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Short Communication

Altered pre-existing SARS-CoV-2-specific T cell responses in elderly individuals

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A B S T R A C T

Pre-existing SARS-CoV-2-specific T cells, but not antibodies, have been detected in some unexposed individuals. This may account for some of the diversity in clinical outcomes ranging from asymptomatic infection to severe COVID-19. Although age is a risk factor for COVID-19, how age affects SARS-CoV-2-specific T cell responses remains unknown. We found that pre-existing T cell responses to specific SARS-CoV-2 proteins, Spike (S) and Nucleoprotein (N), were significantly lower in elderly donors (>70 years old) than in young donors. However, substantial pre-existing T cell responses to the viral membrane (M) protein were detected in both young and elderly donors. In contrast, young and elderly donors exhibited comparable T cell responses to S, N, and M proteins after infection with SARS-CoV-2. These data suggest that although SARS-CoV-2 infection can induce T cell responses specific to various viral antigens regardless of age, diversity of target antigen repertoire for long-lived memory T cells specific for SARS-CoV-2 may decline with age; however, memory T cell responses can be maintained by T cells reactive to specific viral proteins such as M. A better understanding of the role of pre-existing SARS-CoV-2-specific T cells that are less susceptible to age-related loss may contribute to development of more effective vaccines for elderly people.

1. Introduction

There is extensive individual variation in severity of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), ranging from asymptomatic infection to fatal pneumonia [1]. Various factors, including age, sex, and comorbidities such as obesity and diabetes, influence the risk of severe COVID-19 [2–4]. For example, morbidity and mortality among the elderly are significantly higher than among the young [2]. Consideration of protective measures for individuals vulnerable to COVID-19 should be particularly important to control the pandemic [5]. However, cellular and molecular bases of variable risk of COVID-19 disease remain poorly understood.

T cells are assumed to mediate both protective and pathogenic immune responses to SARS-CoV-2 infection [6, 7]. The magnitude and quality of T cell responses induced by SARS-CoV-2 infection are highly heterogeneous and are likely associated with COVID-19 clinical outcomes. For example, SARS-CoV-2-specific T cell numbers and their interferon-γ (IFN-γ) expression in severe COVID-19 patients are lower than in mild COVID-19 patients [8, 9]. Furthermore, asymptomatic COVID-19 patients tend to have increased SARS-CoV-2-specific T cells expressing higher levels of IFN-γ compared to symptomatic patients [10]. This individual variation in T cell responses may be partly explained by heterogeneity in levels of pre-existing SARS-CoV-2-reactive T cells.

Some individuals who have not been exposed to SARS-CoV-2 have nonetheless acquired SARS-CoV-2-reactive T cells, probably through exposure to other common cold coronaviruses [11–13]. Pre-existing CD4 and CD8 memory T cells, specific to various SARS-CoV-2 proteins, including the structural proteins, Spike (S), Membrane (M), and Nucleoprotein (N), have been detected with significant individual variation, and these pre-existing SARS-CoV-2-reacive T cells are likely associated with immune protection against COVID-19 [14]; however, in other cases, they may exacerbate COVID-19 severity [15, 16]. As many of the current vaccines express the SARS-CoV-2 S protein, only pre-existing S-reactive T cells are activated by these vaccines [17, 18]. Several studies have reported age-related differences in SARS-CoV-2-specific T cell

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responses in COVID-19 patients [19,20]; however, the effect of age on pre-existing SARS-CoV-2-reactive T cells remains unknown.

In this study, we compared frequencies of T cells reactive to SARS-CoV-2 S, N, and M antigens between young and elderly donors. The relatively elderly Okinawan population, and the moderate rate of SARS-CoV-2 infection in Okinawa, allowed us to examine pre-existing SARS-CoV-2-specific T cells in a cohort of elderly (>70 years old) individuals. We found that pre-existing T cell responses to S and N antigens are significantly impaired in elderly donors compared to young donors, but a proportion of elderly donors exhibit significant, high levels of M-reactive T cell responses. These data provide new insights into age-related alteration of pre-existing SARS-CoV-2-specific T cells.

2. Methods

2.1. Subjects

The study design was approved by the Okinawa Institute of Science and Technology, Graduate University (OIST) human subjects ethics committee (applications HSR-2020–024, HSR-2020–028). All donors provided informed written consent. Young (20 to 50 years of age, $n = 66$) and elderly volunteers (over 70 years of age, $n = 52$) were recruited in Okinawa, Japan, between October 2020 and April 2021. 90 unexposed donors (48 young and 42 elderly) had no history of COVID-19, while 28 recovered COVID-19 patients (18 young and 10 elderly) tested positive by PCR for COVID-19 1–3 months before blood collection.

2.2. Peripheral blood mononuclear cells (PBMCs) and plasma isolation

Blood samples were collected in heparin-coated tubes (TERUMO; VP-H100K). PBMCs and plasma were separated using Leucosep tubes pre-filled with Ficoll-Paque Plus (Greiner; 163,288). After adding 5 mL of blood and 3 mL of AIM-V medium (Thermo; 12,055,091), Leucosep tubes were centrifuged at 1000 g at room temperature for 10 min. The white layer containing PBMCs was collected, washed with 10 mL AIM-V medium and centrifuged for 7 min at 600 g, followed by a second washing with centrifugation for 7 min at 400 g. PBMC pellets were re-suspended in 500 μL CTL test medium (Cellular Technology Limited (CTL); CTLT-010). Fresh PBMCs were used for IFN-γ ELISpot assays.

2.3. Immunochromatographic SARS-CoV-2 antibody test

Plasma from each donor was first tested using Cellex qSARS-Cov-2 IgG/IgM Cassette Rapid Tests (Cellex 5513C). Plasma from donors who had positive test results in the first test was subsequently retested using SARS-CoV-2 Antibody Detection Kits (KURABO RF-NC001, RF-NC002).

2.4. IFN-γ ELISpot assay

Peptide pools for SARS-CoV-2 S (JPT; PM-WCPV-S-1), N (Miltenyi;130–126–698), and M (Miltenyi;130–126–702) proteins dissolved in DMSO (500 μg/mL for S) or water (50 μg/mL for N and M) were used for cell stimulation. IFN-γ ELISpot assays were performed using Human IFN-γ Single-Color Enzymatic ELISpot kits (CTL; hIFNgp-2 M), according to the manufacturer's instructions. Briefly, freshly isolated PBMCs (1–4 $\times$ 10^5 cells per well) were stimulated with 1 μg/mL peptide solutions for each SARS-CoV-2 protein for 18–20 h. For each sample analysis, negative controls (cells treated with equimolar amounts of DMSO) and positive controls (cells treated with 20 ng/mL phorbol 12-myristate 13-acetate (PMA) and 100 ng/mL ionomycin) were included. After incubation, plates were washed and developed with detection reagents included in the kits. Spots were counted using a CTL ImmunoSpot S6 Analyzer. Antigen-specific spot counts were determined by subtracting background spot counts in a negative control well from wells treated with peptide pools. If >30 spot forming units (SFU)/10^6 PBMCs in the negative control well or <30 SFU/10^6 PBMCs in the positive control well were detected, those sample data were excluded from analysis. 26 SFU/10^6 PBMCs (the mean of negative control SFU + 3 standard deviations) was used as the cut-off for positivity in the ELISpot test.

2.5. Statistical analysis

Mann-Whitney U tests were performed using GraphPad Prism 9.1.0 software. Statistical details are provided in figure legends.

3. Results

3.1. Age-related differences in SARS-CoV-2-specific T cell responses

To assess whether there are age-related differences in SARS-CoV-2-specific T cell responses, we collected peripheral blood from young (20 to 50 years of age) and elderly (>70 years of age), unexposed donors and recovered COVID-19 patients in Okinawa between October 2020 and April 2021. 0% (0/109) of unexposed donors and 53.5% (15/28) of recovered COVID-19 patients were consistently tested positive for anti-SARS-CoV-2 antibodies in two different immunochromatographic tests.

We first compared SARS-CoV-2-specific T cell responses between age groups by Interferon-γ (IFN- γ) ELISpot assays using freshly purified peripheral blood mononuclear cells (PBMCs) stimulated with each of 4 peptide pools covering the major viral structural proteins [N-terminal S (S-1), C-terminal S (S-2), Nucleoprotein (N), or Membrane (M)]. When we tested unexposed individuals with stimulation of S-2 and N peptide pools, we detected IFN-γ spot-forming units (SFU) over a positivity cut-off (26 SFU/10^6 PBMCs) in a substantial proportion of young, but not elderly donors (13.0% vs 0% for S2 and 36.9% vs 8.8% for N) (Fig. 1A). Consistent with this, frequencies of S-2- and N-reactive T cells were significantly lower in elderly than in young persons in the unexposed group (Fig. 1A), suggesting age-related defects in acquisition or maintenance of pre-existing memory T cells specific to S-2 and N antigens. However, there was no remarkable difference in the percentages of positive test results for M-specific T cell responses (32.6% vs 35.3%) and the frequency of M-reactive T cells between unexposed young and elderly donors (Fig. 1A). Only a few unexposed donors (4.3% young and 2.9% elderly) tested positive for T cell responses to the S-1 peptide pool (Fig. 1A). PMA and ionomycin stimulation induced comparably high IFN-γ expression in PBMCs from donors who tested positive and negative for T cell responses to SARS-CoV-2 antigens (data not shown).

Consistent with previous studies [11], percentages of positive test results for T cell responses to S, N and M were higher in recovered COVID-19 patients than in unexposed donors (Fig.1B). 58.6% (27 out of 46) of young unexposed donors, 41.1% (14 out of 34) of elderly unexposed donors, 92.3% (12 out of 13) of young recovered COVID-19 patients, and 100% (9 out of 9) of elderly recovered COVID-19 patients tested positive for T cell responses to at least one of the antigens we tested. In contrast to the unexposed group, frequencies of not only M-specific T cells, but also S- and N-specific T cells were comparable between young and elderly donors in the recovered COVID-19 patient group (Fig. 1B).

We next analyzed intraindividual immunodominance of each SARS-CoV-2 antigen in the donors who tested positive for T cell responses to SARS-CoV-2. 33.3% (9 out of 27) of young and 14.2% (2 out of 14) of elderly unexposed donors who tested positive exhibited T cell responses to multiple viral antigens (Fig. 2A). Remarkably, only M-reactive T cells were detected in the majority of elderly unexposed donors who tested positive (Fig. 2A). In contrast, both young and elderly recovered COVID-19 patients who tested positive exhibited mixed T cell responses to multiple viral antigens (Fig. 2B). Taken together, these data suggest that although SARS-CoV-2 infection can induce comparable T cell responses to various viral antigens in both young and elderly individuals, pre-existing memory T cell responses specific to SARS-CoV-2 S and N antigens are impaired in elderly people, while M-specific memory T cell responses are maintained.,

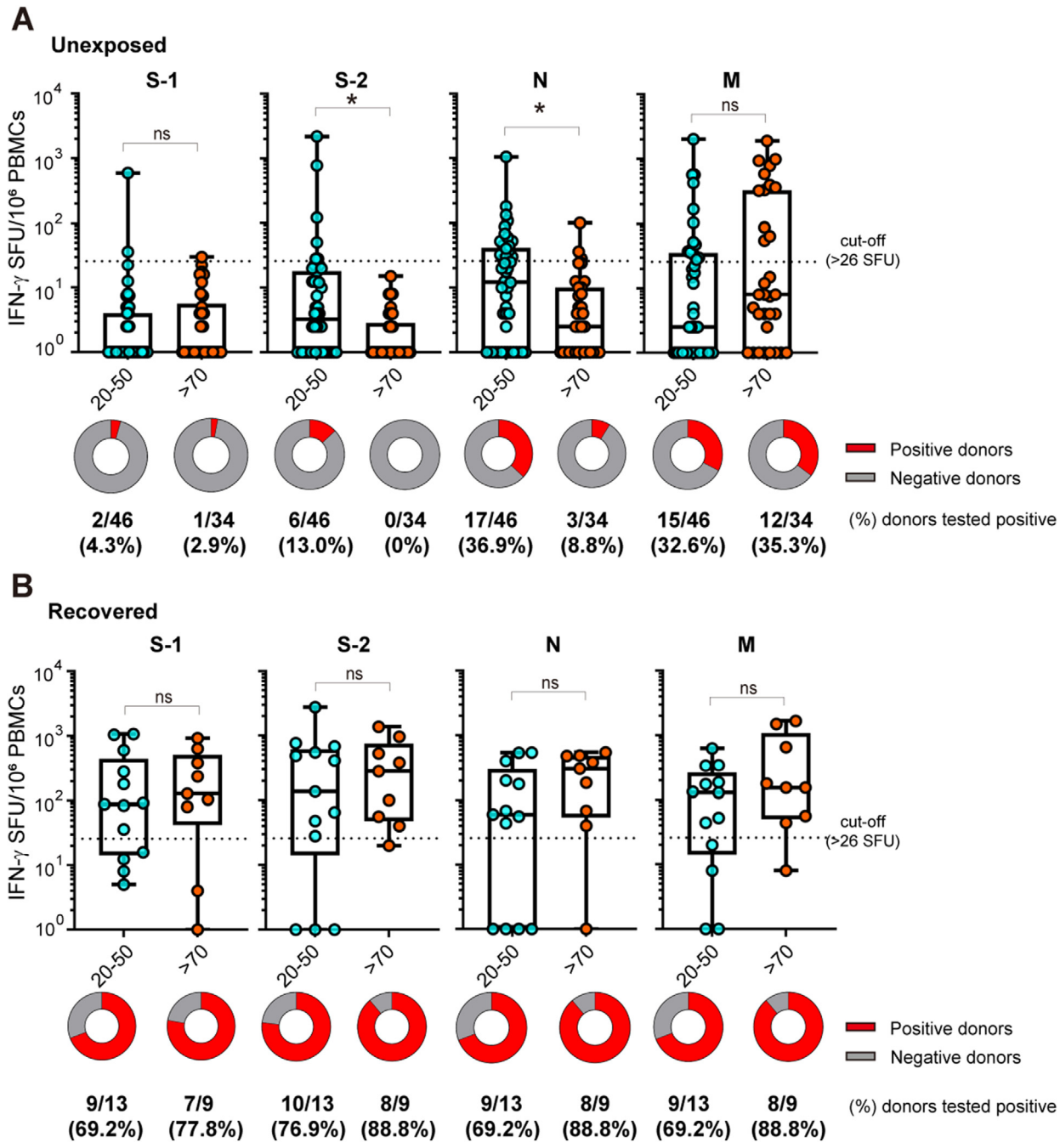

Fig 1. Altered pre-existing T cell responses to SARS-CoV-2 structural proteins in elderly donors. PBMCs isolated from young (20–50 years of age) and elderly (>70 years of age), unexposed donors (A) and recovered COVID-19 patients (B) were stimulated with peptide pools for SARS-CoV-2 S, N, and M proteins and subjected to IFN-γ ELISpot analysis. Spot-forming units (SFU) representing the frequency of IFN-γ-secreting cells in young unexposed donors ($n = 45$), young recovered COVID-19 patients ($n = 35$), elderly unexposed donors ($n = 14$), and elderly recovered COVID-19 patients ($n = 10$) are shown. Box-and-whisker plots show quartiles and range. Circle below shows the percentage of donors who tested positive for T cell responses to each peptide pool. Statistical comparisons between age groups utilized the Mann-Whitney test. $^{*}p<0.05$, ns: not significant.

4. Discussion

Our data indicate that a fraction of elderly donors possess significantly high levels of pre-existing SARS-CoV-2 M-specific T cell responses, though the frequency of pre-existing SARS-CoV-2 S- and N-specific T cells is lower in elderly than young donors. Other recent studies have also reported an age-related decline of S-specific pre-existing CD4 T cells [18, 21]. On the other hand, another study reported that there are no significant differences in the frequency of the pre-existing CD4 and CD8 T cells that are responsive to mixed peptide pools covering S, N, and M proteins between young and elderly donors, although the SARS-CoV-2-responsive naïve CD8 T cells are less numerous in elderly than young [22]. These data suggest that pre-existing T cells specific for SARS-CoV-2 are heterogeneously affected by age in a target antigen-dependent manner and that diversity of the target antigen repertoire for pre-existing T cells may decline with age, but the magnitude of pre-existing T cell responses can be maintained with T cells specific to certain viral proteins, such as M.

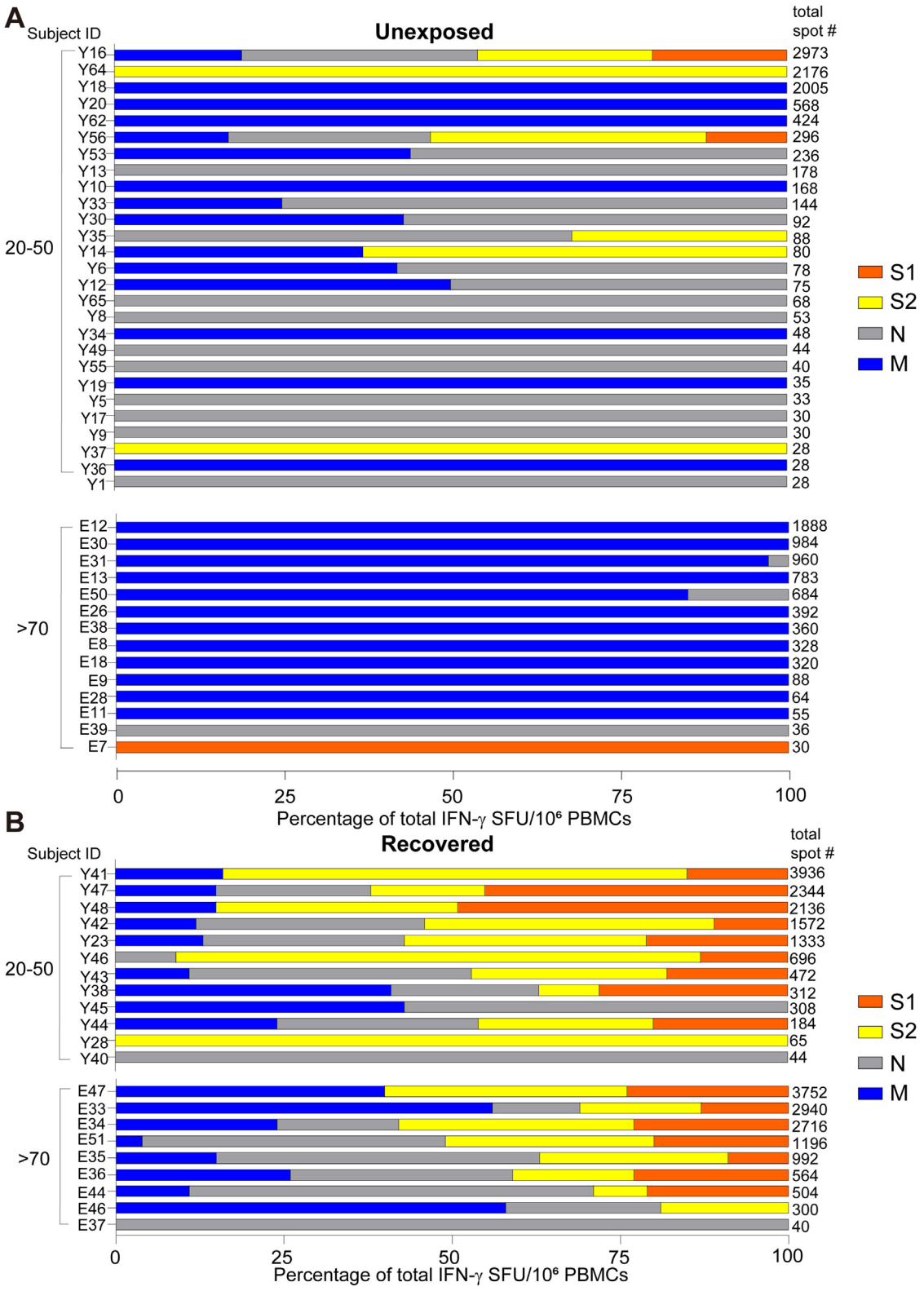

Fig 2. M-specific pre-existing T cell responses predominate in elderly donors. Ratios of spots formed by cells stimulated with SARS-CoV-2 S, N, and M peptide pools in ELISpot data (Fig. 1) were analyzed in unexposed donors (A) and recovered COVID-19 patients (B) donors who tested positive for T cell responses to at least one of the peptide pools.

As the frequency of pre-existing T cells specific to viral structural proteins S, N, and M is likely associated with protection from SARS-CoV-2 infection [14], focused M-specific T cell responses might be particularly important for protection of elderly individuals who have lower T cell responses to S and N. We speculate that pre-existing M-specific T cells provide protection in SARS-CoV-2 infection by mediating cellular immunity through IFN-γ production and by providing T cell help to S- and N-specific B cells via linked recognition. However, we cannot exclude the possibility that pre-existing M-specific T cells are harmful for some elderly individuals. Several studies suggest that pre-existing SARS-CoV-2-specific T cells are detrimental in COVID-19 [23]. In particular, the frequency of M-specific T cells in COVID-19 patients is thought to be a risk factor, as it is correlated with age and severity of disease [8], although how pre-existing M-specific T cells affect magnitude, kinetics and functions of M-specific T cell responses in COVID-19 patients remains unclear. Thus, both protective and pathogenic functions of pre-existing M-specific T cells can be speculated. A longitudinal comparison of susceptibility and symptom severity of COVID-19 between individuals with and without high pre-existing M-specific T cell responses may provide insights into this issue.

Despite defects in pre-existing T cell responses to S and N, most elderly donors who recovered from mild COVID-19 had abundant T cells specific to S and N antigens at levels comparable to those of young donors, suggesting that elderly individuals can induce T cell responses against S and N antigens upon SARS-CoV-2 infection. However, the relationship between age-related alteration of pre-existing T cells and T cell responses during infection in patients with diverse clinical outcomes of COVID-19 should be investigated in a larger, statistically valid test population. Furthermore, whether diverse SARS-CoV-2-induced T cell clones can mediate long-lasting memory responses and how they are affected by age should be addressed in future studies.

Interestingly, SARS-CoV-1 infection induces long-lasting (>11 years) CD8 memory T cells specific to $M_{141–155}$ peptide [24]. SARS-CoV-2 $M_{141–155}$ peptide shows high homology (80% amino acid sequence identity) with SARS-CoV-1 $M_{141–155}$ peptide and can be targeted by CD4 T cells in convalescent COVID-19 patients [25], suggesting that SARS-CoV-2 $M_{141–155}$ may also be a potent inducer of long-lasting memory T cells.

What induces pre-existing M-specific T cells? Common cold coronaviruses may induce pre-existing SARS-CoV-2-specific T cell [26, 27]. Amino acid sequence identity between SARS-CoV-2 and other common cold coronaviruses is relatively high for M (NL63: 27.9%, OC43: 38.6%), S-1 (NL63: 14.8%, OC43: 19.8%), S-2 (NL63: 31.8%, OC43: 39.9%), and N (NL63: 25.1%, OC43: 33.0%)[21]. Importantly, several peptides derived from these coronaviruses are cross-reactive with SARS-CoV-2-specific pre-existing T cells [27]. The decrease of memory T cells specific to common cold coronavirus S proteins in elderly donors [21] can account for the decrease of SARS-CoV-2-specific pre-existing T cells. We speculate that memory T cells specific to common cold coronavirus M proteins, which are cross-reactive to SARS-CoV-2 M, might be resistant to age-related decline.

It is worth considering the potential of novel COVID-19 vaccines to induce M-specific immunity. Current vaccine strategies are to induce S-specific antibody and T cell responses [28,29]. Recent studies reported a correlation between the frequency of pre-existing S-specific T cells and vaccine-induced S-specific T cell responses [18], which suggests a role of pre-existing S-specific T cells in cognate T cell help. However, elderly individuals likely would not benefit fully from pre-existing S-specific T cells. To enhance vaccine efficacy among the elderly, it might be reasonable to consider a strategy to induce not only S-specific, but also M-specific immunity, using vaccines based on inactivated viruses or M-fused S antigens. Linked recognition of M-specific T helper cells by S-specific B cells can promote S-specific antibody production by overcoming the defect of cognate T cell help in elderly individuals. Further characterization of M-specific T cells in young and elderly may provide new insights into vaccine-induced immunity that is less affected by age.

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