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REVIEW

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Replicative senescence of CD8 T cells: potential effects on cancer immune surveillance and immunotherapy

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Abstract The process of replicative senescence, which stringently limits the proliferative potential of normal T cells, constitutes a potential problem for cancer immunotherapy. The ability of CD8 T cells to recognize and destroy tumor cells has been well-established, but the requirement for massive, prolonged proliferative T-cell expansion and maintenance of functional integrity poses a significant obstacle to the success of cancer immunotherapy. Cancer immune surveillance may also be compromised by the long-term exposure of T cells to tumor antigens, particularly those of latent viruses, which could drive certain T cells to replicative senescence. This review summarizes the major characteristics of T-cell replicative senescence and raises the possibility that this process has the potential to affect both cancer development and treatment. Experimental strategies aimed at preventing T-cell replicative senescence are discussed in the context of cancer immunotherapy and vaccines.

Keywords Cancer immunotherapy · CD28 · Replicative senescence · T cells · Telomeres

Introduction

Cancer therapy approaches that take advantage of the immune system's capacity to recognize and eliminate tumor cells are central to the treatment of a variety of malignancies. However, these approaches may ultimately be handicapped by the intrinsic barrier to unlimited proliferation that is a strict characteristic of all normal human cells, including T cells. Indeed, active immunotherapy, i.e., immunization with genetically manipulated autologous tumor cells, tumor extracts, or synthetic peptides, requires that the patient's own immune system generate a normal immune response to tumor antigens [1, 33, 47, 68, 85]. Once generated, the effector cells must undergo significant and prolonged clonal expansion in order to maintain control over the tumor. Similarly, passive immunotherapy, using adoptive transfer of in vitro–expanded immune effector cells, also requires the generation of large numbers of functional anti-tumor T cells [5, 10, 11, 19, 39, 58, 81, 92]. Moreover, even after in vivo transfer, the limited proliferative ability of T cells may prevent them from undergoing additional expansion upon encounter with tumor cells. Thus, the process of replicative senescence, which comprises irreversible cell cycle arrest, as well as alterations in gene expression and effector function, may pose a formidable barrier to successful cancer immunotherapy. Replicative senescence may also hamper immune surveillance, particularly in the case of tumors involving those latent viruses to which the immune system has been exposed for prolonged periods. In this review, we will describe some of the basic features of replicative senescence in CD8 T cells, analyze the possible relationship of this cellular process to the development and treatment of cancer, and, finally, provide examples of strategies to overcome this process that may be applicable to cancer immunotherapy.

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What is replicative senescence?

Most higher eukaryotic cells that are capable of cell division in vivo—i.e., that are not post-mitotic—cannot do so indefinitely. The process of replicative senescence was described almost 40 years ago for cell cultures of human fibroblasts, and has been extended to a variety of cell types, including T cells [20]. It is now widely accepted by cell biologists that replicative senescence is a fundamental feature of normal somatic cells. Although germline cells are capable of continuous replication, at

some point during embryonic development, somatic cells acquire the restriction of replicative senescence [12].

In adult organisms, some stem cells still retain the ability to divide indefinitely, but most other normal somatic cells are restricted in replicative potential. After undergoing a finite number of cell divisions, normal human cells are arrested with a G1 DNA content and cannot be stimulated to enter the S phase of the cell cycle by any known mitogenic signals. Replicative senescence is associated with selective repression of several genes whose expression is required for G1 progression and DNA synthesis. Conversely, senescent fibroblasts and T cells have been shown to overexpress two negative growth regulators: the p21 and p16 inhibitors of cyclin-dependent protein kinases [75].

The process of replicative senescence is particularly stringent in human cells, which, in contrast to mouse cells, rarely, if ever, undergo spontaneous immortalization. However, oncogenic viruses or chemical/environmental carcinogens may permit some human cells to escape replicative senescence and acquire an indefinite replicative life-span [73]. Escape from senescence is a rare event, but many malignant tumors appear to contain at least some cells with an infinite replicative life-span. This observation has led to the speculation that replicative senescence may actually constitute a tumor suppressive mechanism [12].

Telomeres and the mechanism of senescence

The underlying signaling cascade responsible for replicative senescence has not been determined, but based on the known biochemistry of DNA replication, telomere shortening is currently the most viable candidate for the "clock" that registers numbers of cell divisions. Telomeres are the repetitive sequences at the ends of linear chromosomes. Telomere length can be estimated from the terminal restriction fragment (TRF), which contains the telomere TTAGGG region plus some nontelomeric sequences. The importance of telomere length measurements is underscored by the observation that, regardless of the starting TRF length of a cell population, the initial TRF is predictive of the replicative life-span of cells. For example, naïve T cells have longer TRF lengths than memory T cells and have correspondingly greater proliferative potential in cell culture [87]. Because TRF lengths vary within a population of cells and even within the different chromosomes of an individual cell, senescence may be signaled when one or more TRFs reaches a critical length. This length might actually be less than the average 6 kb length observed for senescent cultures [14], possibly due to most or all of the telomeric TTAGGG repeats being lost from the critical chromosome.

Germline cells, many malignant tumor cells, and certain stem cells do not undergo replicative senescence and have stable chromosome lengths despite extensive replication, due to the constitutive activity of telomerase, a ribonuclear protein enzyme. This enzyme contains an RNA component that can direct the resynthesis of telomere sequences onto chromosome ends. Telomerase had been assumed to be absent from all normal cells, but recent evidence indicates that cells of the immune system are an exception. Indeed, it is now known that telomerase is highly active during T-cell development and following antigenic stimulation [43, 88].

If T cells can up-regulate telomerase in response to activation, why, then, do they ultimately undergo replicative senescence? Analysis of telomere/telomerase dynamics in cell culture and in vivo has provided novel insights into this facet of T-cell replicative senescence. First, it has been shown that the overall loss of telomere sequences, which occurs at a rate of 50–100 bp / cell division [84], is actually retarded during the period of high telomerase activity that coincides with early activation [7]. Telomere length stabilization has also been documented in vivo during acute infection with Epstein-Barr virus (EBV), where high telomerase activity in the tetramer-positive cells correlates with telomere length maintenance [52].

The second insight into the paradox of why telomerase-positive cells nevertheless undergo telomere shortening and ultimately reach senescence emerged from studies of repeated antigenic stimulation of purified CD8 T cells in long-term culture. This longitudinal analysis demonstrated that the high telomerase activity induced in response to the first and second encounters with antigen was not sustained during subsequent stimulations. In fact, by the fourth antigenic stimulation, CD8 T cells show no detectible telomerase activity. Interesting, CD4 T cells from the same donors and subjected to identical rounds of antigenic stimulation retain high levels of telomerase activity even as late as the seventh antigenic stimulation [83]. At the point when telomerase was undetectable in the CD8 T-cell cultures, the cells had undergone the same number of population doublings as the CD4 T-cell cultures, suggestive of an intrinsic difference in telomerase dynamics between the two T-cell subsets.

The premature cessation of telomerase induction in the CD8 versus CD4 subset seen under the controlled conditions of cell culture may explain the in vivo preponderance of CD8 T cells versus CD4 T cells with characteristics suggestive of senescence during aging [8] and chronic infection [31]. Indeed, studies on EBV infection have shown that telomere length of antigen-specific CD8 T cells is maintained during the acute infection stage, but once the virus establishes latency, these cells do undergo telomere shortening [42, 52], presumably due to the same phenomenon observed in telomerase down-regulation in repeatedly stimulated cultured CD8 T cells.

Senescence as a physiologic process

Growth arrest is the most easily discernible characteristic of senescent cells, and this trait may in fact explain

the low incidence of spontaneous transformation in human cells. Indeed, replicative senescence and cancer have been proposed to be "opposite sides of the same coin" [23]. Nevertheless, despite the putative protective effect in terms of preventing tumor development, there are negative aspects of replicative senescence that are becoming increasingly evident, as will be discussed below.

In addition to irreversible growth arrest, senescent cells acquire resistance to apoptotic stimuli and show increased levels of bcl2 expression [13]. These characteristic have been well-documented in senescent CD8 T cells, where antibodies to CD3 and Fas, as well as low-dose gamma irradiation and staurosporine, all of which cause robust apoptosis in early passage T cells, do not have the same effect in the senescent descendants of those T cells [76]. The underlying mechanism responsible for the reduced ability of senescent cells to undergo apoptotic death remains unknown, but may relate to their inability to initiate cell cycle entry.

A second important feature of replicative senescence is altered function. T cells that reach replicative senescence in cell culture show markedly different cytokine profiles from those of early passage cells, with striking increases in the production of two major proinflammatory cytokines, TNF-α and IL-6. Though the ability to up-regulate the α chain of the IL-2 receptor in an antigen-specific manner is retained, senescent T cells are unable to proliferate even in the presence of increasing doses of IL-2 [62]. There is also preliminary evidence that replicative senescence of CD8 T cells in cell culture is associated with markedly reduced virus-specific cytotoxicity and diminished ability to suppress viral replication [26].

Replicative senescence in T cells seems to be distinct from anergy, despite sharing certain characteristics, such as the inability to secrete IL-2. The growth arrest experienced by senescent T cells cannot be reversed by any of the following: antigenic restimulation, stimulation with phorbol esters and calcium ionophores, a combination of monoclonal antibodies (mAb) specific for CD3 and CD28, or response to IL-2. By contrast, anergic cells retain responsiveness to IL-2, and a period of growth with IL-2 renders them nonanergic again [6]. Replicative senescence is also distinct from tolerance, since it is the outcome of extensive rounds of normal antigen-induced responsiveness. Finally, senescent cells are not dead; indeed, with adequate feeding they can survive in a quiescent nondividing state for many weeks in culture.

As T cells progress toward senescence in culture, there is a diminished ability to up-regulate the gene encoding Hsp70 in response to heat shock [30]. This change represents a potentially important defect in vivo, in view of the involvement of the Hsp70 family of cytoplasmic proteins in the response to oxidative stress, to viral infection, and in antigen processing and presentation pathways. There is also evidence that T-cell senescence in culture is associated with the acquisition of certain other functions, including major histocompatibilty complex (MHC)-unrestricted cytotoxicity (natural killing) and negative-regulatory effects on other T cells (suppression) [61]. Thus, senescence does not constitute a general breakdown of normal function, but rather may comprise selected genetic and phenotypic alterations, resulting not only in loss, but also in gain of function. Interestingly, one of the characteristics acquired by fibroblasts that reach replicative senescence in cell culture is their ability to enhance the proliferation of transformed, but not normal, epithelial cells [48]. This feature, also documented in vivo, may play a role in the increased cancer incidence during aging, since elderly persons have increased numbers of senescent fibroblasts [14].

CD28 and T-cell replicative senescence

Cultures of senescent T cells show no alterations in the expression of surface markers reflecting cell lineage, adhesion pathways, activation antigens, and receptor structures [62]. To date, the single well-established exception is the costimulation receptor molecule, CD28, which delivers the essential second activation signal to the T cell when ligated by its natural ligands (CD80 or CD86) on antigen-presenting cells. In bulk cultures of CD8 T cells, CD28 is expressed on progressively fewer cells as the number of population doublings increases, so that at senescence $>95\%$ of the cells are $CD28^-$ [29].

Identification of loss of CD28 expression in cultures of T cells that were driven to replicative senescence in cell culture by repeated rounds of antigen-driven proliferation provided a key strategy to determine if replicative senescence might be occurring in vivo. Using flow cytometry, it has been shown that $>99\%$ of human T cells express CD28 at birth [4]. However, over the human life-span, there is a progressive increase in the proportion of peripheral blood T cells that are $CD28^-$ [8].

T cells lacking CD28 expression are associated not only with normal aging, but with certain diseases as well. Persons infected with viruses that establish latency, such as HIV and CMV, have high proportions of $CD28^-$ T cells [31, 51, 89]. In both aging and chronic infection, the majority of T cells that lack CD28 expression are within the CD8 (cytotoxic T lymphocyte, CTL) subset, the cells that control viral infection and cancer. Indeed, in some elderly persons and in individuals infected with HIV, more than 50% of the peripheral blood CD8 T-cell pool is $CD28^-$ [8]. Interestingly, some types of cancer are also associated with increased proportions of CD8 T cells that are $CD28^-$. For example, patients with head and neck cancer have an increased frequency of $CD8^+CD28^-$ T cells compared with age-matched controls [82].

The relationship between $CD28^+$ and $CD28^-$ T cells has been addressed in several studies. In cell culture, it has been clearly demonstrated that the $CD28^-$ T cells are the progeny of $CD28^+$ cells, and not a separate lineage of cells that arose and expanded over time. Telomere

measurements on long-term T-cell cultures further confirm that the $CD28^-$ cells are derived from $CD28^+$ cells as a consequence of extensive rounds of cell division. The TRF of human T-cell cultures is progressively reduced from 10–11 kb to 5–7 kb at senescence [84], at which time the culture is >99% $CD28^-$. A similar telomere relationship has been observed between $CD28^+$ and $CD28^-$ CD8 T cells isolated from peripheral blood samples. When CD8 T cells are sorted into $CD28^+$ and $CD28^-$ fractions, the latter invariably has shortened telomeres compared with the $CD28^+$ T cells [31, 56]. In addition, it has been shown that that the clonal populations of $CD28^-$ T cells share T-cell receptor genes with $CD28^+$ T cells [67]. Thus, we and others have proposed that the $CD28^-$ within the CD8 subset present in vivo arose by the same mechanism as those present in senescent cultures, namely, as a consequence of repeated rounds of antigen-driven proliferation [27, 28].

Whereas a great deal of research on replicative senescence has focused on the subset of human memory CD8 T cells that lack CD28 expression, it should be noted that several recent studies have suggested that changes in expression of additional markers, such as CD27, CCR7, and CD57, may be relevant [17, 41, 55, 69, 90]. For example, telomere length analysis of purified CD8 T-cell subpopulations isolated from HIV-infected persons indicates that the true end stage senescent cell not only lacks CD28 expression, but is also $CD57^+$ [9]. Interestingly, expanded populations of $CD28^-CD57^+$ cells within the CD8 T-cell pool have in fact been detected in myeloma patients [78]. Additional markers, such as CD27 and CCR7, reflecting chronic activation and memory/effector functions may also distinguish true end-stage from pre-senescent CD8 T cells. Doubtless, more definitive characterization of the phenotypic profiles associated with CD8 T-cell replicative senescence will be necessary in order to determine whether they play a role in cancer biology.

Potential deleterious immune effects of senescent CD8 T cells

Assuming that CD8 T-cell replicative senescence is in fact occurring in vivo, how might these cells affect immune function? The most obvious defect of cells that have reached senescence would be the inability to undergo further clonal expansion in response to antigenic stimulation. Interestingly, $CD8^+CD28^-$ T cells isolated ex vivo and stimulated by either antibodies and IL-2 or by PMA and ionomycin, which bypass cell surface receptors, are unable to proliferate [4, 31]. This observation is consistent with extensive research on replicative senescence in a variety of cell types documenting the irreversible nature of the proliferative block, and its association with up-regulation of cell cycle inhibitors and p53-linked checkpoints [13]. Thus, even if senescent virus-specific CD8 T cells are still capable of cytotoxic function, as suggested [3], their cell cycle arrest will prevent the requisite clonal expansion for effective control of the infection or the tumor.

T-cell recirculation and cell trafficking patterns are additional activities that could be affected by the presence of large numbers of $CD28^-$ T cells. Since CD28 ligation enhances the binding affinity of T cells to endothelial cells [74], T cells lacking CD28 may be altered in their trafficking patterns between tissue and blood. Indeed, memory CD8 T cells in humans that are categorized according the chemokine receptors (CCR7) and reacquisition of the CD45RA (naïve T cell) marker do in fact show different homing characteristics [17], with the more "differentiated" population being non-proliferative. Interestingly, some of the end-stage $CD28^-$ that arise in repeatedly stimulated long-term cultures also reexpress the CD45RA marker [65].

The quality and composition of the pool of "antigen-experienced" human T cells may be markedly altered as a consequence of the progressive accumulation of putatively senescent CD8 T cells. Once generated, senescent CD8 T cells do not disappear. Indeed, CD8 T cells that are $CD28^-$ show increased expression of bcl2 and are resistant to apoptosis when tested immediately ex vivo [67], similar to senescent cells that arise in cell culture [76, 86]. Since homeostatic mechanisms are believed to independently regulate the memory and naïve T-cell pools [36], a high proportion of senescent cells will result in a reduced proportion of proliferation-competent, non-senescent memory cells, a situation that may also indirectly influence other memory T cells of unrelated specificities. Moreover, $CD28^-$ T cells are often present as part of oligoclonal expansions [66, 72], a feature that would cause a reduction in the overall spectrum of antigenic specificities within the T-cell pool.

There is accumulating evidence that $CD8^+CD28^-$ T cells may function as suppressor cells, influencing a variety of immune functions. For example, MHC class I-restricted $CD8^+CD28^-$ T cells generated in the course of in vitro and in vivo immunizations induce antigen-presenting cells to become tolerogenic to helper T cells with the cognate antigen specificity [22]. Expanded populations of $CD8^+CD28^-$ T cells also correlate with a more severe disease course in ankylosing spondylitis patients [71]. In addition, high proportions of $CD8^+CD28^-$ T cells are significantly correlated with poor antibody responses to influenza vaccination in the elderly [38, 70]. Moreover, this same subset has been specifically implicated in the tolerance to allogeneic organ transplants. Donor-specific $CD8^+CD28^-$ T cells are detectable in the peripheral blood of those patients with stable function of heart, liver, and kidney transplants, whereas no such cells were found in patients undergoing acute rejection [22]. The above clinical observations raise the possibility that $CD8^+CD28^-$ T cells with suppressor functions might affect immunity to cancer, either at the level of antigen presentation or effector functions. This issue may be particularly relevant in the context of the development of cancer vaccines.

Immune surveillance and cancer

One of the fundamental questions in the field of cancer biology is whether immune surveillance plays a role in tumor initiation and progression. Although this issue has not been resolved, there is accumulating evidence, at least for those cancers that have a viral etiology, that unsuccessful immune control over the virus may be involved in tumorogenesis [50]. Indeed, 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 [45, 59]. Kaposi's sarcoma is consequent to latent infection with another herpesvirus infection (HSV 8), and cervical cancer, which also increases during immune suppression, is associated with certain strains of human papillomavirus.

Viruses that persist and establish latency develop a complex relationship with the immune system, involving both viral strategies to evade immune recognition, as well as virally driven physiologic effects on the T cells themselves [63]. It is clear that the initial primary infection with these viruses does elicit an immune response. For example, during primary acute infectious mononucleosis (EBV-infection), antigen-specific CD8 T cells become activated and show high telomerase activity. However, once latency is established, these same T cells show evidence of having experienced chronic antigenic stimulation, as indicated by the observed telomere shortening seen in the tetramer-binding CD8 T cells 1 year after infection [52]. Thus, at least in the case of EBV, there is evidence consistent with prolonged antigen-specific proliferation in vivo. EBV is involved not only in lymphomas, but also in invasive breast cancer as well as in some tumors of the prostate and of the liver [45], consistent with the potential involvement of T-cell replicative senescence in the development of a broad spectrum of tumor types.

Functional analysis of CD8 T cells that arise in vivo during chronic HIV, CMV, and EBV infections suggests that the chance to eradicate these infections by T-cell-mediated cytolysis may be undermined once the infection becomes chronic [94]. Indeed, such fundamental CD8 T cell protective functions as secretion of interferon γ and perforin expression by CD8 T cells are in fact impaired in patients with certain EBV-associated nasopharyngeal tumors [93]. In patients with EBV-associated nasopharyngeal carcinoma, reduced EBV-specific CTL precursor frequency has also been documented, and importantly, the deficit correlated with plasma viral burden [21]. Since the limiting dilution assay used to detect precursor frequency is critically dependent on proliferation, the above observation is consistent with a role for proliferative exhaustion. In addition, EBV-associated lymphomas are correlated with high TNF-α serum levels [57]. These clinical observations on CD8 T cells are reminiscent of characteristics of CD8 T cells that reach replicative senescence in cell culture as a consequence of repeated rounds of antigen-driven proliferation [32]. In sum, there is increasing evidence lending support to the hypothesis that chronic exposure to tumor antigens, some of which may comprise antigens of latent viruses (e.g., EBV or HPV), may facilitate tumor progression and metastasis by driving the relevant antigen-specific T cells to senescence.

Even nonviral tumor-associated antigens have the potential to drive relevant antigen-reactive T cells to replicative senescence. For example, prostate-specific antigen (PSA), whose elevated blood levels are observed during prostate cancer, is also present in normal prostate tissue, and is thus an antigen to which T cells have had prolonged exposure [46]. CD8 T cells from patients with prostate cancer do in fact show reactivity to PSA peptides immediately ex vivo [16], consistent with the notion that they have been previously primed in vivo to this antigen. Thus, like antigens of viruses that establish latency, tumor-associated antigens have the potential to cause chronic T-cell activation, possibly driving some antigen-specific cells to senescence.

Altered expression of CD28, which, as noted above, is the signature change of T-cell replicative senescence in cell culture, has been associated with the clinical outcome of certain nonviral cancers. For example, in advanced cases of renal carcinoma, the proportion of CD8 T cells that are $CD57^+$ (a marker present on the majority of $CD28^-$ T cells), was recently reported to have predictive value with respect to patient survival [18]. Similarly, in biotherapy-treated metastatic melanoma patients, melanoma-specific antigens, which cause chronic activation of T cells, have been suggested to play a role in the loss of CD28 expression [40]. Finally, 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 [82]. Thus, replicative senescence of CD8 T cells, already implicated in defective immunity to chronic viral infections [2], may also play a role in the failed immune surveillance that may facilitate the development or metastasis of certain types of cancer.

Reversal/prevention of replicative senescence

The role of replicative senescence in cancer immune surveillance may be controversial, but its relevance to cancer immunotherapy is more firmly established, since sustained control over the tumor requires extensive T-cell proliferation and maintenance of functional integrity. The impediment of replicative senescence has already been documented in the case of EBV, where in vitro expansion of EBV-specific CD8 T cells for the purpose of cancer immunotherapy is associated with loss of cytolytic function [15, 77], a change that precisely parallels observations from cell culture studies on

replicative senescence [26]. It therefore seems critical that efforts aimed at optimizing those forms of cancer immunotherapy requiring extensive T-cell proliferation be coupled with development of strategies to manipulate the process of replicative senescence.

The reversal and/or prevention of replicative senescence is already a central aspect of gerontology research. In particular, gene transduction with the catalytic component of human telomerase (hTERT) has been extensively analyzed in human fibroblasts, epithelial cells, and keratinocytes. The transduced cells show unlimited proliferation, telomere length stabilization, normalization of function, and, importantly, no evidence of altered growth or tumorogenesis in immunodeficient (SCID) mice. In CD8 T cells, the same genetic strategy reverses some, but not all, of the components of the replicative senescence program. Specifically, although unlimited proliferative capacity has been reported for hTERT-transduced tumor-specific and HIV-specific CD8 T cells, the loss of CD28 expression is not prevented by this strategy [26, 44]. The importance of retaining expression of CD28 in tumor-specific CD8 T cells is underscored by research on antitumor vaccines and other forms of immunotherapy, where expression of B7, the ligand of CD28, has been shown to be critically important [34, 37, 54, 80].

In addition to enhancing proliferative potential, effective modulation of replicative senescence must also incorporate strategies that lead to the retention of key antitumor immune function characteristics. For example, although hTERT resulted in the retention of virus-specific cytotoxic function in HIV-specific CD8 T-cell clones that had been originally selected for high CTL function, this transduction failed to reverse the senescence-associated loss of this important function in unselected, bulk cultures of HIV-specific CD8 T cells [25]. Thus, results from a variety of hTERT transduction studies on T cells have shown that this approach fails to prevent loss of CD28 expression and does not reproducibly result in retention of CTL function [53].

Alternative vector delivery systems, as well as the use of additional genes, may provide alternative genetic approaches for manipulating T-cell replicative senescence. All the previous hTERT transductions used a Moloney murine leukemia virus-based vector, which has hitherto been the most popular gene delivery system. The newer lentivirus-based vectors offer the advantage of greater transduction efficiency, as well as the ability to transduce primary cells. In addition, based on the uniform failure to maintain CD28 expression in strategies using the telomerase gene alone, it is possible that combining telomerase with CD28 transduction may be required. It is even possible that, since CD28 signal transduction is central to telomerase up-regulation in T cells, use of the CD28 gene alone in transduction may be appropriate. Indeed, it has recently been reported that gene transduction of CD8 T cells that were $CD28^{-}$, with the CD28 gene was able to restore IL-2 production, suggestive of full activation [79].

Nongenetic approaches may provide alternative strategies for modulating T-cell replicative senescence. Indeed, there is an intriguing connection between several aspects of estrogen activity and T-cell effector functions. Cells of the immune system contain estrogen receptors, and the original radioactive-estrogen–binding studies suggested that CD8 T cells in particular, bind estrogen with high affinity [24]. Although little is known about the spectrum of T-cell genes that are modulated by estrogen, an estrogen-responsive element has been documented in the promoter region of IFN-γ [35], a cytokine that is often monitored in evaluating immune responses to malignant diseases [64]. Interestingly, IFN-γ has also been recently shown to up-regulate the enzyme telomerase in T cells [91], suggesting an additional link between tumor control by CD8 T cells and avoidance of replicative senescence.

Estrogen can also directly modulate telomerase activity; there is an estrogen-responsive element in the promoter of the hTERT gene in a variety of reproductive tissues [49]. Estrogen also affects calcium mobilization in T cells. Thus, evidence from a variety of systems suggests that estrogen has the potential to modulate several T-cell functions that are altered in senescent cells, and may therefore constitute a novel type of nongenetic strategy to modulate senescence. Clearly, application of these hormone-based approaches to cancer immunotherapy will require identifying designer estrogens, which specifically affect T cells but not estrogen-sensitive tumor cells. Finally, research on nonhormonal modulators of T-cell telomerase activity may provide additional approaches to modulating replicative senescence, thereby expanding the efficacy of cancer immunotherapy.

Concluding remarks

It has been suggested that immunology is the key to successful cancer therapy [60]; the immune system is also central to cancer surveillance and tumor progression. Because T cells are subject to the process of replicative senescence, chronic exposure to tumor antigens—either during tumor development or during treatment protocols—may hinder the ability of the immune system to prevent cancer initiation or to sustain control over the tumor. A more comprehensive understanding of the mechanisms and signaling pathways involved in the genetic, functional, and phenotypic changes associated with T-cell replicative senescence is therefore essential to the development of strategies that capitalize on the pivotal role of the immune system in the multiple facets of cancer.

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References

1. Alexandroff AB, McIntyre CA, Porter JC, Zeuthen J, Vile RG, Taub DD (1998) Sticky and smelly issues: lessons on tumour cell and leucocyte trafficking, gene and immunotherapy of cancer. Br J Cancer 77:1806
2. Appay V, Rowland-Jones S (2002) Premature ageing of the immune system: the cause of AIDS? Trends Immunol 23:580
3. Appay V, Dunbar PR, Callan M, Klenerman P, Gillespie GM, Papagno L, Ogg GS, King A, Lechner F, Spina CA, Little S, Havlir DV, Richman DD, Gruener N, Pape G, Waters A, easterbrook P, Salio M, Cerundolo V, McMichael AJ, Rowland-Jones SL (2002) Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. Nat Med 8:379
4. Azuma M, Phillips JH, Lanier LL (1993) CD28- T lymphocytes: antigenic and functional properties. J Immunol 150:1147
5. Ballen K, Stewart FM (1997) Adoptive immunotherapy. Curr Opin Oncol 9:579
6. Beverly B, Kang S, Lenardo M, Schwartz R (1992) Reversal of in vitro T cell clonal anergy by IL-2 stimulation. Int Immunol 4:661
7. Bodnar AG, Kim NW, Effros RB, Chiu CP (1996) Mechanism of telomerase induction during T cell activation. Exp Cell Res 228:58
8. Boucher N, Defeu-Duchesne T, Vicaut E, Farge D, Effros RB, Schachter F (1998) CD28 expression in T cell aging and human longevity. Exp Gerontol 33:267
9. Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BJ, Crotty LE, Casazza JP, Kuruppu J, Migueles SA, Connors M, Roederer M, Douek DC, Koup RA (2003) Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. Blood 101:2711
10. Brenner MK, Heslop HE, Rooney CM (1998) Gene and cell transfer for specific immunotherapy. Vox Sang 74[Suppl 2]: 87
11. Brocker T, Karjalainen K (1998) Adoptive tumor immunity mediated by lymphocytes bearing modified antigen-specific receptors. Adv Immunol 68:257
12. Campisi J (1997) The biology of replicative senescence. Eur J Cancer 33:703–709
13. Campisi J (2001) From cells to organisms: can we learn about aging from cells in culture? Exp Gerontol 36:607
14. Campisi J (2003) Cancer and ageing: rival demons? Nat Rev Cancer 3:339–349
15. Carlens S, Liu D, Ringden O, Aschan J, Christensson B, Levitsky V, Dilber MS (2002) Cytolytic T cell reactivity to Epstein-Barr virus is lost during in vitro T cell expansion. J Hematother Stem Cell Res 11:669
16. Chakraborty NG, Stevens RL, Mehrotra S, Laska E, Taxel P, Sporn JR, Schauer P, Albertsen PC (2003) Recognition of PSA-derived peptide antigens by T cells from prostate cancer patients without any prior stimulation. Cancer Immunol Immunother 52:497
17. Champagne P, Ogg GS, King AS, Knabenhans C, Ellefsen K, Nobile M, Appay V, Rizzardi GP, Fleury S, Lipp M, Forster R, Rowland-Jones S, Sekaly RP, McMichael AJ, Pantaleo G (2001) Skewed maturation of memory HIV-specific CD8 T lymphocytes. Nature 410:106
18. Characiejus D, Pasukoniene V, Kazlauskaite N, Valuckas KP, Petraitis T, Mauricas M, Den Otter W (2002) Predictive value of CD8highCD57+ lymphocyte subset in interferon therapy of patients with renal cell carcinoma. Anticancer Res 22:3679
19. Cheever MA, Chen W (1997) Therapy with cultured T cells: principles revisited. Immunol Rev 157:177
20. Chiu CP, Harley CB (1997) Replicative senescence and cell immortality: the role of telomeres and telomerase. Proc Soc Exp Biol Med 214:99–106
21. Chua D, Huang J, Zheng B, Lau SY, Luk W, Kwong DL, Sham JS, Moss D, Yuen KY, Im SW, Ng MH (2001) Adoptive transfer of autologous Epstein-Barr virus-specific cytotoxic T cells for nasopharyngeal carcinoma. Int J Cancer 94:73
22. Cortesini R, LeMaoult J, Ciubotariu R, Cortesini NS (2001) CD8+CD28- T suppressor cells and the induction of antigen-specific antigen-presenting cells-mediated suppression of Th reactivity. Immunol Rev 182:201
23. Cristofalo V (1985) The destiny of cells: mechanisms and implications of senescence. Gerontologist 25:577
24. Cutolo M, Sulli A, Seriolo B, Accardo S, Masi AT (1995) Estrogens, the immune response, and autoimmunity. Clin Exp Rheumatol 13:217
25. Dagarag MD, Effros RB (2003) Ectopic telomerase expression prevents functional and phenotypic alterations associated with replicative senescence in virus-specific CD8 T cells. Clin Immunol (FOCIS Meeting Abstract Book)
26. Dagarag MD, Ng H, Lubong R, Effros RB, Yang OO (2003) Differential impairment of lytic and cytokine functions in senescent HIV-1-specific cytotoxic T lymphocytes. J Virol 77:3077
27. Effros RB (1998) Replicative senescence in the immune system: impact of the Hayflick limit on T-cell function in the elderly. Am J Hum Genet 62:1003
28. Effros RB, Pawelec G (1997) Replicative senescence of T lymphocytes: does the Hayflick limit lead to immune exhaustion? Immunol Today 18:450–454
29. Effros RB, Boucher N, Porter V, Zhu X, Spaulding C, Walford RL, Kronenberg M, Cohen D, Schachter F (1994) Decline in CD28+ T cells in centenarians and in long-term T cell cultures: a possible cause for both in vivo and in vitro immunosenescence. Exp Gerontol 29:601
30. Effros RB, Zhu X, Walford RL (1994) Stress response of senescent T lymphocytes: reduced hsp70 is independent of the proliferative block. J Gerontol 49:B65
31. Effros RB, Allsopp R, Chiu CP, Wang L, Hirji K, Harley CB, Villeponteau B, West M, Giorgi JV (1996) Shortened telomeres in the expanded CD28-CD8+ subset in HIV disease implicate replicative senescence in HIV pathogenesis. AIDS/Fast Track 10:F17
32. Effros RB, Cai Z, Linton PJ (2003) CD8 T cells and aging. Crit Rev Immunol 23:45
33. Fernandez N, Duffour MT, Perricaudet M, Lotze MT, Tursz T, Zitvogel L (1998) Active specific T-cell-based immunotherapy for cancer: nucleic acids, peptides, whole native proteins, recombinant viruses, with dendritic cell adjuvants or whole tumor cell-based vaccines: principles and future prospects. Cytokines Cell Mol Ther 4:53
34. Foss FM (2002) Immunologic mechanisms of antitumor activity. Semin Oncol 29:5
35. Fox HS, Bond BL, Parslow TG (1991) Estrogen regulates the IFN-gamma promoter. J Immunol 146:4362
36. Freitas AA, Rocha B (2000) Population biology of lymphocytes: the flight for survival. Annu Rev Immunol 18:83
37. Gitlitz BJ, Belldegrun AS, Figlin RA (2001) Vaccine and gene therapy of renal cell carcinoma. Semin Urol Oncol 19:141
38. Goronzy JJ, Fulbright JW, Crowson CS, Poland GA, O'Fallon WM, Weyand CM (2001) Value of immunological markers in predicting responsiveness to influenza vaccination in elderly individuals. J Virol 75:12182
39. Grimm EA, Bruner JM, Carinhas J, Keoppen JA, Loudon WG, Owen-Schaub L, Steck PA, Moser RP (1990) Characterization of interleukin-2-initiated versus OKT3-initiated human tumor-infiltrating lymphocytes from glioblastoma multiforme: growth characteristics, cytolytic activity, and cell phenotype. Cancer Immunol Immunother 32:391
40. Hakansson A, Hakansson L, Gustafsson B, Krysander L, Rettrup B, Ruiter D, Bernsen MR (2002) Biochemotherapy of metastatic malignant melanoma: on down-regulation of CD28. Cancer Immunol Immunother 51:499

41. Hamann D, Baars PA, Rep MH, Hooibrink B, Kerkhof-Garde SR, Klein MR, Van Lier RA (1997) Phenotypic and functional separation of memory and effector human CD8+ T cells. J Exp Med 186:1407
42. Hathcock KS, Weng NP, Merica R, Jenkins MK, Hodes R (1998) Antigen-dependent regulation of telomerase activity in murine T cells. J Immunol 160:5702
43. Hiyama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyama E, Piatyszek MA, Shay JW, Ishioka S, Yamakido M (1995) Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. J Immunol 155:3711
44. Hooijberg E, Ruizendaal JJ, Snijders PJ, Kueter EW, Walboomers JM, Spits H (2000) Immortalization of human CD8+ T cell clones by ectopic expression of telomerase reverse transcriptase. J Immunol 165:4239
45. Israel BF, Kenney SC (2003) Virally targeted therapies for EBV-associated malignancies. Oncogene 22:5122
46. Kennedy-Smith AG, McKenzie JL, Owen MC, Davidson PJ, Vuckovic S, Hart DN (2002) Prostate specific antigen inhibits immune responses in vitro: a potential role in prostate cancer. J Urol 168:741
47. Kitchingman GR, Rooney C (1998) Cytotoxic T cells and immunotherapy [comment]. Pediatr Radiol 28:489
48. Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J (2001) Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proc Natl Acad Sci U S A 98:12072
49. Kyo S, Takakura M, Kanaya T, Zhuo W, Fujimoto K, Nishio Y, Orimo A, Inoue M (1999) Estrogen activates telomerase. Cancer Res 59:5917–5921
50. Lanier LL (2001) A renaissance for the tumor immunosurveillance hypothesis. Nat Med 7:1178
51. Looney RJ, Falsey A, Campbell D, Torres A, Kolassa J, Brower C, McCann R, Menegus M, McCormick K, Frampton M, Hall W, Abraham GN (1999) Role of cytomegalovirus in the T cell changes seen in elderly individuals. Clin Immunol 90:213
52. Maini MK, Soares MV, Zilch CF, Akbar AN, Beverley PC (1999) Virus-induced CD8+ T cell clonal expansion is associated with telomerase up-regulation and telomere length preservation: a mechanism for rescue from replicative senescence. J Immunol 162:4521
53. Migliaccio M, Amacker M, Just T, Reichenbach P, Valmori D, Cerottini JC, Romero P, Nabholz M (2000) Ectopic human telomerase catalytic subunit expression maintains telomere length but is not sufficient for CD8+ T lymphocyte immortalization. J Immunol 165:4978
54. Mitchell MS (2002) Cancer vaccines, a critical review–part I. Curr Opin Investig Drugs 3:140
55. Mollet L, Sadat-Sowti B, Duntze J, Leblond V, Bergeron F, Calvez V, Katlama C, Debrae P, Autran B (1998) CD8hi+CD57+ T lymphocytes are enriched in antigen-specific T cells capable of down-modulating cytotoxic activity. Int Immunol 10:311
56. Monteiro J, Batliwalla F, Ostrer H, Gregersen PK (1996) Shortened telomeres in clonally expanded CD28-CD8+ T cells imply a replicative history that is distinct from their CD28+CD8+ counterparts. J Immunol 156:3587
57. Mori A, Takao S, Pradutkanchana J, Kietthubthew S, Mitarnun W, Ishida T (2003) High tumor necrosis factor-alpha levels in the patients with Epstein-Barr virus-associated peripheral T-cell proliferative disease/lymphoma. Leuk Res 27:493
58. O'Reilly RJ, Small TN, Papadopoulos E, Lucas K, Lacerda J, Koulova L (1998) Adoptive immunotherapy for Epstein-Barr virus-associated lymphoproliferative disorders complicating marrow allografts. Springer Semin Immunopathol 20:455
59. Pardoll D (2001) T cells and tumours. Nature 411:1010–1012
60. Pawelec G (2003) Editorial statement from Graham Pawelec. Cancer Immunol Immunother 52
61. Pawelec G, Schneider EM, Wernet P (1986) Acquisition of suppressive activity and natural killer-like cytotoxicity by human alloproliferative "helper" T cell clones. J Immunol 136:402
62. Perillo NL, Naeim F, Walford RL, Effros RB (1993) The in vitro senescence of human lymphocytes: failure to divide is not associated with a loss of cytolytic activity or memory T cell phenotype. Mech Ageing Dev 67:173
63. Petersen JL, Morris CR, Solheim JC (2003) Virus evasion of MHC class I molecule presentation. J Immunol 171:4473
64. Pittet MJ, Zippelius A, Speiser DE, Assenmacher M, Guillaume P, Valmori D, Lienard D, Lejeune F, Cerottini JC, Romero P (2001) Ex vivo IFN-gamma secretion by circulating CD8 T lymphocytes: implications of a novel approach for T cell monitoring in infectious and malignant diseases. J Immunol 166:7634
65. Porter V (1994) Cellular senescence of T lymphocytes in vitro: a model system for the study of the age associated decline in immune functioning. Dissertation, UCLA Medical School
66. Posnett DN, Sinha R, Kabak S, Russo C (1994) Clonal populations of T cells in normal elderly humans: the T cell equivalent to "benign monoclonal gammopathy". J Exp Med 179:609
67. Posnett DN, Edinger JW, Manavalan JS, Irwin C, Marodon G (1999) Differentiation of human CD8 T cells: implications for in vivo persistence of $CD8^+$ $CD28^-$ cytotoxic effector clones. Int Immunol 11:229
68. Salas AP, Chang AE (1998) Genetic approaches to adoptive cellular immunotherapy. Surg Oncol Clin N Am 7:487
69. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A (1999) Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 401:708
70. Saurwein-Teissl M, Lung TL, Marx F, Gschosser C, Asch E, Blasko I, Parson W, Bock G, Schonitzer D, Trannoy E, Grubeck-Loebenstein B (2002) Lack of antibody production following immunization in old age: association with CD8(+)CD28(-) T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. J Immunol 168:5893
71. Schirmer M, Goldberger C, Wurzner R, Duftner C, Pfeiffer KP, Clausen J, Neumayr G, Falkenbach A (2002) Circulating cytotoxic CD8(+) CD28(-) T cells in ankylosing spondylitis. Arthritis Res 4:71
72. Schwab R, Szabo P, Manavalan JS, Weksler ME, Posnett DN, Pannetier C, Kourilsky P, Even J (1997) Expanded CD4+ and CD8+ T cell clones in elderly humans. J Immunol 158:4493
73. Shay J, Wright W (1996) Telomerase activity in human cancer. Curr Opin Oncol 8:66
74. Shimizu Y, Van Seventer G, Ennis E, Newman W, Horgan K, Shaw S (1992) Crosslinking of the T cell-specific accessory moleculesCD7 and CD28 modulates T cell adhesion. J Exp Med 175:577
75. Smith JR and Pereira-Smith OM (1996) Replicative senescence: implications for in vivo aging and tumor suppression. Science 273:63
76. Spaulding CS, Guo W, Effros RB (1999) Resistance to apoptosis in human CD8+ T cells that reach replicative senescence after multiple rounds of antigen-specific proliferation. Exp Gerontol 34:633
77. Straathof KC, Bollard CM, Rooney CM, Heslop HE (2003) Immunotherapy for Epstein-Barr virus-associated cancers in children. Oncologist 8:83
78. Sze DM, Giesajtis G, Brown RD, Raitakari M, Gibson J, Ho J, Baxter AG, Fazekas de St GB, Basten A, Joshua DE (2001) Clonal cytotoxic T cells are expanded in myeloma and reside in the CD8(+)CD57(+)CD28(-) compartment. Blood 98:2817
79. Topp MS, Riddell SR, Akatsuka Y, Jensen MC, Blattman JN, Greenberg PD (2003) Restoration of CD28 expression in CD28- CD8+ memory effector T cells reconstitutes antigen-induced IL-2 production. J Exp Med 198:947
80. Townsend SE, Allison JP (1993) Tumor rejection after direct costimulation of CD28+ T cells by B7-transfected melanoma cells. Science 259:368
81. Tsai V, Kawashima I, Keogh E, Daly K, Sette A, Celis E (1998) In vitro immunization and expansion of antigen-specific cytotoxic T lymphocytes for adoptive immunotherapy using peptide-pulsed dendritic cells. Crit Rev Immunol 18:65

82. Tsukishiro T, Donnenberg AD, Whiteside TL (2003) Rapid turnover of the CD8(+)CD28(-) T-cell subset of effector cells in the circulation of patients with head and neck cancer. Cancer Immunol Immunother 52:599
83. Valenzuela HF, Effros RB (2002) Divergent telomerase and CD28 expression patterns in human CD4 and CD8 T cells following repeated encounters with the same antigenic stimulus. Clin Immunol 105:117
84. Vaziri H, Schachter F, Uchida I, Wei L, Zhu X, Effros R, Cohen D, Harley C (1993) Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. Am J Hum Genet 52:661
85. Wang RF, Rosenberg SA (1996) Human tumor antigens recognized by T lymphocytes: implications for cancer therapy. J Leukoc Biol 60:296
86. Wang E, Lee MJ, Pandey S (1994) Control of fibroblast senescence and activation of programmed cell death. J Cell Biochem 54:432
87. Weng NP, Levine BL, June CH, Hodes RJ (1995) Human naive and memory T lymphocytes differ in telomere length and replicative potential. Proc Natl Acad Sci U S A 92:11091
88. Weng NP, Levine BL, June CH, Hodes RJ (1996) Regulated expression of telomerase activity in human T lymphocyte development and activation. J Exp Med 183:2471
89. Wikby A, Johansson B, Olsson J, Lofgren S, Nilsson BO, Ferguson F (2002) Expansions of peripheral blood CD8 T-lymphocyte subpopulations and an association with cytomegalovirus seropositivity in the elderly: the Swedish NONA immune study. Exp Gerontol 37:445
90. Wills MR, Okecha G, Weekes MP, Gandhi MK, Sissons PJ, Carmichael AJ (2002) Identification of naive or antigen-experienced human CD8(+) T cells by expression of costimulation and chemokine receptors: analysis of the human cytomegalovirus-specific CD8(+) T cell response. J Immunol 168:5455
91. Xu D, Erickson S, Szeps M, Gruber A, Sangfelt O, Einhorn S, Pisa P, Grander D (2000) Interferon alpha down-regulates telomerase reverse transcriptase and telomerase activity in human malignant and nonmalignant hematopoietic cells. Blood 96:4313
92. Yee C, Riddell SR, Greenberg PD (1997) Prospects for adoptive T cell therapy. Curr Opin Immunol 9:702
93. Zanussi S, Vaccher E, Caffau C, Pratesi C, Crepaldi C, Bortolin MT, Tedeschi R, Politi D, Barzan L, Tirelli U, De Paoli P (2003) Interferon-gamma secretion and perforin expression are impaired in CD8+ T lymphocytes from patients with undifferentiated carcinoma of nasopharyngeal type. Cancer Immunol Immunother 52:28
94. Zhang D, Shankar P, Xu Z, Harnisch B, Chen G, Lange C, Lee SJ, Valdez H, Lederman MM, Lieberman J (2003) Most antiviral CD8 T cells during chronic viral infection do not express high levels of perforin and are not directly cytotoxic. Blood 101:226