Telomerase in T Lymphocytes: Use It and Lose It?

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*J Immunol* 2007; 178:6689-6694; doi: 10.4049/jimmunol.178.11.6689

http://www.jimmunol.org/content/178/11/6689
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The enzyme telomerase counteracts telomere loss in proliferating cells and extends their capacity for replication. The importance of telomerase is highlighted by the award of the 2006 Albert Lasker Prize for Basic Medical Research for its discovery. Malignant cells subvert telomerase induction to their advantage, and up-regulation of this enzyme provides these populations with unlimited proliferative potential with obvious detrimental consequences. However this enzyme is also essential for the lifelong maintenance of normal cell populations that have a high rate of turnover. Thymic involution in early adulthood dictates that memory T cell populations have to be maintained by continuous proliferation. This highlights the inherent paradox that telomerase down-regulation in T cells may protect against malignancy yet also lead to replicative exhaustion of repeatedly activated memory T cells. In this article, we review the data on telomerase regulation in T lymphocytes and the implications this has for the maintenance of T cell memory. The Journal of Immunology, 2007, 178: 6689–6694.

Telomeres are repeating hexameric sequences of DNA that are found at the ends of linear chromosomes in association with a complex of proteins; their role is to maintain chromosome integrity and stability (1). Telomeric DNA is lost due to the incomplete terminal synthesis of the lagging DNA strand during cell division (1–3). In the absence of mechanisms that compensate for this, growth arrest of the cell occurs when progressive telomere erosion reaches a critical point, a phenomenon known as replicative senescence (reviewed previously (1, 4)). Telomeres and telomerase regulation have been studied extensively from the standpoint of malignant cell proliferation. However, more recently, telomere measurement has been exploited in two ways in the study of T lymphocytes. First, the comparison of relative telomere length between different T cell subsets informs on their proliferative history for example; naive T cells have been shown to have longer telomeres than memory populations (5). Second, since replicative senescence in human cells is reached when telomeres shorten to ~4 kb, the telomere length of a population has been used to assess its proximity to replicative senescence (6, 7).

The rate of telomere loss is retarded by the enzyme telomerase, a RNA-dependent DNA polymerase, that synthesizes telomeric repeats and thus maintains telomeres during cell replication (4). This enzyme complex consists of a catalytic reverse transcriptase protein (telomerase reverse transcriptase; TERT),3 a RNA template, and a number of associated proteins (reviewed in Ref. 1). Initial studies on telomerase regulation focused on the regulation of proliferative capacity of malignant cells, and >90% of all tumor cell lines were found to express this enzyme constitutively (8). However, the importance of this enzyme extends to the maintenance of normal cells such as gut and skin epithelium (9), hemopoietic stem cells (10), and both T and B lymphocytes (1) that proliferate extensively. All these cell types can up-regulate telomerase (11), and the capacity to do so may determine their ability to persist after repeated re-stimulation in vivo.

Telomere erosion in T cells: aging or chronic stimulation? Telomeres have been shown to erode at the rate of ~50 bp/year in human CD4+ and CD8+ T cells (12), and T cell populations of old individuals have shorter telomeres than those from young subjects (1, 13). This telomere loss could potentially be due to the repeated activation of specific T cells or to cumulative oxidative damage during aging (14). However, the observation that memory T cells in both the CD4+ and CD8+ compartments have shorter telomeres than their naive counterparts suggests that cellular proliferation is the primary stimulus for telomeric attrition (6, 12, 15–17). Support for this is provided by the study of young patients with an immunodeficiency known as X-linked lymphoproliferative syndrome (XLP), who experience excessive T cell stimulation after activation due to defective SAP, a molecule coded for by the SH2D1A gene (18). The CD8+ T cells from these young individuals have short telomeres in the range of those found in old subjects, indicating that telomere erosion is likely to be driven by excessive proliferation and not the aging process itself (16). Indeed, the shorter telomeres in CD4+ and CD8+ T cells from older subjects and patients with XLP was associated with significantly decreased replicative capacity after repeated stimulation in vitro (6, 16). Further evidence for telomere shortening as a consequence of

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3 Abbreviations used in this paper: TERT, telomerase reverse transcriptase; hTERT, human TERT; XLP, X-linked lymphoproliferative syndrome.

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Received for publication February 2, 2007. Accepted for publication March 21, 2007.

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excessive T cell stimulation comes from patients with chronic diseases, e.g., during HIV infection (19), rheumatoid arthritis (20), psoriasis, and atopic dermatitis (21). In HIV-infected individuals in particular, it has been shown that continuous antigenic challenge leads to accelerated telomere shortening and premature senescence in the CD8+ cytotoxic lymphocytes (19). This is likely to play an important role in the eventual failure of cellular immunity in these patients.

**Telomerase activity in T cells after activation in vitro and in vivo**

Telomerase can be investigated in T cells in two ways. First, the activity of the enzyme can be measured by the telomeric repeat amplification protocol assay, and second, the presence of human TERT (hTERT) and/or other proteins of the enzyme complex may be determined (22). However, until recently, the available Abs to hTERT and associated proteins have been suboptimal. Furthermore, since the expression of this protein does not necessarily correlate with enzyme activity, most studies have relied on the telomeric repeat amplification protocol assay to study telomerase activity (22).

Telomerase activity is extremely low or absent in unstimulated human peripheral blood T cells, but activation in vitro induces high levels of enzymatic activity but only a modest increase in hTERT expression (2, 15, 23). These studies were extended by the observation that high levels of telomerase was present in the expanded EBV-specific CD8+ T cell population during acute infectious mononucleosis (24, 25). This was associated with telomere preservation in these cells (24, 25). This telomerase activation and telomere elongation was also observed in EBV-specific CD8+ T cells in lymphoid tissue during acute infectious mononucleosis (26).

Telomerase was also induced after activation in murine T cells, both in vitro and in vivo (1). The CD8+ T cells from mice infected with lymphocytic choriomeningitis virus maintain telomere length despite extensive clonal expansion in vivo due to up-regulation of telomerase activity (27). Thus, some T cells are able to maintain their telomere length and replicative capacity by up-regulating telomerase activity. Since the majority of expanded CD8+ T cells that are found during acute infection are programmed to die by apoptosis (28–30), telomerase up-regulation preserves, at least initially, the replicative capacity of T cells that are selected from the primary response to enter the memory pool (Fig. 1A).

The loss of telomerase activity in T cells after repeated activation

Although high telomerase activity in EBV-specific CD8+ T cells during a primary infection prevents telomere erosion (24, 25, 27), telomeric shortening was observed in these specific T cells when the same individuals were studied between 15 mo and 14 years later (25). Telomerase induction is therefore insufficient to maintain telomeres indefinitely in repeatedly activated memory T cells in vivo (Fig. 1B). This is supported by studies in vitro, which show that upon repeated stimulation, T cells progressively lose the ability to induce telomerase activity, resulting in telomere erosion and replicative senescence (15, 16, 31, 32). The regulation of telomerase activation in T cells therefore changes during their progressive differentiation (Fig. 1C).

To study differentiation-related changes in telomerase activity, it is essential to discriminate between undifferentiated and highly differentiated T cell populations, and changes in surface marker expression have been used for this purpose. It has been shown that sequential loss of the costimulatory receptors CD27 and CD28 and the chemokine receptor CCR7 occurs as T cells differentiate, and this is accompanied by a parallel decrease in telomere length (reviewed in Ref. 33). Relatively undifferentiated T cells within both CD4+ and CD8+ subsets are CD27+CD28+CCR7+ and have long telomeres, and naive and central memory populations fall into this category (6, 16). There is a sequential loss of these receptors, and CD4+ T cells at an intermediate stage of differentiation are CD27−CD28−CCR7− while those in the CD8− subset are CD27+CD28−CCR7− (33). These intermediate populations have telomere lengths between that found in undifferentiated and highly differentiated cells (6, 16, 34). Highly differentiated T cells in both CD4+ and CD8+ subsets are CD27−CD28−CCR7− and have the shortest telomeres, and effector memory, as well as CD45RA-re-expressing effector T cells, fall into this category of differentiation (6, 16). Furthermore, studies of the rate of proliferation of human T cell subsets in vivo indicate that T cells, which have the highest proliferative activity, have the shortest telomeres and express the highly differentiated CD27−CD28−CCR7− phenotype (6, 16, 35).

Telomerase activity after activation was found to be highest in undifferentiated T cells, lower in the intermediate, and very low in the highly differentiated CD27−CD28−CCR7− T cell populations (6, 16, 34). Therefore, the ability to induce this enzyme is lost as T cells differentiate progressively (Fig. 1). Thus, highly differentiated effector T cells have short telomeres and a limited replicative lifespan (6, 16), and these cells accumulate within CD4+ and CD8+ T cell subsets of old individuals and patients with persistent infection with EBV (16, 36), CMV (6, 37, 38), and HIV (39). This raises the question of the mechanism that inhibits telomerase activity during T cell differentiation and whether this process is reversible.

**FIGURE 1.** Model of senescence induction. A, During the first encounter with an Ag, specific T cells from the naive T cell pool will be induced to proliferate, leading to an expansion of Ag-specific T cells. During the expansion, Ag-specific cells up-regulate telomerase, therefore maintaining telomere length. After the expansion phase, the T cell pool will contract again and most of the activated T cells will die, leaving a small pool of memory T cells that retain replicative capacity. B, Memory T cells have shorter telomeres compared with the naive population and are still able to up-regulate telomerase, albeit at a lower level, when they encounter Ag. Repeated stimulation will lead to the generation of highly differentiated memory cells. C, Highly differentiated memory cells have short telomeres and have lost the ability to up-regulate telomerase. Nevertheless, these cells can still proliferate. Following further encounter with Ag, further expansion of these cells leads to replicative senescence. This will reduce the ability to respond to reinfection/reactivation.
A direct link between loss of telomerase activity and telomere erosion in T cells has been demonstrated in animals or patients with defects in this enzyme (40, 41). Although murine model systems have been used to study in vitro and in vivo regulation of telomeres and telomerase, they do not always reflect the physiological status in humans. Common laboratory strains of mice (e.g., C57BL/6 or BALB/c) have telomeres that are 5- to 10-fold longer than those in humans (42–44), and these cells have significantly higher levels of constitutive telomerase activity (45). In addition, the murine lifespan is much shorter than in humans (42–44). Mice that are engineered to have a deficiency in the telomerase RNA template are normal at first (40). However, after six successive generations, these animals exhibit a premature aging phenotype (46). The delayed effect of telomerase ablation was due to the much longer telomeres found in mice (42, 47). Therefore, telomere attrition is unlikely to be a major constraint on immunity in mice compared with humans.

In humans, patients with a syndrome known as dyskeratosis congenita have deficient telomerase activity (48). Patients with the X-linked form of the disease have a defect in the gene coding for a telomerase RNA template-associated protein known as dyskerin, whereas the autosomal-dominant form of the disease arises directly from a defect in the telomerase RNA template itself (48). The leukocytes from patients with both forms of the disease have short telomeres and reduced T and B cell numbers compared with age-matched controls (49), which further underscores the link between lack of telomerase activity and telomere erosion in lymphocytes. Furthermore, 50% of these patients have reduced circulating T cell numbers, while 80% have reduced or absent responses to recall Ags (49). These patients were susceptible to recurrent infections and even opportunistic infections such as Pneumocystis carinii pneumonia (49). This suggests that the their T cells are functionally impaired, possibly as a consequence of telomere erosion (48).

Since lack of telomerase may lead to premature replicative senescence of T cells, an obvious question was whether the reintroduction of telomerase could reverse this process. Various groups have transduced human T cells with hTERT and compared their replicative potential with nontransduced parental T cell populations (16, 31, 50–52). In all these studies, upon repeated activation, telomerase activity was present in the transduced T cells, even though it was lost in the parental population. Consequently, the telomere length in hTERT-transduced cells was stabilized, and their proliferative lifespan was extended considerably (16, 31, 50–52). Furthermore, the low proliferative capacity of T cells from patients with XLP that had short telomeres could also be reversed by transduction with hTERT, but not SAP, the molecule that is defective in these patients (16). Despite the considerably enhanced replicative capacity, however, hTERT-transduced cells eventually lose the ability to expand after activation in the majority of studies due to cumulative oxidative damage (53) or loss of genomic stability (31, 52). Collectively, these studies indicate that proliferative potential is directly linked to telomere maintenance and telomerase activation in T cells. In addition, it is clear that, while telomerase activation enables Ag-specific T cells to retain replicative capacity after primary responses, the down-regulation of enzyme activity in specific T cells during persistent infection leads to telomere erosion and predisposition to replicative senescence (Fig. 1).

**Mechanism of telomerase down-regulation in highly differentiated T cells**

The mechanism for the decrease in telomerase activity during T cell differentiation is a largely unexplored area. Although resting human CD4⁺ (22) and CD8⁺ T cell populations (25) do not express telomerase activity without activation, they express hTERT, the catalytic component of the enzyme (22, 80). This indicates that activity of the enzyme is not only regulated at the level of protein expression. Signals via the TCR and also costimulatory molecules such as CD28 are required for the induction of telomerase activity, which peaks after 4–5 days of stimulation and then decreases to baseline by 10 days (2, 15, 16). As T cells are repeatedly activated in vitro, both the peak and duration of telomerase activity are decreased (16), and highly differentiated CD27⁻ CD28⁻ CCR7⁻ T cells that are freshly isolated and activated in vitro express low telomerase activity at all time points measured (16). This raises the question of whether the decreased enzymic activity in these cells is due to decreased hTERT synthesis or to posttranslational regulation of this enzyme.

The induction of telomerase activity is associated with a modest but significant increase in hTERT protein expression (54, 55). However, the phosphorylation of hTERT was crucial for the translocation of the enzyme from the cytoplasm to the nucleus (22). hTERT is a substrate of the kinase Akt, which itself requires phosphorylation at two different sites for activity (56). In CD8⁺ T cells, there was a specific defect in Akt phosphorylation at the Ser⁷³ site in the highly differentiated
CD8+CD28−CD27− T cell subset but not in less differentiated populations (80). The upstream enzyme complex that phosphorylates AktSer473 after activation has been described recently (57), and it will be crucial to investigate whether this complex is functionally defective in highly differentiated CD8+CD28−CD27− and/or CD4+CD28−CD27− T cells. Collectively, these results suggest that telomerase down-regulation in highly differentiated T cells is not only achieved by transcriptional control but is also related to changes in posttranslational modification of this enzyme.

Since loss of telomerase activity does not seem to be controlled by gene-silencing mechanisms, it is possible that the loss of enzyme activity may be reversed under appropriate conditions. Although CD8+CD28−CD27− and CD4+CD28−CD27− T cells bear the hallmarks of populations that are approaching replicative senescence (6, 16), they express functional activity after stimulation in vitro (6, 58, 59). Therefore, despite the lack of telomerase induction, these cells are not equivalent to an exhausted, functionally deficient population that has been described for specific T cells from mice (60) and humans that have ongoing viral infections (61). Nevertheless, it will be important to investigate whether reversal of the exhausted functional state of T cells, which is maintained though PD-1 signaling (61, 62), can also enable the re-expression of telomerase in highly differentiated CD8+CD28−CD27− or CD4+CD28−CD27− T cells (6).

The regulation of telomerase activity in T cell by cytokines

Apart from Ag-driven expansion, T cells can also be induced to proliferate by certain cytokines in the absence of TCR signaling, a process known as homeostatic proliferation, that is involved in the nonspecific maintenance of T cells numbers in vivo (63). While IL-7 is important in the homeostatic regulation of the CD4+ T cell pool, IL-15 regulates homeostatic expansion of both naïve (64) and memory CD8+ T cells (63, 65–67). The induction of proliferation of CD4+ and CD8+ T cells in vitro by IL-7 and IL-15, respectively (64), was associated with the induction of telomerase activity, which was sufficient to prevent telomere erosion in adult (64, 66) but not in cord blood T cell populations (68). The exact signaling pathways by which this occurs is not clear; however, it has been shown that IL-15 acts via a Jak3 and PI3K pathway to induce telomerase activity (66). However, in these studies, the effect of IL-7 and IL-15 on the expression of hTERT protein itself was not determined.

Cytokines have also been shown to inhibit telomerase activity. In a human model of a T cell memory response, where antigenic rechallenge was performed in the skin, it was found that there was accelerated telomere loss in specific T cells responding at the site of stimulation in vivo (69). This was found to be due to the presence of type 1 IFN (IFN-α) that induced the reversible inhibition of telomerase activity in vivo. This mechanism may restrict the overexpansion of activated T cells in certain tissues (69). This inhibitory effect of IFN-α was also shown in a second study where this cytokine, secreted by CMV Ag-stimulated plasmacytoid dendritic cells, inhibited the telomerase activity of Ag-specific CD4+ T cells in vitro (6). However, it remains to be determined whether IFN-α regulates the transcription or phosphorylation of hTERT. Collectively, these results demonstrate that telomerase in T cells may be controlled by cytokines. This indicates that the rate of telomere erosion of T cells may be determined by the nature of the activating stimulus and the tissue microenvironment where the response is taking place. Furthermore, IL-2 or IL-15 only partially restores the low telomerase induction in highly differentiated CD8+CD28−CD27− T cells, indicating that this is a separate defect that cannot be reversed by cytokines (80).

Do memory T cells differentiate at the same rate during aging?

The short answer to this question is no. It has been shown that, on the basis of loss of costimulatory receptors and relative telomere lengths, CMV-specific T cells are more differentiated than those specific for varicella zoster, EBV, HSV, influenza virus, and also tuberculin-purified protein derivative (6). As described above, this may be due in part to the effects of IFN-α, which is induced by the triggering of plasmacytoid dendritic cells by CMV (6). Furthermore, CMV-specific CD4+ T cells that had poor ability to up-regulate telomerase showed severely decreased capacity to expand in culture (6). These differences were more pronounced when the T cells from old instead of young subsets were investigated, suggesting that CMV-specific CD4+ T cells in healthy carriers are continuously being driven toward replicative exhaustion more rapidly than T cells of other specificities. The question of how this state of replicative exhaustion would manifest itself in vivo and whether it is detrimental for the maintenance of immunity and health of old individuals therefore needs to be addressed.

Manifestation of replicative exhaustion of memory T cells in vivo

If a certain population of T cells, such as those that are specific for CMV, have short telomeres and are driven toward exhaustion, then it is important to determine whether there is a reduction of these cells within the T cell pool of old individuals. Therefore, the initial observations seemed contrainuitive since highly differentiated CMV-specific CD4+ T cells were found to accumulate rather than decrease in old subjects (6, 59, 70, 71). One explanation for this paradoxical observation is that clonal evolution occurs during persistent viral infection (72), which drives the specific T cell clones with the highest avidity and/or functional activity to replicative exhaustion (Fig. 2A). These may be replaced by other populations of less efficient cells in the memory pool (72–74). Thus, the first manifestation of replicative senescence in memory T cells as a result of lifelong stimulation may be the increase rather than decrease of specific T cells as more of the less efficient cells are required to control virus reactivation (Fig. 2B). This hypothesis is consistent with the observation that the expanded, highly differentiated CMV-specific CD8+ T cells that are found in old subjects have decreased functional activity (71). This accumulation of suboptimal highly differentiated CMV-specific T cells (59, 71) would lead to overcrowding of the memory T cell pool, leading to the constriction and loss of other unrelated memory T cell populations (Fig. 2B). Furthermore, this may account for the expansion of large oligoclonal populations of T cells during aging (75, 76). The most severe manifestation of Ag-specific T cell exhaustion may occur when even the suboptimal T cells are lost through replicative senescence. When this occurs, the prediction is that first, specific T cells would diminish in number, and second, for organisms such as CMV that cause severe pathology, there would be an increased incidence of CMV-mediated disease. However, this has not been observed in old subjects.
thus far but cannot be ruled out in the future as life expectancy continues to increase (13). This suggests that it may be important to consider whether targets that CMV replication such as antiviral therapy or anti-CMV vaccination may be used to preserve immune function during aging (77).

Conclusion

Certain memory T cell populations in humans are already being driven toward replicative senescence, and this is linked to loss of telomerase activity. We are already experiencing the effects of telomere erosion driven by CMV infection in old subjects (6), the manifestation of which may be the observed accumulation of highly differentiated CMV-specific T cells with suboptimal function. Nevertheless, at present, there is still sufficient CMV-specific immunity in old subjects to prevent CMV reactivation. The human life expectancy at birth has doubled in the last 150 years and continues to increase; therefore, memory T cell populations will have to be maintained for even longer than eight decades in the future (13). While telomere erosion and telomerase regulation was unlikely to restrict the maintenance of T cell memory in the past, current data suggest that it may have an increasing role in doing so in the future. A better understanding of telomerase regulation and senescence is therefore a matter of some urgency as the telomere clock in T cells from old humans continues to wind down.

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BRIEF REVIEWS: TELOMERASE IN T LYMPHOCYTES


