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Review

Telomere and adaptive immunity

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Abstract

The adaptive immune response relies on the ability of lymphocytes to undergo periodic massive expansion. It is an enigma how lymphocytes are able to undergo this seemingly unlimited number of cell divisions. Telomeres and telomerase play a critical role in regulation of the replicative lifespan of cells, providing a potential mechanism which lymphocytes may employ. Here I will review the recent progress of the role of telomeres and telomerase in lymphocyte differentiation, function, and aging.

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Keywords: Human; Telomere; Telomerase; Aging; T cells; B cells

1. Introduction

A typical adaptive immune response starts with the selection of the best antigen-binding naïve T and B lymphocyte(s) and ends with an enormous expansion of this selected lymphocyte to combat an antigen/pathogen. It has been estimated that a typical immune response involves in 15–20 cell divisions, from a single naïve cell to approximately a million of its activated clones, namely effector cells. Once the antigen/pathogen is cleared, the majority of these effector cells undergo apoptosis and a small number of them survive to become memory cells. Memory cells are long-lived and are capable of further rapid expansion upon re-encounter with the same antigen (Dutton et al., 1998; Kaech et al., 2002). For a common pathogen, such as influenza, the periodic expansion of the influenza-recognizing lymphocytes occurs through the entire life of a person. Thus, the cumulative numbers of cell divisions of these antigen-specific lymphocytes that occur over a lifetime could be an astronomical figure. The question is what mechanism(s) lymphocytes employ to allow them to achieve this.

The telomere is a specialized structure at the end of a chromosome. As the cap of the chromosome, telomeres protect the integrity of chromosomes and ensure the complete replication of chromosomes (Cech, 1994; Blackburn, 2001). Telomeres consists of tandem hexanucleotide repeats

(5'-T₂AG₃-3' over 1000 copies in human) and several telomere DNA binding proteins including telomere repeat binding factors 1 and 2 (TRF1 and TRF2), protection of telomere 1 (POT1), etc. and collectively called Shelterins (de Lange, 2005). Due to the inability of conventional DNA polymerase to completely replicate the 3' ends of chromosomes, loss of a portion of telomere repeats occurs after each round of genome replication. Without an adequate compensatory mechanism, telomere lengths are substantially shortened after substantial cell divisions. Once telomeres are critically shortened, whether it occurs to a single chromosome end or to the ends of multiple chromosomes, cells cease to divide and become senescent or undergo apoptosis (Allsopp et al., 1992; Hao et al., 2005). This function of telomeres as a means for counting the number of cell division provides a mechanism for limiting cell divisions by normal somatic cells.

Telomerase is a unique reverse transcriptase that equips with an RNA template containing a complementary sequence to synthesize telomeric DNA repeats. Telomerase binds to the 3' ends of the chromosome and extends telomeres, and thus could compensate for the telomere loss that results from chromosomal replication. Two core components of telomerase have been identified: (1) the telomerase RNA template (TR, TERC) and (2) telomerase reverse transcriptase (TERT) (Blasco et al., 1995; Nakamura et al., 1997; Meyerson et al., 1997; Hahn et al., 1999). While TERC is ubiquitously present in all human cells, the expression of TERT appears to be strictly regulated and thus is considered as a rate limiting factor for telomerase activity. Telomerase activity is detected in germ

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cells and in some stem or progenitor cell types but is low to undetectable in most somatic cells (Kim et al., 1994; Wright et al., 1996; Morrison et al., 1996). Most normal somatic cells exhibit capacity for only a finite number of divisions in vitro before reaching replicative senescence, a well-known phenomenon called the Hayflick limit. Recent studies demonstrate that these senescent cells have significantly shortened telomeres as compared to their young counterparts. This is partly cumulative telomere loss from cell divisions is due partly to the absence or insufficient levels of telomerase activity, suggesting that telomeres play an essential role in determining the replicative lifespan of these cells. Although lymphocytes are normal somatic cells, they exhibit a capacity for expression of telomerase that differs from many somatic cells and resembles the ability of stem cells to express telomerase.

This review will focus on the role of telomeres and telomerase in human adaptive immune responses. In particular, it will describe what is known about the telomere and telomerase during T and B cell differentiation and about telomere function under normal conditions as well as abnormal situations. The review will conclude with a discussion of the in vivo role of telomeres in the alterations of the adaptive immune response that occurs with aging.

2. Telomeres and telomerase in lymphocyte differentiation and function

The adaptive immune response relies on the functions of two types of lymphocytes: T and B cells. T cells can be further divided into CD4 (“helper”) and CD8 (cytotoxic) T cells. CD4 T cells are responsible for facilitating the ability of CD8 T cells to kill target cells and the ability of B cells to produce antibodies. CD8 T cells are responsible for killing cells infected with intracellular pathogens, while B cells are responsible for secreting pathogen-recognizing antibodies and facilitating the subsequent destruction of these pathogens. Both CD4 and CD8 T cells are derived from bone marrow progenitors that home to the thymus where they differentiate. New thymic migrants from the thymus, are mature CD4 and CD8 T cells which have not yet encountered foreign antigens, are called naïve T cells and are found circulating in peripheral blood and lymphoid organs (Spits, 2002). Upon encounter with antigen, naïve T cells are activated and expanded to become effector cells. After clearance of the antigen, the majority of these effector cells undergo apoptosis, while some survive to become memory T cells that are long-lived. Memory T cells can be subsequently activated by the same antigen and go through similar phases of effector and memory stages with a much rapid expansion phase.

In contrast, B cells derive from bone marrow progenitor cells and mature in the bone marrow. B cells also undergo an ordered sequence of differentiation during lineage development and during activation of an immune response. In a T cell-dependent immune responses, mature antigen-naïve B cells differentiate in a unique germinal center (GC) environment into GC B cells, then to memory B cells or plasma cells (Heyzer-Williams and Heyzer-Williams, 2005). In the GC, substantial cell division occurs during a differentiation process which comprises several

important events including somatic hypermutation of variable domains of immunoglobulin (Ig) genes, clonal selection of mutated antibody-producing B cells with high antigen-binding affinity, and Ig isotype switching. Only those B cells with the best affinity/avidity are selected for further expansion and differentiation to become plasma cells (professional antibody producers) and memory B cells. Like memory T cells, memory B cells are long-lived and are capable of re-activation by the same antigen.

2.1. Telomeres in T cell functions

As described above, T cells undergo numerous cell divisions during the differentiation from naïve to memory cells. The first evidence of shorter telomeres in memory CD4 T cells than in naïve CD4 T cells was reported over a decade ago (Weng et al., 1995). Human naïve and memory CD4 T cells isolated from the peripheral blood of normal adult donors (aged from 25 to 70, $n = 20$) based on their surface phenotype show different length of telomeres: naïve CD4 T cells have consistently longer telomeres than do memory CD4 T cells. Subsequently, Rufer et al. analyzed a larger cohort (over 500 donors aged from 0 to 90) and confirmed that naïve CD4 T cells have consistently longer mean telomere lengths than memory CD4 T cells (Rufer et al., 1999). In addition, Rufer et al. also show that naïve CD8 T cells have longer telomere than do memory CD8 T cells. As both naïve and memory T cells are heterogeneous in nature, a more precise analysis of telomere attrition during clonal expansion can be accomplished by examining antigen-specific T cells. Burns et al. reported that antigen-expanded memory T cells specific for tetanus toxoid and *Candida Albicans* have significantly shorter telomeres than those of the naïve T cell populations (Burns et al., 2000). Together, these findings confirm that clonal expansion of T cells during the differentiation of naïve to memory T cells in vivo results in the loss of telomere repeats. However, it is unclear to if cumulative loss of telomere would eventually affect memory T cell function.

The rate of telomere attrition during T cell differentiation and division in vivo is unknown. Culture of primary T cells in vitro allows the recording of the number of cell divisions and therefore provides a means to directly assess the relationship between telomere attrition and cell divisions. Under culture systems using either cross-linking of TCR and co-stimulatory receptor with antibodies or mitogen (PHA) plus Interleukin 2 (IL-2), T cells at the end of culture have shorter telomere length than in the beginning of culture for both CD4 and CD8 T cells and naïve and memory T cells (Weng et al., 1995; Effros and Pawelec, 1997). A close look at telomere length during these long-term cultures suggests that loss of telomere length is not a linear function of cell division. At the beginning of culture, telomere shortening appears to be minimal, while shortening of telomere length is more evident at the late stage of culture. Furthermore, under culture systems using homeostatic cytokines such as IL-7 and IL-15, the cell division rates are slower than those cross-linking TCR or mitogen and there is little loss of telomere repeats of T cells (Li et al., 2005; Wallace et al., 2006). This raises the question of how telomere length is regulated and maintained in T cells.

The importance of replication for lymphocyte function suggests that lymphocytes may employ mechanisms for better telomere maintenance. Indeed, studies of telomerase expression in T cells show that telomerase activity is highly regulated during T cell development and differentiation (Weng, 2002). During T cell development in the thymus, high levels of telomerase activity are detected in all subsets of thymocytes but the immature CD4⁻CD8⁻ and CD4⁺CD8⁺ populations have higher levels of telomerase activity than the single positive (either CD4 or CD8) mature thymocytes (Weng et al., 1998). In the periphery, little to no telomerase activity is detected in mature resting naïve CD4 and CD8 T cells (Weng et al., 1996). However, telomerase activity is rapidly activated in T cells upon stimulation. In the case of CD4 T cells stimulated by cross-linking of the TCR and co-stimulatory receptor (CD28) antibodies, telomerase activity was detected around 12–16 h after stimulation, reached peak around 3–5 days, and gradually decreased to undetectable levels after 15–30 days (Bodnar et al., 1996; Weng et al., 1997b). When these stimulated T cells are stimulated again with anti-CD3/CD28 antibodies, up-regulation of telomerase activity is observed, but the level of telomerase activity is lower than that induced by the first stimulation. The level of telomerase activity induced by successive rounds of stimulation becomes progressively lower. This pattern of telomerase expression correlates with the pattern of telomere attrition in long-term cultured T cells, i.e. telomere length was stable when telomerase activity was high, whereas telomere length shortened when telomerase activity was low to undetectable in T cells. Furthermore, Epstein-Barr virus (EBV)-specific cytotoxic CD8⁺ T cells from patients with acute infectious mononucleosis (AIM) express high levels of telomerase activity and have stable telomere lengths despite considerable expansions (Maini et al., 1999; Plunkett et al., 2001). These findings suggest that telomerase activity plays a significant role in the maintenance of telomere length in T cells.

The ability to activate telomerase activity in T cells declines after each round of stimulation along with the decrease of T cell proliferation in vitro. The question is: can enhanced telomerase activity restore the replicative ability in these repeatedly stimulated T cells? Using a retroviral mediated infection method to ectopically express TERT in human CD4 and CD8 T cells, several groups observed that TERT-transduced T cells expressed high levels of telomerase activity and maintained telomere length. While Hooijberg et al. and Rufer et al. independently observed a significant increase of proliferative capacity of TERT-transduced CD8 T cells (Hooijberg et al., 2000; Rufer et al., 2001), Migliaccio et al. failed to observe a proliferative advantage in TERT-transduced CD8 T cells (Migliaccio et al., 2000). In CD4 T cells, Roth et al. show that TERT-transduced CD4 T cells express high levels of telomerase and reduced rates of telomere shortening (Roth et al., 2003). A significant increase of replicative lifespan was observed in these TERT-transduced CD4 T cells. The ability of telomerase (TERT) to extend replicative lifespan and yet maintain normal cell function gives hope that enhanced telomerase activity may have therapeutic applications, for example in modifying antigen-specific T cells used in immune-based adoptive therapy

for cancer and autoimmune diseases. However, a recent report by Schreurs et al. shows that development of minor chromosomal aberrations is observed in TERT-transduced CD8 T cells (Schreurs et al., 2005). Similarly, Roth et al. also observed genetic instability with CD4 T cell lines and clones transduced with hTERT after long-term culture. These findings raise the question of whether these changes were due to telomerase over-expression and/or side-effect of retroviral integration and/or long-term culture will require further studies. In addition, whether enhanced telomerase can prevent senescence also remains open. Menzel et al. report that reduced proliferative responses are observed in TERT-transduced CD8 T cells with extended replicative capacity. In the late passages, these TERT-transduced CD8 T cells accumulate the cyclin-dependent inhibitors p16^{Ink4a} and p21^{Cip1} that have largely been associated with in vitro growth arrest (Menzel et al., 2006). As there are multiple levels of control of cell growth and arrest, extended maintenance of telomeres by telomerase alone may be able to extend cell replicative lifespan to a certain degree until other limiting factors kick in, such as p16^{Ink4a} and p21^{Cip1} pathways.

2.2. Telomeres in B cell functions

In contrast to the findings in T cells, it appears that there is no significant loss of telomere length during the differentiation of naïve B cells to memory B cells. Son et al. analyzed 53 adults (aged from 17 to 67) and found no obvious and consistent difference of telomere length between naïve and memory B cells from peripheral blood (Son et al., 2003), which is in agreement with an early report of no obvious telomere shortening from naïve to memory B cells from tonsil (Weng et al., 1997a). However, Martens et al. report an analysis of 3 young adults (aged from 20 to 27) and show an average 1.8 kb shorter mean telomere length in memory B cells than in naïve B cells from peripheral blood (Martens et al., 2002). Since these two reports used the same method of telomere measurement and the same phenotypic markers for defining naïve and memory B cells, the different conclusions will require further analysis of a large number of subjects for resolution. Although substantial cell divisions occur during the differentiation of naïve T and B cells to memory T and B cells, one of the differences between T and B cells during their differentiation is that B cells differentiate in a unique microenvironment, the GC. In the tonsil, GC B cells have the longest telomeres, followed by memory and naïve B cells (Weng et al., 1997a). If lengthening of telomeres occurs during naïve B cell differentiation in the GC, this could explain the fact that there is little difference in telomere length between naïve and memory B cells in tonsil and possibly in peripheral blood as well. The mechanism of this unique telomere lengthening in the course of B cell differentiation concurrent with substantial cell division is further supported by the analysis of telomerase expression in these B cell subsets.

Like T cells, telomerase is not expressed in resting B cells but is rapidly activated after mitogenic stimulation (Hiyama et al., 1995; Weng et al., 1997a). Interestingly, studies of GC B

cells show that telomerase activity is strictly regulated. Low levels of telomerase activity are detected in naïve and memory B cells, while high levels of telomerase activity are detected in GC B cells (Norrback et al., 1996; Igarashi and Sakaguchi, 1997; Weng et al., 1997b). Strikingly, the levels of telomerase activity in naïve, GC, and memory B cells correlate well with the lengths of telomere in these subsets. Thus, these findings show that telomerase activity is not only regulated during B cell differentiation but is also capable of lengthening telomeres in vivo.

3. Telomeres in lymphocyte aging

The set point for telomere length of an individual appears to be controlled at the genetic level (Slagboom et al., 1994). More recently, studies indicate that telomere length displays a paternal heritage (Nordfjall et al., 2005; Njajou et al., 2007). Whether differences in the genetically determined set point of telomere length influence the lifespan of an individual is not known. Regardless of the initial length of telomeres, lymphocytes, especially memory cells, exhibit telomere shortening as the number of cell divisions increases with age. The question thus arises whether the telomere shortening that occurs in lymphocytes with aging becomes a cause for age-associated defects of lymphocyte function.

3.1. Cross-sectional analysis

Shortening of telomeres is found to occur as a function of age in cross-sectional population analysis of peripheral blood mononuclear cells (PBMC) (Slagboom et al., 1994), T cells (Weng et al., 1995; Rufer et al., 1999; Son et al., 2000), and B cells (Son et al., 2000, 2003; Martens et al., 2002). However, the rates of telomere shortening appear to be different among CD4 and CD8 T cells and B cells from these cross-sectional analyses (Table 1). With age, telomere loss is 33 bp/year for CD4 T cells and 26 bp/year for CD8 T cells. Among CD4 T cells, the rates of telomere attrition with age are 39 bp/year for naïve cells and 51 bp/year for memory cells. For CD8 T cells, the rates of telomere attrition with age are 34 bp/year for naïve cells and

54 bp/year for memory cells. The rate of telomere attrition with age for B cells is 15–19 bp/year. Slightly higher rates are found for B cell subsets: naïve B cells (29 bp/year) and memory B cells (40 bp/year), than for total B cells. Interestingly, while the difference in telomere length between naïve and memory CD4 T cells remains relatively constant with age, it appears that there an increasing difference in telomere length between naïve and memory CD8 T cells with age. This higher rate of telomere attrition in memory CD8 T cells with age may reflect accumulation of memory CD8 T cells, especially those CD28⁻ CD8 T cells due to recurrent antigen exposure. However, the significance of the different rates of telomere attrition in CD4 and CD8 T cells and B cells will need further study.

3.2. In vitro study

Culture of primary T cells in vitro provides a direct means to assess the role of telomere length in T cell senescence. As described above, under repeated or continuous stimulation, T cells undergo many rounds of cell division accompanied with shortening of telomere length. When T cells reach replicative senescence, telomeres are shorter in these T cells than those at the beginning of the culture. In CD4 T cells, naïve cells have longer telomeres than do memory cells and also are capable of undergoing a greater number of cell divisions than memory cells in vitro (Weng et al., 1995). One senescence-associated change in CD8 T cells is the loss of expression of the co-stimulatory receptor, CD28, which is found in aged individuals in vivo and in senescent T cells in vitro. Indeed, those CD28⁻ CD8 T cells have shorter telomere length than their CD28⁺ counterparts ex vivo (Monteiro et al., 1996; Allsopp et al., 1996) as well as in vitro (Effros et al., 1996; Effros, 2003). These findings demonstrate some similarities between T cell aging in vivo and in vitro models. A remaining question is whether shortened telomeres in these “aged” or “senescent” T cells act as a causal factor in limiting their functions in aging.

4. Telomeres in abnormal conditions: genetic disorders and chronic stress

Over the past decade, a great deal has been learned about the role of telomeres and telomerase in lymphocyte function through the study of “abnormal” conditions. Discovery of genetic defects in the TERC and TERT genes indicate the critical role of telomerase in hematopoietic cell proliferation and growth. Also, analysis of individuals who undergo prolonged psychological stress also suggests that declines in lymphocyte functions in these individuals are associated with accelerated telomere shortening.

4.1. Genetic disorders

Dyskeratosis congenita (DC) is an inherited bone marrow failure syndrome and has two genetic forms: X-linked and autosomal (dominant and recessive) (Marrone et al., 2005,

Table 1
Rates of telomere attrition with age in lymphocytes^a

Type of cell	Rate of telomere loss (bp/year)	Sample size	References
CD4 naïve	39	127	Rufer et al.
CD4 memory	51	109	Rufer et al.
CD4	35	121	Son et al.
CD8 naïve	34	126	Rufer et al.
CD8 memory	54	77	Rufer et al.
CD8	26	121	Son et al.
T cell	33	51	Martens et al.
B cell	15	51	Martens et al.
B cell	19	121	Son et al.
B cell naïve	29	53	Son et al.
B cell memory	40	53	Son et al.

^a The rates of telomere attrition with age were calculated based on the cross-sectional analyses.

2007; Walne et al., 2007). In the X-linked form of DC, the gene involved encodes dyskerin, a protein that is involved in telomerase RNP assembly (Mitchell et al., 1999), while in the autosomal form of DC, there are mutations in the TERC and TERT genes (Vulliamy et al., 2004; Walne et al., 2007; Marrone et al., 2007). In both the X-linked and autosomal forms of DC, lymphopenia and infections are present in the majority of patients, and dramatically reduced telomere lengths are found in blood lymphocytes, providing a mechanistic link of the replicative failure and premature senescence of lymphocytes in DC patients (Knudson et al., 2005). More recently, a null mutation in motif D of TERT has been found in autosomal DC patients. This mutation leads to haploinsufficiency of telomerase and telomere shortening (Armanios et al., 2005). Analysis of patients with other bone marrow failure syndromes has identified additional mutations in both TERC and TERT (Xin et al., 2007). These mutations cause a compromised telomerase function by haploinsufficiency and shortened telomeres. Together, these findings indicate the importance of telomere maintenance and telomerase dosage for the normal function and homeostasis of lymphocytes.

4.2. Chronic stress

The consequence of chronic stress on telomere length and telomerase activity of peripheral blood mononuclear cells (PBMC) has recently been reported. Higher oxidative stress, lower basal level of telomerase activity, and shorter telomere length of PBMC are found in mothers of chronically ill children (Epel et al., 2004), and shorter telomere length of PBMC in chronically stressed individuals with mood disorders (Simon et al., 2006). Damjanovic et al. examined T cell functions in the caregivers of Alzheimer's disease (AD) patients who endure chronic stress and have significantly higher depressive syndrome than do their controls (Damjanovic et al., 2007). Correspondingly, caregivers have significantly lower T cell proliferation but higher production of immune-regulatory cytokines (TNF- α and IL-10) than controls in response to stimulation in vitro. Most strikingly, caregivers have significantly shorter telomere lengths in peripheral T cells than do the controls. Furthermore, this telomere attrition in caregivers is not due to an increase in T cell subsets that possess shorter telomeres (such as memory T cells or CD28⁻ CD8⁺ T cells). Finally, the basal telomerase activity in T cells is significantly higher in caregivers than in controls ($p < 0.0001$), pointing to an unsuccessful attempt of cells to compensate for the excessive loss of telomeres in caregivers. How chronic stress affects altered T cell function and leads to accelerated aging of T cells will require further study.

5. Conclusion

In the past decade or so, we have learned a great deal of the important role of telomeres and telomerase in lymphocyte differentiation and function as well as under abnormal conditions. It also raises questions regarding the particular role of telomeres in lymphocyte function during in vivo aging.

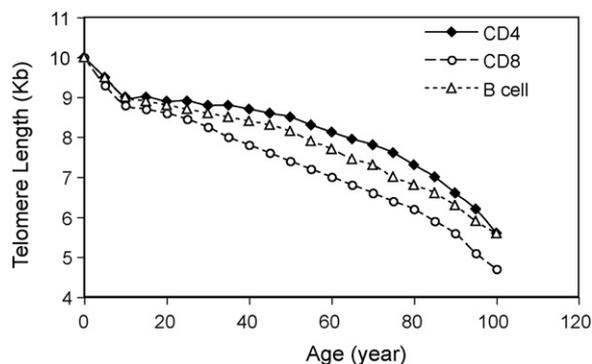


Fig. 1. Model of telomere attrition in T and B cells with age. Loss of telomere length is rapid during the first decade of life and decreases during most of adult life. At advanced age, the rate of telomere shortening may increase. The graph projects the telomere attrition in CD4, CD8, and B cells in vivo based on the cross-sectional analysis of telomere length in lymphocytes with age. Whether significantly shortened telomeres in advanced age cause declined function of lymphocytes will need further study.

Based on cross-sectional analysis, the rate of telomere attrition is ranges from 20 to 60 bp/year in lymphocytes of human adults. Over the course of 80 years life, 2–5 kb of telomere loss could be expected in lymphocytes plus 1–2 kb loss of the first decade of life and thus reduces telomere length to the range of 3–5 kb long as projected based on cross-sectional analyses, assuming that the initial average length of telomere is 10 kb (Fig. 1). The important question posed by these findings is whether the remaining telomere length is sufficient to sustain telomere function in lymphocytes. Obviously, more studies are needed, especially longitudinal analysis of telomere length and telomerase activity, to better understand the in vivo role of telomere in lymphocyte function during aging.

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