Kleemeier Award Lecture 2008—The Canary in the Coal Mine: Telomeres and Human Healthspan

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Telomeres, the repeated series of DNA sequences that cap the ends of linear chromosomes, become shorter during cell division and oxidative stress. Shortened telomeres have been documented in a wide variety of pathologies associated with aging and are also predictive of early mortality in the very old. However, telomere shortening—like the canary in the coal mine—is not the cause of the deleterious effects, but rather, the harbinger of increased health risk. Using immune responses to infection as a model system to further analyze the link between telomeres and age-related disease, we have demonstrated that the end-stage T cell with shortened telomeres is reduced in antiviral immune function and secretes large amounts of so-called proinflammatory factors. Our research has documented that maintaining high levels of the telomere-extending enzyme, telomerase, by either genetic manipulation or exposure of T cells to chemical telomerase activators, not only retards telomere loss but also restores a more youthful functional profile to the T cells. These observations suggest possible novel telomerase-based therapeutic approaches to enhancing healthspan in the elderly population.

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TELOMERES, protective structures at the termini of human chromosomes, shorten with cell division due to the inability of the DNA-copying mechanism to synthesize the very end of the DNA sequence. When telomeres reach a critically short length, a DNA damage signal is transmitted, leading to an irreversible cell cycle arrest (1). The process of proliferation-induced telomere shortening has been studied extensively in cell culture models of replicative senescence. These studies have underscored a variety of functional and genetic changes, which may be features of replicative senescence that are just as important as the inability to divide (2). Subsequent studies on human blood samples have shown that during normal aging and in certain disease states, there is an accumulation of T cells with characteristics that mirror those observed in T cells that reach replicative senescence in cell culture following multiple rounds of antigen-driven activation or proliferation (3).

This article will focus on a particular type of immune cell, the so-called cytotoxic or CD8 T cells. The main features of human T-cell replicative senescence identified in cell culture will be reviewed, followed by examples of cells with similar features that have been documented in the context of many age-associated diseases. The dynamic T-cell changes associated with chronic stimulation will be described, leading to a discussion of possible telomerase-based approaches to prevent the inflammatory and functional changes associated with chronic immune activation.

CLONAL EXPANSION IS CRITICAL TO NORMAL T-CELL FUNCTION

The generation of antigen receptors on T cells by an intricate DNA recombination mechanism allows just a few hundred different gene segments to combine in a variety of ways to create thousands of receptor chains. Moreover, because functional antigen receptors comprise two different receptor chains, each encoded by distinct sets of gene segments, an additional level of diversity is added during the random pairing of two different chains. By these mechanisms, a small amount of genetic material is used to generate at least $10^8$ different specificities. The unique antigen specificity of each individual lymphocyte helps to create an immune system that is capable of responding to the nearly infinite number of foreign antigens that could be encountered over a lifetime.

When an antigen interacts with receptors expressed on a mature T cell, the T cell becomes activated and starts dividing, giving rise to a clone of identical progeny bearing identical receptors for antigen. Antigen specificity is thereby maintained as the dividing cells continue to proliferate and differentiate into effector cells that function to eliminate the foreign pathogen. Once antigen is cleared, a small number of memory cells persist, all expressing the same antigen receptor. If the same antigen is encountered again, the process of activation and clonal expansion is repeated. From the aforementioned description, it is clear that adequate proliferative potential is critical to normal immune function.

Under most circumstances, a limit in the number of cell divisions imposed by the replicative senescence "program" would not exhaust a T cell's overall proliferative potential. Indeed, from the in vitro data, it could be argued that the number of cell divisions achievable by each T cell is sufficiently large that the finite replicative life span would not be biologically meaningful in vivo. Thus, an average life span of 35 population doublings, which if all daughter cells continue to grow unchecked will result in more than $10^{10}$ cells, seems at first glance to be more than adequate. However,
because T-cell expansion occurs in waves of proliferation followed by elimination of excess cells, the finite replicative capacity of T cells may have important implications, particularly in the elderly population, who have markedly reduced ability to generate new T cells, due to thymic involution (4).

EXTENSIVE ANTIGEN-DRIVEN DIVISION LEADS TO REPLICATIVE SENOSENCE

Given the importance of adequate clonal expansion in generating T-cell responses, the potential barrier of replicative senescence would be predicted to play a pivotal role in immune function, especially by old age. To test this possibility, cell culture models of T-cell replicative senescence were established by several different groups, with the goal of identifying the specific characteristics associated with lymphocyte senescence (5,6). Similar to other cell types that reach replicative senescence in vitro, cultures of senescent T cells show irreversible growth arrest, increased expression of several cell cycle inhibitors, inability to undergo apoptosis, and telomere shortening (7–9).

As T cells progress to senescence in culture, they also show altered secretion patterns of several key soluble mediators, known as cytokines. Specifically, with increasing numbers of population doublings in cell culture, T cells secrete progressively higher titers of two proinflammatory cytokines, namely, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), and reduced levels of a critical antiviral cytokine, interferon-gamma (IFN-γ) (3,7). Importantly, senescent T cells no longer express a key signaling surface receptor, CD28. After undergoing multiple rounds of antigen-driven cell division, cultures initiated from T cells that all expressed CD28 become nearly 100% CD28-negative (CD28-) at senescence (10).

SENEGENT T CELLS ARE PRESENT IN VIVO

The identification of absence of CD28 expression as a biomarker of T-cell replicative senescence in cell culture paved the way for analysis of blood samples for the presence of similar cells. Several groups have shown that older persons have significant increases in the proportion of T cells lacking CD28 expression, with some aged individuals having more than 60% CD28-negative cells within the CD8 T-cell subset, compared with the mean young adult value of less than 10% (10). These putatively senescent T cells are often part of oligoclonal expansions, suggesting that they arose as a consequence of the immune response to specific antigens. Telomere studies have confirmed that the CD28- cells had undergone extensive cell division, compared with other T cells from the same individual (11,12).

The underlying mechanism for the accumulation of senescent T cells in elderly persons is not known, but it seems likely that long-term chronic infection with herpes viruses such as cytomegalovirus (CMV), Epstein–Barr virus (EBV), and varicella zoster may be involved (13). Once a person is infected with any of these viruses, the virus persists within that individual throughout life. The proportion of infected individuals gradually increases with age, such that the majority of elderly people are CMV+, EBV+, and varicella+. It is rare for overt reactivation to occur, even in the very elderly population, but the “work” required to maintain immune control over these latent infections is costly. Indeed, the continuous immunosurveillance results in the accumulation of dysfunctional senescent virus-specific cells, which fail to be eliminated from the system. This may explain the documented association between high proportions of senescent T cells and early mortality in the elderly population.

It should be emphasized that the cells lacking CD28 expression do not suddenly appear in old age; there is a progressive increase over the life span in the proportion of T cells that lack CD28 expression (14), which may relate to the gradual increase in the proportion of individuals harboring infection with persistent viruses. Moreover, chronologically age is not unique in its association with T-cell replicative senescence. T cells with the characteristics associated with senescence are seen in a variety of clinical situations that involve chronic stimulation of the immune system. Persons with rheumatoid arthritis, an autoimmune disease involving abnormal reactivity of T cells to self-antigens within the affected joints, have increased proportions of T cells lacking CD28. These cells secrete high titers of TNF-α, one of the cytokines associated with replicative senescence in cell culture studies. Chronic infection with human immunodeficiency virus-1 (HIV-1) is a dramatic example of the progressive accumulation over time of CD8 T cells that are CD28- and have shortened telomeres (11,15).

THE IMMUNE SYSTEM AND DISEASES OF AGING

The beneficial effects of the immune system, which is devoted to the neutralization of harmful environmental agents early in life, may become detrimental during old age. Indeed, a variety of age-related pathologies are now understood to involve the immune system. Inflammation, for example, is a critical component of the immune system’s defense against foreign pathogens. Nevertheless, chronic elevation of inflammatory mediators is known to have negative influences on a variety of organ systems and, indeed, on life span itself. Several studies have shown that increased levels of circulating proinflammatory cytokines, such as IL-6 and TNF-α (the same ones produced by senescent T cells in culture), are strong independent risk factors of morbidity and mortality in the elderly population (15). Data from Swedish longitudinal studies on elderly humans highlight the importance of low-grade inflammation in predicting mortality very late in life (16).

Inflammatory cytokines, many of which are produced by T cells, have also been implicated in age-related bone loss. Activated T cells secrete large amounts of RANKL (receptor
activated by TNF-α, IL-6, and IFN-γ. Other factors produced by immune cells, such as TNF-α, IL-6, and IFN-γ, also play a role in bone homeostasis, by tipping the balance between osteoclast and osteoblast activity. Reduced bone mass within the oral cavity is also seen during aging. Interestingly, CD8 T cells lacking CD28 expression have been documented in inﬂamed gingival tissue (17). Because the CD8+/CD28− phenotype is associated with replicative senescence, it is possible that chronic antigenic stimulation may play a role in alveolar bone loss.

Similar associations between inﬂammatory cytokines have been documented in other age-related diseases. For example, the levels of IL-6 are known to increase within the central nervous system of persons with Alzheimer’s disease (AD) (18), and IL-6 is directly involved in regulation of production of the Alzheimer’s beta amyloid precursor protein (19,20). The concentration of another cytokine produced by T cells, TNF-α, is increased in the serum of centenarians with dementia (21). Finally, T-cell telomere shortening and lymphocyte telomere dysfunction have both been reported in patients with AD (22,23). Interestingly, persons with Down syndrome, who universally develop AD by age 40, show accelerated lymphocyte telomere loss compared with age-matched controls (8).

A ﬁnal example of the T-cell telomere link with age-related disease is cancer. There is increasing evidence that exhaustion of immunosurveillance may play a role in tumor initiation and progression. Tumors associated with latent viral infections frequently arise in persons with immunodeﬁciency. For example, in immunosuppressed individuals, virtually all lymphomas are EBV in origin, presumably resulting from the failure of T cells to effectively control EBV infection (24,25). Kaposi’s sarcoma is consequent to latent infection with another herpes virus infection (HSV 8), and cervical cancer, which also increases during immune suppression, is associated with certain strains of human papillomavirus. In some cases, CD8 T cells with markers of chronic activation or senescence, or both, have been isolated from tumors.

Telomeres and Mortality

Recent longitudinal studies further underscore the notion that telomere shortening may constitute a biomarker for deleterious health status. In one study, lymphocyte telomere length at age 60 was shown to be predictive of subsequent life span. The T-cell involvement in these associations is underscored by that fact that the individuals with telomere lengths in the shortest quartile at age 60 had a 7- to eightfold greater risk for dying of infection as compared with those in the longest telomere quartile group (26).

The Swedish so-called OCTO and NONA longitudinal studies on elderly cohorts examined a variety of physiologic parameters associated with life span and identiﬁed a cluster of immune parameters that was strongly predictive of early mortality, independent of the cause of death (27,28). This so-called immune risk phenotype consisted of a reversal of the normal ratio between helper and cytotoxic T cells, reduced T-cell proliferative potential, increased proportion of CD8 T cells lacking CD28 (ie, senescent T cells), and evidence of CMV infection. The inclusion of high proportions of senescent T cells in the immune risk phenotype has been viewed as a clinical conﬁrmation of the importance of replicative senescence in human organismic aging (4).

The underlying mechanism for the associations between immune proﬁles and mortality is not known. Clearly, if the senescent T cells present in vivo have proinflammatory cytokine patterns that are similar to cells that are driven to senescence in cell culture, they may play a role in the mortality data and in other deleterious outcomes in elderly people. Consistent with this notion, several studies have documented associations between high proportions of senescent CD8 T cells and poor antibody responses to inﬂuenza vaccination (29,30).

Telomerase Activators: A Cure for Age-Related Pathologies?

As previously noted, telomere shortening plays a pivotal role in the replicative senescence program, and there are numerous associations between shortened telomeres and deleterious health effects during aging. For this reason, efforts to prevent or retard replicative senescence have largely focused on the telomere-extending enzyme, telomerase. Early studies, using gene transduction with the catalytic component of human telomerase (hTERT), were performed on a variety of nonimmune cell types, including human ﬁbroblasts, epithelial cells, and keratinocytes (31). In these experiments, introduction of the hTERT gene led to unlimited proliferation, telomere length stabilization, normalization of function, and, importantly, no evidence of altered growth or tumorigenesis in immunodeﬁcient mice.

HIV or acquired immunodeﬁciency syndrome (AIDS) is increasingly recognized as a model of premature immunologic aging (32). For this reason, our own studies aimed at retarding or preventing replicative senescence and telomere shortening have focused on HIV-speciﬁc CD8 T cells isolated from persons infected with HIV-1. Experiments on both cloned (33) and uncloned (7) populations of CD8 T cells showed that the continuous expression of telomerase activity, mediated by retrovirally encoded hTERT, led to telomere length stabilization and reduced expression of several cell cycle inhibitors. Importantly, the transduced HIV-speciﬁc CD8 T cells were able to maintain the production of IFN-γ for extended periods and showed signiﬁcantly enhanced capacity to inhibit HIV replication. These proof-of-principle studies provide evidence that maintenance of telomerase activity in virus-speciﬁc CD8 T
cells may be a useful therapeutic strategy for persons with HIV disease.

Based on the gene therapy results, we next tested a chemical telomerase activator (TAT2) for its effects on HIV-specific CD8 T cell function. Our studies showed that exposure of virus-specific CD8 T cells to TAT2 led to significantly enhanced telomerase activity, retardation of telomere loss, and, importantly, dramatically increased antiviral function (34). If in vivo experiments confirm the utility of telomerase enhancers in antiviral immune function, they will be developed further for clinical use as a treatment for HIV or AIDS as well as to enhance viral immunity and response to vaccines in the elderly population. Finally, cancer immunotherapy, which requires prolonged and continuous proliferation and function of tumor-specific CD8 T cells, may also benefit from treatment using telomerase activators.

**Concluding Remarks**

Telomere shortening, in either total white blood cells or in purified T cells, has been documented in a variety of age-related diseases. Chronic activation of immune cells, involving increased inflammation or exhaustion of antiviral responses and the associated telomere loss, may be indicative of a variety of age-related pathological conditions and may even portend imminent death. A more complete understanding of the factors responsible for telomere loss may lead to novel therapeutic approaches for many diseases of aging. One promising strategy, namely, exposing immune cells to small-molecule telomere activators may be a possible approach to retard or prevent replicative senescence and its associated negative consequences.

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