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Lineage-Specific Telomere Shortening and Unaltered Capacity for Telomerase Expression in Human T and B Lymphocytes with Age

Ni Huiping Son,* Shannon Murray,* Jack Yanovski,‡ Richard J. Hodes,†§ and Nan-ping Weng1*

Age effects on telomere length and telomerase expression in peripheral blood lymphocytes were analyzed from 121 normal individuals age newborn to 94 years and revealed several new findings. 1) Telomere shortening was observed in CD4+ and CD8+ T and B cells with age. However, the rate of telomere loss was significantly different in these populations, 35 ± 8, 26 ± 7, and 19 ± 7 bp/year for CD4+ and CD8+ T and B cells, respectively. In addition, CD4+ T cells had the longest average telomeres at all ages, followed by B cells, with CD8+ T cell telomeres the shortest, suggesting that these lymphocyte populations may have different replicative histories in vivo. 2) Telomerase activity in freshly isolated T and B cells was indistinguishably low to undetectable at all ages but was markedly increased after Ag and costimulatory receptors mediated stimulation in vitro. Furthermore, age did not alter the magnitude of telomerase activity induced after stimulation of T or B lymphocytes through Ag and costimulatory receptors or in response to PMA plus ionomycin treatment. 3) The levels of telomerase activity induced by in vitro stimulation varied among individual donors but were highly correlated with the outcome of telomere length change in CD4+ T cells after Ag receptor-mediated activation. Together, these results indicate that rates of age-associated loss of telomere length in vivo in peripheral blood lymphocytes is specific to T and B cell subsets and that age does not significantly alter the capacity for telomerase induction in lymphocytes. The Journal of Immunology, 2000, 165: 1191–1196.

The impact of aging on immune function is well documented (1). At the cellular level, evidence suggests that aging is associated with decreases in the numbers of circulating CD4+ T and B lymphocytes in peripheral blood (2) and with changes in composition of T lymphocyte subsets (3). Because the function of the immune system is highly dependent on the capacity for extensive cell division and clonal expansion of lymphocytes, it is of great interest to understand whether aging-related processes result in a loss of the replicative capacity of lymphocytes.

Telomere length and its regulation by telomerase have drawn considerable attention for their potential roles in the control of cellular replication (4). Telomeres are specialized terminal chromosomal structures consisting of highly conserved TTAGGG repeats (5) and telomere-binding proteins (6, 7). Because conventional DNA polymerase cannot completely replicate the ends of linear chromosomes, each cell division will result in a loss of terminal telomere DNA in the absence of compensatory mechanisms (8, 9). Studies in human fibroblasts and other normal somatic cells suggest that a minimum length of telomere is essential for the integrity of chromosomes and viability of cells (10). Thus, the length of telomeres serves as a useful indicator for the replicative history of a cell as well as for estimating its residual replicative potential. Telomerase is a telomere-synthesizing reverse transcriptase that can compensate for the loss of telomere associated with cell divisions (11, 12). Telomerase is constitutively expressed in germline cells and in a majority of malignant tumor cells (13). In contrast, telomerase expression is highly regulated in lymphocytes during development and activation (14) and is absent in most normal human somatic cells (13). This differential expression of telomerase provides a molecular explanation for the immortality or unlimited replicative capacity of the germline and of malignant cells and for the limited replicative capacity of normal somatic cells. Indeed, introduction of telomerase into normal human somatic cells, such as fibroblasts and retinal pigment epithelial cells that do not normally express telomerase, prolongs the replicative life span of these cells, indicating that telomerase plays an essential role in regulating cellular replicative capacity (15).

Telomere shortening with age has been reported in human peripheral blood leukocytes (16, 17), in PBMC (18), and in T cells (19, 20). The rate of telomere shortening appears not to be uniform throughout life. Loss of telomeres was reported to be most rapid from newborn to 4 years of age, with that rate gradually declining between age 4 and 39 years, and with telomere shortening continuing at a relatively stable and low rate between age 40 and 95 years (17, 20). Telomerase activity in PBMC also varied with age and was detectable in many newborns and in some children before adolescence, decreasing to low or undetectable levels after age 20 (18). In addition, lymphocytes are capable of expressing induced telomerase activity after antigenic activation both in vivo (21, 22) and in vitro (23–25), in what appears to be a specifically regulated process. However, a detailed analysis of whether or not age affects telomere length changes in T and B cell subpopulations, whether age affects the inducibility of telomerase in resting blood lymphocytes, and whether there is an interplay between telomerase activity and telomere length in lymphocytes with age has not been reported.

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We report here an analysis of telomere length and telomerase activity in human peripheral blood CD4+ and CD8+ T and B lymphocytes of 121 normal donors of ages from newborn to 94 years ex vivo as well as after in vitro stimulation. Both T and B lymphocytes exhibit an age-associated loss of telomeres at rates that are different and specific to each subset. The magnitude of telomerase induction in T and B lymphocytes after Ag and co-stimulatory receptor-mediated activation in vitro also differs among lymphocyte lineages in a fashion that correlates inversely with in vitro telomere loss. In addition, it is observed that the capacity for induced telomerase expression in T and B cells is undiminished with age.

Materials and Methods

Isolation of CD4+ and CD8+ T cells and B cells from peripheral blood

Heparinized peripheral blood of 121 normal donors of ages from newborn to 94 years was collected from Baltimore Longitudinal Study of Aging participants (n = 70, mostly age >40), from the National Institutes of Health blood bank (n = 45, mostly age <50), and from the Johns Hopkins Bayview Hospital (n = 6, cord blood). All donors were healthy with no known malignancy. Blood samples were collected through Institutional Review Board approved protocols. The procedures for isolation of CD4+ and CD8+ T cells and B cells were previously described (22, 24). We used sequential positive selection to isolate CD4+, CD8+ T cells and CD19+ B cells by an immunomagnetic separation method (Dynal, Lake Success, NY) based on manufacturer’s instructions. The purity of subsets of lymphocytes was generally >95%.

Stimulation of peripheral blood CD4+ and CD8+ T cells and B cells in vitro

The protocol for in vitro stimulation of T and B cells was described previously (22, 24). Peripheral blood CD4+ and CD8+ T cells and B cells were stimulated in vitro for 3 days. T cells were stimulated with PMA plus ionomycin (PMA/ionomycin) (Calbiochem, La Jolla, CA), or with anti-CD3 plus anti-CD28 Ab-conjugated magnetic beads, and B cells were treated with PMA/ionomycin or with anti-IgM (Jackson ImmunoResearch, West Grove, PA) plus anti-CD40 Abs (Caltag, South San Francisco, CA) (anti-IgM/CD40). The concentrations of stimulators and the time of harvest of cells were based on conditions previously optimized on T cells (22).

Measuring telomere length by flow cytometry

Telomere length was measured by a flow cytometry-based method as previously described (26). In brief, 6 × 10^6 cells per sample were suspended in PBS, 0.1% BSA (ICN Biomedicals, Costa Mesa, CA), and each sample was divided equally into two Eppendorf tubes, one for propidium iodide (PI)2 (Sigma) staining alone and the other for telomere and PI staining. Samples were incubated at 4°C overnight in the dark and then transferred to Falcon 2058 tubes (Becton Dickinson, Mountain View, CA) for analysis by FACScan flow cytometry (Becton Dickinson). The net fluorescence intensity was calculated by subtraction of background from the specific staining and used to express telomere fluorescence. A standard curve for the conversion of the quantity of telomere fluorescence to actual telomere-terminal fragment length was established from a comparative analysis of telomere fluorescence and terminal length fragments by Southern analysis of the same donors (n = 25) (data not shown). The conversion factor is 1 fluorescence arbitrary unit (AU) = 57 bp.

PCR-based telomerase assay

Telomerase activity was detected by a modified telomeric repeat amplification protocol (TRAP) as described previously (27). Cell lysates were prepared with ice-cold 0.5% choloramidopropidimethylnamino propanesulfonate (Calbiochem) lysis buffer at 100 μl/10^6 cells. Five microliters of each extract were used for telomere synthesis at 10 μl total volume with incubation at 22°C for 1 h. Two microliters of the newly synthesized telomeres (10,000 cell equivalents) were amplified in a DNA Engine Tetra (MJ Research, Cambridge, MA) for 27 rounds (94°C for 30 s, 94°C for 15 s, and 60°C for 15 s). One-third of the amplified products (3333 cell equivalents) was then loaded on a 12% polyacrylamide gel (Novex, San Diego, CA) and stained with SYBR green I (Molecular Probes, Eugene, OR). Telomerase activity was measured by Storm (Molecular Dynamics, Sunnyvale, CA) using blue fluorescence collection, and the results were analyzed by ImageQuat software (Molecular Dynamics). Telomerase products and the internal control showed a ladder of bands starting from 50 bp and a single band of 36 bp, respectively. For quantification of telomerase activity, a serial 3-fold dilution of cell lysate was combined with the measurement of the intensity of telomerase products (from 50 to ~200 bp). Telomerase activity of cell line 293 was used as a standard for normalization, and over repeated measurements was 231 ± 36 AU. We calculated the lowest dilution with clear telomere products for quantification using the formula [TTP (AU) = (SP - NC) × (NIC/SIC)], in which TTP = total telomerase product, SP = sample telomerase products, NC = negative control background, NIC = negative internal control, and SIC = sample internal control.

Proliferation assay

Cell proliferation was assayed by [3H]thymidine incorporation as previously described (22, 24). Purified CD4+ cells were stimulated with anti-CD3/CD28 as described above. Cells (1 × 10^5 per well) were seeded in quadruplicate in flat-bottom 96-well microtiter plates and cultured at 37°C for 48 h. Then, 1 μCi [3H]thymidine (New England Nuclear, Boston, MA) was added and incubated for another 20 h before harvest. [3H]thymidine incorporation was measured by liquid scintillation counting (Wallac, Gaithersburg, MD).

Statistical analysis

Separate paired r tests were applied to compare the means of telomere length for CD4+ vs CD8+, CD4+ vs CD19+, and CD8+ vs CD19+. To account for the repeated measurements on the samples, a mixed effects analysis using ProcMixed in SASS/STAT (Cary, NC) software was used to assess the difference in the rates of telomere length shortening with age and in induced telomerase activity with age among CD4+, CD8+, and CD19+ cells (28). The correlations between telomerase activity and cell proliferation, and between telomerase activity and telomere length change were assessed by Pearson correlation coefficient.

Results

Telomere length dynamics in human peripheral blood CD4+ and CD8+ T and CD19+ B cells

To determine the telomere length in T and B cells as a function of age, we isolated CD4+ and CD8+ T cells and CD19+ B cells from peripheral blood of 121 normal donors ages 0–94 years. A reduction of telomere length was observed in all three subsets of lymphocytes as a function of increasing age (Fig. 1). The rates of telomere shortening were: 35 ± 8 bp/year for CD4+ T cells, 26 ± 7 bp/year for CD8+ T cells, and 19 ± 7 bp/year for CD19+ B cells. The rate of telomere shortening in B cells was significantly slower than that in CD4+ T cells (p < 0.05). There were no gender differences in telomere length or in the rate of telomere shortening with age in these three subsets of lymphocytes (data not shown).

The relative length of telomeres among T and B cell subsets exhibited variability in this group of donors studied here. On average in the total population of donors, CD4+ T cells (7.6 ± 2.1 kb) have longer telomeres than CD8+ T (6.3 ± 2.0 kb) and CD19+ (7.1 ± 1.9 kb) B cells, and CD8+ T cells have the shortest telomeres on average (Table I). The differences in average telomere length were statistically significant among these three populations at all ages.

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5 Abbreviations used in this paper: PI, propidium iodide; TRAP, telomeric repeat amplification protocol; AU, arbitrary unit; hTERT, transcriptional level of telomerase catalytic subunit.
Telomerase expression in peripheral blood CD4⁺ and CD8⁺ T and CD19⁺ B cells

Because telomere length can be influenced by telomerase activity, the effect of age on telomerase expression in freshly isolated CD4⁺ and CD8⁺ T and CD19⁺ B cells was assayed. Telomerase activity was undetected in the majority of donor CD4⁺ (75%) and CD8⁺ (83%) T cells and B (91%) cell populations. Low levels of telomerase activity were detected in some donors and did not appear to be restricted to the young donors as previously reported (18) or to either gender (Fig. 2). We then examined the activity of telomerase in CD4⁺ and CD8⁺ T and B cells after in vitro stimulation. Stimulated cells were collected on day 3 based on our previous determination that telomerase activity reached a peak on days 3–6 after stimulation (24). It has been reported that age-associated changes occur in the surface expression of Ag and costimulatory receptors (29, 30). We therefore stimulated T and B cells with both receptor-dependent (anti-CD3/CD28 for T cells and anti-IgM/CD40 for B cells) and receptor-independent (PMA/ionomycin for both T and B cells) stimuli to assess capacity for induction of telomerase. Telomerase activity was induced in CD4⁺ and CD8⁺ T and B cells after stimulation (Fig. 3). The levels of induced telomerase activity after treatment with PMA/ionomycin were similar in CD4⁺ and CD8⁺ T and B cells. In contrast, Ag receptor and costimulatory receptor-mediated stimulation induced ~4.7- and 1.5-fold higher levels of telomerase activity in CD4⁺ and in CD8⁺ T cells than did PMA/ionomycin stimulation (872 ± 53 vs 187 ± 71 AU for CD4⁺, p = 0.0001; and 183 ± 22 vs 123 ± 27 AU for CD8⁺, p = 0.0001) (Fig. 3).

There was no statistically significant age-associated loss of telomerase induction in CD4⁺ and CD8⁺ T cells in response to anti-CD3/CD28 stimulation (Fig. 3). Although B cells appeared to have a reduced telomerase response to anti-IgM/CD40 stimulation as a function of age, this trend was not statistically significant with the number of samples analyzed (Fig. 3). No decline was observed with age in induced telomerase induction by PMA/ionomycin treatment in either T or B cells (Fig. 3).

Relationship between telomerase activity and cellular proliferation in CD4⁺ T cells

To determine the relationship between cellular proliferation and the levels of induced telomerase in peripheral blood lymphocytes, we measured [³H]thymidine incorporation as an indicator for cellular proliferation and compared it with the levels of telomerase activity. When CD4⁺ T cells were analyzed after anti-CD3/CD28 stimulation, a correlation was suggested between activation-induced cellular proliferation and telomerase activity in CD4⁺ T cells (R = 0.23, p = 0.086) (Fig. 4). CD8⁺ T cells and B cells could not be analyzed due to inadequacy of recovered cell numbers.

Correlation between telomerase activity and telomere length changes in CD4⁺ T cells

Telomere length changes in lymphocytes after activation may be influenced by multiple factors including preferential selection and expansion of certain cells, the extent of cell division after activation, and the level of induced telomerase activity. To determine

Table I. Telomere length of peripheral blood CD4⁺ and CD8⁺ T, and CD19⁺ B lymphocytes in different age groups

<table>
<thead>
<tr>
<th>Lymphocyte Subsets</th>
<th>Mean Telomere Length (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–94 yr (n = 121)</td>
</tr>
<tr>
<td>CD4⁺</td>
<td>7.7 ± 2.1b</td>
</tr>
<tr>
<td>CD8⁺</td>
<td>6.3 ± 2.0b</td>
</tr>
<tr>
<td>CD19⁺</td>
<td>7.1 ± 1.9b</td>
</tr>
</tbody>
</table>

* Values are average ± SD.

b Statistical analysis was done by separate paired t tests. Difference of average telomere length between lymphocyte subsets in 0 to 4-year group is significant. CD4⁺ vs CD8⁺ (p < 0.05), CD4⁺ vs CD19⁺ (p < 0.05), CD8⁺ vs CD19⁺ (p < 0.05).
whether telomerase activity induced after activation is capable of protecting telomere loss in lymphocytes, we compared changes of telomere length and telomerase activity in CD4\(^+\) T cells before and 3 days after anti-CD3/CD28 stimulation in vitro. It was found that telomere length changes were correlated with the telomerase activity induced in CD4\(^+\) T cells (\(R = 0.265, p = 0.012\)) (Fig. 5). A correlation was observed between the level of induced telomerase activity and change in telomere length, such that telomere lengthening rather than telomere loss was found in CD4\(^+\) T cells when telomerase activity reached relatively high levels; conversely, telomere loss was evident when telomerase activity was low. The calculated regression line for this relation describes fully compensated telomere length (no change with activation) when telomerase activity was ~1340 AU.

**Discussion**

Telomere shortening in normal somatic cells with age is a well-documented phenomenon. The majorities of normal somatic cells do not express telomerase and undergo progressive telomere shortening with age (31). Lymphocytes exhibit a similar trend of telomere shortening with age despite expressing telomerase under tight regulation during development and activation (32). The relationship between age and telomerase expression and the precise relationship between telomerase activity and telomere length changes in lymphocytes have not been systematically addressed. The results presented here show that 1) telomere shortening occurs in CD4\(^+\) and CD8\(^+\) T and B cells and that the rate of telomere loss is different in these lymphocyte populations; 2) there is no significant loss in the capacity for induction of telomerase activity with age in T or B cells after in vitro stimulation; and 3) the magnitude of induced telomerase activity correlates with cellular proliferation and telomere length changes after activation of in CD4\(^+\) T cells, suggesting a quantitative relationship between telomerase activity and telomere length dynamics in lymphocytes.

**FIGURE 2.** Telomerase activity in freshly isolated peripheral blood CD4\(^+\) and CD8\(^+\) T and CD19\(^+\) B cells with age (n = 106). Telomerase activity was measured by a modified TRAP assay as described in Materials and Methods. The majority of freshly isolated CD4\(^+\) (75%) and CD8\(^+\) (83%) T cells and CD19\(^+\) (91%) B cells did not express a detectable level of telomerase activity, and only a small number of lymphocyte populations showed detectable but weak telomerase activity (arbitrary unit ranges from 20 to 50).

**FIGURE 3.** Induced telomerase activity in human peripheral blood CD4\(^+\) and CD8\(^+\) T cells and CD19\(^+\) B cells as a function of age after Ag receptor or non-Ag receptor-mediated activation. CD4\(^+\) (n = 78 for anti-CD3/CD28 and n = 90 for PMA/ionomycin) and CD8\(^+\) (n = 41 for anti-CD3/CD28 and n = 54 for PMA/ionomycin) T cells and CD19\(^+\) (n = 21 for anti-IgM/CD40 and n = 39 for PMA/ionomycin) B cells were stimulated in vitro for 3 days. Telomerase activity was calculated based on cell equivalents. After Ag receptor or non-Ag receptor stimulation, no significant age-associated telomerase loss was observed in either T or B cells.

**FIGURE 4.** Correlation between telomerase activity and cellular proliferation in human peripheral blood CD4\(^+\) T lymphocytes (n = 55). \(^{[3]}\)HThymidine incorporation in CD4\(^+\) T cells after anti-CD3/CD28 stimulation was measured and compared with the levels of telomerase activity in the same individuals (\(R = 0.236, p = 0.084\)).
Previous reports indicated that telomere shortening occurs with age in human peripheral blood leukocytes, PBMC, and T cells (16–20). Frenck et al. (17) and Rufer et al. (20) both reported that telomere length decreases at a rapid rate from newborn to 4 years of age relative to the less rapid rate of loss during later years of life. The study reported here did not include sufficient donors between age 0 and 4 years to permit a similar analysis. However, the present study has provided novel information by 1) analyzing telomere length in T cell subsets as well as B cells as a function of age and 2) analyzing the relationship between telomere induction and telomere length maintenance in activated CD4⁺ T cells. The rates of overall telomere loss with age in CD4⁺ and CD8⁺ T cells are slightly lower than recently reported by Rufer et al. (39–51 and 34–54 bp/year for CD4⁺ and CD8⁺ T cells, respectively) (20), but are within the ranges of other reports (16, 19). Analysis of telomere length as a function of age has not been reported previously in normal B cells. Interestingly, the rate of overall telomere loss reported here in B cells with age was significantly slower than that in CD4⁺ T cells. Thus, it appears that overall age-related changes in telomere length are cell lineage specific. Previous study of telomere length changes in B cells suggested that telomerase was capable of lengthening telomeres during the process of differentiation of naive B cells to germinal center B cells in vivo concurrent with extensive cell division (22). However, telomere length changes in proliferating T cells during an immune response in vivo have not been analyzed. It remains to be directly demonstrated whether telomerase-associated protection of telomeres during B cell differentiation is responsible for the slower rate of overall telomere loss in the B cell lineage with age or whether such differences reflect distinct rates of cell division in T and B cell subsets or differential selection or survival of cells with shorter or longer telomeres.

Regulation of telomerase activity is a complex and tissue-specific process. In most normal somatic cells, it appears that telomerase activity is controlled at the transcriptional level of telomerase catalytic subunit (hTERT) (33, 34). In contrast, lymphocytes appear to regulate telomerase activity through mechanisms other than the quantitative level of hTERT mRNA (35). Age influence on telomerase activity in PBMC has been reported recently (18). It was found that telomerase activity in freshly isolated PBMC markedly declined from newborn to adolescence and was stable at low to undetectable levels in all adult life (18). Because PBMC consist of multiple cell lineages, such differences in telomerase activity may reflect changes such as decreased numbers of telomerase-expressing stem cells and naive T cells newly derived from the thymus. A systematic analysis of age effects on telomerase expression and regulation in lymphocytes has not been previously reported. The finding that there is no obvious loss of the induced telomerase activity in T or B cells after in vitro stimulation suggests that the capacity for telomerase expression is stable and does not change with age in T and B cells.

The observation that telomere shortening can occur in lymphocytes in the presence of telomerase activity has raised the question of whether telomerase is in fact related to telomere length maintenance in these cells. Recent reports of telomere changes in limited numbers of long term-cultured T cells imply that high levels of telomerase were capable of protecting telomere length loss (36, 37). Study of B cell in vivo differentiation shows that a high level of telomerase activity may only protect against loss of telomeres but also lengthen telomeres (22). The results presented here examined the quantitative relationship between telomerase activity and telomere length changes in CD4⁺ T cells isolated from 88 normal individuals. The quantitative level of telomerase induced in CD4⁺ T cells through Ag receptor-mediated stimulation was observed to be correlated with cellular proliferation and telomere loss or gain observed in these activated cells. The mechanism that mediates the relationship observed in the present study between telomerase activity and telomere length maintenance after T cell activation is not established. The best characterized action of telomerase is the function of this enzyme in adding telomeric repeats, thereby protecting against telomere shortening with cell replication. It is possible that this is the predominant mechanism, accounting for the observation that telomere length is better maintained, or even increased, in those activated T cell populations that express the highest levels of telomerase. However, recent studies suggest additional possible functions of telomerase that are distinct from its role in telomere elongation. It has been proposed that telomerase activity is critically important in protecting cellular proliferative capacity in T-Ag-transformed human fibroblasts (38) and in preventing apoptosis in neuronal cells (39). Such activities might, by altering the relative survival and clonal expansion of telomerase-expressing cells, contribute to the observed overall correlation of telomerase expression with telomere length.

The dynamics of telomere length and telomerase activity in lymphocyte populations are likely influenced by multiple factors, such as the conditions used for activation, the strength of signals, induced telomerase levels, numbers of responding cells, proliferation rate, and number of apoptotic cells. Although we did not observe any age-related alteration of telomerase induction in lymphocyte subsets after short term stimulation, it is not clear whether or not sustained induction of telomerase activity changes with age. Previous reports have shown an age-associated increase of activation-induced apoptosis (40–42) and changes in subset composition (3, 43) and in immune functions (1). It is possible that some of these age-related factors may affect the sustained expression of telomerase in lymphocytes during long term culture or in vivo.

Our analysis of the dynamics of telomere length and telomerase expression in lymphocyte subsets provides information on changes in telomere length and telomerase expression by T and B lymphocytes in relationship to age. Because the variability of telomere length in blood leukocytes (16) and the levels of activation-induced telomerase activity in lymphocytes (44) are strongly influenced by genetic factors, it will be informative to conduct longitudinal studies of normal individuals to determine the true influence of age. The results derived from such longitudinal analysis will lead to better understanding of the influence of aging on...
the dynamics of telomere length and telomerase expression in lymphocytes.

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References