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Review

T Cell Receptor Gene Therapy for Cancer

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Abstract

T cell-based adoptive immunotherapy has been shown to be a promising treatment for various types of cancer. However, adoptive T cell therapy currently requires the custom isolation and characterization of tumor-specific T cells from each patient—a process that can be not only difficult and time-consuming but also often fails to yield high-avidity T cells, which together have limited the broad application of this approach as a clinical treatment. Employing T cell receptor (TCR) gene therapy as a component of adoptive T cell therapy strategies can overcome many of these obstacles, allowing autologous T cells with a defined specificity to be generated in a much shorter time period. Initial studies using this approach have been hampered by a number of technical difficulties resulting in low TCR expression and acquisition of potentially problematic specificities due to mispairing of introduced TCR chains with endogenous TCR chains. The last several years have seen substantial progress in our understanding of the multiple facets of TCR gene therapy that will have to be properly orchestrated for this strategy to succeed. Here we outline the challenges of TCR gene therapy and the advances that have been made toward realizing the promise of this approach.

Introduction

The ability of T cells to eradicate tumor cells in patients with cancer is well documented in the setting of allogeneic hematopoietic cell transplantation for leukemia, in which allogeneic donor T cells can induce graft-versus-leukemia effect (GVL) after recognition of antigens expressed by the leukemic cells, including major histocompatibility antigens (in HLA-mismatched transplants), minor histocompatibility (H) antigens, and "leukemia-specific" (mutated or aberrantly expressed) antigens (Fefer *et al.*, 1987; Bleakley and Riddell, 2004). Unfortunately, the desired GVL effect is often accompanied or overshadowed by harmful side effects including graft-versus-host disease (GVHD) (Kolb *et al.*, 1995; Collins *et al.*, 1997). One way to harness the antitumor activity of donor T cells and to reduce toxicity is to target the malignant cells by adoptive transfer of $CD8^+$ cytotoxic T lymphocytes (CTLs) specific for antigens uniquely or preferentially expressed by the cancer cells. Adoptive T cell therapy can also be pursued in an autologous setting, in which T cells reactive against a selected antigen are isolated from the patient, expanded to large numbers *in vitro*, and reinfused into the patient. Potent antitumor effects after such therapy have been observed clinically, particularly in patients with melanoma (Yee *et al.*, 2002; Dudley *et al.*, 2005; Hunder *et al.*, 2008). Several tumor-associated antigens (TAAs) are currently being targeted by adoptive T cell therapy in clinical trials, including MART-1 (melanoma antigen recognized by T cells-1), gp100, and tyrosinase in melanoma and WT1 (Wilms tumor-1) in acute myeloid leukemia. Although adoptive T cell therapy has thus far been effective in a limited number of patients and only for a limited number of diseases, the number of identified TAAs that might be targeted is continually increasing.

Despite some dramatic successes, several factors currently limit the efficacy and broad application of adoptive T cell immunotherapy, including the inability of transferred T cells to persist at high levels *in vivo* after infusion, the difficulty of reproducibly isolating high-affinity T cells that recognize relevant TAAs, and the relatively long time frame required to isolate and expand these T cell clones. Transfer of T cell receptor (TCR) genes into primary T cells provides a strategy to impart specificity for a desired target antigen that can circumvent some of these obstacles. It was first demonstrated that transfer of TCRα and TCRβ genes into T cells could redirect the specificity of those T cells more than 20 years ago (Dembic *et al.*, 1986). By the early part of the twenty-first century, several groups had built on this concept, using TCRs specific for various viruses or TAAs to confer specificity for the selected antigen to mature peripheral T cells *in vitro* (Clay *et al.*, 1999; Cooper *et al.*, 2000), and the feasibility of TCR gene therapy in animal models had been demonstrated (Kessels *et al.*, 2001; Morris *et al.*, 2005; Abad *et al.*, 2008; Coccoris *et al.*, 2008; de Witte *et al.*, 2008; Dossett *et al.*, 2009). A clinical trial published by Rosenberg and colleagues has highlighted the

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promise of TCR gene therapy as a treatment for cancer, as well as the challenges associated with this approach. In that trial, autologous polyclonal T cells were transduced with a MART-1-specific TCR, expanded *in vitro*, and then infused into lymphodepleted patients with metastatic melanoma (Morgan *et al.*, 2006). Importantly, tumor regression was clearly observed in 2 of 17 patients treated. However, at the time of infusion, 42% of $CD8^+$ T cells expressed the MART-1-specific TCRβ chain, and only 17% bound MART-1 tetramer, indicating that the introduced TCR chains were being expressed at insufficient levels and/or mispairing with endogenous TCR chains. One month after infusion, the retroviral transgene could be detected by polymerase chain reaction (PCR) in 26% of the patients' peripheral T cells, but expression of the MART-1-specific TCRβ chain was detected on only 8%, and less than 1% expressed sufficient levels of introduced TCR to bind the MART-1 tetramer (Morgan *et al.*, 2006). These findings suggest that the retroviral transgene underwent transcriptional silencing in a large proportion of the transduced T cells, with resultant loss of MART-1 specificity, and highlight some of the challenges of developing TCR gene therapy as a viable clinical strategy.

The Challenges of TCR Gene Therapy

A long-standing problem in the field of TCR gene therapy is that TCR-transduced T cells often have lower avidity than the TCR "donor" cell, as wild-type levels of TCR gene expression are rarely achieved in transduced cells. Moreover, the expression levels of the introduced TCR chains generally decline further when the transduced T cells become quiescent and are no longer being triggered by the antigen, making any persisting memory cells largely ineffective (Dossett *et al.*, 2009). Suboptimal expression of one or both of the TCR chains, or inefficient pairing and expression of the introduced TCR on the cell surface, has a major impact on the functional avidity of the transduced T cell, which is proportional to the surface expression of the introduced TCR (Cooper *et al.*, 2000; Labrecque *et al.*, 2001).

The introduction of a second TCRα and TCRβ chain into a mature T cell also introduces the significant risk of autoimmunity due to mispairing of the introduced TCR chains with their endogenous counterparts, which not only reduces expression of the desired TCR pair but can create a new TCR with unknown specificity that can potentially cause autoimmunity (Fernandez-Miguel *et al.*, 1999). Several strategies have been devised to decrease mispairing between the introduced and endogenous TCR chains, as discussed in detail later.

Even when the introduced TCR chains are expressed at high levels and pair efficiently, several other factors will influence the efficacy of TCR gene therapy in the clinic, including the affinity of the introduced TCR, the maintenance of TCR gene expression over time, and the persistence of the TCR-transduced T cells *in vivo*. Significant progress has been made toward improving TCR expression cassettes to make them more likely to be successful in the clinic. In this review we discuss these advances in the field, and highlight the issues that remain to be resolved.

Overview of TCR Gene Therapy

The first step in TCR gene therapy is to isolate a T cell clone that expresses a TCR with high affinity for the target antigen. This is often accomplished by culturing tumor-infiltrating lymphocytes (TILs) or peripheral blood mononuclear cells (PBMCs) in the presence of antigen-presenting cells (APCs) pulsed with a peptide representing an epitope known to elicit a dominant T cell response when presented in the context of a defined HLA allele. High-affinity clones can be selected on the basis of MHC–peptide tetramer staining and/or the ability to recognize and lyse target cells pulsed with low titrated concentrations of cognate peptide antigen. Once a clone has been selected, the TCRα and TCRβ chains are identified and isolated by molecular cloning. The TCRα and TCRβ gene sequences are then used to generate an expression construct that ideally promotes stable, high-level expression of both TCR chains in human T cells. The transduction vehicle, generally a gammaretrovirus or lentivirus, is then generated and tested for functionality (antigen specificity and functional avidity), and used to produce a clinical lot of the vector. An aliquot of the final product is then used to transduce the target T cell population (generally purified from patient PBMCs), which is expanded before infusion into the patient (Fig. 1).

Designing the Expression Construct

TCR chains introduced into a peripheral T cell must compete with endogenous TCR chains for association with the CD3 complex, which is necessary for TCR surface expression. Because a high level of TCR surface expression is essential to confer appropriate sensitivity for triggering by cells expressing the target tumor antigen (Cooper *et al.*, 2000; Labrecque *et al.*, 2001), strategies that enhance TCRα and TCRβ gene expression levels are an important consideration in TCR gene therapy. A number of promoter sequences have

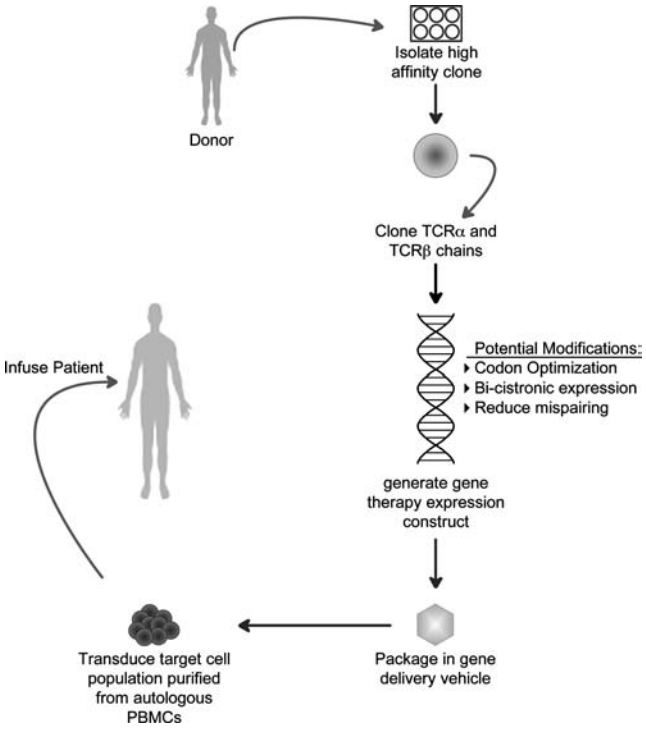

FIG. 1. Overview of TCR gene therapy. PBMCs, peripheral blood mononuclear cells; TCRs, T cell receptors.

been investigated for their ability to drive a high level of gene expression in T cells. In addition to retroviral long terminal repeats (LTRs), which are an essential component of retroviral vectors that can promote high levels of gene expression in human T cells (Cooper *et al.*, 2004), investigators have compared the utility of a number of viral and cellular promoter elements, including cytomegalovirus (CMV), murine stem cell virus (MSCV) U3, phosphoglycerate kinase (PGK), β-actin, ubiquitin, and a simian virus 40 (SV40)/CD43 composite promoter (Cooper *et al.*, 2004; Jones *et al.*, 2009). In these studies, the viral LTR in combination with an interferon (IFN)-β scaffold attachment region (SAR), and the MSCV U3 promoter appeared to provide the highest level of stable gene expression. However, successful TCR gene transfer and high-level expression have been reported with other promoters as well, including elongation factor (EF)-1α (Tsuji *et al.*, 2005) and the spleen focus-forming virus (SFFV) promoter (Joseph *et al.*, 2008). In addition to strong promoters, many TCR expression cassettes contain additional elements that can enhance transgene expression, including a central polypurine tract (cPPT), which promotes the nuclear translocation of lentiviral constructs (Follenzi *et al.*, 2000), and the woodchuck hepatitis virus posttranscriptional regulatory element (wPRE), which increases the level of transgene expression by increasing RNA stability (Zufferey *et al.*, 1999). Another modification that has proven to be beneficial for increasing TCR transgene expression is codon optimization. Redundancy in the genetic code allows some amino acids to be encoded by more than one codon, but certain codons are less "optimal" than others because of the relative availability of matching tRNAs as well as other factors (Gustafsson *et al.*, 2004). Modifying the TCRα and TCRβ gene sequences such that each amino acid is encoded by the optimal codon for mammalian gene expression, as well as eliminating mRNA instability motifs or cryptic splice sites, has been shown to significantly enhance TCRα and TCRβ gene expression (Scholten *et al.*, 2006).

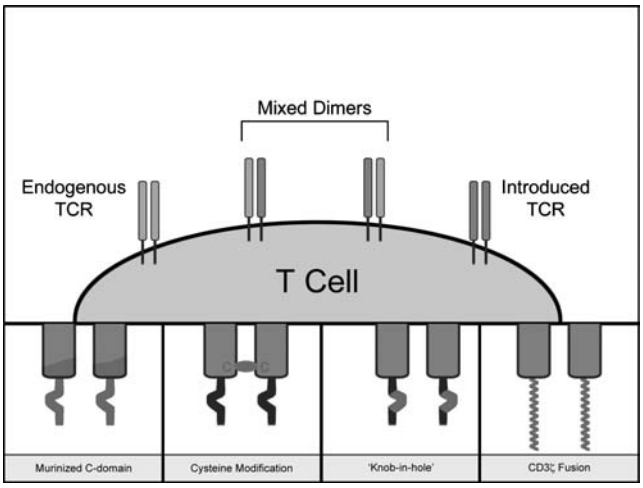

FIG. 2. Strategies to reduce TCR mispairing. T cells modified by TCR gene therapy to express a second pair of TCR chains have the potential to form four unique TCRs on their cell surface, consisting of: the endogenous TCRα and TCRβ chains (orange), the introduced TCRα and TCRβ chains (blue), and mixed dimers of the endogenous TCRα or TCRβ chain paired with the introduced TCRβ or TCRα chain, respectively (*top*). Strategies to reduce mispairing generally involve modifying the C domain of the introduced TCR chains in order to promote interchain affinity, while decreasing the ability of the introduced chains to pair with the endogenous TCR (*bottom*). These strategies include replacing the human TCRα/β C domains with their murine counterparts (murinized C domain); generating a second interchain disulfide bond in the C domain by introducing a second cysteine residue into both the TCRα and TCRβ chains of the introduced TCR (cysteine modification); swapping interacting residues in the TCRα- and TCRβ-chain C domains ("knob-in-hole"); and fusing the variable domains of the TCRα and TCRβ chains directly to CD3ζ (CD3ζ fusion).

Approaches to Reducing TCR Chain Mispairing

Several still unpublished studies with both human and mouse TCR-transduced T cells have revealed, *in vitro* and *in vivo*, respectively, that mispairing between the introduced and endogenous TCR chains can indeed result in the acquisition of specificities that pose a significant risk for autoimmunity. Furthermore, the formation of mixed TCR dimers reduces the number of CD3 molecules available to form properly paired TCR complexes, and therefore can significantly decrease the functional avidity of the cells expressing the introduced TCR (Kuball *et al.*, 2007). Therefore, several strategies have been employed to reduce the likelihood of mixed TCR dimer formation (Fig. 2). In general, this involves modifying the constant (C) domains of the TCRα and TCRβ chains to promote the preferential pairing of the introduced TCR chains with each other, while rendering them less likely to successfully pair with endogenous TCR chains. One approach that has shown some promise *in vitro* involves replacement of the C domain of human TCRα and TCRβ chains with their mouse counterparts. Interestingly, human T cells expressing both human and mouse TCR chains preferentially express the mouse TCR chains on the cell surface (Sommermeyer *et al.*, 2006), due in part to the murine TCR chains, which preferentially pair with each other, exhibiting increased stability with the human CD3 complex compared with fully human complexes (Cohen *et al.*, 2006). These "murinized" TCRs (containing the mouse C_α and C_β domains) are expressed at high levels on the surface of human T cells (although some mixed dimer formation with endogenous human TCR chains does occur) and appear to signal properly (Cohen *et al.*, 2006; Sommermeyer *et al.*, 2006; Voss *et al.*, 2008). Retroviral transfer of nonself-protein sequences into human T cells has been shown to elicit immune rejection (Riddell *et al.*, 1996). Thus, although murinized TCRs appear to effectively overcome the problem of TCR mispairing, and function normally *in vitro*, the potential immunogenicity of the murine C domains, which could lead to rejection of the transferred autologous T cells by the host, is a significant concern with this system.

A second approach to minimize TCR chain mispairing is to increase interchain affinity by engineering a second disulfide bond into the extracellular domain of the expressed TCR via introduction of an additional cysteine residue in both the α- and β-chain C domains (Kuball *et al.*, 2007). This modification is modeled on a similar design that was found to stabilize interchain pairing for the development of soluble TCR molecules (Boulter *et al.*, 2003). We have demonstrated that, in T cells expressing the cysteine-modified but not the wild-type

TCR, up to 95% of the expressed introduced TCR chains were properly paired, indicating that the addition of a second cysteine bond substantially increased the propensity of the introduced TCR chains to pair with each other (Kuball *et al.*, 2007). In addition to promoting preferential pairing of the introduced TCR chains, cysteine modification also resulted in a significant increase in the total expression level of each introduced TCR chain, perhaps due to a competitive advantage for assembly with components of the CD3 complex. Because this cysteine modification represents a single amino acid change, the likelihood of an antigenic peptide being produced is much reduced. Indeed, a similar modification of murine TCR chains did not elicit an antibody response in B6 mice after several injections of TCR gene-modified T cells (T.M. Schmitt and P.D. Greenberg, unpublished observations). An alternative but similar strategy, which also inserts point mutations into the α- and β-chain C domains, has exploited evidence from the crystal structure revealing that the TCRα/β C domain interface is maintained in part by a "knob-in-hole" interaction resulting from a protruding arginine side chain from the TCRβ C domain inserting into a cavity created by a glycine residue in the TCRα C domain—by swapping the chain on which these interacting residues are expressed before introducing the TCR, the resulting TCR chains preferentially pair with each other (Voss *et al.*, 2008).

A distinct approach has been the development of CD3-independent chimeric antigen receptors, in which the variable domains of the TCRα and TCRβ chain are fused directly to CD3ζ. These receptors do not pair with endogenous TCR chains, and therefore do not pose a risk of forming potentially self-reactive mixed TCR dimers (Sebestyen *et al.*, 2008). Furthermore, surface expression of these receptors is not limited by the fixed pool of CD3 molecules, and therefore can be expressed at high levels on the T cell surface. These chimeric molecules necessarily have junctional sequences that, similar to the point mutations described previously, have the potential to elicit a host immune response, which could lead to rejection of the transduced T cells *in vivo*. However, of greater concern is the fact that these receptors are uncoupled from several important signaling pathways, and appear to have a decreased rather than the desired increased sensitivity to low antigen concentrations (Sebestyen *et al.*, 2008).

Coordinated Expression of TCRα and TCRβ Chains

Achieving high-level TCR surface expression requires that both the α and β chains of the introduced TCR be transcribed at high levels. It is generally desirable that both be introduced within a single expression vector rather than separate vectors, because this greatly increases the frequency of T cells expressing both chains and reduces the risk of insertional mutagenesis resulting from a second retroviral insertion event. Studies using an internal promoter between the TCRα and TCRβ chains to drive expression of one of the chains demonstrated the relative inefficiency of using an LTR and an internal promoter for achieving high-level expression of both TCR chains (Dossett *et al.*, 2009). However, the use of bicistronic constructs in a single vector has been shown to be capable of overcoming this obstacle. The use of a viral intraribosomal entry site (IRES) between the TCRα and TCRβ chains results in the coordinated expression of both chains, with detectable tetramer binding by the transduced T cells. However, constructs containing an IRES generally have significantly lower expression of the gene downstream of the IRES than of the gene preceding the IRES (Mizuguchi *et al.*, 2000; Yu *et al.*, 2003), and this has also been observed for constructs containing two TCR chains separated by an IRES (Jones *et al.*, 2009). To address this obstacle, Vignali and colleagues developed a system for expressing two genes linked by viral 2A peptides (Szymczak *et al.*, 2004). These peptide sequences, identified as components of several viral genomes, allow two separate proteins to be generated from one long composite polypeptide through a ribosome-skipping mechanism that terminates the first protein and permits initiation of translation of the second protein (Ryan *et al.*, 1991; Palmenberg *et al.*, 1992; Donnelly *et al.*, 2001). 2A peptides have the advantages of adding little to the overall size of the insert, as they are substantially smaller (18–22 amino acids or $\sim$60 bp of DNA) than most IRES sequences, and, because the TCRα and TCRβ chains are generated from a single transcript that is broken into two proteins during translation, ensuring that an equal molar ratio of TCRα and TCRβ chains are produced. One concern associated with the use of 2A elements in gene therapy is that much of the 2A peptide remains attached to the translated 5′ gene, which could interfere with function or elicit an immune response. However, 2A peptides have been used extensively for TCR gene transfer in animal studies, and neither of these adverse events has yet been reported (Holst *et al.*, 2006).

Delivery Systems for TCR Gene Therapy

The gene delivery vehicle used to transduce target T cells for gene therapy can influence the safety and function of the TCR expression cassette. The most commonly used vehicles are gammaretroviral and lentiviral systems. These vectors have a number of benefits that make them attractive for TCR gene therapy. In particular, gammaretroviral and lentiviral constructs integrate into the host genome, and thus have the potential to provide long-term stable expression of the transgene; encode no vector proteins, and thus are not immunogenic; and have a relatively large packaging capacity. The gammaretroviruses have been used extensively for gene therapy, and several packaging lines are available from which stable transgene-specific packaging lines can be generated. This makes retroviral systems ideal for the large-scale production of virus necessary for a clinical trial. However, retroviruses present some expression and safety issues that require consideration. Retroviruses preferentially integrate near the transcriptional start site of active genes, which can result in insertional mutagenesis with dysregulation of gene expression (Mikkers and Berns, 2003; Wu *et al.*, 2003). This problem was highlighted by the results of an X-linked SCID (severe combined immunodeficiency) gene therapy trial, in which gammaretrovirus-mediated insertion of a gene into $CD34^+$ hematopoietic stem cells resulted in integration near an oncogene and the development of a lymphoproliferative disease in three patients (Deichmann *et al.*, 2007); the risk was likely greatly increased in this clinical trial setting, in which the few transferred transduced T cell precursors were driven to proliferate to fill an empty T cell compartment. Furthermore, many retroviral constructs, particularly those based on the murine leukemia virus (MLV) (Pannell and Ellis, 2001), have a tendency to undergo transcriptional silencing, particularly in

cells not actively cycling (Quinn *et al.*, 1998; Rubinstein *et al.*, 2009). This represents a paradox for establishing a persistent response after TCR gene therapy, because activation through the transgenic gene product (the TCR) is necessary to drive the proliferation that is required to maintain transgene expression. These latter issues are being addressed by modifying elements within the retroviral LTRs that mediate silencing (Ellis, 2005), or by the incorporation of an IFN-β SAR into the retroviral vector to sustain TCR expression in resting T cells (Cooper *et al.*, 2004).

Lentiviruses have several advantages over gammaretroviruses, and an increasing number of gene therapy studies are using lentiviral systems for gene delivery. Lentiviruses do not have the same propensity to integrate near transcriptional start sites as gammaretroviruses, and appear unlikely to lead to oncogenesis. Indeed, one study using a tumor-prone mouse model demonstrated that gammaretroviral but not lentiviral vectors promoted tumorigenesis in a dose-dependent fashion (Montini *et al.*, 2006). Lentiviral vectors also appear to be less susceptible to transcriptional silencing (Pfeifer *et al.*, 2002). Finally, unlike gammaretroviral vectors, lentiviral systems are capable of transducing nondividing cell types, such as hematopoietic stem cells and minimally activated, nonproliferating T cells (Ailles *et al.*, 2002; Cavalieri *et al.*, 2003), which are both considered promising target cells for TCR gene therapy (Gattinoni *et al.*, 2005). However, one substantial limitation to employing lentiviral vector systems is the current absence of stable packaging lines, which increases the complexity of scale-up for clinical trials or establishing a reproducible stock for multiple trials.

An alternative, nonviral gene transfer system, which bypasses many of the obstacles and costs to produce Good Manufacturing Practice (GMP) viral vectors, involves the use of the *Sleeping Beauty* transposon to transfer the TCR expression cassette. In addition to the transposon plasmid containing the TCR genes, mRNA expressing the helper transposase is concurrently transduced into the target T cells. Using this approach, levels of TCR gene transduction in human T cells equivalent to those of the viral approaches discussed previously have been reported (Cooper *et al.*, 2004; Peng *et al.*, 2009). Although these results appear promising, further studies to optimize gene transfer and to carefully analyze potential integration site bias will need to be performed before considering broad application in the clinic.

Methods for Increasing Avidity

One of the major challenges in harnessing cellular immunity to treat cancer has been the difficulty of isolating T cells with adequate avidity for the selected TAA to mediate an effective response. Because the mature T cell repertoire consists only of those T cells with a sufficiently low avidity for self-antigens to have avoided negative selection and deletion, isolating high-avidity T cells reactive against TAAs can often be frustratingly difficult (De Visser *et al.*, 2003). However, in the context of gene transfer, it is possible to increase the functional avidity of a TCR either by modifying the physical properties of the TCRα and TCRβ chains to increase the affinity for its cognate peptide–MHC, or by manipulating proximal TCR signaling pathways in order to increase the sensitivity of a lower affinity TCR to antigenic stimulation. Techniques for *in vitro* affinity maturation of TCRs have been developed that use yeast or phage display technology to screen mutated chains (Kieke *et al.*, 1999; Li *et al.*, 2005), similar to methods previously developed for *in vitro* antibody affinity maturation (McCafferty *et al.*, 1990). Yeast display has yielded affinity increases of up to 1000-fold to the low nanomolar range (Holler *et al.*, 2000), and phage display has yielded TCRs with affinities in the picomolar range (Li *et al.*, 2005).

Another strategy for increasing TCR avidity is to modify the α- and β-chain sequences to remove sites of N-glycosylation (Kuball *et al.*, 2009). Glycosylation of T cells is known to diminish the sensitivity of T cells to antigen (Daniels *et al.*, 2002), and this appears to reflect in part glycosylation of TCR chains. Selective removal of TCR glycosylation sites has resulted in up to a 1-$\log_{10}$ increase in the functional avidity of TCR-transduced T cells (Kuball *et al.*, 2009).

An alternative approach is to leave the TCR chains unchanged, but to disrupt T cell signaling pathways that normally act to diminish TCR signals and increase the threshold for activation. For example, T cells transduced to express a dominant-negative form of Cbl-b or that are deficient for SHP-1 (SH2-containing protein tyrosine phosphatase-1) exhibit an augmented T cell response to antigen (Lorenz *et al.*, 1996; Fawcett and Lorenz, 2005; Zha and Gajewski, 2007), and preliminary studies with T cells deficient for either of these molecules have suggested increased efficacy *in vivo* (our unpublished data).

Target Cell Choice for TCR Gene Therapy

T cell therapy is often limited by the ability of transferred T cells to expand and persist *in vivo* after transfer, and the intrinsic properties of the T cells from which infused cells are derived contributes to their fate *in vivo.* In this regard, one advantage of TCR gene therapy is that it provides the opportunity to choose the cell type from which the T cells used for adoptive cell therapy are derived. Conventional $CD8^+$ T cells can be divided into naive T cells (T_N) and antigen-experienced memory T cells (T_M). Memory T cells can be further divided into central memory T cell (T_{CM}) and effector memory T cell (T_{EM}) subsets, which have distinct transcriptional programs that dictate homing, phenotype, and function (Sallusto *et al.*, 2004). When T_N and T_{EM} cells are stimulated *in vitro*, they expand and differentiate largely into short-lived effector cells, which effectively kill targets, but generally fail to persist for long periods *in vivo* (Sallusto *et al.*, 2004; Gattinoni *et al.*, 2005; Klebanoff *et al.*, 2005; Berger *et al.*, 2008). Although T_{CM} cells also largely expand and differentiate into effector cells in response to *in vitro* stimulation, these effector cells appear to retain some of the beneficial properties of the parent T_{CM} cell from which they were derived. In a nonhuman primate model, Riddell and colleagues stimulated CMV-specific $CD8^+CD45RO^+CD62L^+$ T cells (T_{CM}) and $CD8^+CD45RO^-CD62L^-$ T cells (T_{EM}) with CMV peptide and derived, after extensive *in vitro* expansion, CMV-specific T cell clones. The clones from both T cell compartments had a typical effector T cell phenotype and function at the time of transfer to the macaques. The T_{EM}-derived clones, which were detected in the blood the day after transfer, became undetectable in the blood on days 5 and 14, and were similarly undetectable in the lymph nodes or bone marrow 14 days after infusion. On the other

hand, infused clonal T cells derived from the T_{CM} subset were detected at a higher frequency in the blood on day 1 (26.3% of $CD8^+$ T cells vs. 16.3% for T_{EM}-derived T cells), were readily detectable in the lymph nodes and bone marrow, and remained detectable more than 11 months after the infusion. A fraction of the persisting T_{CM}-derived T cells also reexpressed typical phenotypic markers for T_{CM} (CD62L, CCR7, CD28, and CD127) and retained their ability to respond to antigen challenge *in vivo* without a requirement for exogenous cytokines (Berger *et al.*, 2008). Therefore, the introduction of TCR genes into T_{CM} is an attractive strategy for TCR gene therapy, because T_{CM}-derived cells appear not only to function and survive better after transfer, but can be expanded *in vivo* by immunization, which may facilitate the generation and maintenance of large numbers of persistent, tumor-specific, gene-modified T cells.

Patient Conditioning to Increase the Efficacy of T Cell Infusion

When T cells are infused into a patient, therapies that either decrease tumor or T cell-derived suppressive signals, or stimulate the infused T cells, can increase the efficacy of adoptive T cell therapy. In murine leukemia models, the transfer of leukemia-specific T cells has been shown to be effective in mice with established disease only when the leukemia burden has been reduced by chemotherapy before T cell infusions (Greenberg, 1991), supporting the notion that cytoreductive therapy to reduce tumor burden before T cell infusion is likely to improve the efficacy of adoptive T cell therapy. However, patients with metastatic melanoma have had substantial responses to adoptive T cell therapy even in the context of a large tumor burden. Lymphodepletion before transfer of T cells, using fludarabine or cyclophosphamide, has been shown in clinical trials to increase the efficacy of infused T cells, potentially by promoting both the *in vivo* expansion of transferred cells by increasing the availability of cytokines such as interleukin (IL)-7 and IL-15 that promote the homeostatic proliferation of the existing T cell compartment, and by decreasing the number of T-regulatory cells (Muranski *et al.*, 2006). An additional strategy is the coadministration of cytokines, including IL-2, which has been shown clinically to increase the persistence and antitumor activity of transferred T cells (Yee *et al.*, 2002), and IL-15, which can expand and maintain memory $CD8^+$ T cells and has been shown in mice and nonhuman primates to have a feasible safety profile (Klebanoff *et al.*, 2004; Berger *et al.*, 2009). CTLA4 (cytotoxic T lymphocyte antigen-4) and PD-1 (programmed death-1) are receptors on T cells that have an inhibitory effect on T cell activation, and monoclonal antibodies that recognize these receptors have demonstrated activity as single agents in clinical trials for patients with various metastatic malignancies (Hodi *et al.*, 2003; Brahmer *et al.*, 2009). Coadministration of these antibodies with tumor-specific T cells will need to be investigated as a potential means to increase the therapeutic efficacy of adoptive T cell therapy.

Conclusions

TCR gene therapy promises to afford an exquisite level of control to T cell-based cellular immunotherapy, potentially mitigating many of the difficulties that have limited the development and application of this clinical strategy. The isolation, characterization, and expansion of TAA-reactive T cells for adoptive immunotherapy is a technically challenging process that is often prohibitively time-consuming, especially in the case of more aggressive malignancies. TCR gene therapy provides the means to turn a single well-characterized TCR into a broadly applicable therapeutic agent for the treatment of cancer. The α and β chains of such a TCR can be transduced into large numbers of individual patient T cells that can then be expanded to numbers appropriate for infusion in a relatively short period of time. We have seen enormous progress in increasing the safety and efficacy of this approach, and many of the advances discussed in this review are currently being implemented in new gene therapy trials. In addition to the issues discussed here, progress in this field will rely heavily on continued efforts to identify new TAAs that allow more specific targeting of various malignancies, and on the accumulation of TCR libraries that encompass diverse antigens and diverse class I-restricting elements. Indeed, streamlining the methods for TCR gene therapy in concert with such advancements has the potential to transform this strategy into a readily accessible and effective cancer therapy.

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Author Disclosure Statement

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