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Cytomegalovirus Infection Reduces Telomere Length of the Circulating T Cell Pool

Pablo J. E. J. van de Berg,*,† Stephen J. Griffiths,‡ Si-La Yong,* Richard Macaulay,‡ Frederike J. Bemelman,† Sarah Jackson,‡ Sian M. Henson,‡ Ineke J. M. ten Berge,† Arne N. Akbar,‡ and René A. W. van Lier*

Short telomeres of circulating leukocytes are a risk factor for age-related diseases, such as atherosclerosis, but the exact mechanisms generating variations in telomere length are unknown. We hypothesized that induction of differentiated T cells during chronic CMV infection would affect T cell telomere length. To test this, we measured the amount of differentiated T cells and telomere length of lymphocytes during primary CMV infection as well as CMV-seropositive and -seronegative healthy individuals. After primary CMV infection, we observed an increase in highly differentiated cells that coincided with a steep drop in telomere length. Moreover, we found in a cohort of 159 healthy individuals that telomere shortening was more rapid in CMV-seropositive individuals and correlated with the amount of differentiated T cells in both $CD4^+$ T cells and $CD8^+$ T cells. Finally, we found that telomere length measured in blood leukocytes is correlated with lymphocyte telomere length. Thus, CMV infection induces a strong decrease in T cell telomere length, which can be explained by changes in the composition of the circulating lymphocyte pool. *The Journal of Immunology*, **2010, 184: 3417–3423.**

Telomeres are gene-poor regions at the end of chromosomes containing long repeats of TTAGGG sequences that protect chromosomes from inappropriate DNA repair and recombination. Because the 3′ end of linear DNA cannot be duplicated by DNA polymerase, telomeres shorten after each cell division. Therefore, decreasing telomere length can be regarded as a biological clock: when a critical length is reached, telomeres cannot function anymore, and cells will either become senescent or apoptotic (1). Although telomeres steadily decrease in somatic cells with aging, large interindividual variations are seen among people of the same age (2). The origin of this variation is largely unknown, but genetic factors, oxidative stress, and chronic inflammatory challenges have been implicated (3, 4). Because short leukocyte telomere length has been shown to be a risk factor for mortality and age-related diseases, such as cardiovascular disease, aging, and Alzheimer's disease (5–8), it is important to identify factors that contribute to the interindividual variations.

The telomere length of leukocytes measured in blood resembles the combined mean telomere length of all nucleated cells in blood, comprising the lymphoid and myeloid cells. Adaptive immune responses come with huge expansions of Ag-specific lymphocytes but leave the myeloid compartment unaltered. Although activated lymphocytes increase telomerase expression during primary infection (9), the ability to induce this enzyme is lost as T cells differentiate progressively (10), and, as a consequence, the mean telomere length of the Ag-primed T cell population is shorter than that of the naive subset (11, 12). During a primary viral infection, virus-specific naive T cells are activated and go through a number of divisions, forming a large pool of virus-specific, highly cytotoxic effector cells. After viral clearance, the pool of effector cells will decrease, leaving a relatively small pool of long-lived memory cells. Although the primary immune response appears to be comparable for many viruses, persistent viruses may leave specific imprints in both $CD4^+$ and $CD8^+$ T cell compartments during the latency phase (13, 14). Human CMV infection is one of the most common persistent viral infections in the Western world and in contrast to other persistent viruses generates a vast pool of highly differentiated, resting cytotoxic $CD8^+$ T cells that are formed already early during primary infection and are being maintained as a relatively stable population throughout the latency period (15). These cytotoxic $CD8^+$ T cells can be characterized by the expression of CD57 and the loss of the costimulatory receptors CD27 and CD28 (13, 16). During CMV latency, these CMV specific cells do not cycle (e.g., lack expression of the nuclear marker Ki67) and show no signs of recent activation (17, 18). Additionally, CMV latency is associated with the specific accumulation of cytotoxic $CD4^+CD28^-$ T cells that can secrete IFN-γ and execute cytotoxicity in a CMV-specific and HLA class II-restricted fashion (19). Both $CD8^+CD28^-CD27^-$ and $CD4^+CD27^-CD28^-$ T cells have been documented to have short telomeres (10, 20). Because of these rapid and permanent changes from a relatively small number of naive T cells with long telomeres to a large pool of highly differentiated T cells with decreased telomeres, we tested the impact of acute and chronic CMV infection on lymphocyte telomere length.

Materials and Methods

Subjects

We studied 31 renal transplant recipients who were CMV-seronegative prior to the transplantation. A total of 19 patients received a kidney of a CMV-positive donor (average age, 57 ± 18 y) and 12 patients from a CMV-negative donor (average age, 53 ± 17 y). Patients receiving a kidney of a seropositive donor all developed a primary CMV infection post-transplantation and were monitored weekly for vireamia until there was no detectable CMV DNA present (within 8 wk after the peak of CMV DNA

*Department of Experimental Immunology and †Renal Transplant Unit, Division of Nephrology, Department of Internal Medicine, Academic Medical Center, Amsterdam, The Netherlands; and ‡Division of Infection and Immunity, Department of Immunology, University College London, London, United Kingdom

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Address correspondence and reprint requests to Dr. P. J. E. J. van de Berg, Department of Experimental Immunology, Academic Medical Center, Meibergdreef 9, 1100 AZ Amsterdam, The Netherlands. E-mail address: p.j.vandeberg@amc.nl

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Abbreviations used in this paper: FISH, fluorescence in situ hybridization; PNA, protein nucleic acid.

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measurement). Ganciclovir (5 mg/kg i.v. twice daily, adjusted for renal function) was only given to symptomatic patients with CMV viremia (n = 2). The underlying renal diseases and immunosuppressive treatments are shown in Table I. During the course of the study, none of the patients had signs of graft rejection. Blood samples were collected just before and at regular intervals posttransplantation. From three patients, blood samples were collected annually up to 3 y posttransplantation. All patients signed an informed consent, and studies were approved by the medical ethical committee of the Academic Medical Center Amsterdam.

To investigate the relation between T cell differentiation status and telomere length, a group of 23 healthy volunteers, 12 CMV-seronegative (average age, 35 ± 11 y) and 11 CMV-seropositive individuals (average age, 37 ± 11 y), was recruited.

Additionally, to study the relation between latency and telomere length, 159 healthy volunteers from the age of 20–95 y were studied. Of these, 70 were CMV negative and 89 CMV positive. All samples were obtained in accordance with the ethical committee of Royal Free and University College Medical School. Old donors did not have any comorbidity, were not on any immunosuppressive drugs, and retained physical mobility and lifestyle independence.

Cell isolation

To obtain PBMCs, blood was diluted twice in HBSS (Cambrex Corporation, East Rutherford, NJ) with 0.025 M Tris (pH 7), layered on Lymphoprep (Lucron Bioproducts, De Pinte, Belgium; 1.077 g/ml), and centrifuged (400 × g for 20 min at 25°C) without break. The WBC ring was isolated and washed twice in HBSS with 4% FCS and 0.025 M Tris. Subsequently, cells were cryopreserved according to standard procedures.

For the combined analysis of total leukocytes, granulocytes, and lymphocytes, we collected blood samples of 10 healthy volunteers and purified half of the blood volume by Lymphoprep sedimentation as described above. Subsequently, the mononuclear fraction as well as the granulocyte pellet was collected. We then lysed the erythrocytes in the total leukocyte, granulocyte, and mononuclear fractions and performed the telomere staining and analysis according to protocol.

PBMCs of one donor were stained with fluorchrome-labeled Abs for CD3, CD8, CD27, CD45RA, and CMV-pp65 tetramer. Subsequently, cells were sorted into naive, $CD27^+CD45RA^+CD8^+CD3^+$ cells, $CD27^-CD45RA^+CD8^+CD3^+$ cells, and $tetramer^+CD8^+CD3^+$ cells. After sort, cells were labeled with protein nucleic acid (PNA)-telomere probe using the flow-fluorescence in situ hybridization (FISH) method and subsequently analyzed.

Viral diagnostics

Quantitative PCR for CMV DNA was performed in EDTA whole blood samples. To determine CMV serostatus, anti-CMV IgG was measured in serum using the AxSYM microparticle enzyme immunoassay (Abbott Laboratories, Abbott Park, IL) according to the manufacturer's instructions. Measurements were calibrated relative to a standard serum.

Immunofluorescent staining and flow cytometry

Mononuclear cells were stained in PBS with 1% BSA and 0.01% sodium acetate for 30 min on ice, using saturating amounts of CD8, CD4, CD28, CD45RA (R&D Systems, Minneapolis, MN), and CD27 (PeliCluster, Amsterdam, The Netherlands) mouse mAbs. Samples were acquired on a BD FACSCanto using FACSDiva Software (BD Biosciences, San Jose, CA). Analysis was done using FlowJo MacV8.6.3 (Tree Star, Ashland, OR).

Measurement of telomere length

Telomere length was measured by flow-FISH (12, 21), a FACS-based in situ hybridization assay where telomeres are hybridized to a labeled PNA probe complementary to the telomere sequence and subsequently analyzed by FACS. The telomere length in individuals experiencing primary CMV infection was measured using a commercially available kit (DakoCytomation, Glostrup, Denmark). In short, prehybridization, all samples were treated twice with ice-cold erythrocyte-lyses buffer (0.15 M NH_4Cl, 0.01 M $KHCO_3$, and 0.1 mM EDTA) for 10 min. DNA of the sample cells was denatured for 10 min at 82°C in the presence of hybridization solution with or without the PNA probe. Hybridization took place overnight at room temperature. Cells were then washed twice at 40°C, stained with propidium iodide for DNA staining, and analyzed by FACS. Based on propidium iodide staining, cells in $G_{0/1}$ phase of cell cycle were gated and used for analysis. As an internal control for variances between tests, samples contained sample cells and control cells. As control cells, we used the 1301 cell line, a subline of the EBV genome-negative T cell leukemia line CCRF-CEM that has long telomeres. These cells are tetraploid, relatively large, and granular compared with lymphocytes and can therefore be distinguished by forward and side scatter. The relative telomere length value was calculated as the ratio between the mean intensity of the FITC-labeled PNA probe of mononuclear cells and the 1301 cell line multiplied by two as a correction for the tetraploidy of the 1301 cell line. All samples were measured in duplo, and the averages of these two measurements were calculated: relative telomere length = 2 × 100% × (mean FL1 mononuclear cells with probe − mean FL1 mononuclear cells without probe)/(mean FL1 1301 cells with probe − mean FL1 1301 cells without probe).

Telomere length of PBMCs in the cohort of 159 healthy individuals (age 20–95 y) was measured using a modified two-color flow-FISH protocol (9). The cells were stained with either CD4- or CD8-biotin (Immunotech, Praha, Czech Republic) followed by streptavidin-Cy3 (Cedarlane Laboratories, Burlington, Ontario, Canada), after which samples were fixed and permeabilized (Fix & Perm Cell Permeabilization Kit; Caltag Laboratories, Burlingame, CA). After washing in hybridization buffer, cells were incubated with 0.75 μg/ml PNA telomeric (C3TA2)3 probe conjugated to Cy5. Samples were heated for 10 min at 82°C, rapidly cooled on ice, and hybridized for 1 h at room temperature in the dark. Samples were washed and analyzed immediately by flow cytometry. Fluorescently labeled beads (DakoCytomation) were used to standardize the cytometer settings. Two cryopreserved PBMC samples with known telomere fluorescence were used as standards to ensure consistency of the results. Results were obtained as median fluorescensence intensity values, which could be converted to telomere length in kilobases using a standard curve. The standard curve was constructed using 30 samples of varying telomere length analyzed both by flow-FISH and telomeric restriction fragment analysis (22).

Statistical analysis

The average ± SD is depicted in all diagrams. All comparisons between groups were done using unpaired Student t test. For correlations, the Pearson correlation test was used.

Results

Primary CMV induces the formation and accumulation of differentiated T cells

Most of the primary CMV infections go unnoticed because they occur early in life, and infection in immunocompetent individuals is normally mild with hardly any specific clinical symptoms. To characterize the primary immune response to human CMV, we have been studying CMV-seronegative recipients of CMV-positive donor kidneys (Table I) (17). Typically, posttransplantation, CMV DNA becomes detectable in blood and increases over time with a peak viremia 2 wk posttransplantation. In the following months, the immune system mounts a response that leads to the clearance of CMV from the blood and induces viral latency (17). In this model, we can study the concomitant impact of primary CMV infection on both lymphocyte differentiation status and telomere length.

CMV-specific effector $CD8^+$ T cells are derived from the unprimed, naive fraction (defined as $CD45RA^+CD27^{bright}$ lymphocytes) (16) through successive cell divisions (17). After resolution of the primary infection, a considerable pool of highly differentiated $CD8^+CD27^-$ T cells remains, reflecting a permanent change of the lymphocyte pool composition from an unprimed, naive state to a more differentiated state. Importantly, original observations by Appay et al. (13) have previously documented that CMV-specific

Table I. *Patient characteristics*

	Primary CMV (n = 19)	CMV Seronegative (n = 12)
Underlying renal disease (n)		
Vascular	3	2
Glomerular	4	5
Diabetes	0	1
Unknown	1	2
Congenital/hereditary	4	0
Interstitial nephropathy	7	2
Immunosuppression (%)		
Calcineurin inhibitor	100	100
Mycophenolate	89	100
aCD25	67	75
Prednisolone	100	100

T cells, but not EBV- or HIV-reactive ones, prototypically have a CD27$^-$ phenotype. The instant and strong change in the composition of the circulating CD8$^+$ T cell compartment is exemplified for a typical patient in Fig. 1*A*.

To quantify the changes that CMV induced in the lymphocyte pool, we calculated the ratio of unprimed, naive CD27^{bright} cells versus primed, nonnaive CD27$^-$ cells within the CD8$^+$ T cell population. Indeed, CMV infection induced the appearance of a high percentage of differentiated CD8$^+$ T cells within the circulating lymphocyte pool in all CMV-seronegative renal transplant patients. These changes were not attributable to side effects of the transplant procedure nor to immune suppressive treatment, as the lymphocyte pool of CMV-seronegative patients receiving an organ of a CMV-seronegative donor did not alter (Fig. 1*B*). The slight increase in the CD27^{bright}/CD27^{dull} ratio in these CMV-negative patients could possibly be explained by the attraction of CD27^{dull} cells toward the transplanted kidney. These nonnaive T cells may leave the bloodstream responding to chemokines produced the renal allograft (23). As previously reported (19), in parallel with the increase in cytotoxic CD8$^+$ T cells, a similar rise in cytotoxic CD28$^-$ T cells was observed within the CD4$^+$ population during primary CMV infection (data not shown).

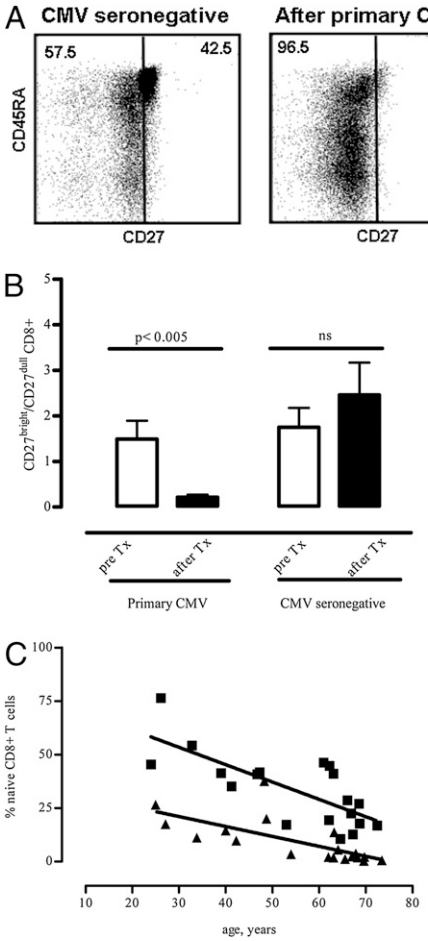

FIGURE 1. CMV induces marked changes in the differentiation status of the lymphocyte pool by decreasing naive T cells and expanding nonnaive T cells. *A*, Representative plots of CD8$^+$ T cells of a renal transplant patient. Samples are drawn pretransplantation and 1 y after primary CMV infection. *B*, Changes in the differentiation status of CD8$^+$ T cells are the result of primary CMV infection. Shown are CMV-seronegative patients that develop primary CMV infection following transplantation with a kidney from a CMV-seropositive donor (n = 19) or stay CMV seronegative posttransplantation of a kidney from a CMV-seronegative donor (n = 12). Bars represent means ± SD. *C*, CMV induces a sudden drop in the percentage of naive, CD45RA$^+$ CD27^{bright} CD8$^+$ T cells, irrespective of age. Shown are naive CD8$^+$ T cells of renal transplant patients before and 1 y after primary CMV infection. Squares in the graph show the percentage of naive CD8$^+$ T cells measured pretransplantation in relation to age (n = 19; r = −0.74; p = 0.0003). Triangles show the frequency of naive CD8$^+$ T cells for the same patients but 1 y after primary CMV infection (n = 19; r = −0.69; p = 0.001).

The changes in differentiation status of the lymphocyte pool are a consequence of a decrease in naive CD8$^+$ T cell production, an increase in nonnaive, highly differentiated effector cells, or both. With increasing age, naive T cell numbers decrease as a result of involution of the thymus, which starts immediately after birth and results in a severely decreased lymphocyte production after the fifth decade of life (24). To study if age is a contributing factor that influences the changes seen during primary CMV infection, we examined the percentage of naive CD8$^+$ T cells before and 1 y after primary CMV infection in patients with ages ranging from 21–73 y. The percentage of naive CD8$^+$ T cells was found to diminish with age. However, after primary CMV, an acute and steep drop is seen that appeared to be independent of age (Fig. 1*C*). The latter observation indicated that in this transplantation cohort, the differentiation status of the CD8$^+$ lymphocyte pool is more strongly influenced by CMV infection then by age.

Changes in the lymphocyte pool, induced by CMV, are reflected in mean telomere length

To investigate if primary CMV infection also had an immediate and lasting influence on telomere length, we studied patients that developed a primary CMV infection up to 3 y postinfection. In parallel with the decrease in unprimed naive T cells and increase in primed T cells, the mean lymphocyte telomere length dropped shortly after primary CMV infection, whereas telomeres did not change significantly posttransplantation of a kidney from a CMV-seronegative donor (Fig. 2*A*). When cell separations were performed, we found, in agreement with previous observations, that CD45RA$^+$CD27$^-$ cells had shorter telomeres than naive CD8$^+$ T cells (Supplemental Fig. 1), indicating that the drop of lymphocyte telomere length of primary infection is related to the change in subset distribution (25). Moreover, the telomere length of CMVpp65-tetramer binding cells was indistinguishable from that of total CD45RA$^+$CD27$^-$ CD8$^+$ T cells (Supplemental Fig. 1). There was no correlation between the viral load and observed telomere shortening (data not shown). Changes in the lymphocyte pool that developed over the initial course of primary CMV infection remained unaltered during the complete follow up of 3 y (Fig. 2*B*).

The immune response to CMV is a major cause of variation in lymphocyte telomere length in healthy individuals

Immune suppressive treatment in renal transplant patients may exaggerate the CMV- induced changes in the immune system (19). To investigate if CMV also influenced telomere length in immunocompetent people, we examined the impact of CMV infection in healthy individuals ranging from 20–95 y of age. Telomere length was found to strongly correlate with age in all lymphocyte populations studied, and this attrition was exacerbated in CMV-positive individuals (Fig. 3). The rate of telomere loss was found to be greatest in CD8$^+$ T cell populations with CMV-positive individuals losing more bp per year: 94 ± 9 compared with CMV-negative donors, 77 ± 9. However, this CMV-related attrition in telomere length was also seen within live lymphocytes (CMV$^+$: 61 ± 6; CMV$^-$: 45 ± 9) and CD4$^+$ T cells (CMV$^+$: 65 ± 3; CMV$^-$: 47 ± 8), but not CD4$^-$CD8$^-$ lymphocytes (Fig. 3*A*). When the data are grouped by age (young, <40 y, old, >60 y), it becomes apparent that persistent CMV infection causes a significant shortening of telomere

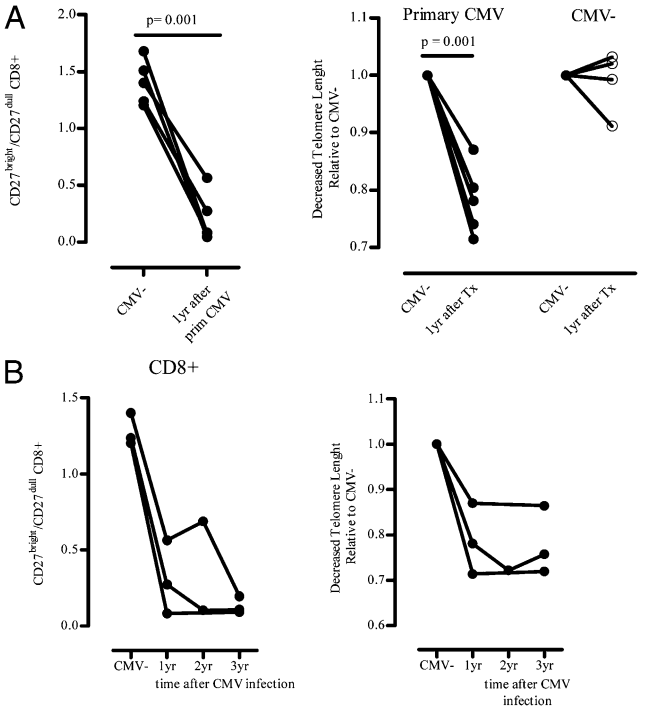

FIGURE 2. Together with the change in differentiation status of the lymphocyte pool, a sudden and lasting drop in telomere length is seen after primary CMV infection. *A*, The differentiation status of $CD8^+$ T cells of CMV-seronegative patients developing a primary CMV infection (*left panel*), and the lymphocyte telomere length of renal transplant recipients developing a primary CMV infection before (CMV^-) and after the primary infection (CMV^+) ($n = 5$) and CMV seronegative patients transplanted with a donor kidney of a CMV-seronegative donor ($n = 4$; *right panel*). At these later time points, the CMV PCR was negative again. *B*, Changes in $CD8^+$ T cell pool and telomere length after primary CMV infection during a follow-up period of 3 y ($n = 3$).

length (Fig. 3*B*). This is in line with the work of Kahn et al. (26), in which the authors show that the amount of CMV-specific cells with a highly differentiated phenotype increases over time, which leads to an increase of CMV-specific cells in elderly individuals up to 23% of the total lymphocyte population.

Having established that also in healthy individuals CMV had an impact on mean telomere length, we next investigated whether in this group the T cell differentiation correlated with telomere length. Indeed, a decrease of naive T cells and an increase in differentiated T cells in the peripheral blood of CMV-seropositive healthy individuals in both $CD8^+$ and $CD4^+$ T cells was observed (Fig. 4*A*). The variation in telomere length was found to be strongly related to the differentiation status of both $CD8^+$ T cells ($r = 0.68$; $p = 0.004$; Fig. 4*B*) and $CD4^+$ T cells ($r = 0.55$; $p = 0.007$; Fig. 4*C*).

Variation in leukocyte telomere length is correlated to the variation in telomere length of the lymphocyte pool

Previous studies reporting short telomeres as a risk factor for atherosclerosis have measured telomere length in full blood (6–8). Because myeloid and lymphoid cells are the only nucleated cells in the blood, the average telomere length of both populations will determine the leukocyte telomere length. Because we showed in this study that the telomere length of the lymphocytes can vary depending on the differentiation status of the T cell pool, and granulocytes belong to a short-lived, relatively homogeneous population, we hypothesized that the variation in the telomere length measured in blood is dependent mainly on telomere length of the T cell pool. To investigate this, we compared the telomere lengths of the circulating leukocytes with the telomere length of the lymphocyte and granulocyte pool ($n = 10$). We found a nonsignificant correlation between the total leukocyte pool and granulocytes (data not shown). In contrast, a significant correlation between the total leukocyte pool and the lymphocyte pool fraction ($r = 0.74$; $p = 0.01$) was seen (Fig. 4*D*). Thus, the differentiation status of the circulating lymphocyte pool may be a major contributor to the differences in telomere length as measured in blood leukocytes between individuals.

Discussion

CMV has infected over 70% of the adult individuals globally, and although this generally remains unnoticed in immunocompetent individuals, CMV carriership has been associated with a number of diseases (27, 28), immunosenescence in elderly, and a decrease in longevity (29, 30). Further, CMV latency is associated with a decrease in vaccination efficiency and concomitant increase in infection-related mortality (31). It is, however, unclear if these associations are inflicted by direct cytopathic effects of CMV or rather related to the exceptionally potent and chronic effector responses evoked by the virus (32). CMV infection changes the composition of the T cell pool by inducing a decrease of naive T cells and a permanent increase of highly differentiated T cells. We now show that this shift is accompanied by a rapid and sustained decrease in telomere length. It is an important question if and why these changes are exclusively induced by CMV. After resolution of the acute infection, CMV, like many other persistent pathogens, remains lifelong latent. However, in contrast to other common persistent viruses, such as EBV, only in CMV infection are high numbers of fully differentiated, effector-type T cells found during the latency phase of infection (13). We and others have shown previously that these CMV specific cells have predominantly a $CD27^-CD45RA^+$ phenotype in $CD8^+$ T cells and $CD27^-CD28^-$ phenotype in $CD4^+$ T cells. Also, cells with this phenotype are specifically increased in CMV-seropositive individuals, and the amount of $CD27^-CD45RA^+$ cells is correlated with percentage of CMV tetramer-specific cells (18, 33, 34). Furthermore, in healthy as well as immunocompromized renal transplant recipients, over 15% of the total $CD8^+$ T cells can bind to a single CMVpp65 tetramer (32). Given the fact that this is only one peptide-MHC combination out of the numerous possible combinations, it is perceivable that the many of the remaining $CD27^-CD45RA^+$ $CD8^+$ T cells are reactive toward CMV peptides. Indeed, elegant studies using overlapping peptide pools covering most open reading frames of CMV have shown that both CD4 and CD8 responses occupy an unprecedented part of the T cell response (32). Both for the induction and maintenance of highly differentiated T cells, chronic or intermittent antigenic stimulation appears to be necessary (35, 36). Additionally, as has been found in chronic murine CMV infection, the recruitment of new CMV-specific cells from the naive and memory T cells populations may replenish the pool of highly differentiated cells (37). It is debated whether CMV ever reaches true latency or rather if the virus continuously reactivates at a low frequency (38). Moreover, because of the tropism of CMV for cells of the myeloid lineage and endothelium, a constant Ag presentation to circulating lymphocytes can occur. Both frequent reactivation and the specific cellular tropism of CMV may provide the necessary requirements for chronic T cell stimulation, thereby inducing and maintaining a vast population of highly differentiated virus-specific T lymphocytes. It has been shown by others that the pool of CMV tetramer-specific as well as the pool of cells with a highly differentiated phenotype accumulates with increasing age (26, 39) and thereby accounts for the increased telomere loss in elderly CMV-seropositive individuals observed in our study.

CMV is known to increase the risk for restenosis after coronary angioplasty (40) and transplant vascular sclerosis after heart or

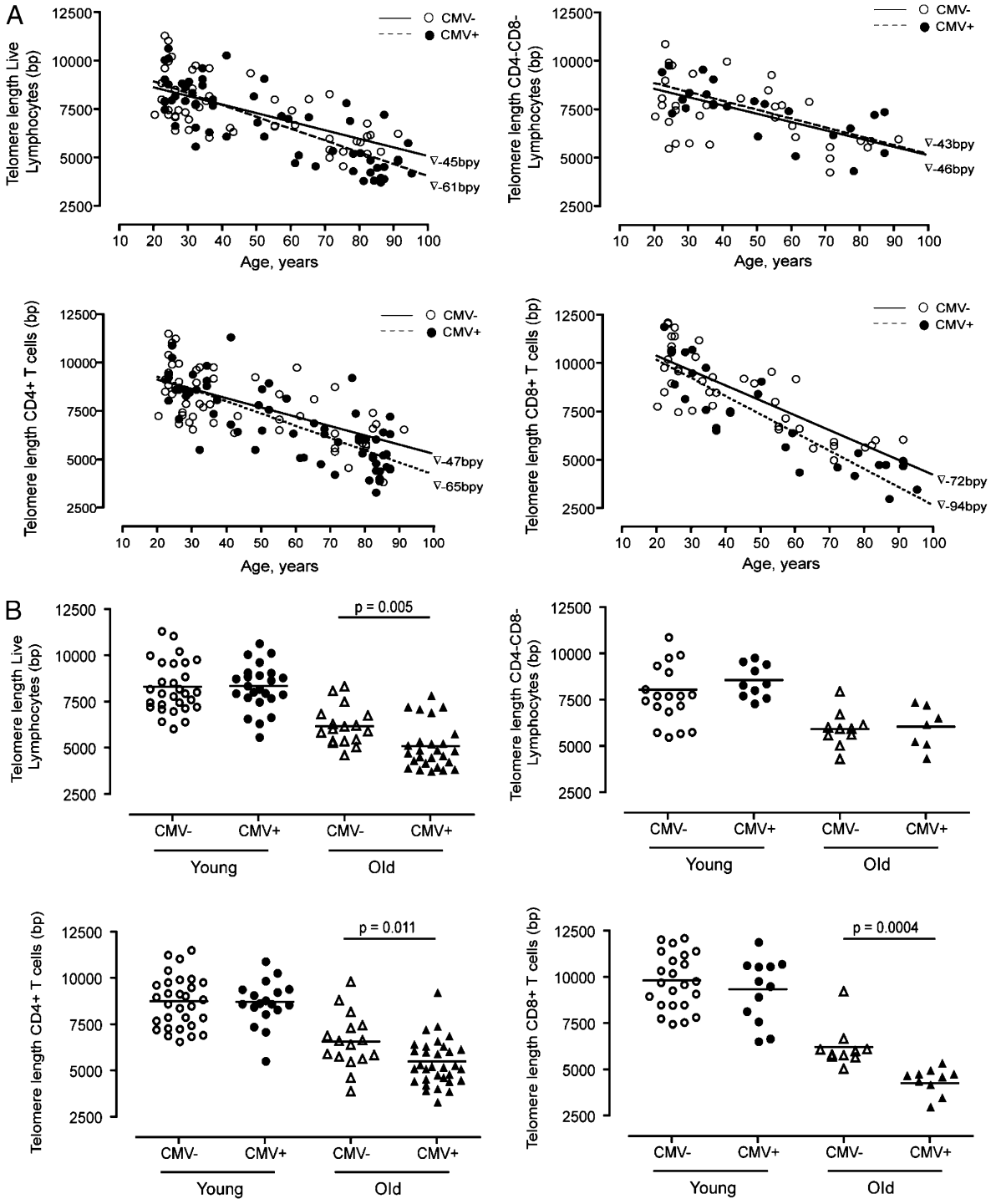

FIGURE 3. CMV accelerates telomere attrition in T cell populations from healthy individuals. *A*, Correlation between telomere length and age in total lymphocytes (CMV negative: $n = 56$, $r = -0.64$, $p < 0.0001$; CMV positive: $n = 58$; $r = -0.78$; $p < 0.0001$), $CD4^+$ (CMV negative: $n = 52$; $r = -0.64$; $p < 0.0001$; CMV positive: $n = 64$; $r = -0.76$; $p < 0.0001$), $CD8^+$ (CMV negative: $n = 40$; $r = -0.79$; $p < 0.0001$; CMV positive: $n = 29$; $r = -0.89$; $p < 0.0001$), and $CD4^-CD8^-$ T cells (CMV negative: $n = 42$; $r = -0.51$; $p = 0.0015$; CMV positive: $n = 64$; $r = -0.71$; $p = 0.0002$) from CMV-positive and -negative healthy individuals. The rate of telomere attrition, represented as loss of bp per year (bpy) was calculated from the gradient and is shown on the graphs. Open circles and an undashed line represent CMV-negative individuals; filled circles and a dashed line represent CMV-positive individuals. *B*, Telomere length of these individuals is represented by grouping via age (young, <40 y; old, >60 y) and CMV status.

kidney transplantation (41). Although the contribution of CMV in the pathogenesis of cardiovascular disease in otherwise healthy people is still debated (42–44), a link between chronic inflammation and progression of atherosclerosis is well established (45). In extensive cohort studies (5–8), relative short telomeres have been associated with an increased risk for vascular disease. Recently, Spyridopoulos et al. (46) reported that the telomere length of leukocytes was similar for all myeloid cells among patients with chronic heart disease and age-matched controls. Only the telomere length of $CD8^+$ T cells was lower in patients with chronic heart disease and coincided with an increase in differentiated $CD8^+$ T cells. However, they did not analyze the contribution of these cells to the mean blood telomere length and the impact that ongoing latent CMV infection had on the decrease in telomere length.

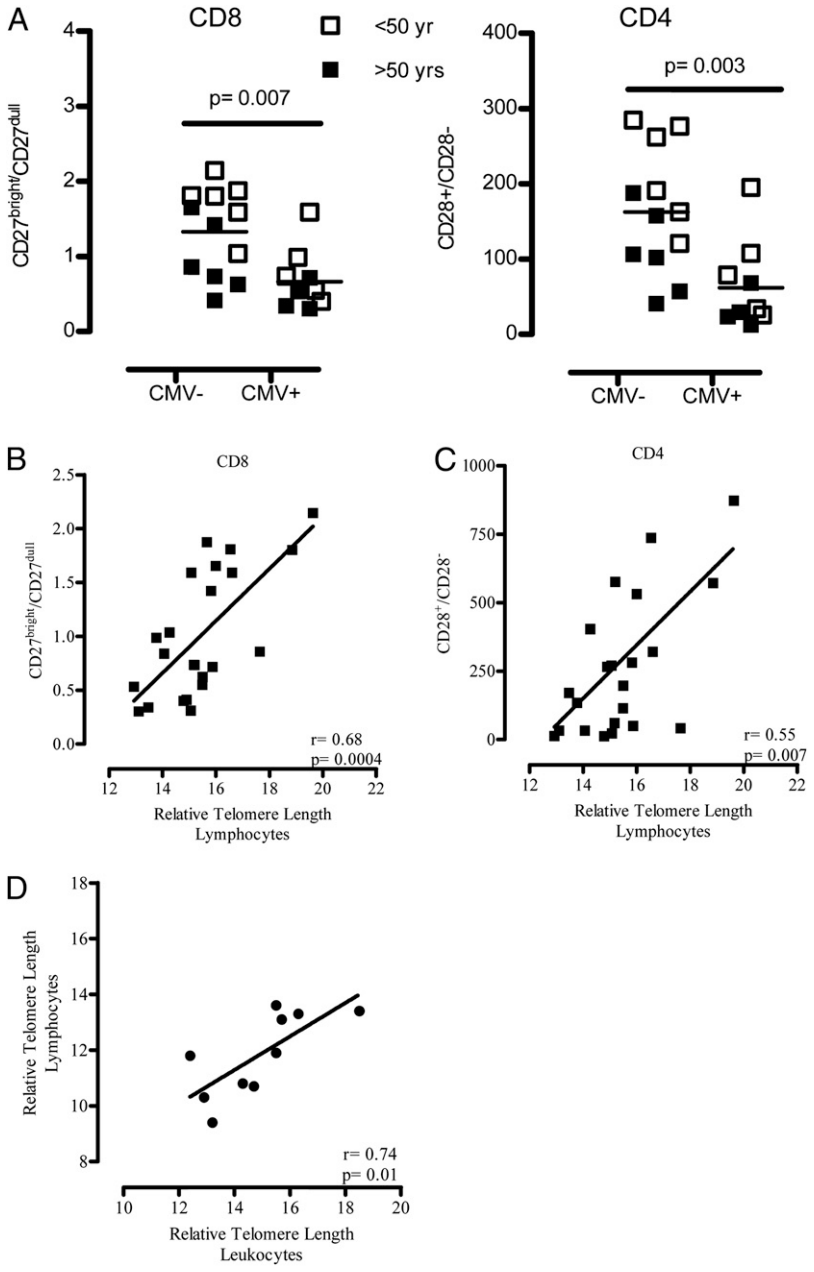

FIGURE 4. Differentiation status of the lymphocyte pool is correlated to the lymphocyte telomere length. *A*, Shown are the differentiation status of $CD8^+$ (ratio $CD27^{bright}$:$CD27^-$) and $CD4^+$ T cells (ratio $CD28^+$:$CD28^-$) in CMV-seropositive ($n = 11$; average age, 37 ± 11 y) and CMV-seronegative ($n = 12$; average age, 35 ± 11 y) healthy volunteers. *B*, Correlation between differentiation state $CD8^+$ T cells and lymphocyte telomere length ($n = 23$; $r = 0.68$; $p = 0.004$). *C*, Correlation between differentiation status of $CD4^+$ T cells and lymphocyte telomere length ($n = 23$; $r = 0.55$; $p = 0.007$). *D*, Variations in telomere length measured in blood are correlated with the telomere length of the lymphocyte pool. Telomere length was measured on total leukocytes and correlated to the telomere length of lymphocytes of 10 healthy volunteers ($r = 0.74$; $p = 0.01$).

Importantly, Spyridopoulos et al. (46) showed that telomere length of $CD8^+CD45RA^+$ T cells correlated with cardiac function. These $CD8^+CD45RA^+$ T cells comprise naive, $CD27^{bright}$, and highly differentiated $CD27^{dull}$ T cells, and the negative correlation would be in line with increasing amount of $CD27^{dull}$ cells. We show in this study that the cellular response to CMV is a major factor in generating telomere length variation among individuals. The contribution of differentiated T cells that emerge after CMV infection in the progression of vascular disease has to be defined, but it may be important that, in contrast to naive and memory-type T cells, they are able to home and adhere to endothelial cells that are activated by stressors as for instance disturbed blood flow, oxidized lipids, or inflammatory cytokines. These particular cells have this ability because of the expression of a unique set of adhesion molecules and chemokines, notably CX3CR1 that binds to fractalkine on activated endothelium (45, 47). Furthermore, highly differentiated $CD4^+$ $CD28^-$ T cells, functionally and phenotypically similar to the CMV induced $CD28^-$ T cells, are the dominant type of T cells in atherosclerotic plaques and can induce plaque rupture by inducing apoptosis in vascular smooth muscle cells in an Ag-independent way based on TRAIL and killer Ig-like receptor triggering (48, 49). In addition, both $CD4^+$ and $CD8^+$ fully differentiated T cells are equipped with cytotoxic and proinflammatory proteins that give them the capacity to induce local inflammation and promote the influx and activation of macrophages, which may ultimately lead to an increased progression of atherosclerotic plaque formation and plaque growth.

Reports showing an association between leukocyte telomere length and age-related diseases have not provided an explanation for this association (5–8, 50). Our data show that CMV infection has a major impact on the composition and telomere length of the lymphocyte pool and suggest that these changes have a major impact on the leukocyte telomere length. Because of the association of short telomere length with age-related diseases, we propose that CMV-induced chronic immune activation is a major determinant in the generation of these ailments. Indeed, interference with the long-term side effects of continuous immune activation could have a major positive impact on public health.

Disclosures

The authors have no financial conflicts of interest.

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