Arterial Stiffness Is Associated With Cytomegalovirus-Specific Senescent CD8^{+} T Cells

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Background—Arterial stiffness is a well-known predictor of future cardiovascular events. Search for the underlying mechanism of arterial stiffening is still under way. We investigated the relationship between arterial stiffness and cytomegalovirus infection in terms of T-cell senescence.

Methods and Results—Arterial stiffness was evaluated using pulse wave velocity measurements in 415 Koreans (age 59 ± 12 years). We also investigated the frequency of CD57^{+} or CD28^{null} senescent T cells in peripheral blood lymphocytes and analyzed which immune parameters were correlated with pulse wave velocity. Furthermore, cytomegalovirus-specific T cells were stimulated with overlapping peptides covering pp65 protein, and T-cell function was evaluated by intracellular cytokine staining of interferon-γ, tumor necrosis factor-α, and CD107a. In a multivariate analysis, it was found that the frequency of CD57^{+} cells in the CD8^{+} T-cell subset was independently correlated with pulse wave velocity after adjusting for traditional cardiovascular risk factors such as age, sex, diabetes mellitus history, smoking history, body mass index, blood pressure, serum creatinine, high-density lipoprotein cholesterol, and high-sensitivity C-reactive protein. Cytomegalovirus pp65-specific T cells were more frequently observed in the CD8^{+}CD57^{+} population than in the CD8^{+}CD57^{-} population, and multivariate analysis revealed that the frequency of cytomegalovirus pp65-specific interferon-γ^{+}, tumor necrosis factor-α^{+}, or CD107a^{+} cells in the CD8^{+} T-cell subset was independently correlated with pulse wave velocity as well.

Conclusions—We demonstrate that arterial stiffness is associated with senescent CD57^{+} T cells and CMV pp65-specific T cells in the CD8^{+} T-cell subset. The precise role of cytomegalovirus-specific, senescent T cells in vascular aging needs to be further investigated. (*J Am Heart Assoc*. 2017;6:e006535. DOI: 10.1161/JAHA.117.006535.)

Key Words: arterial stiffness • cytomegalovirus • immunosenescence • pulse wave velocity • T cells

Growing evidence from recent animal and human studies suggests that T cells contribute to the development of hypertension.[1–4] Previously, we demonstrated that hypertensive patients have an increased frequency of replicative senescent CD8^{+} T cells in peripheral blood; these cells are characterized by the loss of CD28 and the acquisition of CD57 on their surface.[4] CD28 loss in T cells is one of the most prominent changes associated with aging in humans and is caused by the repetitive antigenic stimulation of T cells.[5] CD57 expression is known to occur during the late stage of T-cell differentiation and might be a distinct measure of replicative senescence in T cells.[6] Compared with CD28^{+} or CD57^{-} T cells, CD28^{null} or CD57^{+} T cells produce more proinflammatory cytokines and exert greater cytotoxicity.[7]

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Clinical Perspective

What Is New?

- Previously, immunologic mechanisms for arterial stiffening have not been well addressed.
- This is the first study to reveal the relationship between cytomegalovirus-specific, senescent CD8$^+$ T cells and arterial stiffness in humans.

What Are the Clinical Implications?

- Cytomegalovirus-related T-cell aging may contribute to age–associated arterial stiffness and hypertension.
- Future studies should address specific mechanisms by which cytomegalovirus-specific, senescent T cells contribute to arterial stiffening and therapeutic interventions to prevent this process.

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These senescent T cells are known to be associated with various inflammatory diseases in humans including cardiovascular disease.[8–10]

It has been also known that cytomegalovirus infection is involved in the accumulation of CD28^{null} or CD57$^+$ senescent T cells. In humans, cytomegalovirus is known to be one of the most important antigens for repetitive T-cell stimulation,[11] and latent infection with cytomegalovirus has been shown to strongly exert age-associated changes on peripheral T cell homeostasis.[12] The cytomegalovirus-seropositive population has a higher frequency of CD28^{null} or CD57$^+$, replicative senescent T cells than the cytomegalovirus-seronegative population.[13] Moreover, cytomegalovirus infection is associated with a variety of chronic inflammatory processes in cardiovascular disease[14,15] such as hypertension.[16] However, it has not been elucidated how cytomegalovirus infection and senescent T cells contribute to the pathogenesis of cardiovascular disease.

Increased arterial stiffness is one of the major mechanisms underlying the pathogenesis of hypertension.[17] Arterial stiffness is increased in the presence of conventional cardiovascular risk factors including aging.[18,19] The degree of arterial stiffness is known to be associated with various markers of inflammation,[20,21] suggesting that immune responses likely play a role in increasing arterial stiffness. Furthermore, a recent study has reported that activated T-cell markers are associated with arterial stiffness among HIV-infected patients.[22] Therefore, we investigated whether T-cell senescence is associated with arterial stiffness in the general population, as assessed using pulse wave velocity (PWV) measurements. Next, considering the antigen reactivity of senescent T cells, we examined cytomegalovirus-specific T-cell response and analyzed the relationship between these results and the degree of arterial stiffness. Because increased arterial stiffness is characteristic of the vascular aging process, we attempted to elucidate the relationship between vascular aging and T-cell aging.

Methods

Study Participants

The study population consisted of 415 Koreans who were recruited from subjects initially registered in the Yonsei Cardiovascular Genome cohort. They were recruited from the outpatient clinic of Severance Cardiovascular Hospital irrespective of their disease status. The study population includes those with hypertension, coronary artery disease, diabetes mellitus (DM), and healthy subjects. The average age of the cohort members was 59.4±11.7 years (20–82 years). Hypertension was defined as either a documented systolic blood pressure of >140 mm Hg or a diastolic blood pressure of >90 mm Hg over 3 visits before the use of antihypertensive medication. DM was defined as having fasting plasma glucose levels >126 mg/dL, HbA1c >6.5%, or a history of DM therapy. Coronary artery disease was defined as 1 or more lesions with a >50% diameter reduction either by invasive coronary angiography or CT coronary angiography. At the time of enrollment, patients underwent a complete physical examination and laboratory assessment. Peripheral blood pressure was measured in both arms after 5 minutes of rest in a sitting position using an OMRON HEM 780 device (OMRON Healthcare, Kyoto, Japan),[23] and the higher values were used for the analysis. Patients with any of the following conditions were excluded from this study: significant systemic disease, malignant debilitating disease, severe hypertension (>200/140 mm Hg), a history of overt chronic inflammatory disease and/or were receiving anti-inflammatory medications. The baseline clinical characteristics and laboratory data regarding the study participants are summarized in Table 1. The medications taken by the patients are summarized in Table S1. All the measurements in this study including PWV and the immunologic parameters were obtained while the patients were taking their prescribed medications. This study received prior approval from the Institutional Review Board of the Yonsei University College of Medicine, and the procedures followed were in accordance with institutional guidelines (IRB number: 4-2001-0039, 4-2010-0500). All participants provided informed consent before enrollment.

PWV Measurements

PWV was measured as heart-femoral PWV using a VP-2000 pulse wave unit (Nippon Colin Ltd, Komaki City, Japan) as previously described.[24] The method was validated in 948 patients with hypertension or coronary artery disease by

Table 1. Baseline Characteristics of the Study Population (n=415)

Age, y	59.4±11.7
Male sex	273 (65.8%)
CAD	281 (67.7%)
Hypertension	247 (59.5%)
DM	148 (35.7%)
Hyperlipidemia	179 (43.1%)
Current smoker	51 (12.3%)
BMI, kg/m^2	25.2±3.1
SBP, mm Hg	131.3±17.1
DBP, mm Hg	80.8±11.0
Creatinine, mg/dL	1.0±0.7
Total cholesterol, mg/dL	163.1±41.1
HDL-cholesterol, mg/dL	48.2±11.9
LDL-cholesterol, mg/dL	88.1±34.7
Triglyceride, mg/dL	135.8±122.2
hsCRP, mg/L	1.5±3.1
hfPWV, cm/s	1.016.6±234.6
Frequency of T-cell subset, %	
CD57$^+$ cells in CD4$^+$ T cells	6.1±6.0
CD28^{null} cells in CD4$^+$ T cells	4.4±5.7
CD57$^+$ cells in CD8$^+$ T cells	43.0±17.1
CD28^{null} cells in CD8$^+$ T cells	41.9±17.6

Data are presented as the means±SD or n (%). BMI indicates body mass index; CAD, coronary artery disease; DBP, diastolic blood pressure; DM, diabetes mellitus; HDL, high-density lipoprotein; hfPWV, heart-femoral pulse wave velocity; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

showing high correlation and good agreement with the conventional SphygmoCor system.[25] Briefly, carotid and femoral artery pressure waveforms were recorded in multi-element tonometry sensors at the left carotid and left femoral arteries with patients in a supine position. The ECG was monitored from electrodes placed on both wrists. Heart sounds S1 and S2 were detected by placing a microphone on the left edge of the sternum at the third intercostal space. The waveform analyzer measured the time intervals between S2 and the notch of the carotid pulse wave (Thc) and between the carotid and femoral artery pulse waves (Tcf). The sum of Thc and Tcf represents the time required for pulse waves to travel from the heart to the femoral artery (Thf). The heart-femoral PWV, a marker of central aortic stiffness,[26] was calculated as Lhf/(Thc+Tcf), where Lhf is the distance from the heart to the femoral artery. Lhf and the distance between the sampling points were calculated based on patient height and then divided by the time interval for the waveform from each measuring point.

Immunophenotype Analysis of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells from anticoagulated blood were isolated using Ficoll-Hypaque (GE Healthcare, Uppsala, Sweden) density gradient centrifugation. For surface staining, peripheral blood mononuclear cells were incubated with directly conjugated monoclonal antibodies for 20 minutes at 4°C. The antibodies used were anti-CD3 (horizon V500), anti-CD4 (PE-Cy7), anti-CD8 (APC-H7), anti-CD19 (PerCP-Cy5.5), anti-CD28 (APC) (all from BD Biosciences, San Jose, CA), and anti-CD57 (eFluor 450) (Biolegend, San Diego, CA). Multicolor flow cytometry was performed using an LSR II instrument (BD Biosciences), and FlowJo software (Treestar, San Carlos, CA) was used to analyze the data.

In Vitro Stimulation of T Cells and Intracellular Cytokine Staining

To analyze the specific antigen reactivity of T cells, peripheral blood mononuclear cells were stimulated with overlapping peptides from cytomegalovirus pp65 (0.6 nmol of each peptide/mL; Miltenyi Biotec), which could directly bind to MHC class I or II molecules on antigen-presenting cells and be presented to CD8$^+$ or CD4$^+$ T cells in peripheral blood mononuclear cell cultures, for 6 hours in the presence of phycoerythrin (PE)-conjugated anti-CD107a (BD Biosciences). The CD107a molecule is exposed to the cell surface during degranulation of cytotoxic proteins such as perforin and granzymes, and therefore PE-conjugated anti-CD107a was added to the cultures for the detection of T cells with cytotoxic activity.[27,28] After 1 hour of incubation, brefeldin A (GolgiPlug, BD Biosciences) and monensin (GolgiStop, BD Biosciences) were added to accumulate cytokine protein intracellularly. Following surface staining with anti-CD3 (horizon V500), anti-CD4 (PerCP-Cy5.5), anti-CD8 (APC-H7), anti-CD28 (horizon V450), and anti-CD57 (APC), the cells were fixed and permeabilized using a Fixation/Permeabilization Buffer Kit and further stained for intracellular cytokines with anti-interferon-γ (IFN-γ) (fluorescein isothiocyanate conjugated) and anti-tumor necrosis factor-α (TNF-α) (PE-Cy7) (both from BD Biosciences). All samples were assessed using an LSR II Flow Cytometer (BD Biosciences), and the data were analyzed using FlowJo software (Treestar, San Carlos, CA). This assay enables enumeration of cytomegalovirus pp65-specific T cells in both T-cell compartments, CD8$^+$ and CD4$^+$ T cells.[28,29]

Measurement of Serum Anti-Cytomegalovirus IgG Antibody Titers

Serum anti-cytomegalovirus immunoglobulin G (IgG) titers were determined using a commercially available

chemiluminescent microparticle immunoassay (Abbott Laboratories, Chicago, IL) in accordance with the manufacturer's instructions. A titer of 6.0 antibody units/mL of IgG or greater was considered cytomegalovirus IgG-seropositive.

Statistical Analysis

Continuous variables are summarized as the mean±SD. Categorical variables are summarized as a percentage of the group total. Continuous variables were compared using Student t test and ANOVA. Intragroup comparisons were summarized using the paired t test, and the Wilcoxon signed-rank test was used to verify the results. Pearson's correlation analysis was used for the simple correlation between continuous variables. To examine the association of senescent T cells and cytomegalovirus-specific T-cell responses with arterial stiffness, multiple linear regression models were used, using heart-femoral PWV as the dependent variables and the frequencies of senescent or cytomegalovirus-specific T cells as the main independent variables of interest. All P values were 2-sided and considered significant at the 0.05 level. All statistical analyses were performed using SPSS 13.0 (SPSS Inc, Chicago, IL).

Results

The Frequency of CD8$^+$CD57$^+$ T Cells Is Independently Correlated With Arterial Stiffness

In the present study, we investigated the significance of T-cell senescence in arterial stiffness. The study population consisted of 415 Koreans who were registered in the Yonsei Cardiovascular Genome cohort. The baseline characteristics and laboratory data of the study participants are summarized in Table 1. The mean frequencies of CD57$^+$ and CD28^{null} T cells among the peripheral blood CD8$^+$ T-cell population were 43.0±17.1% and 41.9±17.6%, respectively. In Figure 1A, representative flow cytometry plots are presented for CD57 and CD28 expression in the CD8$^+$ T-cell subset from young and old subjects. As expected, the frequency of CD57$^+$ (r=0.410, P<0.001) or CD28^{null} (r=0.391, P<0.001) cells among the CD8$^+$ T-cell subset was positively correlated with age (Figure 1B). The study population showed a significant, positive relationship between age and systolic blood pressure (Figure 1C). We analyzed the relationship between senescent CD8$^+$ T cells and blood pressure (Figure 1D) and found that the frequency of CD8$^+$CD57$^+$ T cells was positively correlated with systolic blood pressure (r=0.103, P=0.036). Next, we analyzed the relationship between the frequency of senescent T cells and PWV. The frequencies of CD8$^+$CD57$^+$ (r=0.322, P<0.001) and CD8$^+$CD28^{null} (r=0.300, P<0.001) T cells were both positively correlated with PWV (Figure 1E and 1F).

Because PWV is strongly affected by age and blood pressure, we performed a multiple linear regression analysis of the association between the frequency of senescent CD8$^+$ T cells and PWV after adjusting for traditional cardiovascular risk factors including age, sex, systolic blood pressure, medical history, and laboratory findings (Table 2). Based on this analysis, the frequency of CD8$^+$CD57$^+$ T cells was independently correlated with PWV (B=0.037, 95% confidence interval [CI], 0.001–0.073, P=0.047).

Cytomegalovirus pp65-Specific T Cells Are More Frequently Observed in the CD57$^+$ T-Cell Population

Because a major population within CD8$^+$CD57$^+$ T cells is known to be reactive to cytomegalovirus,[30] we analyzed cytomegalovirus-specific immune response parameters. First, cytomegalovirus serostatus was evaluated in a subgroup of 123 individuals, and all of these individuals were seropositive for cytomegalovirus (data not shown). This result confirms previous reports showing high (96–98%) cytomegalovirus seroprevalence in Korean adults.[31,32] Next, we investigated cytomegalovirus-specific T-cell responses by stimulating peripheral blood mononuclear cells with overlapping peptides covering cytomegalovirus pp65 protein in all 415 individuals. T-cell function was evaluated using intracellular cytokine staining of IFN-γ and TNF-α. In addition, cytotoxic function of T cells was simultaneously evaluated by CD107a staining, which measures the degranulation of cytotoxic proteins such as perforin and granzymes.[27] Figure 2A shows representative flow cytometry plots for cytomegalovirus pp65-specific IFN-γ and TNF-α secretion and CD107a staining in the CD8$^+$ T-cell population against CD57 expression. Figure S1 shows the specificity of this assay by representative flow cytometry plots with or without stimulation with cytomegalovirus pp65 overlapping peptides. As previously found, cytomegalovirus pp65-specific T cells were more frequent in the CD8$^+$CD57$^+$ population than in the CD8$^+$CD57$^-$ population (Figure 2B), and the degree of cytomegalovirus pp65-specific CD8$^+$ T-cell responses was positively correlated with increasing age (Figure 2C). Interestingly, cytomegalovirus pp65-specifically IFN-γ-producing cells represented >10% of the CD8$^+$CD57$^+$ T-cell population in certain subjects (Figure 2B). We demonstrated that a major population of CD8$^+$CD57$^+$ T cells was derived from cytomegalovirus infection and that cytomegalovirus-specific CD8$^+$ T cells are preferentially present in the senescent CD57$^+$ population.

Cytomegalovirus-Specific CD8$^+$ T Cells Are Independently Correlated With Arterial Stiffness

The relationship between cytomegalovirus pp65-specific CD8$^+$ T-cell responses and the degree of arterial stiffness was
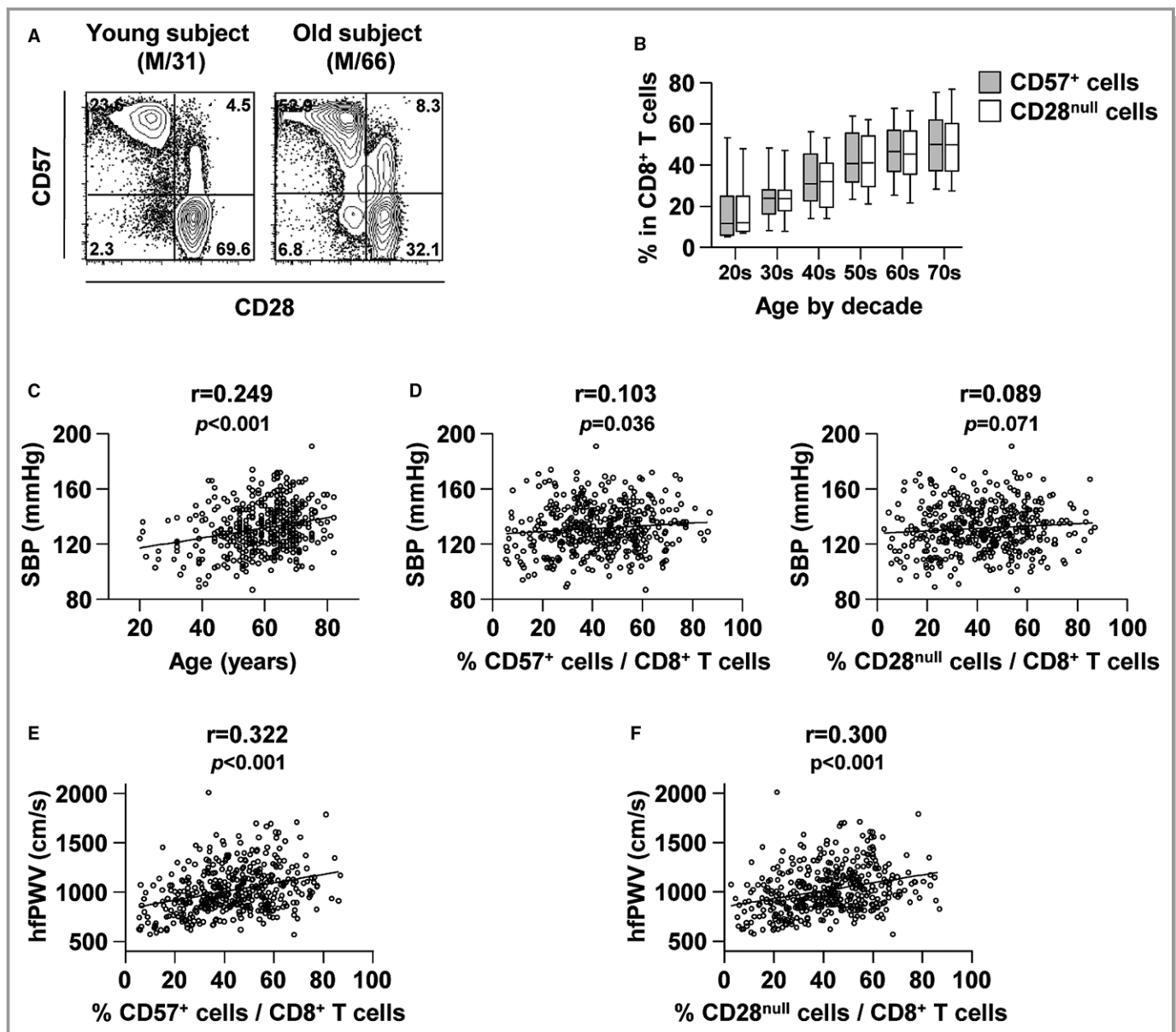
Figure 1. The significance of the frequency of senescent CD8+ T cells in blood pressure and PWV. Immunophenotyping of peripheral blood mononuclear cells obtained from 415 individuals was performed. A, Representative flow cytometry plots are presented for CD57 and CD28 expression in CD8+ T cells in a young (left) and an old subject (right). B, The frequency of CD57+ (gray) or CD28null (white) cells in CD8+ T cells is plotted against age. C, Pearson's correlation scatterplot of SBP and the age of all participants. D, Pearson's correlation scatterplot of SBP and the frequency of CD57+ (left) or CD28null (right) cells in CD8+ T cells. E and F, Pearson's correlation presented by plotting hfPWV against the frequency of CD57+ (E) or CD28null (F) cells in CD8+ T cells of the overall population. hfPWV indicates heart-femoral pulse wave velocity; PWV, pulse wave velocity; SBP, systolic blood pressure.

assessed as well. The frequencies of cytomegalovirus pp65-specific IFN-γ and TNF-α secretion and CD107a staining in CD8+ T cells were positively correlated with PWV (r=0.202, P<0.001 for IFN-γ; r=0.213, P<0.001 for TNF-α; and r=0.172, P=0.001 for CD107a) (Figure 2D). We also performed multiple linear regression analysis of the association between cytomegalovirus pp65-specific CD8+ T-cell responses and

PWV after adjusting for traditional cardiovascular risk factors (Table 3). The frequency of cytomegalovirus pp65-specific IFN-γ and TNF-α secretion and the cytotoxic function of CD8+ T cells were independently correlated with PWV (B=0.020, 95% CI, 0.007–0.033, P=0.003 for IFN-γ; B=0.021, 95% CI, 0.006–0.036, P=0.006 for TNF-α; and B=0.033, 95% CI, 0.010–0.056, P=0.006 for CD107a).

Table 2. Multiple Linear Regression Analysis for Determinants of PWV

	Regression Coefficient	95% CI	P Value
CD8$^+$CD57$^+$ T cells (R^2=0.513)			
Age, y	0.004	0.003 to 0.005	<0.001
Female sex	−0.020	−0.039 to −0.001	0.041
DM history	0.025	0.009 to 0.041	0.002
Smoking history	0.007	−0.005 to 0.019	0.264
BMI, kg/m^2	−0.002	−0.004 to 0.001	0.150
SBP, mm Hg	0.002	0.001 to 0.002	<0.001
Creatinine, mg/dL	0.004	−0.006 to 0.015	0.409
HDL-cholesterol, mg/dL	0.000	−0.001 to 0.000	0.576
hsCRP, mg/L	0.001	−0.001 to 0.003	0.314
Percent CD57$^+$ in CD8$^+$ T cells*	0.037	0.001 to 0.073	0.047
CD8$^+$CD28^{null} T cells (R^2=0.512)			
Age, y	0.004	0.003 to 0.005	<0.001
Female sex	−0.021	−0.040 to −0.002	0.031
DM history	0.025	0.009 to 0.040	0.003
Smoking history	0.007	−0.005 to 0.020	0.249
BMI, kg/m^2	−0.002	−0.004 to 0.001	0.149
SBP, mm Hg	0.002	0.001 to 0.002	<0.001
Creatinine, mg/dL	0.005	−0.006 to 0.015	0.404
HDL-cholesterol, mg/dL	0.000	−0.001 to 0.000	0.576
hsCRP, mg/L	0.001	−0.001 to 0.003	0.323
Percent CD28^{null} in CD8$^+$ T cells*	0.029	−0.004 to 0.062	0.086

BMI indicates body mass index; CI, confidence interval; DM, diabetes mellitus; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; PWV, pulse wave velocity; SBP, systolic blood pressure.
*Log-transformed.

Anti-cytomegalovirus Humoral Immune Response Is Not Correlated With Arterial Stiffness

Finally, we analyzed the relationship between the anti-cytomegalovirus humoral immune response and arterial stiffness. As described above, all individuals were positive for anti-cytomegalovirus IgG antibody in a subgroup of 123 individuals. When we divided the individuals into 3 groups according to anti-cytomegalovirus IgG antibody titer, no significant relationship was found between anti-cytomegalovirus IgG antibody titer and age (Figure 3A) or PWV (Figure 3B). In addition, anti-cytomegalovirus IgG antibody titer was not associated with the intensity of cytomegalovirus-specific T-cell responses (Figure 3C). In summary, in view of the immune response to cytomegalovirus, we found that arterial stiffness is related only to T-cell–mediated immunity

and not to the humoral immune response against cytomegalovirus. Taken together, our data suggest that cytomegalovirus-specific, replicative senescent T cells, but not cytomegalovirus-specific antibody, might contribute to increased arterial stiffness in the general population.

Discussion

Our findings demonstrate that the frequency of senescent CD8$^+$CD57$^+$ T cells in peripheral blood is independently correlated with arterial stiffness. Cytomegalovirus-specific T-cell responses were analyzed because cytomegalovirus is a major driving antigen for replicative senescence in T cells.[11] We found that cytomegalovirus-specific CD8$^+$ T cells were more frequently observed in the CD57$^+$ population, and cytomegalovirus-specific IFN-γ and TNF-α-secretion and the cytotoxic degranulation of CD8$^+$ T cells were independently associated with PWV. In the present study, we evaluated cytomegalovirus pp65-specific T-cell activity not only by IFN-γ and TNF-α staining but also by CD107a staining. CD107a molecule is exposed to the cell surface during degranulation of cytotoxic proteins such as perforin and granzymes, and therefore we can detect T cells with cytotoxic activity by adding fluorophore-conjugated anti-CD107a to the culture of T cells during antigen stimulation.[27,28] Our data showed that the frequency of cytomegalovirus pp65-specific CD107a-stained cells among CD8$^+$ T cells was independently correlated with PWV. These data suggest that immune aging, especially T-cell senescence that is linked to cytomegalovirus infection, might play a role in the progression of vascular aging.

Recently, several research groups have reported the existence and relevance of senescent T cells in various cardiovascular diseases. In particular, the role of senescent T cells in atherosclerotic disease has been extensively studied. Accelerated telomere shortening in T cells and other leukocyte subpopulations has been reported in patients with coronary heart disease.[33] It is not yet clear whether T cells with shorter telomeres lead to increased cardiovascular risk or telomere shortening in T cells is caused by the chronic inflammation associated with cardiovascular disease. The former explanation is more likely because proinflammatory cytokines (such as interleukin-6 and TNF-α, which are secreted by senescent T cells) are also found in atherosclerotic plaque lesions.[34] Other studies reported that increased senescent memory CD4$^+$ T cells are associated with subclinical atherosclerosis, as assessed based on carotid artery intimal media thickness and coronary artery calcification,[35] particularly in patients with rheumatoid arthritis.[36] In addition, a higher frequency of CD8$^+$CD28^{null}CD57$^+$ T cells was found to be correlated with an increased prevalence of carotid artery
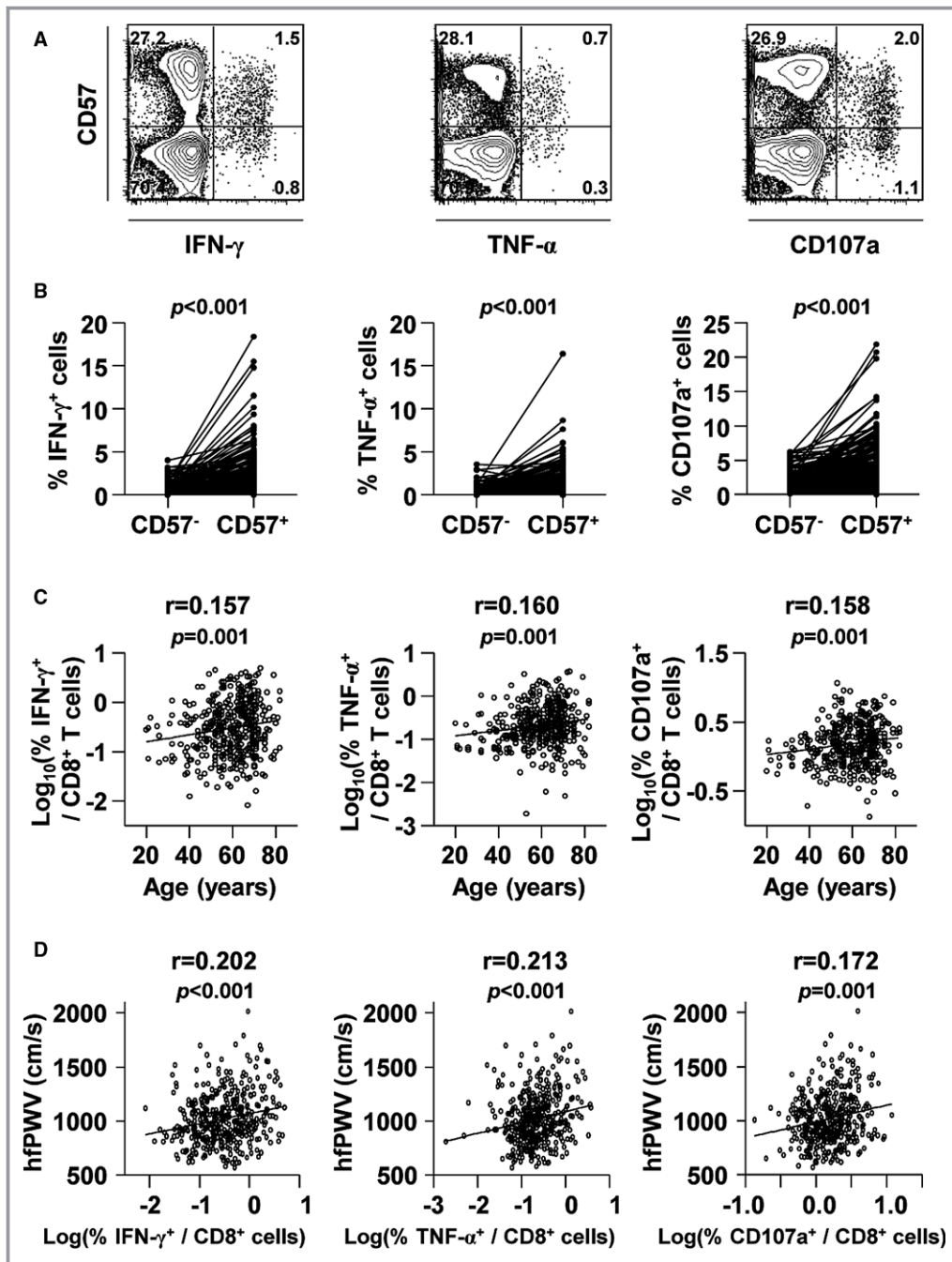
Figure 2. The significance of cytomegalovirus pp65-specific CD8^{+} T-cell responses in the CD57^{+} population and PWV. Peripheral blood mononuclear cells were stimulated with cytomegalovirus pp65 overlapping peptides, and intracellular staining for IFN-γ, TNF-α, and CD107a was performed. A, Representative flow cytometry plots are presented for IFN-γ and TNF-α secretion and for CD107a expression in the CD8^{+} T-cell populations. B, The frequencies of cytomegalovirus pp65-specific IFN-γ-, TNF-α-, and CD107a-expressing cells were compared between CD8^{+}CD57^{+} and CD8^{+}CD57^{−} T-cell populations. Cytomegalovirus pp65-specific responses were more frequently observed in CD8^{+}CD57^{+} T cells than in the CD8^{+}CD57^{−} population. The *P* value was calculated using the paired *t* test. C, Pearson's correlation presented as a scatterplot of age and the frequency of cytomegalovirus pp65-specific IFN-γ-, TNF-α-, and CD107a-expressing cells (log-transformed) in CD8^{+} T cells. D, Pearson's correlation presented as a plot of hfPWV against the frequency of cytomegalovirus pp65-specific IFN-γ-, TNF-α-, and CD107a-expressing cells (log-transformed) in CD8^{+} T cells of the overall population. hfPWV indicates heart-femoral pulse wave velocity; IFN-γ, interferon- γ; TNF-α, tumor necrosis factor- α.

Table 3. Multiple Linear Regression Analysis of the Association Between Cytomegalovirus pp65-Specific CD8$^+$ T Cells and PWV

	Regression Coefficient	95% CI	P Value
Pp65-specific IFN-γ secretion (R^2=0.519)			
Age, y	0.004	0.004 to 0.005	<0.001
Female sex	−0.021	−0.039 to −0.002	0.031
DM history	0.026	0.010 to 0.042	0.001
Smoking history	0.009	−0.003 to 0.021	0.161
BMI, kg/m^2	−0.002	−0.005 to 0.000	0.067
SBP, mm Hg	0.002	0.001 to 0.002	<0.001
Creatinine, mg/dL	0.003	−0.008 to 0.014	0.579
HDL-cholesterol, mg/dL	0.000	−0.001 to 0.000	0.237
hsCRP, mg/L	0.001	−0.001 to 0.003	0.361
Percent IFN-γ$^+$ in CD8$^+$ T cells*	0.020	0.007 to 0.033	0.003
pp65-specific TNF-α secretion (R^2=0.517)			
Age, y	0.004	0.004 to 0.005	<0.001
Female sex	−0.021	−0.040 to −0.002	0.030
DM history	0.025	0.010 to 0.041	0.002
Smoking history	0.008	−0.004 to 0.021	0.181
BMI, kg/m^2	−0.002	−0.005 to 0.000	0.084
SBP, mm Hg	0.002	0.001 to 0.002	<0.001
Creatinine, mg/dL	0.004	−0.007 to 0.015	0.453
HDL-cholesterol, mg/dL	0.000	−0.001 to 0.000	0.256
hsCRP, mg/L	0.001	−0.001 to 0.003	0.351
Percent TNF-α$^+$ in CD8$^+$ T cells*	0.021	0.006 to 0.036	0.006
Pp65-specific CD107a expression (R^2=0.517)			
Age, y	0.004	0.003 to 0.005	<0.001
Female sex	−0.021	−0.040 to −0.002	0.027
DM history	0.027	0.011 to 0.043	0.001
Smoking history	0.009	−0.004 to 0.021	0.174
BMI, kg/m^2	−0.002	−0.004 to 0.000	0.116
SBP, mm Hg	0.002	0.001 to 0.002	<0.001
Creatinine, mg/dL	0.004	−0.007 to 0.015	0.466
HDL-cholesterol, mg/dL	0.000	−0.001 to 0.000	0.330
hsCRP, mg/L	0.001	−0.001 to 0.003	0.314
Percent CD107a$^+$ in CD8$^+$ T cells*	0.033	0.010 to 0.056	0.006

BMI indicates body mass index; CI, confidence interval; DM, diabetes mellitus; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IFN-γ, interferon-γ; PWV, pulse wave velocity; SBP, systolic blood pressure; TNF-α, tumor necrosis factor-α.
*Log-transformed.

lesions in a study of HIV-infected patients.[37] Liuzzo et al[38] reported that the number of CD4$^+$CD28^{null} T cells increased significantly in the peripheral blood of patients with unstable angina. Furthermore, it was shown that increased frequency of CD4$^+$CD28^{null} T cells was associated with the recurrence of acute coronary events.[39] Another study found that the presence of CD4$^+$CD28^{null} T cells is correlated with the occurrence of a first cardiovascular event and with a worse outcome after an acute coronary syndrome in diabetic patients.[40] More recently, a direct role of T cells in hypertension has been demonstrated in angiotensin II-infused humanized mice.[41]

Recent studies have suggested that cytomegalovirus infection is associated with a variety of chronic inflammatory processes in cardiovascular disease.[14,15] An increase in the number of peripheral CD8$^+$CD57$^+$ and CD8$^+$CD28^{null} T cells has been reported in patients with coronary artery disease in relation to cytomegalovirus infection.[33,42] Cytomegalovirus-specific T-cell responses have independently been associated with carotid atherosclerosis in HIV-infected patients.[43] In terms of prognostic implications, the occurrence of cytomegalovirus DNAemia is strongly associated with the death or readmission of patients with acute heart failure.[44] Furthermore, cytomegalovirus-seropositive subjects exhibiting high C-reactive protein levels were at significantly higher risk for all-cause and cardiovascular disease–related mortality among the general population.[45] Cytomegalovirus infection has effects beyond atherosclerotic disease; cytomegalovirus caused a significant increase in blood pressure that was independent of atherosclerotic plaque formation in the aorta in a mouse model.[16] Moreover, recent research has shown that cytomegalovirus seropositivity is independently associated with increased arterial stiffness in patients with chronic kidney disease.[46]

Most studies on cytomegalovirus infection or on the immune response to cytomegalovirus in humans have focused on cytomegalovirus seroprevalence according to anti–cytomegalovirus IgG antibody titers. However, the cytomegalovirus seropositivity of nonwhites tends to be 20% to 30% higher than that of whites, and some nonwhites, especially black and Asian populations, exhibit cytomegalovirus seroprevalences approaching 100%.[47] This is also the case in Koreans.[31,32] Among our study participants, the subgroup of 123 individuals exhibited 100% cytomegalovirus seropositivity; therefore, we were unable to analyze differences in the degree of arterial stiffness according to cytomegalovirus seroprevalence. Instead, we focused on cytomegalovirus-specific T cells and found that the PWV is strongly correlated with the frequency of cytomegalovirus-specific CD8$^+$ T cells but is not correlated with anti-cytomegalovirus IgG antibody titer. To the best of our knowledge, this is the first study to demonstrate an independent correlation between cytomegalovirus-specific
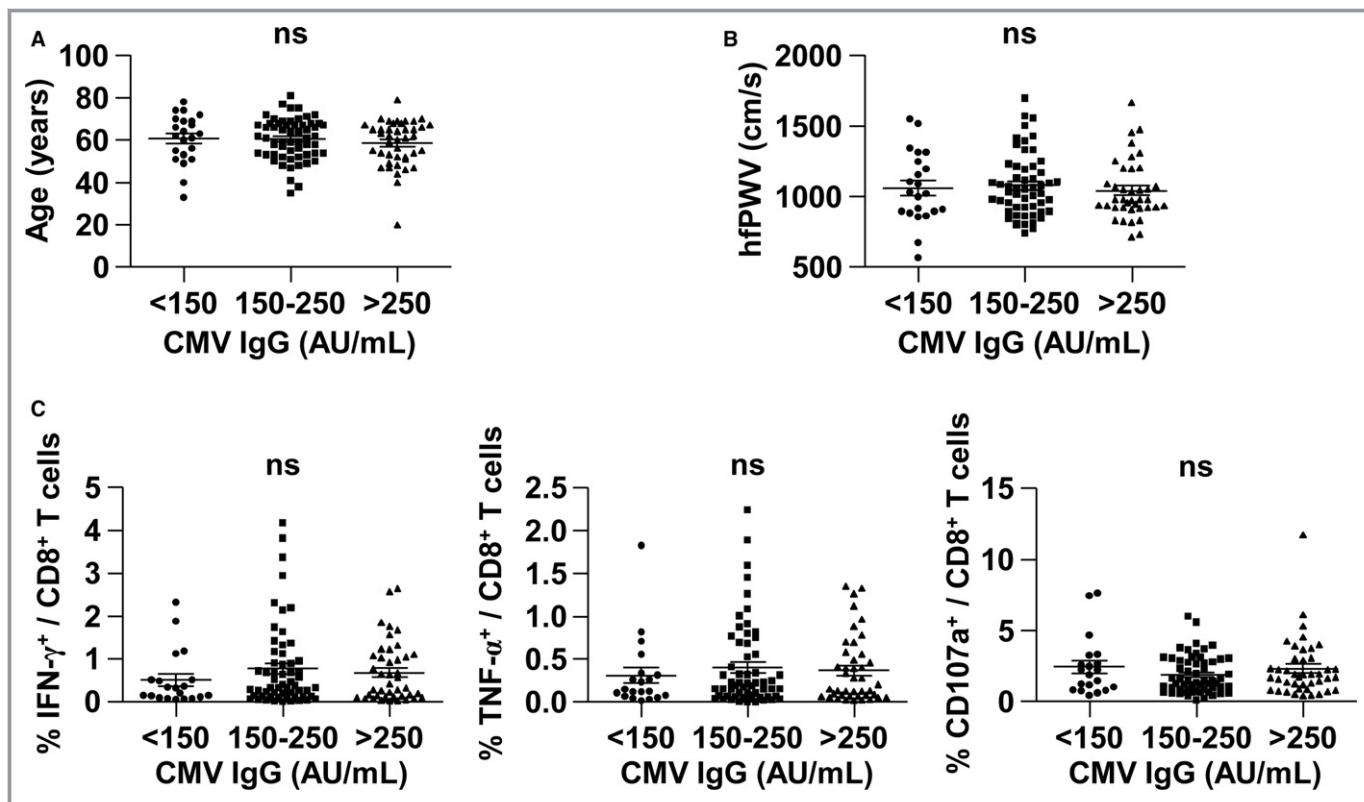
Figure 3. Anti-cytomegalovirus IgG titer is not correlated with age, PWV, or cytomegalovirus pp65-specific CD8^{+} T-cell responses. Serum anti-cytomegalovirus IgG antibody titer was assessed in a subgroup of 123 individuals. The population was divided into 3 groups according to antibody titer (<150, 150–250, and >250 AU/mL). Age (A), hfPWV (B), and cytomegalovirus pp65-specific CD8^{+} T-cell responses (C) were comparable among the 3 groups. A *P*<0.05 was considered significant (ANOVA). AU indicates antibody units; CMV, cytomegalovirus; hfPWV, heart-femoral pulse wave velocity; IFN-γ, interferon- γ; IgG, immunoglobulin G; ns, not significant; TNF-α, tumor necrosis factor-α.

CD8^{+} T-cell response and arterial stiffness. Considering the correlation between cytomegalovirus-specific T cells and arterial stiffness, it is hypothesized that cytomegalovirus-specific T cells are more important than cytomegalovirus itself during vascular pathogenesis in cytomegalovirus-seropositive subjects.

The mechanism by which senescent T cells or cytomegalovirus-specific T cells contribute to vascular aging has not yet been clearly identified. Considering the fact that senescent T cells produce more proinflammatory cytokines than nonsenescent T cells,[48] proinflammatory cytokines such as IFN-γ and TNF-α might injure vessel walls directly or activate other immune cells. Indeed, senescent T cells have been shown to produce large amounts of IFN-γ that induce macrophage activation, thereby releasing metalloproteinases that degrade the extracellular matrix.[49,50] Moreover, senescent T cells exert higher cytotoxicity than nonsenescent T cells.[51] In the current study, arterial stiffness was independently correlated with frequency of CD8^{+}CD57^{+} T cells, but not with CD4^{+}CD57^{+} T cells. This result suggests that CD8^{+} T-cell cytotoxicity might be a major effector function in increasing arterial stiffness. Homing properties of T cells need to be

considered in T-cell–mediated vascular injury. Bolovan-Fritts et al[52] revealed that cytomegalovirus-reactive T cells induce endothelial damage via fractalkine–CX3C motif chemokine receptor 1 (CX3CR1) interaction. We previously confirmed that CD8^{+}CD57^{+} T cells express higher levels of CX3CR1,[53] suggesting that these senescent T cells might participate in endothelial dysfunction progression.

The present study has limitations. First, this is a human observational study, rather than an interventional study. Therefore, it is difficult to identify a direct cause-and-effect relationship. Even though the statistical methods of multivariate analysis in the current study are clear and sound, the principal correlations are relatively weak with limited explanatory power. However, the results of multivariate analysis are not a final outcome in itself, but rather serve as a bridgehead for the pathophysiologic evaluation of arterial stiffening in terms of cytomegalovirus infection. Future studies are required to address specific mechanisms by which cytomegalovirus-specific, senescent T cells contribute to arterial stiffening. Second, we could not use live/dead cell staining in the flow cytometry analysis because of a limitation in the multicolor staining panel. However, we have confirmed that

autofluorescence from dead cells in our analysis was minimal and almost negligible based on our previous experiments.

Conclusion

Arterial stiffness is associated with CD8$^+$CD57$^+$ T cells and cytomegalovirus-specific IFN-γ and TNF-α-secretion and with the cytotoxic degranulation of CD8$^+$ T cells. These findings might provide a new perspective on the immune-related mechanism of arterial stiffness and might lead to a new therapeutic strategy for arresting the progression of human hypertension. The precise role of these CD8$^+$CD57$^+$ T cells and cytomegalovirus-specific CD8$^+$ T-cell responses warrants further investigation.

Sources of Funding

Disclosures

None.

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SUPPLEMENTAL MATERIAL

Table S1. Summary of the medications taken by the study population (n=415)

Types of medication	N (%)
Antihypertensive	293 (70.6%)
Hypoglycemic	67 (16.1%)
Lipid-lowering	272 (65.5%)
Antiplatelet	262 (63.1%)
Anticoagulation	66 (15.9%)
Antiarrhythmic	56 (13.5%)

Figure S1. Representative flow cytometry plots are presented for IFN-γ and TNF-α secretion and for CD107a staining in the CD8^{+} T cell populations with or without stimulation with CMV pp65 overlapping peptides.