein peptide-stimulated peripheral blood mononuclear cells of subjects before and six months after the primary vaccination, and three months after the booster vaccination. Participants are stratified into three subgroups: healthcare workers (HW), outpatients (OP), and residents of long-term care facilities (LTCF). Each dot represents an individual participant, and the lines indicate corresponding pairs. The levels of each cytokine are logarithmically transformed. Boxes span the interquartile range; the line within each box denotes the median, and the whiskers are the largest and smallest values within the range of ±1.5-fold in the interquartile range from the first and third quartiles. P values were determined using Dunnett’s test for multiple comparisons. N.S.; not significant.

Figure 2

Comparison of the spike-specific T-cell response among subgroups

CD4+ T-cell responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein, as examined by measuring (a) interferon (IFN)-γ, (b) tumor necrosis factor (TNF), (c) interleukin (IL)-2, (d) IL-6, (e) IL-10, and (f) IL-4 secreted from the spike protein peptide-stimulated peripheral blood mononuclear cells of subjects before and six months after the primary vaccination, and three months after the booster vaccination. Participants are stratified into three subgroups: healthcare workers (HW), outpatients (OP), and residents of long-term care facilities (LTCF). Each dot represents the individual participant. The levels of each cytokine were logarithmically transformed into a plot. Boxes span the interquartile range; the line within each box denotes the median, and the whiskers are the largest and smallest values within the range of ±1.5-fold in the interquartile range from the first and third quartile. Between-group differences were tested pairwise using Dunn’s test for multiple comparisons. P values are indicated for each plot. N.A.; not applicable.
Figure 3

Correlations between age and IFN-γ, TNF, IL-2, IL-6, and IL-10 levels

The upper and lower rows show the correlations six months after primary vaccination and three months after booster vaccination, respectively. rS: Spearman rank correlation coefficient. Values of rS are shown in bold when P values are less than 0.05: * P<0.05, ** P<0.01, and *** P<0.001. The levels of each cytokine are logarithmically transformed. The 90% confidence ellipse region was drawn assuming a near-Gaussian distribution of the values.
Correlations between cytokine levels and humoral immune responses

Correlations between cytokine levels [interferon (IFN)-γ, tumor necrosis factor (TNF), interleukin (IL)-2, IL-6, and IL-10], serum antibodies to the receptor-binding domain (RBD) of the S1 subunit of the viral spike protein [IgG(S-RBD)] levels, and neutralizing activity of sera against the wild-type virus, Delta and Omicron variants, (a) six months after primary vaccination and (b) three months after booster vaccination. rS: Spearman rank correlation coefficient. Values of rS are shown in bold when P values are less than 0.05: * P<0.05, ** P<0.01, and *** P<0.001. The levels of each cytokine are logarithmically transformed. The 90% confidence ellipse region was drawn assuming a near-Gaussian distribution of the values.

Figure 5

Correlations between IFN-γ, TNF, interleukin IL-2, IL-6, and IL-10 levels

Correlations between IFN-γ, TNF, IL-2, IL-6, and IL-10 levels at (a) six months after primary vaccination and (b) three months after booster vaccination. rS: Spearman rank correlation coefficient. Values of rS are shown in bold when P values are less than 0.05: * P<0.05, ** P<0.01, and *** P<0.001. The levels of each cytokine are logarithmically transformed. The 90% confidence ellipse region was drawn assuming a near-Gaussian distribution of the values.

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