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Telomerase induction in T cells: a cure for aging and disease?

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Abstract

Cells of the immune system are unique among normal somatic cells in that they have the capacity to upregulate the telomere-extending enzyme, telomerase, albeit in a precisely controlled fashion. Kinetic analysis of telomerase activity in long-term T cell cultures has documented that the high level of telomerase induced in concert with activation reaches a peak at 3–5 days, then declines by 3 weeks. The process is recapitulated during secondary antigenic stimulation, but by the third, and all subsequent stimulations in vitro, CD8 T cells are unable to upregulate telomerase. Cell division in the absence of telomerase activity results in progressive telomere shortening, and ultimately, the DNA damage/cell cycle arrest that is signaled by critically short telomeres. Cultures of senescent CD8 T cells show altered cytokine patterns, resistance to apoptosis, and absence of expression of the CD28 co-stimulatory receptor. CD8 T cells with these and other features of replicative senescence accumulate progressively with age, and at an accelerated rate, during chronic infection with HIV-1. Clinical studies have shown that high proportions of CD8 T cells with the senescent phenotype correlate with several deleterious physiologic outcomes, including poor vaccine responses, bone loss, and increased proinflammatory cytokines. $CD8^+CD28^-$ T cells have also been shown to exert suppressive activity on other immune cells. Based on the central role of telomere shortening in the replicative senescence program, we are developing several telomerase-based approaches as potential immunoenhancing treatments for aging and HIV disease. Gene therapy of HIV-specific CD8 T cells with the telomerase catalytic component (hTERT) results in enhanced proliferative capacity, increased anti-viral functions, and a delay in the loss of CD28 expression, with no changes in karyotype or growth kinetics. These proof-of-principle studies have led to screening for pharmacological approaches that might mimic the gene therapy effects, in a more clinically-suitable formulation.

1. Introduction

The search for biomarkers that are able to provide a metric of physiological, as opposed to chronological age, has led to the identification of certain immune parameters that may offer predictive clinical value. One such marker, an increase in the proportion of cytotoxic (CD8) T cells that lack expression of the CD28 costimulatory molecule has recently received a great deal of attention.

Several studies on the elderly have documented that the presence of high proportions of $CD8^+CD28^-$ T cells correlates with blunted responses to influenza vaccines [1,2]. Furthermore, longitudinal studies have shown that this same population of cells is also part of a cluster of parameters, the so-called immune risk phenotype, which is predictive of early all-cause

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mortality in a Swedish octogenarian cohort [3]. The mechanism for these associations remains to be elucidated, but one possible factor may be the suppressor function that has been ascribed to the $CD8^+CD28^-$ subset. A second possible contributory factor may be the effect of this unusual population of memory cells on the homeostatic regulation of the overall T cell pool.

This review will describe experiments that have provided insight into the putative origin of the $CD8^+CD28^-$ T cells, and will then summarize their major characteristics. Finally, several telomerase-based strategies for preventing or at least retarding the generation of $CD8^+CD28^-$ T cells will be described. Although these approaches do not purport to be a *cure* for aging and disease, they may lead to more vigorous immune responses to both infection and to vaccines, thereby enhancing the health and quality of life of affected persons.

2. Replicative senescence: the *in vitro* model

Gerontologists have focused for nearly 10 decades on the relationship between the cellular process of replicative senescence and organismic aging, using mainly fibroblasts and epithelial cell model systems. Ironically, application of the replicative senescence paradigm to cells of the immune system was a relatively recent phenomenon, despite the fact that immunologists had long been aware of the need for immune cells to undergo massive proliferative expansion as part of normal immune responses. It was not until the 1980's—nearly 20 years after the original Hayflick limit had been described—that cells of the immune system were rigorously tested for the occurrence of replicative senescence

Using a long term cell culture model, in which human T cells from young donors were repeatedly stimulated with antigen, it was demonstrated on nearly 100 independent cultures that T cells do, in fact, reach the end- stage of replicative senescence after multiple rounds of cell division [4]. Senescent CD8 T cell cultures are experimentally defined by their inability to enter cell cycle in response to two rounds of antigenic stimulation and/or increasing doses of Interleukin 2 (IL 2). Despite the inability to divide further, senescent cells are, nevertheless, metabolically active, and do respond to antigenic stimulation by upregulating the alpha chain of the IL-2 receptor (CD25) [5]. Possibly related to the inability to enter cell cycle, cells in senescent cultures are unable to initiate programmed cell death (similar to senescent fibroblasts), and once reaching senescence, cells can actually survive for several months in culture, with proper feeding [6].

Whereas the process of replicative senescence is most easily defined by the exhaustion of proliferative potential, some of the functional and genetic alterations associated with senescence may be even more deleterious than the inability to divide. Senescent fibroblasts, for example, enhance the growth of tumor cells both *in vitro* and *in vivo* [7]. In the case of CD8 T cell cultures, senescence is associated with enhanced production of two pro-inflammatory cytokines, IL-6 and TNFα, and reduced secretion of the anti-viral cytokine, interferon γ [8]

Arguably, the most significant genetic and phenotypic alteration associated with T cell replicative senescence in cell culture is the complete and irreversible loss of expression of the CD28 costimulatory molecule [9]. This permanent loss of any detectible CD28 surface and message expression is in contrast to the well-documented up- and down-regulation of the quantity of CD28 surface expression associated with physiological activation [10–14]. Thus, the end stage of replicative senescence can be distinguished by the inability of T cells to upregulate CD28 expression after several cycles of antigenic stimulation.

CD28 is a homodimer that is constitutively expressed on >99% of T cells at birth [15]. In addition to co-stimulation, CD28 also functions in a variety of important cellular processes, including mRNA stabilization, cell trafficking, lipid raft formation and glucose metabolism [16–18]. In addition, the CD28 co-stimulatory signal is required for optimal upregulation of

telomerase activity [19,20]. Thus, T cells lacking CD28 are fundamentally different from T cells that express CD28.

3. Telomere-telomerase dynamics of CD8 T cells

The process of replicative senescence is modulated, in large part, by telomeres, the repeated DNA sequences at the termini of linear chromosomes. Due to the mechanism of DNA replication, telomeres shorten with each cell division. This so-called end replication problem results in the loss of 50–100 bp of telomere sequence with each cell division, ultimately leading to critically short telomeres. Either the shortened telomeres themselves, or the reduced abundance of certain telomere-binding proteins, is perceived by the cell as DNA damage, which signals the cell cycle arrest arrest associated with replicative senescence. Tumor cells are able stabilize their telomere length due the activity of telomerase, an enzyme that is also active in humans during early embryological development. However, after birth, except for certain pluripotent stem cells and germ cells, human cells do not express telomerase. One important exception, however, is within the immune system.

Cells of the immune system are able to upregulate telomerase activity during certain stages of development, as well as in concert with activation [19]. Indeed, stimulation of T cells with antigen, mitogen, or activatory antibodies elicits telomerase activity that is as high as that of tumor cells, but this activity is gradually reduced and is undetectable after approximately three weeks [20]. *In vitro* analysis of the kinetics of T cell telomerase activity has shown that T cells are able to repeat the telomerase upregulation upon secondary stimulation with antigen. Interestingly, CD8 T cells lose this capacity by the third antigenic encounter, and show progressive telomere loss with further antigen-driven proliferation. By contrast, CD4 T cells maintain high telomerase activity in response to even the 7^{th} encounter with antigen. Importantly, optimal telomerase upregulation in T cells requires CD28 co-stimulation; blocking the interaction between CD28 and its B7 ligands on antigen-presenting cells leads to significantly blunted telomerase activity [20]

4. Senescent CD8 T cells accumulate *in vivo*

Identification of the absence of CD28 from the surface of cells in senescent cultures provided a critical marker that enabled us to address the fundamental question of whether replicative senescence was merely a cell culture artifact, or, alternatively, whether it occurred in the context of the whole organism. Cross sectional analysis of T cells from different age groups has clearly documented that the proportion of cells lacking CD28 expression increases progressively with age [21]. During chronic infection with HIV-1, even in younger persons there is a progressive increase in $CD28^-$ T cells [22]. In both situations, the majority of $CD28^-$ T cells are within the CD8 subset. Indeed, during both HIV infection and aging, the proportion of $CD28^-$ T cells within the peripheral blood CD8 T cell pool can be as high as 65% [23].

Telomere length analysis on *ex vivo* blood samples has demonstrated that $CD28^-$ T cells have shorter telomeres that other T cells from the same individual, suggesting a more extensive replicative history [23,24]. Furthermore, in response to both specific antigenic stimuli and to stimuli that bypass the T cell receptor, $CD28^-$ T cells show minimal proliferative capacity [23]. Finally, $CD8^+CD28^-$ T cells tested *ex vivo* show reduced ability to undergo superantigen-induced apoptosis [25]. Thus, by a variety of criteria, these cells are reminiscent of cells that are driven to replicative senescence *in vitro* as a result of multiple rounds of antigen-driven proliferation.

There is increasing evidence that the population of $CD28^-$ T cells isolated *ex vivo* is actually somewhat heterogeneous in nature and that multiple phenotypic markers are required for a more precise definition of the true end stage senescent cell. Several candidate markers that

were not necessarily observed in the *in vitro* long-term culture model, have, in fact, been identified. In studies on T cells isolated from HIV-infected persons, telomere analysis and proliferative assays suggested that surface expression of CD57 on $CD28^-$ T cells defined the ultimate end-stage cell in the senescence pathway [26]. The inhibitory receptor programmed death 1 (PD-1; also known as PDCD1), a negative regulator of activated T cells whose expression is significantly increased on the surface of exhausted virus-specific CD8 T cells in mice, is also present on end stage HIV-specific CD8 T cells from HIV-infected persons. Moreover, expression of PD-1 correlates with impaired HIV-specific CD8 T-cell function as well as predictors of disease progression [27]. Interestingly, this marker was not elevated on CMV-specific CD8 T cells from the same donor, underscoring the importance of the specific virus in determining memory cell differentiation [28]. FOXP3, an antigen previously associated with regulatory CD4 T cells, has now been shown to be present on $CD8^+CD28^-$ T cells from cancer patients [29]. The inhibitory killer cell lectin-like receptor G1 (KLRG1) is expressed on clonal populations of CD8 T cells that are non-proliferative, linking this marker with the senescent phenotype as well [30]. Other cell surface marker changes, including loss of CD27 expression, may also be informative in defining the true end stage CD8 memory cell.

It is clear that using a combination of several phenotypic markers to purify and characterize senescent T cells will be critical for future genetic and mechanistic studies on this population. Nevertheless, even the single criterion of absence of the CD28 molecule has already lead to provocative insights on the potential role of these cells *in vivo.* As noted above, one of the key immune correlates of reduced vaccine responses in the elderly is the presence of high proportions of CD8 T cells that lack CD28 expression. Furthermore, clonal expansions of CD8 T cells that lack CD28 have important clinical predictive value in terms of mortality risk [31]. In addition, the abundance of $CD28^-$ T cells also correlates with disease pathogenesis in certain autoimmune conditions. For example, the proportion of $CD8^+CD28^-$ T cells is increased in patients with ankylosing spondylitis, and their abundance is actually correlated with disease progression [32]. In rheumatoid arthritis, there is an increase in $CD28^-$ T cells within the CD4 subset, which seem to particularly important in patients with non-joint disease manifestations, where they have been proposed to be involved in vascular injury [33]. Moreover, altered expression of CD28 has been associated with the clinical outcome of certain forms of cancers. For example, in patients with head and neck tumors, it has been shown that tumor resection is associated with a reduction in the $CD8^+CD28^-$ T cell subset, which had undergone expansion during the period of tumor growth [34]. Thus, even in the absence of additional markers, lack of CD28 has been an informative biomarker for disease status. It is not clear whether the presence of $CD28^-$ T cells in some of these diseases reflects chronic antigenic stimulation or is an epiphenomenon, but further research on these cells should clarify this issue.

5. Telomerase-based strategies may prevent CD8 T cell exhaustion

Development of methods to reverse and/or prevent replicative senescence is an important facet of aging research, due to its potential use in both maintaining function as well as its possible role in tissue regeneration. Based on the pivotal role of telomere shortening in the replicative senescence program, efforts to manipulate the process have focused attention 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 non-immune cell types, including human fibroblasts, epithelial cells and keratinocytes [35]. 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 tumorogenesis in immunodeficient mice.

In our studies, attention was focused on HIV-specific CD8 T cells isolated from persons infected with HIV-1. Experiments on both cloned [36] and uncloned [37] 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 the $p16^{INK4A}$ and $p21^{WAF1}$ cyclin-dependent kinase inhibitors, suggesting an important role for both proteins in mediating the senescence program. The transduced HIV-specific-specific CD8 T cells were able to maintain the production of IFNγ 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 these results, our current research is testing several pharmacologic telomerase enhancers for their effects on HIV-specific CD8 T cell function. If these experiments confirm the utility of telomerase enhancers in anti-viral immune function, they will be developed further for clinical use as a treatment for HIV/AIDS. Moreover, similar therapeutic approaches may enhance viral immunity and response to vaccines in the elderly. Finally, cancer immunotherapy, which requires prolonged and continuous proliferation and function of tumor-specific CD8 T cells, may also benefit from telomerase-activators [38].

Gene transduction of HIV-specific CD8 T cells also showed prolonged expression of CD28 [37]. However, ultimately, loss of CD28 expression was not prevented by hTERT transduction. Since CD28 signaling is required for optimal telomerase induction in T cells [20], it is possible that strategies to prevent the loss of CD28 may not only lead to sustained CD28 expression but may also allow the maintenance of telomerase activity. Current experiments are addressing this issue.

6. Concluding Remarks

The immune status of elderly humans is the composite outcome of genetic background, thymic involution, and most importantly, a lifelong exposure to a myriad of encounters with foreign pathogens. The immunological history of humans cannot, therefore, be mimicked in laboratory animals, which are subject to minimal exposure to such antigens.

Due to the extraordinarily low frequency of T cells specific for any single antigen, immune responses require a massive degree of proliferation in order to adequately control infection. In situations involving chronic antigenic stimulation, such as infection with certain viruses that establish latency, some of the responding CD8 T cells eventually reach their innate proliferative limit by a telomere-based process known as replicative senescence. Cells with characteristics suggestive of replicative senescence accumulate progressively with age, and, in accelerated fashion, during chronic infection with HIV-1. High proportions of these cells, are correlated with a variety of deleterious health outcomes, including poor control over infection, reduced responses to vaccines, bone loss, and early mortality. In addition, the presence of large numbers of senescent T cells may affect naïve and memory T cell populations, via homeostatic mechanisms that regulate the size and composition of the T cell pool.

Proof-of principle gene therapy studies on cells in culture have suggested that the process of replicative senescence can be retarded or even prevented in virus-specific CD8 T cells by manipulation of the telomere-extending enzyme, telomerase. Based on these observations, ongoing research is testing several pharmacologic modulators of telomerase for their effects on CD8 T cell senescence and function. The ability of reduce the rate of generation of senescent T cells may result in significantly enhanced immune function, and may also correct some of the deleterious effects associated with high proportions of these cells. Although improved immunity cannot be considered a “cure” for aging and disease, the beneficial effects promise

to enhance health and quality of life of the elderly and of persons infected with HIV-1, a disease characterized by accelerated aging of the immune system.

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