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Memory T cell responses in seronegative older adults following SARS-CoV-2 vaccination

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ABSTRACT

Generating memory T cell responses besides humoral immune responses is essential when it comes to the efficacy of a vaccine. In this study, the presence of memory T cell responses after aluminum-adsorbed inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac) in seronegative and seropositive elderly individuals were examined. CD4⁺ and CD8⁺ memory T cell proliferation and IFN- γ production capacities were evaluated. Additionally, clinical frailty scale (CFS) and FRAIL scales of the individuals were scored. CD4⁺ memory T cell responses more prominent than CD8⁺ memory T cells. In seronegative individuals, 80% of them had memory CD4⁺ and IFN- γ , whereas 50% of them had memory CD4⁺ and all of them had IFN- γ responses. Additionally, 40% of seronegative patients and 50% of seropositive patients had memory CD8⁺ responses. To sum up, humoral immune responses are not associated with memory T cell responses, and in seronegative individuals, memory T cell responses can be detected.

Introduction

The SARS-CoV-2 virus has caused a global pandemic since late 2019 and the affect of virus on immune system is still not clearly understood. Effective and safe COVID-19 vaccines are necessary to control the pandemic. The method generally used to understand the effectiveness of vaccination is determination of antibody titers against S1 spike protein of the virus. However, antibody titers following COVID-19 vaccination have been reported inconsistent and not durable; the exact correlation between antibody titers and protection from symptomatic infection is unknown. The response of memory T cells is essential in vaccine-induced immune response assessment and long-term protection against virus. The course of the disease and vaccine responses have been shown to vary due to immunosenescence especially in older adults [1]. With immunosenescence, reduction in T cell proliferation, IL-2 production, antigen recognition, cytokine production, and naive T cell population can occur. Frailty also contributes to immunosenescence. Thus, decreased vaccine response can be seen in older adults [2]. Moreover, memory T

cell responses in SARS-CoV-2 infection may vary due to heterogeneity in infection kinetics [3].

Studies on memory T cell response against SARS-CoV-2 virus has been mainly with mRNA vaccines, adenovirus vaccines, or in COVID-19 convalescents [4]. SARS-CoV-2-specific CD4⁺ and CD8⁺ Memory T cell responses are sustained for 10 months regardless of disease severity in COVID-19 convalescent individuals [5]. SARS-CoV-2-specific T cell response after first doses of mRNA vaccines and adenovirus vaccines has been shown similar [6]. Studies comparing COVID-19 convalescents and those with a single dose of mRNA vaccine found also similar results [7].

CoronaVac is an inactivated SARS-CoV-2 vaccine developed by Sinovac Life Sciences (Beijing, China). Vaccination schedule is two doses of 3 μ g. 14–28 days apart; vaccine is well tolerated, and induced humoral responses in adults aged 60 years and older, despite the relatively low levels than those reported among mRNA-vaccinated individuals [8]. T cell immunity is imperative for long term protection against viruses, and could be a major determinant in combating the COVID-19 pandemic. However, data on memory T cell responses following inactive SARS-CoV-2 are sparse.

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This study aimed to evaluate the cellular immune response in a series of older adults who did not develop a positive humoral immune response following two successive doses of -inactive vaccine, i.e. CoronaVac.

Methodology

The study included 10 adults aged 60 years and older, who were detected to have negative antibody responses following two doses of inactivated SARS-CoV-2 vaccine and 4 adults who had positive antibody responses. Participants were recruited from those admitted to the geriatric outpatient clinic of a university hospital for any reason during April 1 through July 1, 2021. Those vaccinated by any other COVID-19 vaccine or had SARS-CoV-2 infection were excluded from the study group so were those on chemotherapy or immunosuppressive therapy, and/or renal dialysis.

Age, gender and antibody levels of all participants were recorded at admission. For assessment frailty of individuals FRAIL scale, Clinical Frailty Scale (CFS) were performed, and Charlson Comorbidity Index (CCI) were noted for comorbidity burden. FRAIL scale consists of five self-reported components: fatigue, resistance (capacity to climb a flight of stairs), ambulation (difficulty in walking several hundred yards alone and without aid), illnesses, and loss of weight (5% or greater) with in the previous 12 months. The scale score differs from 0 to 5 points with 1 point given to each positive answer. The “frailty status” was further categorized as “non-frail” (0 points), “pre-frail” (1 to 2 points), and “frail” (≥ 3 points) [9]. The Clinical Frailty Scale (CFS), a cumulative frailty scale, was performed by giving a score between 1 and 9 (1: very fit; 2: well; 3: well with the treated comorbid disease; 4: apparently vulnerable; 5: mildly frail; 6: moderately frail; 7: severely frail; 8: very severely frail; and 9: terminally ill) [10]. For detecting the disease burden Charlson Comorbidity Index (CCI) was performed [11].

The seronegativity was defined as a SARS-CoV-2 spike-specific antibody IgG levels less than 1 U/ml, as measured with the Siemens Atellica IM sCOVG kit. For comparison, four other elderly with seropositive humoral response (i.e. an IgG level of 1 U/ml or more) was further recruited, based on the same inclusion and criteria. Study population cannot be expanded due to limited study budget allocated to laboratory analyses.

For assessment of memory T cell responses, peripheral blood samples were freshly collected, and peripheral blood mononuclear cells (PBMC) were isolated by density gradient (Histopaque 1.077 g/mL Sigma, Germany). CD14⁺ monocytes were purified with MACS (Miltenyi, Germany) and monocyte-derived dendritic cells were generated according to a published protocol [12]. Maturation of monocyte-derived dendritic cells were initiated with LPS (1 μ g/mL; Sigma) and S1 spike glycoprotein (10 μ g/mL; Abcam) was loaded. As negative controls, monocyte-derived dendritic cells not loaded with S1 spike glycoprotein were used. From the same individuals, CD56⁻CD19⁻CD45RA⁻ memory T cells were sorted by BD FACS AriaII and labelled with CFSE (BioLegend). The sorting strategy of memory T cells were given in the supplemental data. Then, they were co-cultured with monocyte-derived dendritic cells during 96 hours in round-bottom 96 well plate in RPMI-1640 containing 10% FBS, 1% penicillin-streptomycin, and 5 ng/mL IL-2 (BioLegend). As a positive control condition, memory T cells were stimulated with anti-CD3 monoclonal antibody (HIT3a; 25 ng/mL; BioLegend). At the end of 96 hours, memory T cells were labelled with anti-CD4 and anti-CD8 monoclonal antibodies, and proliferation percentages were determined by BD FACS AriaII. To determine change in proliferation, T cell proliferation percentages obtained from co-cultures with S1 glycoprotein-loaded monocyte-derived dendritic cells were normalized to T cell proliferation percentages obtained from co-cultures with monocyte-derived dendritic cells not loaded with S1 glycoprotein. For the assessment of IFN- γ levels, supernatants were collected from the co-cultures and IFN- γ levels were measured by flow cytometry-based bead assay (IFN- γ LEGENDplex™; BioLegend).

The study was approved by the institutional review board of Hacettepe University (2021/29-10-KA 21130) to be conducted in geriatric outpatient clinics of the Hacettepe University Medical Faculty Hospital, Ankara, Turkey.

Statistical analysis

Statistical analyses were performed using SPSS software package, Version 25. Distribution of continuous variables were analyzed for normality assumption using visual figures and Kolmogorov-Smirnov analysis. The median and interquartile range (IQR) was used for non-normally distributed and ordinal variables and correlation coefficients and the Spearman test were used for potential associations. A 5% type-I error level was used to infer statistical significance.

Results

The median (IQR) age of the patients was 74 (15) years; 8 patients (57.1%) were females and 6 (42.8%) were males. The duration the second dose of CoronaVac vaccine was calculated as 80.5 (40.75) days, for the median value and the IQR. The median (IQR) frailty scores were 1 (2.5) and 4 (1.75), for Frail and CFS, respectively, for total population of study. Among seronegative participants, the median (IQR) spike IgG antibody level was 0.5 (0.16) U/ml whilst, the median of the comparison group was 99.4 (103.53) U/ml. The CD4⁺ Memory T cell response was more prominent, 80% of seronegative individuals and 50% of seropositive individuals were identified with CD4⁺ memory T cell responses. Furthermore, 40% of seronegative individuals and 50% of seropositive individuals were identified with CD8⁺ memory T cell responses (Fig. 1A and Table 1). IFN- γ secretion capacity of memory T cells were measured, IFN- γ production was detected in 80% of seronegative individuals and in all seropositive individuals (Fig. 1C and Table 1). Independent of the antibody response, CD4⁺ Memory T cell proliferation change was negatively correlated with age ($p = 0.033$, $r = -0.673$) and CD8⁺ Memory T cell proliferation change was negatively correlated with CFS scores ($p = 0.044$, $r = -0.545$). IFN- γ levels were negatively correlated with FRAIL scale ($p = 0.029$) and CFS scores ($p = 0.049$) as presented in Table 2.

Discussion

Our study revealed a heterogeneity of T cell responses in seronegative older adults after a median of 80 days following full (i.e., 2 doses) vaccination with inactive SARS-CoV-2 vaccine, CoronaVac. In the study population, CD4⁺ Memory T cell % proliferation decreased with age, and CD8⁺ Memory T cell % proliferation and IFN- γ levels were negatively correlated with frailty status of individuals. The association between seropositivity, and cellular immunity could not be studied with further adjustments for age, and gender given the limited size of participants, though.

Antibody titers have been shown to decrease over time in people infected with SARS-CoV-2 and among those vaccinated, but memory T cells can persist for 6 months after primary infection [13]. Coordination of adaptive immune responses, including CD4⁺ T cell, CD8⁺ T cell and antibody responses is essential for controlling viral infections, but the exact mechanism in response to SARS-CoV-2 and/or vaccines is yet to be clarified. Memory T cell response formation has been shown to be heterogeneous distribution among individuals with COVID-19 infection [3,14]. The literature has revealed higher CD4⁺ T cell response among patients with symptomatic SARS-CoV-2 infection compared to that in their asymptomatic counterparts [15]. SARS-CoV-2 responsive T cells have also been described in COVID-19 naive population, due to previous coronavirus infections, yet the related protection level is not evident. Impaired cellular immune responses can be observed in severe COVID-19 patients [16]. Disease severity has been shown to be inversely correlated with the number of SARS-CoV-2-specific CD4⁺, and CD8⁺ T

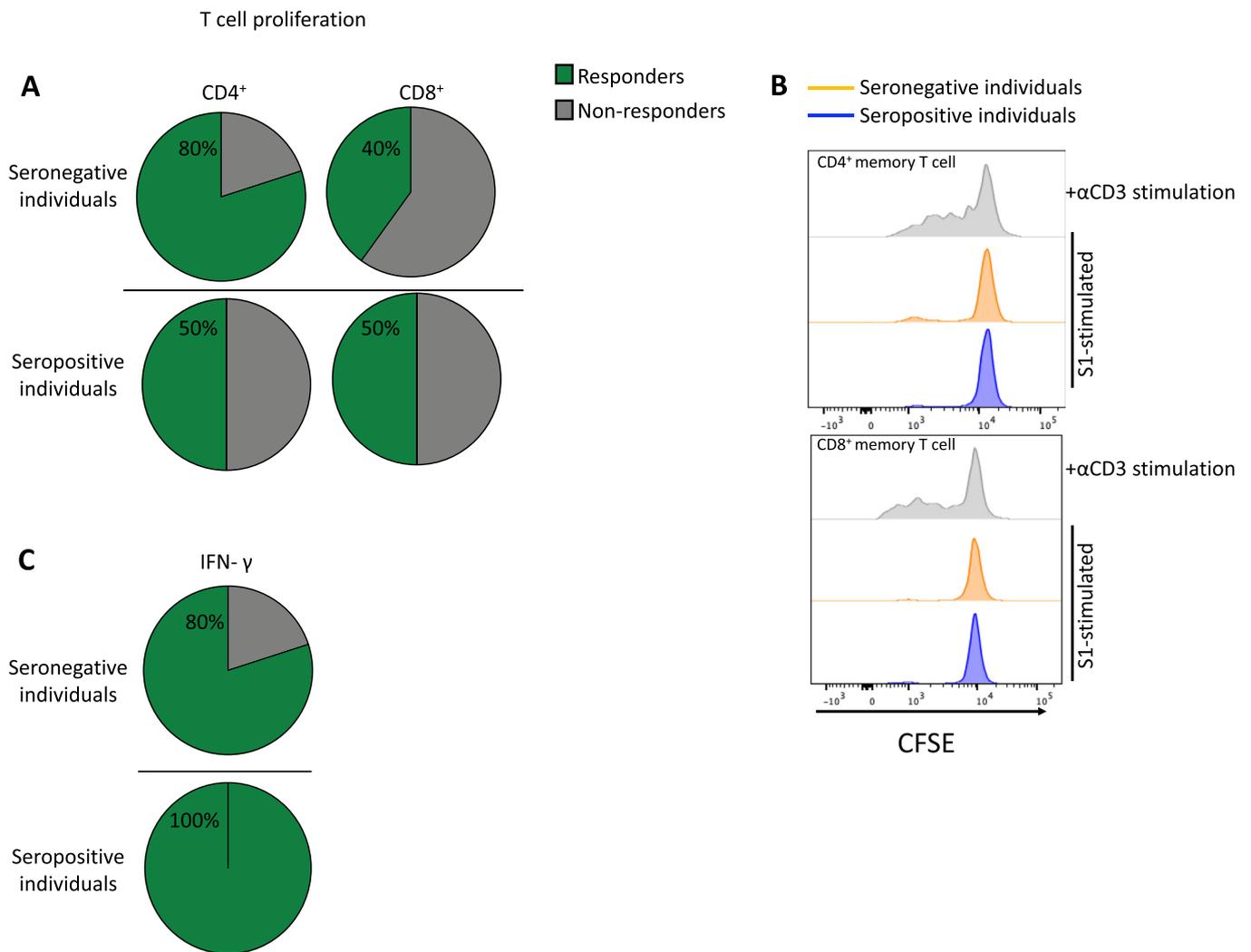


Fig. 1. Functional analyses of memory T cells. (A) Monocyte-derived dendritic cells loaded with S1 spike glycoprotein were co-cultured with purified memory T cells from seronegative and seropositive elderly individuals following SARS-CoV-2 vaccination. Individuals whose memory T cells responded S1 spike glycoprotein were classified as responders. The percentages of responders and non-responders were calculated. (B) According to the change in proliferation percentages, representatives of flow cytometry histograms were given. (C) Individuals whose memory T cells produced IFN- γ were classified as responders. The percentages of responders and non-responders were calculated.

Table 1
T cell % proliferation change and IFN- γ levels according to anti-SARS-CoV-2 IgG antibody response groups.

	Median (IQR) days since 2nd dose of vaccine (day)	IgG spike antibody titers (U/ml)	The change in CD4+ memory T cell proliferation median (range)	The change in CD8+ memory T cell proliferation median (range)	IFN- γ production median (range) (pg/mL)
Seronegative group (n = 10)	86 (35.25)	0.5 (0.16)	31.2 (55.85)	0 (7.28)	173 (1586)
Seropositive group (n = 4)	77.5 (59.25)	99.4 (103.53)	7.21 (15.25)	8.24 (78.3)	551.5 (2455)

Table 2
Correlation of T cell % proliferation changes and IFN- γ levels with age and frailty, regardless of antibody response (n = 14).

	Age (year)	FRAIL scale	CFS	CCI
CD4+ T cell % proliferation change	<i>p</i> = 0.033* <i>r</i> = -0.673	<i>p</i> = 0.343	<i>p</i> = 0.755	<i>p</i> = 0.532
CD8+ T cell % proliferation change	<i>p</i> = 0.268	<i>p</i> = 0.684	<i>p</i> = 0.044* <i>r</i> = -0.545	<i>p</i> = 0.809
IFN-γ levels (ng/ml)	<i>p</i> = 0.693	<i>p</i> = 0.029* <i>r</i> = -0.604	<i>p</i> = 0.049* <i>r</i> = -0.535	<i>p</i> = 0.163

CFS: Clinical frailty scale. CCI: Charlson Comorbidity index
* Spearman correlation

cells [17]. Severe COVID-19 can lead to T cell dysfunction, and T cell apoptosis [18]. Also, it was observed that T-SPOT responses to Spike, Membrane, and Nucleoprotein were higher in seropositive individuals, and there was a little difference in T-SPOT Envelope, and Structural protein responses between seropositive, and seronegative individuals [19]. In a study of convalescent patients with COVID-19, T-cell responses were observed, despite undetectable SARS-CoV-2 IgG levels [20].

Studies with double-dose mRNA vaccines have shown that SARS-CoV-2 specific antibodies are significantly reduced 3 months after the vaccination and a booster dose of vaccine is recommended based on short-lived humoral immune response [21]. Therefore, T cell response is of interest for long-term protection against the virus, over the period of decrease in humoral immunity, in particular.

In our study, the higher CD4⁺ memory T-cell response in the seronegative group suggest a discordance between humoral, and cellular immunity. The fact that approximately 80 days had passed since completion of vaccination among study is a promising finding for protection potential of inactivated vaccine at/after the gradual decrease in humoral immunity. Memory T cell response peaks at approximately 120 days from the symptom onset and this response is sustained 10 months [5]. Accordingly, the apparent increase in the CD4⁺ memory T cell proliferation observed among seronegatives in our study, might suggest an underestimation of the true cellular response among vaccinated yet seronegative individuals. The limited size of the study population, inability for adjustment for age, gender, and other potential confounders across the study groups warrant further research on humoral, and cellular immune response after vaccination with CoronaVac in future studies. More importantly, periodical assessment of cellular, and humoral response in prospective cohorts, over time passed after vaccination, would be crucial for a thorough understanding of immune response, and its association with breakthrough COVID-19 infections [22,23].

Aging may influence the course of viral infections, and vaccine responses may decline over years. Immunosenescence, and inflammaging can lead to a chronic low-intensity inflammation, with decline in functionality, and availability of T cell, and B cell populations in older adults. Also, age related thymic involution reduces the output of naive T cell, and T-cell receptors (TCR). Appraisal of vaccine effectiveness using antibody titers, antibody isotypes, and the ability of the immune system to neutralise pathogens may cause underestimation of the true status in older adults. With advancing age, there is a reduction in naive T cells; CD4/CD8 cells ratio becomes higher; a loss of T cell receptor diversity, and reduced T cell survival can be observed. Altogether, these lead to impaired response to vaccine among elderly [1]. With increasing age, IFN- γ production also decreases, making lung epithelium vulnerable to viral infections [24–26]. In our study, we found that CD4⁺ Memory T cell responses decreased with age, regardless of seropositivity.

Frailty is a multi dimensional condition that reduces individual's response to intrinsic/extrinsic stressors. In our study, we assessed the frailty with the FRAIL and Clinical Frailty Scales (CFS). The FRAIL scale is based on physical frailty that consists of five self-reported components, and CFS is used for assessing cumulative frailty. The effect of frailty on the immune system is obvious. Studies have provided elevated IL-6, and CRP levels in frail older adults. Also relationship of frailty with increased WBC counts (as well as neutrophil, and monocyte levels) have been clarified. There are studies showing higher counts of CD8⁺ Memory T cell, and lower CD4⁺ / CD8⁺ Memory T cell ratio among frail women. High frailty scores are known to be associated with high CCR5⁺ T cell counts, which contribute significantly to several inflammatory conditions [27].

In frail older individuals, reduced vaccine response was reported for pneumococcal and influenza vaccines [28,29] but a similar information is missing for SARS-CoV-2 vaccines. In the literature, it has been shown that frail older adults are more susceptible to COVID-19 and mortality rates are higher [30]. A study published from Turkey revealed lower frequency of antibody positivity in frail geriatric patients after two-dose scheduled vaccination with inactive vaccine (CoronaVac) [31]. Our

finding of a statistically significant negative association between frailty scores and CD8⁺ Memory T cell proliferation, and IFN- γ levels suggest a need for considering frailty for its potential effects on immune responses of older adults, besides their biological ages. Future studies are clearly necessary for a thorough evaluation of vaccine effectiveness across age groups, considering a wide array of potential confounders, including frailty. The main limitation of this study is the modest sample size but there are limited number of studies in the literature evaluating T cell responses, and frailty status in older individuals considering their humoral response.

Conclusion

A T-cell heterogeneity in seronegative older adults following full vaccination with inactive CoronaVac vaccine was revealed in our study, supporting relevant literature. Statistically significant negative association between frailty, and CD8⁺ Memory T cell response, and IFN- γ levels underlines the importance of considering frailty rather than biological age in evaluating immune response in older adults. Therefore, frail individuals should be prioritized in COVID-19 vaccinations, and earlier administration of booster doses should be motivated. Prospective, comprehensive studies in large groups, with immunological analyses are clearly warranted for better understanding of the effectiveness of COVID-19 vaccine candidates, and a thorough understanding of the effects of SARS-CoV-2 infection on immune system.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRedit authorship contribution statement

Merve Hafizoğlu: Investigation, Visualization, Formal analysis, Writing – review & editing. **Arzu Okyar Bas:** Investigation, Visualization, Formal analysis. **Ece Tavukçuoğlu:** Conceptualization, Resources. **Zeynep Sahiner:** Investigation, Visualization, Formal analysis. **Merve Güner Oytun:** Investigation, Visualization, Formal analysis. **Sıla Ulutürk:** Conceptualization, Resources. **Hamdullah Yanık:** Conceptualization, Resources. **Burcu Balam Doğu:** Conceptualization, Writing – review & editing. **Mustafa Cankurtaran:** Conceptualization, Writing – review & editing. **Güneş Esendağlı:** Conceptualization, Resources. **Filiz Akbıyık:** Conceptualization, Resources. **Banu Çakır:** Conceptualization, Writing – review & editing. **Serhat Ünal:** Conceptualization, Resources. **Meltem Gülhan Halil:** Conceptualization, Writing – review & editing, Supervision.

Data Availability

The authors do not have permission to share data.

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Supplementary materials

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