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γ/δ T cell subsets in human aging using the classical α/β T cell model

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ABSTRACT

Aging is associated with an increased susceptibility to infections and diseases. It has also been associated with reduced functionality and altered distribution of immune cells, especially T cells. Whereas classical α/β T cells, especially CD8$^+$ T cells, were shown to be highly susceptible to aging, the effects of viral persistent stimulations on the fate of γ/δ T cells are much less documented. Healthy, elderly individuals of Chinese ethnical background were recruited under the aegis of SLAS-II. In this observational study, γ/δ T cell populations were characterized by flow cytometry and compared with the α/β CD4$^+$ and CD8$^+$ T cells in elderly and young controls. In our study, we identified a reduced frequency of γ/δ T cells but not α/β T cells with aging. The classical markers of α/β T cell aging, including CD28, CD27, and CD57, did not prove significant for γ/δ T cells. The extreme range of expression of these markers in γ/δ T cells was responsible for the lack of relationship between γ/δ T cell subsets, CD4/CD8 ratio, and anti-CMV titers that was significant for α/β T cells and, especially, CD8$^+$ T cells. Although markers of aging for γ/δ T cells are not clearly identified, our data collectively suggest that the presence of CD27 γ/δ T cells is associated with markers of α/β T cell aging. *J. Leukoc. Biol.* 96: 647–655; 2014.

Introduction

Elderly individuals (>65 years old) are usually of lower resilience and are more vulnerable to physical, physiological, metabolic, and psychological challenges [1], which result in a higher susceptibility to diseases and infections [2]. Ryan et al. [3] demonstrated that vaccination remarkably reduced the adverse effects of influenza infection to such an extent that mortality is markedly reduced. However, this intervention is not comprehensive, as some elderly individuals will not respond to flu vaccination [4]. This hyporesponsiveness and the increased susceptibility to infections strongly suggest that the immune response is compromised in the elderly [5, 6]. This age-associated loss in immunity, known as immune senescence, affects both innate and adaptive immune systems [7]. A phenotypic hallmark of immune senescence is the loss of expression of CD28 and other coreceptors in T cells [8, 9]. Notably, persistent infection with CMV may result in hastened aging of the immune system [10]. Individuals with a history of infection with CMV, EBV, or HIV have an increased frequency of cells expressing receptors, such as CD57, KLRG-1, or programmed cell death-1, which exhibit poor proliferation and/or increased exhaustion [11–13]. In the National Health and Nutrition Examination Survey longitudinal study, CMV seropositivity was associated to "all-cause" of mortality, independently of age [14]. Other longitudinal studies suggest that CMV infection associates to the IRP, a cluster of immunological parameters that predicts mortality over 2, 4, and 6 years [15]. The IRP applies to the elderly only and includes CMV seropositivity, CD4/CD8 ratio, frequency of CD8$^+$CD28$^-$ T cells, and B cell number.

Most reports on the role of T cells in immune senescence focus on more abundant classical α/β T cells. The less abundant subset, γ/δ T cells, represents 5–10% of the whole T cell population and is characterized by its restricted antigenic recognition [16]. This restricted recognition highlights its potential role to bridge innate and adaptive immunity [17]. The presence of certain classes of phospho-antigens will induce a rapid response of γ/δ T cells [18]. Experimental models revealed that on Mycobacterium infection, the first cells to reach the lung are the γ/δ T cells, whereas the α/β T cells reach the site of infection later [19]. Despite its importance, the differentiation of this γ/δ T cell population is not well-demonstrated [20]. A major limitation is that classical models accepted for α/β T cells might not be useful for the γ/δ subset.

Abbreviations: APC=allophycocyanin, CRP=C-reactive protein, FSC=forward-scatter, IRP=immune risk profile, KLRG-1=killer-lectin receptor G-1, SLAS=Singapore Longitudinal Aging Study, SSC=side-scatter, T_{CM}=central memory T, T_N=naive T, T_{TE}=terminal effector T

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For example, Vrieling et al. [21], showed that migration of γ/δ T cells was CCR7-independent and may rely on the CD62 ligand instead. Hence, the CCR7/CD45RA model for the definition of naive and memory in classical α/β T cells might not be relevant for the γ/δ T cells. Additionally, the frequency of γ/δ T cells also varies with age, ethnicity, and approach used, and hence, results must be interpreted considering these confounders [22–25].

In the present study, we wanted to evaluate (1) the impact of aging on the frequency of γ/δ T cells, (2) whether these cells share similarities with α/β T cells in terms of coreceptor expression with aging, and (3) how γ/δ T cell subsets are linked to biomarkers associated to persistent stimulation of the immune system.

MATERIALS AND METHODS

Subjects

Elderly individuals were participants from the SLAS. The characteristics of the home-dwelling, healthy elderly participants (>65 years old) of Chinese background are provided in **Table 1**. Elderly participants lived in a very similar environment (nutritional, physical activity, socioeconomic status). The participants were also screened for cognitive functions, and individuals with a Mini-Mental State Examination <23 (classified as cognitively impaired) were excluded [26]. Many chronic conditions may coexist in this population, namely, hypertension, high cholesterol, and diabetes. Four donors display mild neutrophilia, but those with elevated CRP values (>3 mg/L) were excluded from the analysis. Healthy, young participants of Chinese background were recruited from the National University of Singapore students and staff (age 23–35 years old). Individuals with abnormal CRP values were excluded. The study has been approved by the National University of Singapore-Institutional Review Board 04–140, and all participants gave written, informed consent.

Sample preparation

Blood from overnight fasting participants was drawn in CPT tubes (BD Biosciences, San Jose, CA, USA), and after centrifugation at 1650 rpm for 20 min at room temperature, plasma and PBMCs were collected. Plasma was stored in −80°C, whereas PBMCs were washed twice in PBS and cryopreserved in liquid nitrogen. Freezing was performed by keeping cells in 90% FBS containing 10% DMSO. The day of experiments, cryo-vials were thawed rapidly and washed extensively with PBS containing 10% FBS. Samples usually offer a recovery >75%, with no specific loss of immune population [27] Viability was >95%, as tested by trypan blue exclusion. Whole blood samples were used to measure the CD4/CD8 ratio [15]. When compared with frozen samples, the absolute value for CD4/CD8 was different but represented a good linearity with fresh whole blood (data not shown).

TABLE 1. SLAS Participants' Characteristics

Parameter	Mean ± SD
Age (years)	68.0 ± 6.9
Gender	33 M/50 F
History of hypertension	36/83
History of diabetes	16/83
History of cholesterol	31/83
Hemoglobin (g/dL)	13.18 ± 1.67
Glucose fasting (mmol/L)	5.45 ± 1.60
Triglycerides (mmol/L)	1.36 ± 0.54
HDL cholesterol (mmol/L)	1.39 ± 0.40
LDL cholesterol (mmol/L)	3.12 ± 1.01
White blood cells ($\times 10^9$/L)	6.89 ± 2.15
Red blood cells ($\times 10^{12}$/L)	4.44 ± 0.50
Platelets ($\times 10^9$/L)	251.3 ± 81.1
Neutrophils ($\times 10^9$/L)	3.79 ± 1.55
Lymphocytes ($\times 10^9$/L)	2.29 ± 0.75
Monocytes ($\times 10^9$/L)	0.53 ± 0.23
CMV serostatus	9.27 ± 5.12 IU/ml (100% CMV+)

ELISA and Luminex

Frozen plasma samples were thawed and diluted 1:100 in appropriate buffer. Seropositivity to CMV was tested by ELISA (Genesis Diagnostics, McAllen, TX, USA), as performed elsewhere [28]. For semiquantitative determination of anti-CMV IgG, standards of 0 IU/ml, 3 IU/ml, 10 IU/ml, and 30 IU/ml were used. Concentrations <3 IU/ml are considered negative for anti-CMV IgG. Values >3 IU/ml are regarded as positive. No value was >30 IU/ml (saturation). CRP was measured by Luminex in 1:5 dilute plasma with appropriate standards, per the manufacturer's recommendation (Bio-Rad Laboratories, Hercules, CA, USA).

Flow cytometry staining

PBMCs were counted and allowed to rest for 2 h in FACS buffer (PBS containing 10% FCS, 5 mM EDTA, and 2 mM azide). For each staining, 1×10^6 PBMCs were used. The staining includes the following markers: CD3-PE-Cy5.5 (Beckman Coulter, Brea, CA, USA), CD4-PE-Cy7 (BioLegend, San Diego, CA, USA), CD8-APC-Cy7 (BD Biosciences), pan-γ/δ-PE (BioLegend), CD45RA-eFluor605 (eBioscience, San Diego, CA, USA), CD57-Pacific Blue (BioLegend), CD28-PE-Texas Red (Beckman Coulter), and CD27-APC (BioLegend). All staining performed included a Live/Dead marker (Invitrogen, Carlsbad, CA, USA). The gating strategy used for this study is shown in **Fig. 1**. The CD4/CD8 ratio was calculated from 100 μl whole blood, stained with CD3-PE-Cy5.5, CD4-PE-Cy7, and CD8-APC-Cy7, followed by incubation with red cell lysis buffer (eBioscience).

Data analysis

Flow cytometry data were analyzed using FlowJo (Treestar, Ashland, OR, USA), FACSDiva (BD Biosciences), and Kaluza (Beckman Coulter). Samples were compared using GraphPad Prism software (GraphPad, La Jolla, CA, USA). Groups of young and elderly were compared by Mann-Whitney test, and data shown are mean ± SD. Analysis with $P < 0.05$ was considered significantly different between the groups. Spearman's correlations were tested for CMV titer and CD4/CD8 ratio versus T cell subsets.

RESULTS

Perturbation in the main T cell subsets with aging

Age-associated changes in T cell subsets were tested by flow cytometry with CD28, CD57, CD27, and CD45RA markers in $CD4^+$, $CD8^+$, and γ/δ T cells, as shown in Fig. 1A. Lymphocytes were gated based on a FSC/SSC profile and doublets/dead cell exclusion. The cells were gated on the CD3+ population, followed by γ/δ+ cell identification using a pan-γ/δ antibody. The CD3+ γ/δ− population was considered as the $CD4^+$ and $CD8^+$ α/β populations (Fig. 1A). Figure 1 shows a significant increase in the CD4/CD8 ratio with aging, which is likely a result of reduced frequency of $CD8^+$ T cells and increased frequency of $CD4^+$ T cells (Fig. 1B; $P<0.0001$). The frequency of CD3+ bearing the γ/δ TCR was screened, and a typical staining in young and elderly individuals is shown (Fig. 1C). In elderly individuals, the mean frequency of γ/δ T cells

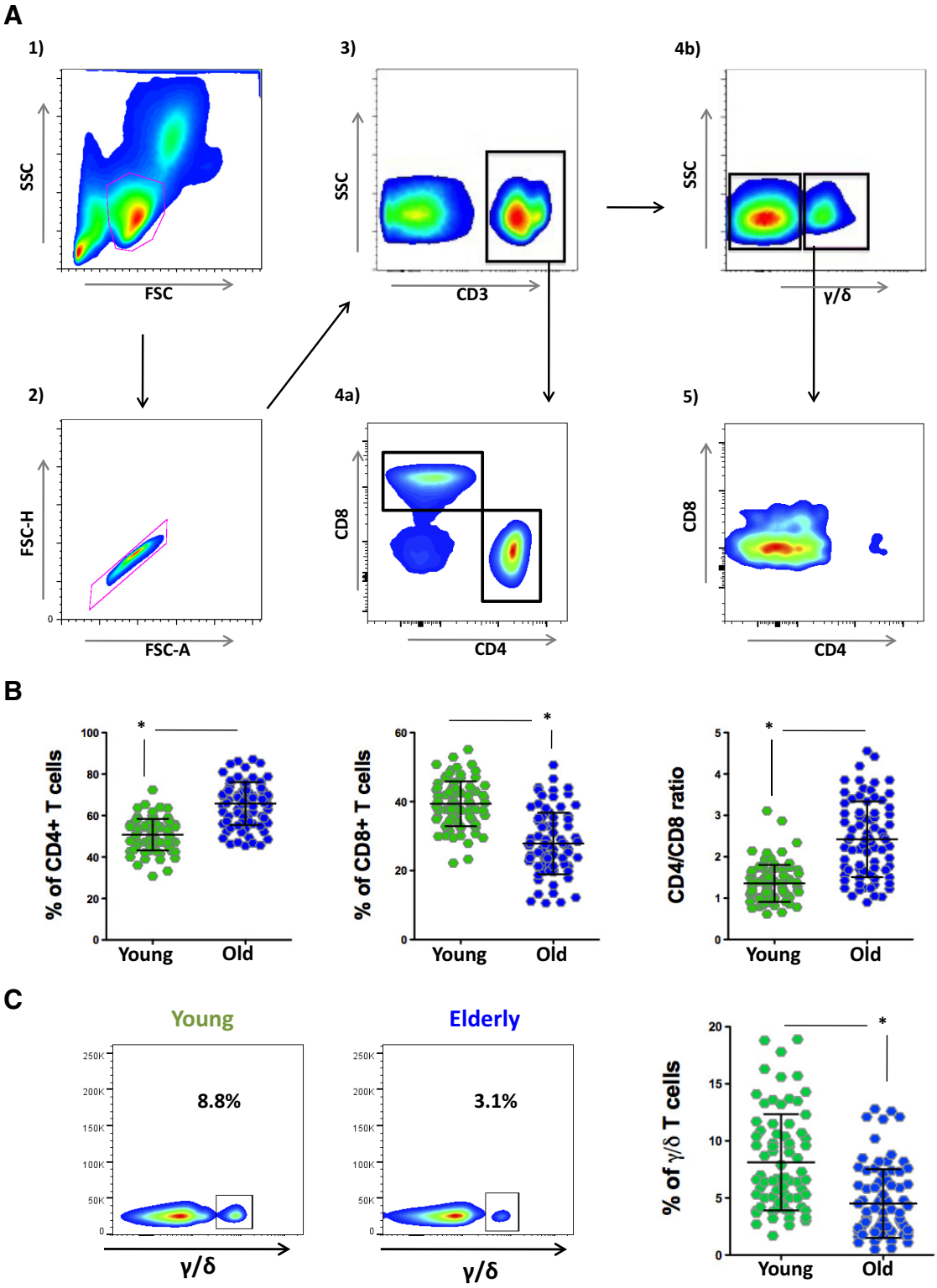

Figure 1. Flow cytometry analysis of α/β and γ/δ T cell frequencies in aging. (A) PBMCs were stained for surface marker expression, including CD3, pan-γ/δ, CD4, and CD8. Cells were acquired by flow cytometry, and data were analyzed by gating the lymphocyte population (1), singlets (2), and CD3+ cell (3); expression of CD4 and CD8 in the total CD3+ population (4a), as well as in cells expressing the γ/δ TCR (4b and 5). This confirms the predominant $CD4^{-}CD8^{-}$ profile of γ/δ T cells. FSC-H, FSC-height; FSC-A, FSC-area. (B) PBMCs from young and elderly participants (green and blue symbols, respectively) were stained as described. The frequency of $CD4^{+}$ and $CD8^{+}$ T cells within the CD3 pool is shown, with $^{*}P < 0.0001$. The CD4/CD8 ratio (right panel) has been calculated and shows significant difference, with $^{*}P < 0.0001$. (C) A typical example of γ/δ T cell staining in a young and an old individual is shown. The frequency of γ/δ T cells is reported and shows significant difference with aging ($^{*}P<0.0001$). For the study, a pool of 74 young and 83 elderly individuals was analyzed.

was 4.74% ± 3.27, whereas in young individuals (n=82, 23–35 years old), it was 7.84% ± 3.35 (P<0.0001). As the number and frequency of total CD3+ T cells remained unchanged with age (69.4%±7.8 in young and 65.9%±9.2 in elderly), the trend observed is that there is a reduced number of γ/δ T cells in the elderly. We have identified that females always represent a higher fraction of the elderly in our SLAS and could bias the analysis as suggested elsewhere [23]. In our data, however, when age-matched males (n=35, mean age 67.4 years old) and females (n=48, mean age 68.4 years old) were tested, no significant difference in the frequency of γ/δ T cells, 4.3 ± 3.4% (males), and 5.0 ± 3.9% (females) was observed (P=0.4411). Analysis of the other markers tested in our study (CD28, CD27, CD45RA, and CD57) did not result in any gender-specific expression (data not shown; all P>0.05); hence, further analysis was not stratified by gender.

Markers of senescence in α/β T cells: where γ/δ T cells stand

We next measured the expression of the three main markers used in the context of T cell aging, namely, CD28, CD27 (costimulatory receptors), and CD57 (marker of senescence in α/β T cells). There was a reduced frequency of CD27+ and CD28+ T cells with aging in both $CD4^+$ and $CD8^+$ T cells (**Fig. 2A** and **B**; P<0.0001). In contrary, the frequency of CD57+ T cells was significantly higher in T cells from elderly individuals (P<0.0001). In Fig. 2C, we show that there is no significant difference in the frequency of CD27+ and CD57+ γ/δT cells with aging. However, we observed a higher frequency of CD28+ γ/δ T cells in elderly individuals compared with young individuals. This contrasts with the reduced frequency of CD28+ α/β T cells in aged individuals. We have calculated that as a result of the fact that the overall frequency of total γ/δ T cells is lower with aging, the higher frequency of CD28+ γ/δ T cells does not lead to a significant increased frequency of CD28+ γ/δ+ T cells within the total CD3+ pool (3.16% vs. 3.38% in young and elderly individuals, respectively; P>0.05). However, similar analysis revealed that the frequency of CD27+ γ/δ T cells within the CD3 pool is reduced significantly with age (3.31% vs. 2.19% in young and elderly individuals, respectively; P<0.01). This shows a selective loss of CD27+ γ/δ T cell numbers and maintenance of CD28+ γ/δ T cell numbers with aging. The populations of $CD4^+$ T cells show a much tighter distribution for the markers tested compared with the $CD8^+$ T cells. A similar trend is observed for γ/δ T cells. Whereas this wide distribution is more prevalent in the elderly for $CD4^+$ and $CD8^+$ T cells, we observe that this pre-exists in young individuals for the γ/δ T cells (Fig. 2C).

Figure 3 displays the subsets: T_N cells (CD45RA+CD27+), T_{CM} cells (CD45RA−CD27+), effector memory T cells (CD45RA−CD27−), and T_{TE} cells (CD45RA+CD27−) of young and elderly individuals—$CD8^+$, $CD4^+$, and the corresponding population in γ/δ T cells. When looking at $CD8^+$ T cells, we show the significantly lower frequency of the T_N compartment with aging (64.08±13.68% vs. 29.26±14.93%), whereas frequency of all memory subpopulations increased with aging. There was a 2.5-fold increase in the frequency of $CD8^+$ T_{CM} cells (P<0.0001) and a 1.5-fold increase in the frequency of $CD8^+$ T_{TE} cells (P<0.0001). The significant decrease in the frequency of $CD4^+$ T_N (51.80±13.91% vs. 36.97±15.45%) is associated with an increase in memory cell percentages, mostly the T_{CM}. Overall, the age-related fold changes in T cell composition were higher in the $CD8^+$ compartment. Figure 3C revealed that for γ/δ T cells, aging is associated with an increased frequency of CD45RA+CD27− (25.69%% ±16.74 and 35.31±21.25; P=0.0031) and CD45RA−CD27+ γ/δ T cells (24.38% ±13.84 and 32.80% ±17.39; P=0.0014). However, CD45RA+CD27+ (18.89% ±11.41 and 13.64% ±9.57; P=0.0008) and CD45RA−D27− γ/δ T cells decreased with aging (31.03% ±14.66 and 18.25% ±13.17; P<0.0001). When looking at the combination between CD57 and CD28 (Fig.

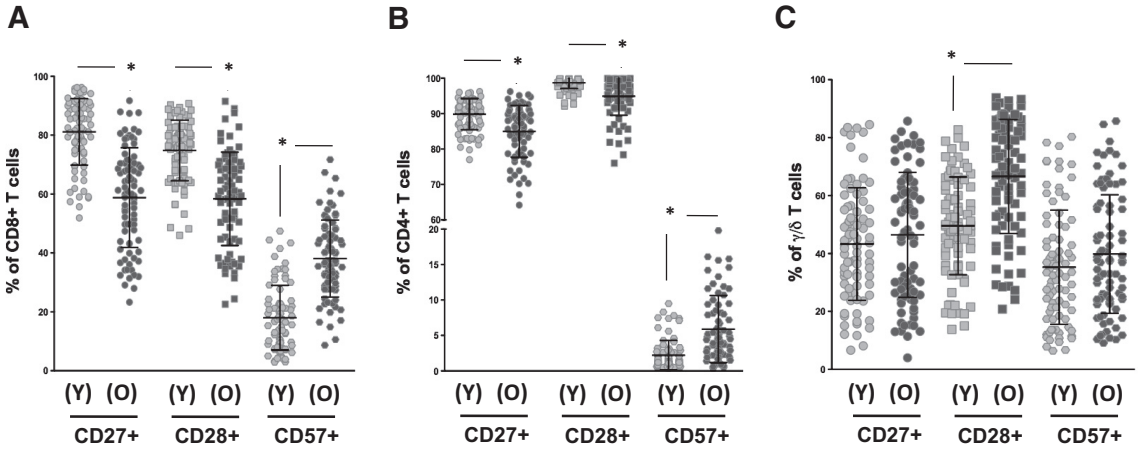

Figure 2. Expression of CD27, CD28, and CD57 in T cells with aging. (A) The frequency of $CD8^+$ T cells expressing CD27, CD28, or CD57 is shown for young (Y; n=74; green symbols) and elderly individuals [old (O); n=83; blue symbols]. Significant difference is depicted by *P < 0.0001. (B) The same analysis was performed in $CD4^+$ T cells (*P<0.0001). (C) Similarly, the frequency of γ/δ T cells expressing CD28, CD27, and CD57 is shown for young and elderly individuals (*P<0.0001).

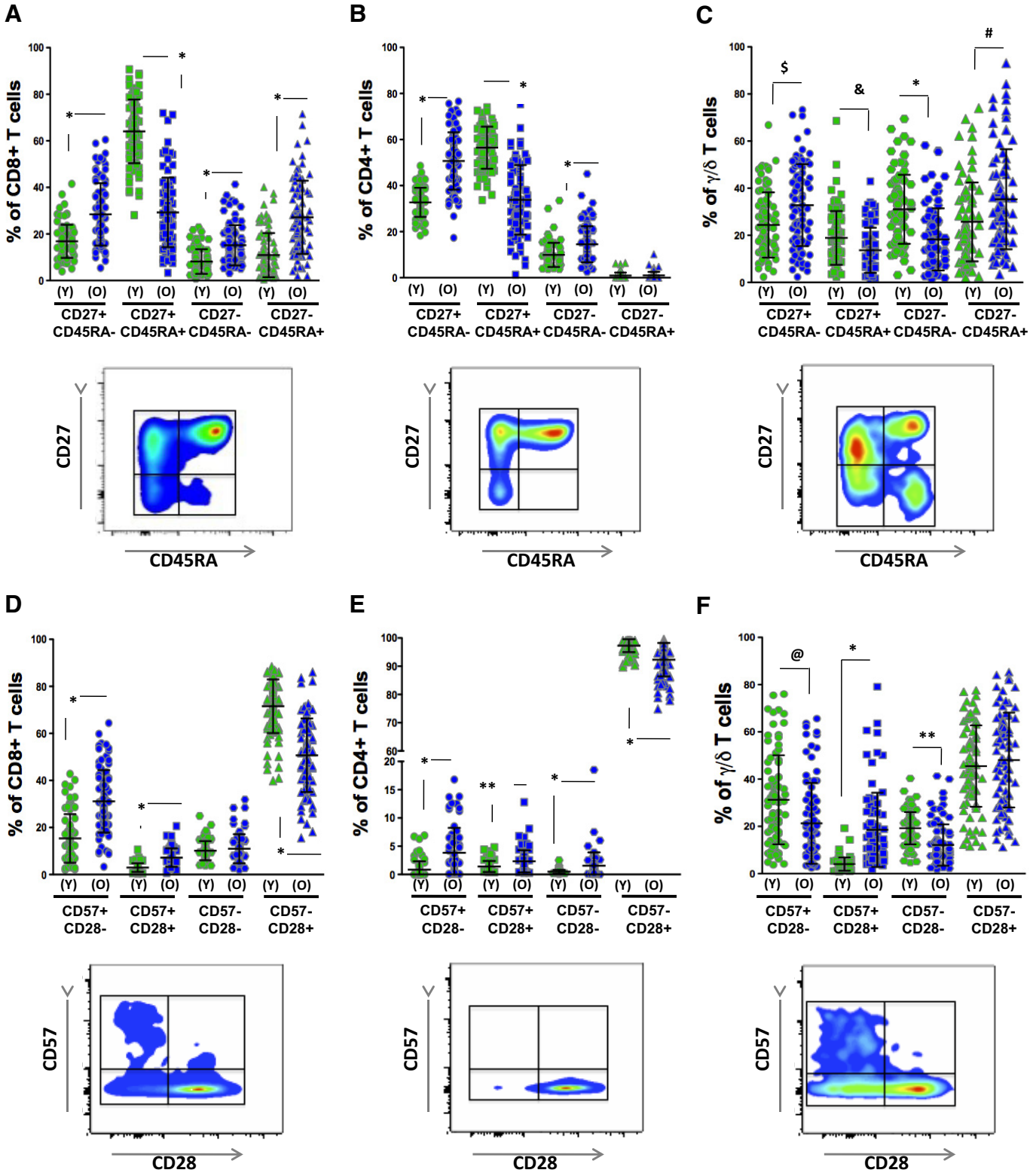

Figure 3. Subsets analysis in T cells with aging. A Boolean analysis is showing the frequency of CD27/CD45RA and CD57/CD28 subsets in $CD8^+$, $CD4^+$, and γ/δ T cells. An example of the profile and gating is provided for each T cell population. The CD27/CD45RA subset distribution is shown for $CD8^+$ (A), $CD4^+$ (B), and γ/δ (C) T cells. Similar analysis was performed for CD57/CD28 (D–F, respectively). Significant differences are depicted by $*P < 0.0001$, $\$P = 0.0014$, $\&P = 0.0008$, $\#P = 0.0031$, $**P < 0.001$, and $@P = 0.0002$.

3D–F), we observe similar trends in $CD8^+$ and $CD4^+$ T cell phenotypes in aging. The frequency of CD57+CD28− T cells was similar to that of CD27−CD45RA+, as these may represent the same population of late, differentiated T cells. As for the γ/δ population, we observed a decreased frequency of CD28−CD57+ (1.8-fold; $P=0.0002$) and CD28−CD57− (1.7-fold; $P<0.001$) and an increased frequency of CD28+CD57+ (5.0-fold; $P<0.0001$) with aging (Fig. 3F). This data on γ/δ contrast clearly with that of α/β T cells.

Relationship between γ/δ and α/β T cells with aging

To show whether markers may follow a parallel evolution in γ/δ and α/β T cells during aging, we provide the significant correlations (Spearman's) of γ/δ population frequencies (CD45RA+, CD27+, CD28+, and CD57+) with the corresponding $CD4^+$ and $CD8^+$ cells (**Table 2**). We identified that the frequency of CD27+ γ/δ T cells correlated with the frequency of various α/β T cell subsets in young and elderly individuals, whereas this was not the case for CD28+ γ/δ T cells. Additionally, the frequency of $CD4^+CD28^+$ and $CD8^+CD28^+$ T cells in the elderly correlated with mostly all γ/δ T cell subsets. Only one correlation present in young individuals became not significant in the elderly ($CD4^+CD45RA^+$), whereas most correlations appear with aging for $CD4^+$ and $CD8^+$ populations. Overall, the CD27+ γ/δ is the γ/δ T cell population that is showing the highest correlation to the α/β T cell subsets, especially in the elderly. This clearly shows a discrepancy in the expression and evolution of CD28 and CD27 in γ/δ compared with α/β T cells during aging.

γ/δ T cells and biomarkers of persistent infection

In our study, all elderly individuals tested were seropositive for CMV and thus, susceptible to fall in the IRP. We thus sought to identify whether the frequency of γ/δ T cells and associated subsets was linked to the CD4/CD8 ratio, probably the most robust parameters of the IRP, and anti-CMV IgG titers (**Fig. 4**). We measured the CD4/CD8 ratio in fresh blood samples, as performed in the original IRP studies [15]. We found no correlation between frequency of total γ/δ T cells and the CD4/CD8 ratio. We show that this was also true for CD57+, CD45RA+, CD28+, and CD27+ γ/δ T cells (Fig. 4A; $P>0.05$). On the other hand, we show that the CD4/CD8 ratio is inversely correlated with the frequency of CD57+ T cells and positively correlated with the frequency of CD28+ and CD27+ T cells in the α/β compartment. This was significant for $CD8^+$ and $CD4^+$ T cells (Fig. 4A, right). We further show an inverse correlation between CD45RA+CD27− $CD8^+$ T cells (identified as T_{TE}) and a positive correlation between CD45RA+CD27+ $CD4^+$ and $CD8^+$ T cells (identified as T_N) with the CD4/CD8 ratio (Fig. 4B). However, none of the γ/δ subset appears significant in our analysis.

A similar approach was tested for anti-CMV antibody titers, and we did not observe any correlation with the frequency of γ/δ T cells (Fig. 4C). However, we show that the frequency of CD27+ γ/δ T cells, as well as CD45RA+ γ/δ T cells, is correlating significantly with the anti-CMV titers (Spearman $r=-0.2695$, and $r=0.2346$, respectively). However, no significance was identified for CD28+ and CD57+ γ/δ T cell subsets. We also show that the frequency of $CD8^+$ T cells expressing CD28+ or CD27+ T cells is inversely correlated with anti-CMV titers and that frequency of CD57+ cells positively correlated with anti-CMV titers (Fig. 4C, right). However, we did not observe similar changes for the CD4 compartment. Finally, we analyzed CD45RA+CD27− and CD45RA+CD27+ cells and found that only γ/δ T cells bearing a CD45RA+CD27− profile related to CMV titers (Fig. 4D; $r=0.2756$).

DISCUSSION

Earlier studies have demonstrated that age and CMV history influence the phenotype and functional capacity of T cells [29,

TABLE 2. Spearman's Correlations between γ/δ and $CD8^+$ or $CD4^+$ T Cell Subset Frequencies

Subset	γ/δ+ CD45RA+	γ/δ+ CD27+	γ/δ+ CD28+	γ/δ+ CD57+
$CD4^+$				
Young CD45RA+	$r = 0.22$, $P = 0.043$			
Elderly CD45RA+		$r = 0.29$, $P = 0.008$		
Young CD28+		$r = 0.23$, $P = 0.035$		
Elderly CD28+	$r = -0.23$, $P = 0.037$	$r = 0.55$, $P < 0.0001$		$r = -0.27$, $P = 0.012$
Young CD27+				
Elderly CD27+	$r = -0.25$, $P = 0.020$	$r = 0.49$, $P < 0.0001$		
Young CD57+				$r = 0.27$, $P = 0.013$
Elderly CD57+		$r = -0.37$, $P < 0.001$		$r = 0.22$, $P = 0.042$
$CD8^+$				
Young CD45RA+	$r = 0.60$, $P < 0.0001$			
Elderly CD45RA+	$r = 0.28$, $P = 0.011$			
Young CD28+				
Elderly CD28+	$r = -0.26$, $P = 0.021$	$r = 0.52$, $P < 0.0001$	$r = 0.26$, $P = 0.0165$	$r = -0.26$, $P = 0.018$
Young CD27+		$r = 0.22$, $P = 0.047$		
Elderly CD27+	$r = -0.26$, $P = 0.019$	$r = 0.46$, $P < 0.0001$		
Young CD57+				$r = 0.25$, $P = 0.021$
Elderly CD57+		$r = -0.44$, $P < 0.0001$		$r = 0.32$, $P = 0.003$

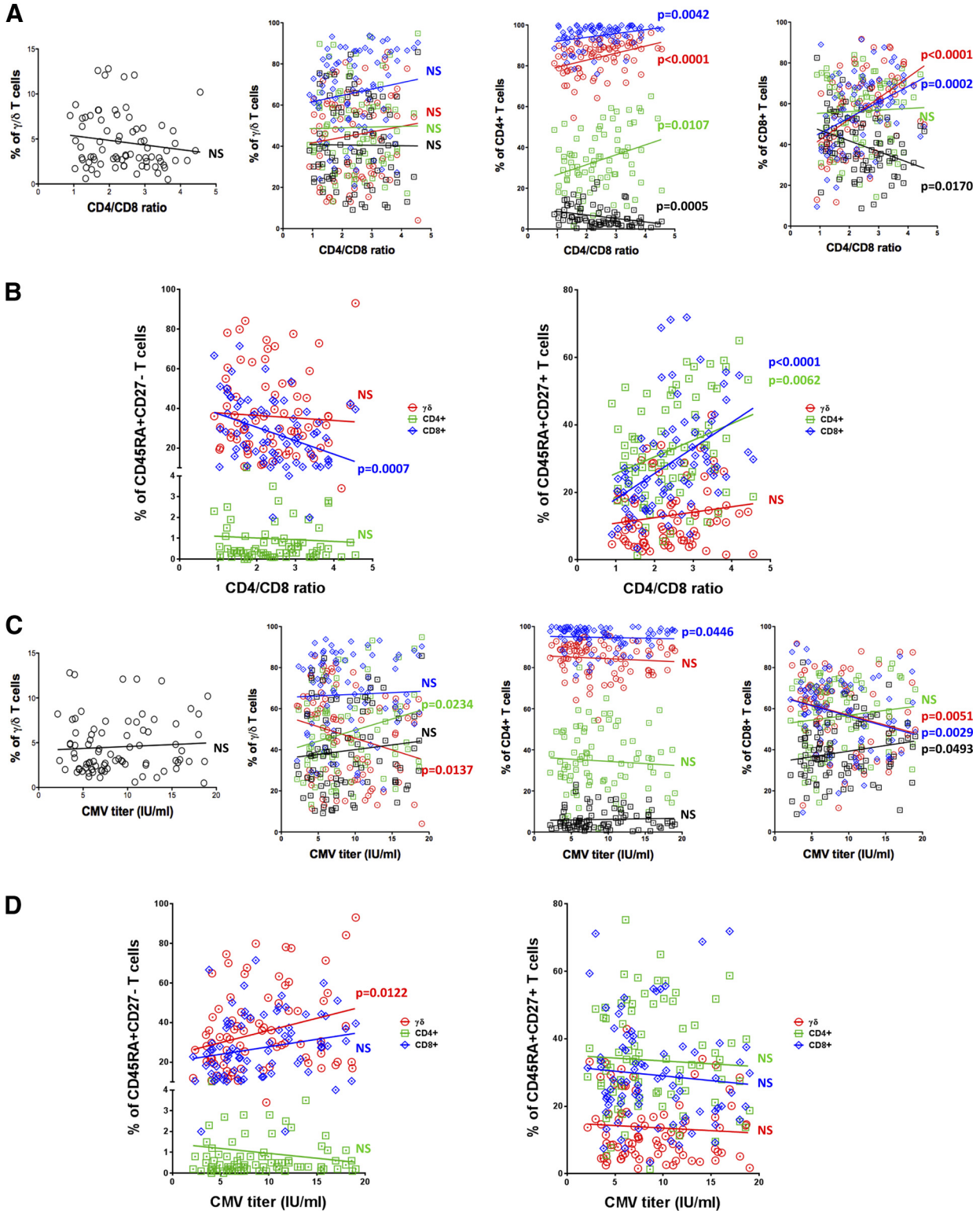

Figure 4. Relationship among γ/δ T cell subsets, the CD4/CD8 ratio, and anti-CMV titer in elderly. (A) The frequency of total γ/δ T cells from elderly individuals (n=83) was compared with the corresponding CD4/CD8 ratio. No significant correlation was identified [nonsignificant (NS)]. The frequency of CD28+ (blue), CD27+ (red), CD57+ (black), and CD45RA+ (green) in γ/δ T cells, $CD8^+$ T cells, and $CD4^+$ T cells was tested for Spearman's correlations against the CD4/CD8 ratio. (B) A similar analysis was performed for CD45RA+CD27− and CD45RA+CD27+ in γ/δ, $CD8^+$, and $CD4^+$ T cells. (C) The frequency of total γ/δ T cells from elderly individuals (n=83) was compared with the corresponding anti-CMV titer. No significant correlation was identified (NS). The frequency of CD28+ (blue), CD27+ (red), CD57+ (black), and CD45RA+ (green) γ/δ, $CD8^+$, and $CD4^+$ T cells was tested for Spearman's correlations against the anti-CMV titers. (D) A similar analysis as in B was performed using CMV titer instead of CD4/CD8 ratio.

30]. We were able to show in this Chinese population age-related changed expression for CD28, CD27, and CD57 in α/β T cells, similarly to what is reported in Caucasian populations. Recent efforts have identified that other immune cells, such as invariant NKT cell (altered frequency) and DC populations (functional level), show alterations with aging [31, 32]. Results from this study show that the frequency and number of γ/δ T cells, in particular, are lower in healthy, elderly compared with younger individuals. A recent study showed that aging and CMV infection impact independently on the $\gamma\delta$ T cell compartment [24]. This is consistent with a possible skewing toward CMV-induced Vδ1 cells that are mostly CD27-negative. CMV seroconversion is associated with expansion of highly differentiated cells from the $CD8^+$ (T_{TE}) and γ/δ (Vδ2−) compartments. Future studies discriminating the different Vδ populations would bring additional information on the potential diversity of γ/δ T cells and their phenotype with aging. This will also require better identification of antigens for the various Vγ and Vδ chains (especially Vδ1 that represents the majority of Vδ2− cells) and how the differentiation of the respective populations is driven. Our data (unpublished) also suggest that Vδ1 T cells display a much differentiated phenotype (CD27−CD45RA+), even in young individuals, suggesting that analysis of total γ/δ T cells may still represent a good strategy to identify age-related changes. This CD27−CD45RA+ population was also associated significantly to anti-CMV titers in our study.

We identified that the CD28+ γ/δ T cells are maintained, whereas CD27+ γ/δ T cells are depleted with age (one-third of the population contracted). Born and O'Brien [33] have shown the importance of CD27 in the maintenance of the Vγ9Vδ2 T cell pool. We can then hypothesize that Vδ2+ T cells are more susceptible to activation-induced cell death, explaining their loss. This is partly demonstrated by Bcl-2 downregulation following mitogen stimulation of Vδ2+ T cells [23]. CD27-expressing Vδ2+ cells were associated with IFN-γ secretion, whereas IL-17 secretion was restricted to CD27− γ/δ T cells, which are mostly Vδ2− [34]. The fact CD27 is involved in the delineation of cytokine profiles suggests that the observed loss of Vδ2+ with age and inflation of Vδ1+ induced by CMV may balance γ/δ immunity toward Th17. The number of T cells present in the circulation represents a minute fraction of the whole T cell pool, and this also applies to γ/δ T cells. It cannot be excluded that CD27− γ/δ T cells are not cleared by apoptosis but are remobilized to aging tissues. It has been shown that senescent T cells identified by KLRG-1 expression were released to the periphery following exercise in the elderly, giving credit to this possibility [35]. As γ/δ T cells are involved in recognition of a limited class of antigens, it is possible that immunosurveillance in the tissue is performed by cells with previous experience or specialized functions, possibly the CD27− γ/δ T cells. Furthermore, the frequency of CD27+ γ/δ T cells was significantly associated with the frequency of $CD4^+$ and $CD8^+$ T cell subsets, especially in the elderly, and anti-CMV titers. To our best knowledge, this is among the first evidence of γ/δ T cell subsets coincident with α/β T cells in the elderly. The high frequency of CD27− γ/δ T cells correlating with a high anti-CMV titer may represent a population recently activated by CMV antigens. This is novel and may bring new avenues in the understanding of the impact of persistent infections on the immune system [36].

Our study provides further evidence for selective depletion in circulating γ/δ T cell subsets that may be multifactorial. The impact of γ/δ T cell alterations in the increased susceptibility to infections and overall vulnerability with aging is still unclear. Evidence suggests that γ/δ T cells (Vδ2) from elderly individuals have impaired capacity to proliferate in response to isopentenyl pyrophosphate stimulation, suggesting that immune senescence is also affecting this compartment [25]. Other classes of antigens inducing γ/δ T cell responses have been identified (MHC class I chain-related gene A/B, UL16-binding protein 4, sulfatides, aminoacyl tRNA synthetases) [37]. The biological role of these cells has also been understood more clearly and included defense against infections, sterile stress, production of cytokines/chemokines, and activation/maturation of immune cells (reviewed in ref. [38]). Such further functional analysis of the different γ/δ T cell subsets is necessary and may show significant alterations in the context of aging and/or persistent infections. Future studies will require the use of recently developed, standardized panels [39] for the identification of common traits of the γ/δ T cell populations with aging.

AUTHORSHIP

A.V., C.T.T.Y., S.A., N.B.S., S.M., and I.L. performed experiments. K.J.P., A.K.A, and O.R. set up the young cohort enrollment, collected blood samples, and reviewed data. M.S.Z.N. and T.P.N. enrolled elderly participants, performed clinical assessment, and collected blood samples. T.F. analyzed data and reviewed the manuscript. A.L. designed the study, analyzed data, and wrote the majority of the manuscript, and all other coauthors reviewed the manuscript.

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DISCLOSURES

The authors declare no conflicts of interest.

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