Association Between Telomere Length and Experimentally Induced Upper Respiratory Viral Infection in Healthy Adults

Sheldon Cohen, PhD
Denise Janicki-Deverts, PhD
Ronald B. Turner, MD
Margaretha L. Casselbrant, PhD, MD
Ha-Sheng Li-Korotky, PhD, MD
Elissa S. Epel, PhD
William J. Doyle, PhD

Telomeres, the DNA-protein complexes at the end regions of chromosomes, decrease in length with every cell division. In primary blood cells, telomeres are partly reconstructed by the activity of telomerase, a specialized intracellular enzyme that adds subunit repeats to telomeres. Despite the activity of telomerase, telomeres continue to shorten with repeated cell divisions, leading to disrupted cell function and eventual cell senescence.

Telomere shortening in leukocytes has implications for immunocompetence and is associated with increased synthesis of proinflammatory cytokines and poorer antibody response to vaccines. Shorter leukocyte telomere length also is associated with aging-related morbidity and mortality from conditions with immune system involvement, including infectious diseases, cancer, and cardiovascular disease.

The rate of progression to senescence differs among lymphocyte subsets, with an advanced rate of telomere shortening in the cytolytic CD8 T cells. This is especially important for cancer and virally induced infectious diseases, because rapid loss of telomere length in cytolytic T cells, are associated with decreased resistance to upper respiratory infection and clinical illness in young to midlife adults.

Main Outcome Measures Infection (virus shedding or 4-fold increase in virus-specific antibody titer) and clinical illness (verified infection plus objective signs of illness).

Results Rates of infections and clinical illness were 69% (n=105) and 22% (n=33), respectively. Shorter telomeres were associated with greater odds of infection, independent of prechallenge virus-specific antibody, demographics, contraceptive use, season, and body mass index (PBMC: odds ratio [OR] per 1-SD decrease in telomere length, 1.71 [95% CI, 1.08-2.72]; n=128 [shortest tertile 77% infected; middle, 66%; longest, 57%]; CD4: OR, 1.76 [95% CI, 1.15-2.70]; n=146 [shortest tertile 80% infected; middle, 71%; longest, 54%]; CD8CD28+: OR, 1.93 [95% CI, 1.21-3.09], n=132 [shortest tertile 84% infected; middle, 64%; longest, 58%]; CD8CD28–: OR, 2.02 [95% CI, 1.29-3.16]; n=144 [shortest tertile 77% infected; middle, 75%; longest, 50%]). The association between CD8CD28– telomere length and infection increased with age (CD8CD28– telomere length × age interaction, b=0.09 [95% CI, 0.02-0.16], P=.01, n=144).

Conclusion and Relevance In this preliminary study among a cohort of healthy 18- to 55-year-olds, shorter CD8CD28– T-cell telomere length was associated with increased risk for experimentally induced acute upper respiratory infection and clinical illness.

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CD8 T cells cause senescence marked by loss of expression of CD28, a costimulatory molecule important for antiviral function.

In this study we assessed whether telomere length in leukocytes is associated with host resistance to experimentally induced viral upper respiratory infection in young to midlife adults. Our expectation was that shorter leukocyte telomere length, especially in CD8CD28− cells, would be associated with an increased risk for infection and clinical illness.

METHODS

Participants

Participants were 152 healthy residents of the greater Pittsburgh, Pennsylvania, area aged 18 through 55 years and recruited by newspaper advertisements to participate in a study of the causes of the common cold. Each received $1000 for participating in the study. The study received approval from the Carnegie Mellon University and University of Pittsburgh human participants review boards, and all participants provided signed informed consent.

Design

Healthy participants who had their blood drawn for telomere assessment were subsequently quarantined, administered nasal drops containing a rhinovirus that causes the common cold (rhinovirus type 39 [RV39]), and monitored for 5 days for development of infection and clinical illness.

Data were collected between 2008 and 2011. Volunteers were screened 6 to 8 weeks before viral administration and enrolled only if they reported no acute or chronic illnesses; were in good health as assessed by a complete physical examination that included examination of the ear, nose, and throat, complete blood and urine panels, and human immunodeficiency virus testing; did not take prescription medications, with the exception of birth control; and had specific neutralizing serum antibody titers to the challenge virus and participants were evaluated for objective signs of illness. Following approximately 28 days after exposure to the virus, blood samples were taken for assay of convalescent serum-specific antibody titer to the challenge virus. Investigators conducting the telomere length assays, which were performed in batch after all trials were concluded, were blinded to all participant data.

Procedures of the Viral Challenge Trial

Infection. Infection was defined as isolation of the challenge virus in nasal secretions (cultured using standard procedures) on any of the postexposure quarantine days or as a 4-fold or greater increase in specific antibody titer to the challenge virus (microtiter neutralization assay) from before to 28 days after exposure.

Clinical Illness. Individuals were considered to have a cold on meeting the criteria for both infection and objective signs of illness. Objective signs of illness were assessed using 2 daily measures: nasal mucociliary clearance time and mucus weight. Nasal mucociliary clearance time—an indicator of nasal congestion—was measured as the time (seconds) needed for a flavored dye administered to the inferior turbinates to arrive at the nasopharynx. Mucus weight (grams)—a marker of nasal secretion production—was measured by weighing sealed plastic bags containing the participants’ used paper tissues and then subtracting the weights of unused tissues and empty plastic bags. These measures were calculated on a daily basis with all daily measures adjusted for baseline by subtracting baseline values (mode values, 0 seconds and 0 g) from the daily values. Negative adjusted values were given a score of 0. The criterion for illness was a total (sum of 5 days) adjusted weight of mucus of 10 g or greater or an average adjusted time of nasal mucociliary clearance of 420 seconds (7 minutes) or longer.

Telomere Length. Because we could not conduct timely cell separations and preservation for the entire sample in a single day, whole blood for telomere length assay was collected from a random one-third of participants on each of 3 days: during the baseline screening visit (6-8 weeks prequarantine), 3 to 5 days prequarantine, or on the baseline day of quarantine. Blood samples were collected by standard venipuncture into 3 green-top (heparinized) collection tubes. Lymphocyte subsets from each blood sample were labeled with fluorochrome conjugated mouse anti-human monoclonal antibodies (BD Bioscience Pharmingen) in RosetteSep cocktails, isolated immediately using an automated immunomagnetic cell separator (RoboSep, StemCell Technologies), and then stored at −80°C.
Total DNA was purified from each cell subset, including peripheral blood mononuclear cells (PBMCs) and CD4, CD8CD28+, and CD8CD28− cells (DNasey Blood & Tissue Kit, Qiagen). Duplicate DNA samples (20 ng) from each cell subpopulation were amplified using primers of telomere (forward, 5'-CCGTTTGTTCGTTGCTCT-3'; reverse, 5'-GGCTTTGCGCTAGGCTCT-3') and a single copy gene (36B4; forward, 5'-CACAAAGTGAGGATGTGAATCC-3'; reverse, 5'-CCCTTCAATCACGTTACAA-3'), separately, on the same plate in 1× SYBR Green master mix (Applied Biosystems) using a real-time quantitative polymerase chain reaction assay (qRT-PCR) (7300 Fast Real Time PCR system, Applied Biosystems) as per a published protocol.18 Applied Biosystems SDS software was used to generate standard curves and to determine the dilution factors of standards corresponding to the telomere (T) and single-copy gene (S) amounts in each sample. From these data, a T:S ratio was computed, providing an index of average telomere length. Coefficients of variation were 12% and 13% for T and S, respectively. In a subsample of 26 participants, qRT-PCR-derived T:S ratio was correlated (r = 0.64, P < .001) with telomere length as determined by terminal restriction fragment Southern blot analysis.

Although the blood volumes used for telomere assays were the same across cell types, samples differed randomly in cell number and thus in DNA concentration. This, in combination with random cell loss during separation and DNA purification, resulted in some samples containing insufficient DNA for telomere assay.

### Statistical Analyses

Pearson correlations (r and P values) were used to test associations between continuous variables, analysis of variance (F and P values) to test differences in telomere length among categorical control variables, and paired (within-person) t tests (t and P values) to compare telomere length differences between cell types. We used multiple logistic regression19 to predict the binary outcomes, incidence of infection, and clinical illness. All regression equations included the 7 control variables (covariates) described in the design section. Telomere length (T:S ratio) was normally distributed in all cell populations and for analysis was standardized (z score) and treated as a continuous variable. Shorter telomere length could be related to risk for clinical colds through an association with either increased risk of infection among all participants, increased risk of developing clinical illness among participants who became infected, or both. We tested the second hypothesis by conducting a multiple logistic regression (including all standard covariates) with clinical illness as the outcome and using data only from the subset of participants who became infected with the challenge virus.

### RESULTS

#### Sample Characteristics

Table 1 presents descriptive information on sample demographic characteristics, other covariates, and telomere length.

### Correlations Between Telomere Lengths in Different Cell Populations

Table 2 presents telomere length correlations among the different cell types. Mean PBMC telomere length was longer than telomere length in all 3 T-cell subsets (PBMC telomere length vs CD4 telomere length, t124 = 12.57, P = .001; PBMC telomere length vs CD8CD28+ telomere length, t110 = 11.67, P = .001; PBMC telomere length vs CD8CD28− telomere length, t121 = 9.51, P = .001).

### Associations of Control Variables

#### With Telomere Length, Infection, and Colds

Older age was correlated with shorter telomere length in PBMCs (n = 128, r = −0.17, P = .05), CD8CD28+ cells

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with shorter telomere length in PBMCs (n=128, r=-0.19, P=0.03) and CD8CD28− cells (n=144, r=-0.16, P=0.02), and higher BMI was correlated

Table 2. Intercorrelations Among Telomere Lengths in the 4 Leukocyte Populations

<table>
<thead>
<tr>
<th>T:S Ratio</th>
<th>PBMC</th>
<th>CD4</th>
<th>CD8CD28</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>Pearson r</td>
<td>0.46</td>
<td>NA</td>
</tr>
<tr>
<td>P value</td>
<td>.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. b</td>
<td>125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8CD28+</td>
<td>Pearson r</td>
<td>0.39</td>
<td>0.36</td>
</tr>
<tr>
<td>P value</td>
<td>.001</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td>No. b</td>
<td>111</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>CD8CD28−</td>
<td>Pearson r</td>
<td>0.50</td>
<td>0.37</td>
</tr>
<tr>
<td>P value</td>
<td>.001</td>
<td>.001</td>
<td>.001</td>
</tr>
<tr>
<td>No. b</td>
<td>123</td>
<td>138</td>
<td>131</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not applicable; PBMC, peripheral blood mononuclear cell.

Table 3. Multivariable Associations of Lymphocyte Telomere Length With Viral Upper Respiratory Infection and Clinical Illness (Colds)

<table>
<thead>
<tr>
<th>T:S Ratio</th>
<th>PBMC (n=128)</th>
<th>CD4 (n=146)</th>
<th>CD8CD28+ (n=132)</th>
<th>CD8CD28− (n=144)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR (95% CI)</td>
<td>1.71 (1.08-2.72)</td>
<td>1.76 (1.15-2.70)</td>
<td>1.93 (1.21-3.09)</td>
<td>2.02 (1.29-3.16)</td>
</tr>
<tr>
<td>RR (95% CI)</td>
<td>1.22 (1.03-1.38)</td>
<td>1.27 (1.07-1.44)</td>
<td>1.26 (1.08-1.40)</td>
<td>1.38 (1.14-1.59)</td>
</tr>
<tr>
<td>F2</td>
<td>0.26</td>
<td>0.19</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>DF</td>
<td>0.05</td>
<td>0.06</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Likelihood ratio testaχ2</td>
<td>5.35</td>
<td>7.24</td>
<td>8.47</td>
<td>11.07</td>
</tr>
<tr>
<td>P value</td>
<td>.02</td>
<td>.007</td>
<td>.004</td>
<td>.001</td>
</tr>
</tbody>
</table>

Clinical Illness

| OR (95% CI) | 1.38 (0.85-2.23) | 1.14 (0.74-1.76) | 1.36 (0.83-2.23) | 1.69 (1.01-2.84) |
| RR (95% CI) | 1.26 (0.88-1.71) | 1.11 (0.79-1.55) | 1.29 (0.85-1.88) | 1.57 (1.01-2.35) |
| F2 | 0.19 | 0.16 | 0.20 | 0.21 |
| DF | 0.02 | 0.02 | 0.02 | 0.04 |
| Likelihood ratio testaχ2 | 1.77 | 0.35 | 1.47 | 4.37 |
| P value | .18 | .55 | .23 | .04 |

Abbreviations: OR, odds ratio; PBMC, peripheral blood mononuclear cell; RR, relative risk; T:S, ratio of telomere to single-copy gene amounts.

Figure 1: Twenty-two percent of the entire sample (n=33) developed a clinical illness (common cold). As shown in Table 3 and Figure 1, only telomere length in the CD8CD28− subset was associated with risk for clinical illness, with shorter telomeres being associated with greater risk.

Pathways Linking CD8CD28− Telomere Length to Clinical Illness. In the analysis testing whether telomere length is associated with the development of signs of illness among infected individuals, limited to the participants who were infected (n=97), there was no association between telomere length and colds (OR, 1.11 [95% CI, 0.59-2.07]).

Telomere Length, Infection, and Clinical Illness, by Age. We tested whether the magnitude of the association of CD8CD28− telomere length with infection and clinical illness varied across age. The magnitude of the association between telomere length and infection increased with increasing age (interaction, b=0.09 [95% CI, 0.02-0.16], P=0.1,
n = 144; $\chi^2 = 9.12$ by likelihood ratio test, $P = .003$). To facilitate the interpretation of the interaction, ORs for separate analyses of each age tertile are presented in Figure 2. For colds, the form of the interaction was similar to that of infection but did not reach statistical significance, and addition of the interaction term did not significantly improve the model fit ($b = 0.05$ [95% CI, −0.001 to 0.10], $P = .09$; $\chi^2 = 3.16$ by likelihood ratio test, $P = .08$).

COMMENT

Leukocyte telomere length has emerged as a predictor of earlier onset of aging-related morbidity and mortality in older adults.8-11 Telomere length traditionally has been thought important in age-related functional decline and development of chronic disease. No studies have examined whether shorter telomere length might also be related to acute functional consequences in younger, healthy populations. In this study, we used a prospective viral-challenge methodology to test whether leukocyte telomere length is associated with host resistance to standardized exposure to a virus that causes the common cold in humans (RV39). Further, because CD8CD28+ T cells appear to play an important role in immune response to immunization and are particularly prone to early telomere shortening, we compared telomere length in these cells with length in several other peripheral blood cell populations.

We found that shorter telomere lengths in all 4 cell types were associated with greater odds of infection following experimental exposure to RV39. However, CD8CD28− telomere length had the largest association with infection. Moreover, after CD8CD28− telomere length was entered into the logistic equation, adding the telomere length for other cell types did not improve the prediction. Further, only CD8CD28− telomere length was associated with clinical illness.

When examining these relations by age, we found that the association between CD8CD28− telomere length and infection increased with increasing age. The increasing importance of leukocyte telomere length with advancing age may be attributable to cells with very short telomeres becoming more prevalent as individuals get older (eg, 31% of 30- to 55-year-olds vs 17% of 18- to 22-year-olds had CD8CD28− telomere lengths in the bottom 20% of the distribution); to a younger immune system more effectively compensating for the level of CD8 cell senescence; or to

**Figure 1. Incidence of Infection and Colds by Tertile of Leukocyte Telomere Length**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>T:S Ratio Range</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood mononuclear cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longest (n = 44)</td>
<td>0.65-1.16</td>
<td>100</td>
</tr>
<tr>
<td>Middle (n = 48)</td>
<td>0.46-0.64</td>
<td>100</td>
</tr>
<tr>
<td>Shortest (n = 48)</td>
<td>0.14-0.45</td>
<td>0</td>
</tr>
<tr>
<td>CD4 lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longest (n = 46)</td>
<td>0.64-1.15</td>
<td>100</td>
</tr>
<tr>
<td>Middle (n = 49)</td>
<td>0.45-0.63</td>
<td>50</td>
</tr>
<tr>
<td>Shortest (n = 51)</td>
<td>0.01-0.44</td>
<td>0</td>
</tr>
<tr>
<td>CD8CD28+ lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longest (n = 48)</td>
<td>0.65-1.16</td>
<td>100</td>
</tr>
<tr>
<td>Middle (n = 48)</td>
<td>0.46-0.64</td>
<td>100</td>
</tr>
<tr>
<td>Shortest (n = 48)</td>
<td>0.14-0.45</td>
<td>0</td>
</tr>
<tr>
<td>CD8CD28− lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longest (n = 48)</td>
<td>0.67-1.87</td>
<td>20</td>
</tr>
<tr>
<td>Middle (n = 48)</td>
<td>0.46-0.66</td>
<td>0</td>
</tr>
<tr>
<td>Shortest (n = 48)</td>
<td>0.10-0.45</td>
<td>0</td>
</tr>
</tbody>
</table>

Ratio of telomere to single-copy gene amounts (T:S ratio) used as an index of average telomere length. Total Ns differ between cell types owing to insufficient DNA content in some blood samples. Data for each cell population are presented based on data from only those participants with complete telomere length data for that cell type. Y-axis segments in blue indicate range y=0 to y=30.
CD8 cell senescence contributing to immune impairment, especially impairment occurring within the context of other age-related biological changes.

This is a preliminary study with a small volunteer sample and modest effect sizes. However, because these analyses were prospective, we can eliminate reverse causation (developing an infection or illness in this study did not cause shortening of telomere length) as an alternative explanation. Use of multiple control variables (age, sex/birth control status, race, prechallenge antibody, season, BMI, day of blood draw to assess telomere length) eliminated many potential spurious explanations. Even so, the possibility remains that alternative unspecified third variables, eg, a common genetic contributor to both telomere length and susceptibility to infection, could account for our results. Also, the generalizability of the results may be limited if study volunteers differ significantly from the wider population.

There are no published functional data (eg, differences in virus-stimulated proliferation or cytokine production) comparing CD8CD28+ and CD8CD28− cells as they pertain specifically to rhinovirus infection. Consequently, the explanations for our findings are speculative and based on a more general understanding of the function of these cells. Because CD8 lymphocytes are important for eliminating virus-infected cells, a decreased ability to replicate would likely contribute to an increased susceptibility to viral infection. CD8CD28− cells with short telomeres are near or have already reached replicative senescence21 and have poor antigen-induced proliferation.21 Thus, the number of effector cells available to respond to the virus may be reduced in persons with a large number of senescent or near-senescent CD8 cells.

There is a close relationship between the CD28 molecule and the telomere/telomerase aging system.18,24 Without CD28, cells can no longer up-regulate telomerase during activation, which is essential for proliferation, cytokine/chemokine production, and antiviral activity.25 Maintaining CD28 (through gene transduction) slows immunosenescence by increasing telomerase activity and cell proliferation and by reducing proinflammatory cytokine expression in vitro.25 Given these in vitro findings, it is reasonable to expect that in vivo loss of CD28 from CD8 cells should impair the host’s ability to fight infection.

Previous research has shown telomere length to be shorter in CD8CD28− cells relative to CD8CD28+ cells, with that research being restricted to samples of patients who are older20 or human immunodeficiency virus–positive25 and to 1 small study (N=10) of 29- to 59-year-olds.26 In contrast, we found no difference in telomere length between CD8CD28− and CD8CD28+ cells in our healthy younger sample (mean age, 29.9 years). Nevertheless, CD8CD28− cells did show a wide range of telomere lengths.

In sum, this study found an association between leukocyte telomere length and resistance to a common virus infection in healthy young and middle-aged adults. These findings are consistent with the wide variance in leukocyte telomere length found in young adults.18 The presence of short telomeres among young people could result from several factors including genetics,29,30 older paternal age at conception,26 poor health behaviors, and oxidative41 and chronic psychological stress.32,33 CD8 cell senescence has also been attributed to the presence of latent viral infections34,35 and proinflammatory environments.36

Implicit in the relationship between telomere length and colds in this study is that telomere length is relatively stable over the course of at least 1 to 2 months. Published data from a small study of 30- to 50-year-olds found that telomere length in leukocytes is quite stable over the course of 7 months (r=0.93),37 whereas a 10-year follow-up of a large sample of older adults (>53 years) found a still substantial but smaller association (r=0.65).38 Evidence of long-term stability is also suggested by correlations of telomere length with stable individual characteristics including genetic markers,29,30 education,29 and stable psychological dispositions.40,41 A provocative possibility is that telomere length is a very stable marker of disease susceptibility, with associations between telomere length and clinical outcomes beginning to emerge in early adulthood.

In this study of healthy young and middle-aged adults, shorter CD8CD28− cell telomere length was associated with upper respiratory tract infection and clinical illness following experimental exposure to rhinovirus. Because these data are preliminary, their clinical implications are unknown.
Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr. Turner reported serving as a consultant for, and receiving grants/ grants pending from, Janssen Research and Development, Inc. before the study was conducted, and holds stock or stock options in, Telomere Health Inc, a telomere measurement company. No other authors reported disclosures.

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Obtained funding: Cohen, Casselbrant, Doyle.

Administrative, technical, or material support: Turner, Li-Korotky.

Study supervision: Cohen, Li-Korotky, Doyle.

REFERENCES