Principles of adoptive T cell cancer therapy

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The transfusion of T cells, also called adoptive T cell therapy, is an effective treatment for viral infections and has induced regression of cancer in early-stage clinical trials. However, recent advances in cellular immunology and tumor biology are guiding new approaches to adoptive T cell therapy. For example, use of engineered T cells is being tested as a strategy to improve the functions of effector and memory T cells, and manipulation of the host to overcome immunotoxic effects in the tumor microenvironment has led to promising results in early-stage clinical trials. Challenges that face the field and must be addressed before adoptive T cell therapy can be translated into routine clinical practice are discussed.

Introduction

Adoptive T cell therapy for cancer is a form of transfusion therapy consisting of the infusion of various mature T cell subsets with the goal of eliminating a tumor and preventing its recurrence. Allogeneic and autologous sources of T cells derived from several anatomic sites have been tested. Indeed, in the 1970s, Chester Southam and colleagues demonstrated that subcutaneous growth of human tumor autografts to patients bearing advanced cancers was inhibited by the cotransfer of autologous leukocytes in about half of the patients (1). This finding suggested that lymphocytes with a specific inhibitory effect on the implantation and growth of cancer cells were present in many patients and could be mined as potential candidates for adoptive immunotherapy. The primary advantage of using $CD8^+$ T cells for adoptive therapy, as opposed to other cytolytic cells, such as NK cells, is their ability to specifically target tumor cells through the recognition of differentially expressed tumor proteins presented on the cell surface. Using T cells for adoptive therapy is also attractive due to the long clonal life span of T cells (2, 3), which allows both therapeutic and immunoprophylactic scenarios to be envisioned. In addition, T cells are well suited for genetic manipulation, which has enabled the evaluation of genetically enhanced or retargeted T cells in pilot clinical trials for cancer as well as other diseases. The intent of this Review on adoptive T cell therapy for cancer is to address current issues facing this field, with a focus on translational issues relevant to human immunotherapy. For earlier references, the reader is referred to previous reviews on adoptive transfer (4–6).

Adoptive T cell therapy depends on the ability to optimally select or genetically engineer cells with targeted antigen specificity and then induce the cells to proliferate while preserving their effector function and engraftment and homing abilities. Unfortunately, many clinical trials have been carried out with adoptively transferred cells that were propagated in what are now understood to be suboptimal conditions that impair the essential functions of T cells. In addition, until recently, many trials were carried out before the complexity of T cell biology was understood. Some important issues that were previously not understood but might have affected the outcome of many of the early trials are discussed in this Review. These issues include the important differences in T cell biology between rodents and humans that have emerged in recent years (7), the observation that T cell populations are heterogeneous and comprise memory cells, effector cells, and Tregs, and the process of immunosenescence. The implications of these findings need to be incorporated into the translation of therapeutic approaches from animal models to the clinic. In addition, although the principles of T cell biology should guide the ex vivo culture process, in some instances it is necessary to compromise in order to achieve a cell manufacturing approach that is robust enough to support large scale trials and is compliant with FDA guidelines and other regulations (8).

Use of the laboratory mouse for adoptive T cell therapy: curse or blessing?

Mouse syngeneic tumor models have been essential for the identification and preclinical optimization of many tumor therapies. However, so far, no mouse tumor model has been a good predictor of a successful human response to immunotherapy. For example, it is instructive to note that in mice, treatment with cytotoxic T lymphocyte–associated antigen 4–specific (CTLA4-specific) antibody induces antitumor responses and is well tolerated (9) whereas in humans, antitumor responses can be accompanied by life-threatening colitis and hypophysitis (10, 11). Over recent years, substantial limitations in some mouse models have emerged that have important implications for adoptive immunotherapy. In particular, the critical demonstration that many mouse tumor antigens are not representative of human tumor antigens (12) has led to improved strategies to generate adoptively transferred human T cells with appropriate tumor specificity. With the exception of virally induced cancers, which account for about 10% of human tumors, clinical trials target self antigens, a technically challenging task since tolerance and TCR repertoire limitations can restrict the quality of the T cell response (13). Furthermore, the seminal discovery of telomerase (14) and the developing field of telomere biology identified fundamental differences in mechanisms of immunosenescence between mice and humans (15–17) that have important implications for adoptive T cell transfer therapeutics. These issues of immunosenescence and limitations of repertoire are discussed in more detail below.

CD28, a membrane glycoprotein that is a costimulatory receptor for TCR-mediated T cell activation, is the main costimulatory mol-

Nonstandard abbreviations used: γ_c, common γ chain; CCR7, CC chemokine receptor 7; MART1, melanoma-associated antigen recognized by T cells 1; T_{CM} cell, central memory T cell; T_{EM} cell, effector memory T cell; TERC, telomerase RNA component; TERT, telomerase reverse transcriptase.

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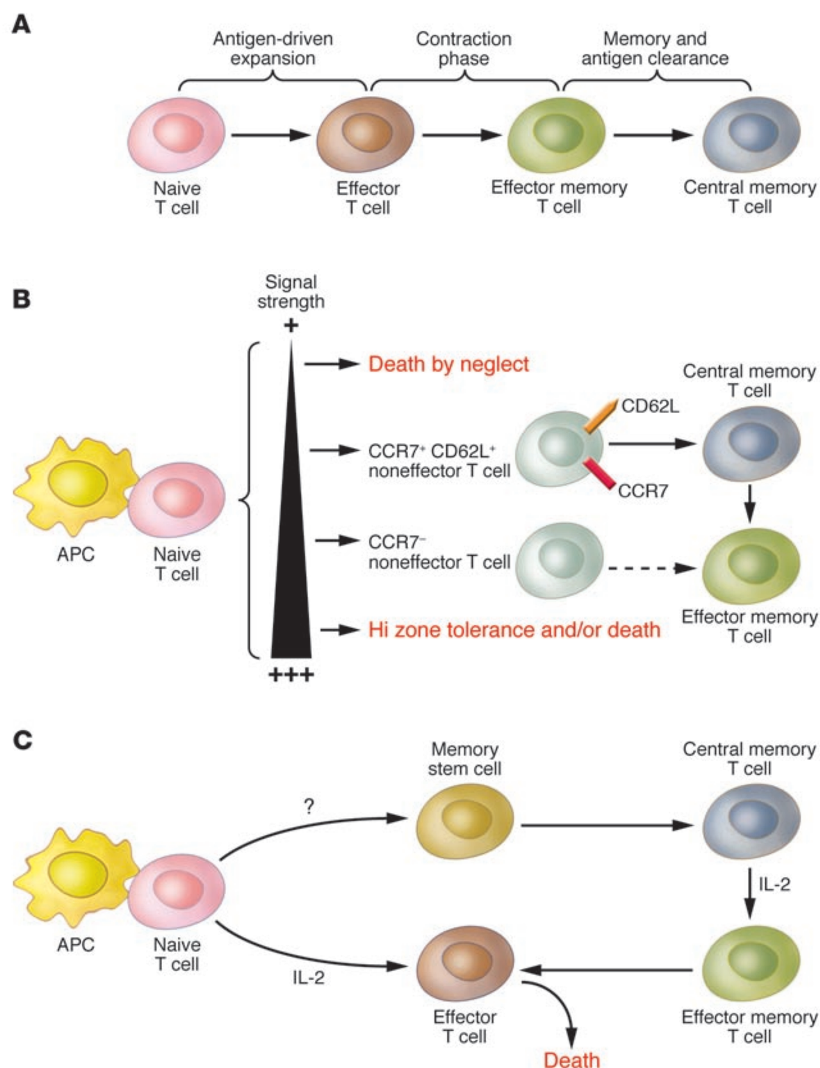

Figure 1
Models of $CD8^+$ T cell differentiation to distinct memory cell subsets. (**A**) In the linear differentiation model, an autonomous antigen-triggered differentiation process consisting of conversion from naive to effector to T_{EM} cell occurs, followed by the appearance of T_{CM} cells after antigen clearance through a process of dedifferentiation (27). (**B**) The signal strength model proposes that naive T cells progress through hierarchical thresholds for proliferation and differentiation as the strength and duration of the interaction with APCs is increased (28, 30). T cells receiving the weakest signals do not survive, whereas high-intensity signaling causes the development of terminally differentiated effector T cells that cannot survive into the memory phase. The T_{CM} cells, being the least differentiated of the antigen-stimulated T cells, retain the developmental options of naive T cells, including their capacity for marked clonal expansion. (**C**) The memory stem cell model proposes that the cells within the T_{CM} cell compartment are self renewing and serve as a source of effector T cells (29, 43).

ecule for T cell activation in mice and humans (18). In humans, but not mice, there is an accumulation of $CD8^+CD28^-$ T cells in the peripheral blood with age and with increasing length of ex vivo culture (19). $CD8^+CD28^-$ T cells are often oligoclonal in nature and do not proliferate well in response to antigenic stimulation (20). Because almost all T cells in the peripheral blood of newborn humans express CD28 and because the accumulation of $CD28^-$ T cells is a gradual process with age, it has been suggested that $CD8^+CD28^-$ T cells are derived from $CD8^+CD28^+$ T cells (21). The mechanism of CD28 downregulation in human T cells is not fully understood but involves decreased *CD28* transcription (22). To the extent that loss of CD28 expression with prolonged in vitro culture models the aging human immune system (23), this suggests the importance of developing strategies to preserve CD28 expression on adoptively transferred human T cells. CD28-specific agonist antibodies have been useful for ex vivo stimulation of human T cells for adoptive therapy (24). However, for reasons that still require clarification, the in vivo administration of CD28-specific agonist antibodies, although well tolerated in rodents, is highly toxic in humans (25). Together, the above examples illustrate the need for improved adoptive T cell transfer models. Recent advances in the development of humanized mouse models might circumvent these issues (26). Furthermore, despite these downsides, syngeneic mouse models have been valuable in determining the optimal lymphocyte subsets and culture conditions for adoptive T cell therapy (5).

Striving for optimal T cell function

T cells exist in several distinct stages of differentiation. Naive $CD4^+$ and $CD8^+$ T cells undergo unique developmental programs after antigen activation, resulting in the generation of effector memory and long-lived central memory T cells, T_{EM} cells and T_{CM} cells, respectively. Three models by which memory $CD8^+$ T cells can be generated have been proposed (Figure 1). In the linear differentiation model, an autonomous antigen-triggered differentiation process consisting of conversion from naive to effector to T_{EM} cell occurs, followed by the appearance of T_{CM} cells after antigen clearance through a process of dedifferentiation (27). The signal strength model proposes that naive T cells progress through hierarchical thresholds for proliferation and differentiation as the strength and duration of the interaction with APCs is increased (28). T cells receiving the weakest signals do not survive whereas high-intensity signaling causes the development of terminally differentiated effector T cells that cannot survive into the memory phase. The T_{CM} cells, being the least differentiated of the antigen-stimulated T cells, retain the developmental options of naive T cells, including their capacity for marked clonal expansion. The stem cell model proposes that the cells within the T_{CM} cell compartment are self renewing and serve as a source of effector T cells (29). Although the details and mechanisms of differentiation remain to be clarified, it is clear that the various T cell memory subsets have specialized roles and that not all the subsets would be efficacious in the setting of adoptive T cell therapy for the treatment of cancer.

At present, naive T cells are not thought to be useful for adoptive transfer as they are unable to kill tumor cells. The demonstration

by Sallusto and colleagues (30) that T_{CM} and T_{EM} cell subsets have discrete trafficking and functional properties has the potential to fundamentally alter approaches to adoptive T cell therapy. In retrospect, previous clinical trials have primarily tested adoptively transferred populations of CD8+ T_{EM} cells (31). This approach was taken because available tissue culture technologies resulted in rapid differentiation of T cells to late-stage effector cells; late-stage T_{EM} cells express CD57 and have poor replicative capacity (32, 33). In vitro, T_{EM} cells are superior to T_{CM} cells at tumor cytotoxicity. However, in vivo, T_{CM} cells exhibit superior therapeutic effects when compared with T_{EM} cells on a per cell basis (31, 34). Therefore, in principle, adoptive T cell transfer strategies are attractive for the ability to generate long-lived populations of T_{CM} cells capable of immune surveillance as well as tumor eradication, which is why efforts to test T_{CM} cells in clinical trials are currently of high priority.

Factors determining the optimal CD8+ T cells for adoptive transfer. The cellular basis of immunologic memory has been one of the central issues of immunology for more than half a century. Many studies in mice indicate that true memory with long-lived T cells is only established in the absence of persistent antigen (35, 36). This raises questions for tumor immunologists as to whether central memory can be established in tumor-bearing patients and, if so, how this establishment would occur. Relevant to this issue is that in patients at risk of developing EBV lymphoma, adoptively transferred gene-marked CTLs persist for years (37), demonstrating that in principle it is possible to establish central memory in cancer patients by adoptive T cell transfer. Therefore, to understand approaches to generating persistent immunity in patients in whom tumor antigens are unlikely to be completely eliminated, it might be more instructive to study the human immune response in patients with chronic persistent viral and parasitic infections (38, 39). A major issue of relevance is the differentiation pathway leading to T_{CM} cells (Figure 1). Some studies indicate that naive T cells first differentiate into effector T cells, a proportion of which then differentiate into T_{CM} cells (40). In contrast to this linear differentiation model, other studies suggest that parallel differentiation occurs, with naive T cells directly differentiating into T_{CM} and effector cells simultaneously through asymmetric division (41, 42). Resolution of the above issues is important so that culture systems can be devised to optimally derive populations of T_{EM} and T_{CM} cells.

In the mouse, a memory stem cell subset of CD8+ T cells has recently been described (43). These cells were identified based on alloreactivity and had extensive replicative potential in vivo but maintained the naive CD44–CD62L+ T cell phenotype. Intriguingly, unlike naive T cells, these newly identified CD8+ T cells expressed high levels of stem cell antigen 1 (SCA1), a glycosylphosphatidylinositol-linked molecule found on self-renewing cells from various tissues. In humans, the existence of T_{CM} cells with stem cell–like qualities has been proposed (28), but these cells have not been clearly identified, preventing the design of adoptive immunotherapy strategies to test this putative subset of cells. Assuming that the existence of the mouse memory stem cell is confirmed, it will be important to determine whether a similar stem cell subset exists in human T cells.

In humans, adoptive T cell therapy approaches to date have used peripheral blood, tumors, malignant effusions, and draining lymph nodes as the anatomic sources of input T cells for adoptive transfer (for examples, see refs. 44–47). Given the recent demonstration that the bone marrow is a major reservoir of self-reactive T cells (48), it is important to determine whether improved antitumor effects are observed when bone marrow–resident T cells are used for adoptive transfer. The bone marrow of breast cancer patients has been shown to contain CD8+ T cells specific for peptide epitopes from the tumor antigens MUC1 and HER2/neu (49), and adoptive transfer of bone marrow–derived human CD8+ memory T cells mediates antitumor activity in mice bearing tumor xenografts (50). Furthermore, bone marrow from patients with either pancreatic cancer or myeloma has also been shown to be enriched for tumor-reactive CD8+ T cells (51–54).

A role for CD4+ T cells in adoptive T cell transfer. Many studies show that the generation and/or maintenance of CD8+ T cell memory requires CD4+ T cell help (55) and that immunity specific for tumors lacking expression of MHC class II molecules is enhanced with CD4+ T cell help (56). It is counterintuitive that CD4+ T cells enhance antitumor effects in hosts bearing tumors that lack MHC class II. However, adoptively transferred CD4+ T cells have the potential to augment tumor immunity by several mechanisms that might enhance the survival and function of CD8+ T cells, including the secretion of essential cytokines such as IL-2 and IL-21 (57) and the expression of CD40L (58). Besides their intimate involvement in priming tumor-specific CTLs, CD4+ cells participate in additional effector functions. Evidence indicates that other cytokines produced by CD4+ T cells can recruit and activate macrophages and eosinophils that, in turn, mediate antitumor effects (59). Clinical adoptive transfer studies also show that the persistence of adoptively transferred cytotoxic CD8+ effector T cells is enhanced with the concomitant administration of IL-2 (60) or CD4+ T cells (61). Recent studies in patients with myeloma show that the adoptive transfer of mixed populations of pathogen-specific CD4+ and CD8+ T cells promoted the establishment of immunity with a robust central memory component (62). However, it is not yet known if this approach enhances the establishment of immunity to self antigens in cancer patients.

The common γ chain (γ_c) is a shared receptor component for the IL-2, IL-4, IL-7, IL-9, and IL-15 receptors. In the mouse, IL-15 is not produced by T cells whereas human CD4+ memory T cells are reported to constitutively produce and proliferate in response to the cytokine IL-15 (63), suggesting different roles for IL-15 in mouse and human CD4+ memory T cell homeostasis (64). Genetic analysis of patients with a γ_c deficiency indicate that the human is absolutely dependent on cytokines that signal through γ_c for T cell development, as T cells are absent in these individuals, whereas in γ_c-deficient mice the spleens of older mice have nearly normal numbers of CD4+ T cells (65), indicating that γ_c-independent signals can support CD4+ T cell development in mice but not humans. In mice, adoptively transferred CD8+ T cells cultured in IL-15 show more tumor cytotoxicity than those cultured in IL-2 (31), so the use of IL-15 for adoptive T cell therapy in humans holds substantial promise of augmenting T cell numbers and effector functions, although species differences can be expected.

At present, one of the most important issues facing the field is the complexity of CD4+ lineage T cells. Until recently, Th cells were separated into two different subsets named *Th1* and *Th2 cells*, based on the pattern of cytokines that they produce when stimulated. However, several types of CD4+ Tregs have now been described in humans (66), and it is probably important to remove Tregs from adoptively transferred T cell populations because they suppress antitumor immunity (67). Since Tregs are often enriched in tumor-infiltrating lymphocytes and the peripheral

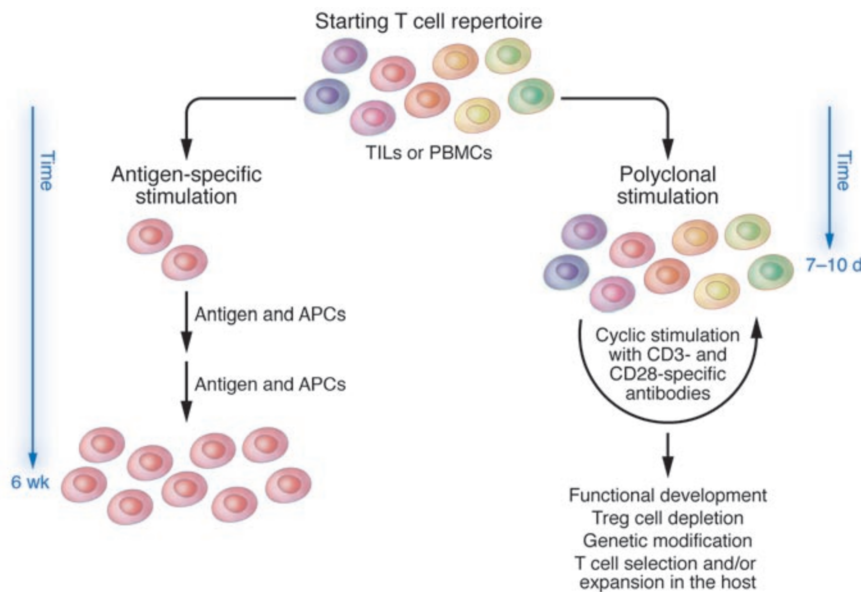

Figure 2
General approaches for ex vivo T cell expansion. Input T cells are obtained from various anatomic sites, including peripheral blood and the tumor itself. Antigen-specific CTLs can be selected and expanded by repeated stimulation with antigen pulsed APCs or tumor cells. This process requires several rounds of stimulation (left). Alternatively, the starting T cell population can be numerically expanded by polyclonal stimulation by several methods to generate cells with enhanced effector function, to deplete Tregs, and to genetically modify T cells while maintaining the TCR repertoire of the input population (right). TIL, tumor-infiltrating lymphocyte.

blood in cancer patients (68), it is possible that the outcomes of previous adoptive T cell therapy clinical trials were compromised because the adoptively transferred T cell populations inadvertently contained Tregs.

Of late, a fourth axis to the CD4+ T cell lineage was recognized, as a new IL-23–driven subset of IL-17–producing CD4+ T cells called *Th17* has been described (69). Th17 cells are proinflammatory, but paradoxically they seem to have tumor immunosuppressive effects. An interesting finding is that tumors express IL-23, which might decrease immune surveillance of tumors by preventing Th1 cell–driven CD8+ T cell infiltration (70). Therefore, a newly recognized form of immune evasion is the diversion by tumors of immune responses from Th1- to Treg- and Th17-dominated CD4+ responses. However, as evidence from several groups indicates that IL-17–producing Th17 cells, rather than, as once was thought, IFN-γ–producing Th1 cells, represent the key effector cells in the induction of autoimmunity (71), it remains possible that adoptive transfer of Th17 cells might augment antitumor responses.

Developing optimal cell culture systems

The only forms of adoptive cellular therapy routinely employed in the practice of medicine are allogeneic bone marrow and peripheral blood stem cell transplantation (72). In this setting, donor leukocyte infusions mediate various potent antitumor effects (73). The adoptive transfer of activated donor (allogeneic) T cells shows promise of augmenting this effect (74, 75). Ex vivo culture approaches to altering the ratio of effector T cells to Tregs has the potential of decreasing the risk of graft-versus-host disease while preserving antitumor effects (76). However, for most patients, comorbidity and logistic issues related to the identification of suitable allogeneic donors mean that autologous approaches are preferred. Therefore, a central issue for the development of clinical adoptive T cell therapy strategies has been the development of culture systems in order to produce adequate numbers of effector T cells for autologous therapy.

Two basic approaches are being tested for clinical adoptive T cell therapy (Figure 2). The first approach is to isolate and activate in vitro antigen-specific T cells from peripheral blood or tumor specimens and then to use repetitive stimulation to clonally expand in vitro the antigen-specific T cells by various approaches. In the second approach, polyclonal ex vivo activation of the T cells is done based on three assumptions: first, tumor-specific T cells are present in the patient; second, the tumor-specific T cells are primed in the patient; and third, the in vivo function of the tumor-specific T cells in the patient is impaired. In the second approach, the cells are activated in a polyclonal fashion by various means in vitro and are then reinfused into the patient with the expectation that they will respond directly to the tumor or to tumor antigens presented by APCs. The first approach guarantees antigen specificity but is costly and labor intensive; the second approach is technically more rapid and feasible. In practical terms, only the second approach has been sufficiently robust to support randomized clinical trials (47, 62), and therefore, only this approach has the potential for regulatory approval. The rationale for the second approach has been substantially strengthened by the realization that many patients are already primed to their tumors (77, 78) and that the major challenge is improving the quality and quantity of the natural immune response (79). However, interest in both approaches has been reinforced by the realization that antigen-independent expansion of the transferred memory T cells can occur in vivo (80) under certain situations.

Different ways to present antigen to T cells ex vivo. The most appropriate methods of ex vivo T cell culture mimic the physiologic processes whereby DCs generate a constellation of antigen-specific and costimulatory signals in the T cells. DCs are the most efficient APCs for the activation of naive T cells. However, although useful for therapeutic vaccination, due to practical considerations such as substantial manufacturing costs and the logistics of maintaining independent culture systems, DCs are not useful as APCs for large-scale adoptive T cell therapy trials. In addition, DCs have limited replicative potential; for ex vivo expansion of autologous T cells, it is desirable to have APCs with extensive replicative potential to facilitate both the scaling up of the process and multiple rounds of T cell stimulation. In addition, as was noted previously, since many patients are already primed to their tumors, other less potent forms of APCs might suffice to induce T cell activation.

The best results to date have been with the rapid expansion method developed by Riddell and coworkers, which uses irradiated allogeneic peripheral blood mononuclear cells as APCs (also known as feeder cells) to expand CTLs for adoptive transfer (81). The main limitation of this approach is in scale-up because conforming to FDA-mandated requirements for the validation and qualification of allogeneic feeder cells can be tedious and expensive. Schultze and coworkers have shown that CD40-stimulated B cells, which have an extensive replicative potential, are an efficient means of propagating antigen-specific T cells (82). Therefore, although currently available tissue culture approaches have provided proof of concept for adoptive T cell therapy, a current priority is to develop alternative approaches that can support the large-scale trials required for FDA approval.

To generate antigen-specific T cells, cell lines and beads can be engineered to create artificial APCs and avoid the need to use autologous APCs for patient-specific cultures (reviewed in ref. 83). General approaches have been to produce artificial APCs either by coating beads with CD3-specific antibodies or peptide-MHC complexes or by transfecting cells that lack endogenous MHC molecules with MHC molecules and costimulatory molecules. Enhanced polyclonal T cell activation and proliferation result when cells are stimulated through the TCR and CD28 (84). In addition, CD28 stimulation maintains telomere length in human T cells, and this might improve engraftment and the persistence of the adoptively transferred T cells (85, 86). This culture system has been adapted for clinical use, and starting with an initial apheresis product, it is possible to generate the number of mature T cells found in an adult within two weeks of ex vivo culture (87, 88).

Magnetic beads coated with MHC class I molecules loaded with specific peptide have been used to elicit antigen-specific T cell propagation (89). Following isolation and expansion, cell populations generated using such beads specifically kill antigen-expressing target cells in vitro and display antiviral therapeutic effects in rodents (90). Others have used nonmagnetic microspheres coated with complexes of recombinant peptide–loaded MHC molecules to successfully generate CTLs ex vivo from naive precursors (91). Peptide-MHC tetramers presenting peptides from the melanoma tumor antigens MART1 (melanoma-associated antigen recognized by T cells 1) and gp100 (glycoprotein 100) have also been used to isolate high-avidity tumor-reactive $CD8^+$ T cells from a heterogeneous population by flow cytometry. The tetramer-reactive cells have been cloned and retained their functional activity upon restimulation (92, 93). Sadelain and colleagues have engineered APCs that can be used to stimulate T cells of any patient expressing a specific HLA allele (94). Mouse fibroblasts were retrovirally transduced with a single HLA class I complex along with the human accessory molecules CD80 (also known as B7-1), CD54 (also known as ICAM-1), and CD58 (also known as LFA-3). These artificial APCs consistently elicited and expanded CTLs from patients of the appropriate haplotype specific for MART1 and gp100. We have also found that artificial APCs that express 4-1BB ligand efficiently expand human $CD8^+$ T_{CM} cells that have potent cytolytic function (89, 95, 96), and others have shown that CD83 expression on artificial APCs enhances the generation of CTLs (97).

Antigen concentration affects T cell stimulation ex vivo. It is critical that the correct concentration of antigenic peptide be used to pulse APCs. Alexander-Miller and colleagues showed that if high concentrations of peptide are used in vitro, only low-avidity T cells are propagated because the high-avidity T cells die by apoptosis (98), which suggests that submaximal concentrations of peptides might be required for in vitro induction of tumor-reactive CTLs. Furthermore, CTLs generated with peptide-pulsed APCs are often peptide reactive but not reactive with tumors that express the gene of interest due to low level expression or impaired antigen processing by the tumor cells. To circumvent this, Greenberg and colleagues have infected autologous APCs with recombinant vaccinia virus encoding the melanoma-associated antigen *tyrosinase* and have generated tyrosinase-specific and melanoma-reactive CTLs from the peripheral blood of 5 out of 8 patients with melanoma (99). In addition, tyrosinase-specific $CD4^+$ T cell clones were isolated from 6 of the 8 patients by stimulation with the autologous engineered APCs, and all these clones were able to recognize autologous tumor cells. This approach and other similar approaches (100) increase the probability that the expanded T cells recognize peptides that are presented by tumors. In addition, because the APCs express full-length protein sequences, this strategy permits the isolation of T cells that recognize epitopes not previously defined, circumventing the problem of not having identified all tumor antigens and not knowing which peptides are most efficiently presented. Furthermore, this approach permits the isolation of $CD4^+$ and $CD8^+$ tumor-reactive T cells from the peripheral blood of patients with cancer, perhaps obviating the need to use tumor-infiltrating T cells as the input population in ex vivo T cell expansion protocols.

Telomeres: size does matter!

Telomeres have been shown to be involved in the control of cell proliferation, the regulation of cell senescence, and the unlimited proliferative capacity of malignant cells (14, 15). Human telomeres are composed of TTAGGG repeats at the chromosomal ends, and they function as tumor suppressors to protect chromosomes from degradation, fusion, and recombination (Figure 3).

Telomerase has been identified as a ribonucleoprotein enzyme that can synthesize telomeric repeats onto chromosomes. Telomerase consists of two essential components, telomerase RNA component (TERC) and telomerase reverse transcriptase (TERT) (14). In all human cells, with the exception of germ cells, stem cells, and some activated lymphocytes, telomeres lose a portion of their noncoding repetitive DNA with each cell division, and this shortening of telomeric DNA is one mechanism that can lead to cellular senescence. By contrast, there is a wide variety of evidence indicating that mice do not use replicative aging as a counting mechanism to induce senescence and to suppress the progression of cancers, as mice retain telomerase competency throughout their life span and cells from telomerase-deficient mice undergo senescence as readily as wild-type cells (101). Therefore, it seems that a telomere-independent mechanism regulates the proliferative life span of rodent cells (15), and, as discussed above, this difference between humans and mice in telomere biology presents a major shortcoming in using standard laboratory strains of mice for preclinical studies to support the development of strategies for human adoptive T cell therapy.

Telomere length in T cells. Patients with various bone marrow failure syndromes have a number of structural and functional abnormalities of telomeres and telomerase (102). Some patients with aplastic anemia have mutations in the gene encoding TERT (103), and haploinsufficiency of *TERC* or *TERT* has been associated with human dyskeratosis congenita (104) and aplastic anemia (103). Human T cells have finite clonal life spans in vitro (105), a phe-

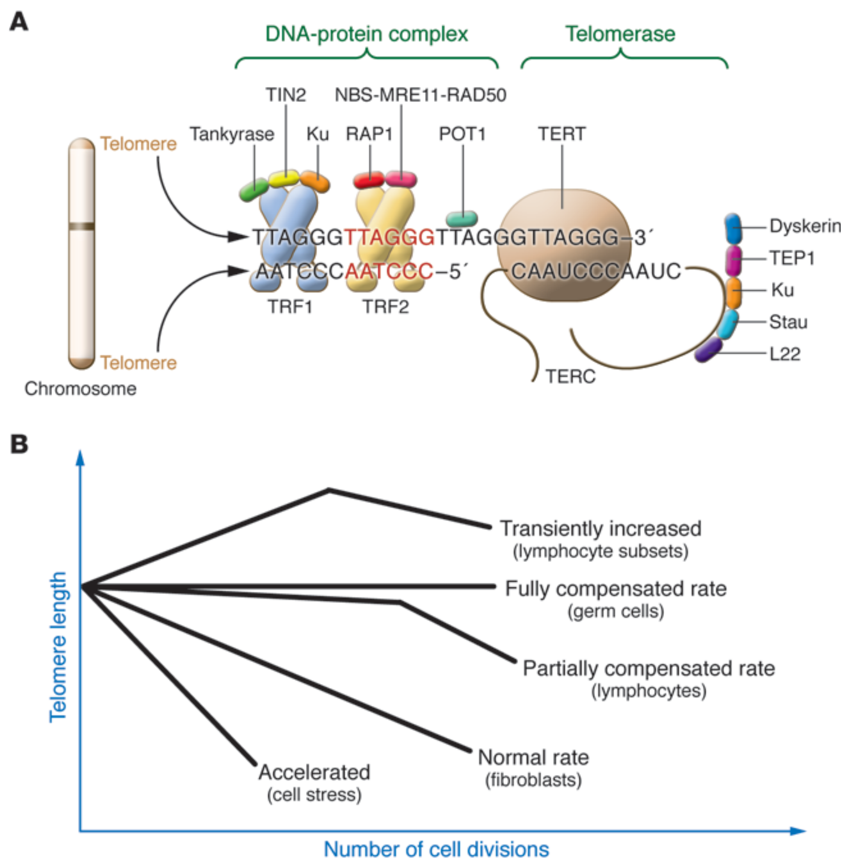

Figure 3
Telomeres and telomerase function in T cell subsets. (**A**) In mammalian cells, telomeres are structures at the ends of all linear chromosomes that consist of hexanucleotide repeats [(TTAGGG)n] and several associated protein complexes. The two components of telomerase are illustrated — TERC and TERT. NBS, Nijmegen breakage syndrome; MRE, meiotic recombination 11 homolog; L22, ribosomal L22 protein; TEP1, telomerase-associated protein 1. (**B**) The relationship of telomere length to cell division is not constant. There is a relatively constant loss of telomere length during normal cell division in the absence of compensatory mechanisms in most cells. However, telomere length can also increase with cell division in some lymphocyte subsets. Human T cells can partially sustain telomere length during cell division. Cellular stress can accelerate telomere loss rates.

nomenon also manifested with other somatic tissue cells and commonly known as the Hayflick limit. In humans, lymphocytes and stem cells belong to a select class of cells that are able to induce telomerase activity (106).

For effector T cells, costimulation is required for the induction of telomerase activity (85, 107). In vitro, the limit of polyclonal expansion for human adult mature $CD4^+$ T cells is about 30–40 doublings of the population size (84, 108). In previous studies, we found that human naive $CD4^+$ T cells have telomeres that are, on average, 1.4 kb longer than those of human memory T cells (109). In more recent studies using CC chemokine receptor 7 (CCR7) and CD27 expression to discriminate human $CD4^+$ memory T cell subsets, telomere length was found to differ substantially among the subsets, with $CD4^+CD27^+CCR7^+$ naive T cells having the longest telomeres, followed by $CD4^+$ $CD27^+CCR7^-$ T_{CM} cells, and then terminally differentiated $CD4^+CD27^-CCR7^-$ T_{EM} cells (110). In human $CD8^+$ memory T cell subsets, the telomeres are shorter in $CD8^+CD27^-CD45RA^+$ T_{EM} and $CD8^+CD27^+CD45RA^-$ T_{CM} cell subsets than in unprimed $CD8^+CD27^+CD45RA^+$ naive T cells (111). In the peripheral blood, more detailed phenotypic analysis indicates that some populations of T_{EM} cells ($CD27^+CD28^-CD45RA^+CCR7^-$ cells) have telomeres that are on average *longer* than those of $CD27^+CD28^+CD45RA^+CCR7^+$ naive T cells, suggesting that telomerase has forestalled telomere degradation in these cells (112). To the extent that relative preservation, or even extension, of telomere length is a biomarker of candidate stem cells, it seems that human memory stem T cells might be contained in this population.

Telomere length and adoptive T cell therapy. It is possible that previous adoptive T cell therapy clinical trials have been unsuccessful because the prolonged ex vivo culture process resulted in a population of cells that had reached, or were near, replicative senescence. Recent studies suggest that preservation of telomere length and replicative capacity correlates with the engraftment efficiency and antitumor efficacy of adoptively transferred T cell lines in patients with melanoma (86). CD28 stimulation maintained telomere length in T cells (106), and cultures that optimize costimulation might improve the engraftment and persistence of adoptively transferred T cells. Recent studies indicate that IL-15 is able to activate telomerase activity in human memory $CD8^+$ T cells through the JAK3 and PI3K signaling pathways (113). Furthermore, IL-15 induces a sustained level of telomerase activity, and this minimizes telomere loss in memory $CD8^+$ T cells after a substantial number of cell divisions (113, 114).

For optimal adoptive T cell therapy, it is probable that preservation of the replicative life span of memory T cells is vital for long-term immune protection. Culture methods that preserve CD28 expression on the transferred T cells might be important because CD28 expression is associated with long telomeres (32) and, following adoptive transfer, there is a correlation between CD28 expression, telomere length, and T cell persistence, with enhanced antitumor effects in patients with metastatic melanoma (86). For effector T cells that have downregulated CD28, it is possible that strategies that employ IL-7 and IL-15 during culture and/or after infusion might be beneficial (113, 114); alternatively, genetic engineering of T cells to restore CD28 expression seems to have promise to rejuvenate T cells, in which reintroduction of the *CD28* gene reconstituted the ability to produce IL-2 and thereby induced autocrine proliferation after antigen stimulation (115).

One way to circumvent the problem of telomere degradation is to express TERT in human T cells. Ectopic expression of TERT in human CD8+ and CD4+ T cells leads to immortalization of these T cells (116, 117). Human T cells constitutively expressing TERT are not overtly leukemogenic, as they display minimal alterations in phenotype, specificity, and functionality and remain dependent on cytokines and antigenic stimulation for in vitro expansion. TERT reprogrammed T cells mount effective immune responses following adoptive transfer into mice bearing tumor xenografts (118). However, although in vitro studies so far indicate that constitutive TERT expression achieves immortalization without malignant transformation, caution is urged with the clinical use of life span–extended human T cells because chromosomal instability, which is associated with tumor development, has been observed in human T cells constitutively expressing TERT (119, 120).

Other strategies might forestall or reverse senescence of memory T cells. Rando and colleagues have used parabiotic pairings (i.e., a shared circulatory system) between young and old mice to demonstrate that the proliferative and regenerative capacity of aged satellite muscle cells is restored by exposure of satellite cells to young mice (121). By extension, to the extent that lymphocyte aging is extrinsically regulated, it is possible that the "parking" of memory T cells in a young environment during tissue culture could restore T cell functions. In contrast, a cell intrinsic form of senescence is mediated by the cyclin-dependent kinase inhibitor p16INK4a, as HSCs have delayed aging and improved ability to support the survival of animals in successive transplants (122). Therefore it is possible that inhibition of p16INK4a could extend the useful life span of some lymphocyte subsets.

Conclusion

Adoptive T cell therapy of rodent malignancies was first reported in 1955 (123), and there are no forms of FDA-approved T cell therapy for cancer available after more than 60 years of research into adoptive immunity for tumors. However, there is increasing optimism that the scientific barriers preventing clinically effective adoptive immunotherapy have been addressed. Evidence in support of the cancer stem cell hypothesis and the idea that these cells present a substantial barrier to complete tumor elimination using cytotoxic chemotherapy raises the hope that it might be possible to target these cells using adoptive T cell therapy (124). Given the success of allogeneic cellular therapy for chemotherapy-resistant hematologic malignancies (125), it is of interest to learn whether T cells can target cancer stem cells, as is suggested by the durability of responses following allogeneic T cell therapies. Advances in the understanding of T cell biology and the tumor microenvironment have provided multiple novel adoptive transfer strategies that are now poised for translation into clinical trials.

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