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T-cell receptor gene transfer for the treatment of leukemia and other tumors

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Almost two decades ago the potency of adoptive T-cell therapy was demonstrated by the success of donor lymphocyte infusions for the treatment of chronic myeloid leukemia after allogeneic stem cell transplantation. Since then several studies have demonstrated the clinical efficacy of adoptively transferred T cells for the treatment of viral infections as well as cancers. The broad application of adoptive immunotherapy using antigen-specific T cells is, however, hampered by the inability to isolate and expand large numbers of T cells with a defined

specificity and phenotype. In addition, the T-cell receptor (TCR) repertoire useful for the generation of T cells directed against specific antigens is often lacking. The lack of an effective T-cell repertoire is especially relevant for tumor-associated self-antigens, since high affinity T cells directed against these self-antigens are mostly deleted from the TCR repertoire due to self-tolerance.

Adoptive immunotherapy of gene-modified T cells

As an alternative approach, the adoptive transfer of TCR gene-modified T cells has been developed with the aim of inducing immune reactivity towards defined antigens. TCR gene transfer enables instantaneous generation of defined T-cell immunity, and allows the introduction of TCR with specificities not present in the T-cell pool. *In vitro* studies have demonstrated that TCR gene-modified T cells are fully functional. TCR with high affinity for their peptide/MHC complex produce high avidity TCR-transferred T cells recognizing target cells presenting endogenously processed antigens, including leukemic cells.[1,2] In preclinical mouse models it has been demonstrated that adoptive transfer of TCR-transduced T cells can mediate protection against growth of tumors expressing the TCR-recognized antigen.[3] In addition, redirected human T cells prevented engraftment of a leukemia cell line as well as autologous primary leukemic cells in NOD/SCIDmice.[4,5]

TCR gene modification of T cells as adoptive immunotherapy for cancer patients has progressed significantly in recent years, with two clinical studies using TCR-modified T cells as cellular immunotherapy in patients with metastatic melanoma.[6,7] These studies, performed by the group of S. Rosenberg, demonstrated the feasibility of clinical implementation of TCR gene therapy. TCR gene-modified T cells persisted in the peripheral blood of all patients for at least 1 month after treatment. Objective cancer regression was seen in 19-30% of patients treated with Mart-1 or gp100-specific TCR, respectively. However, normal melanocytes in the skin, eye, and ear were destroyed, and local steroid treatment was needed to treat uveitis and hearing loss.[6] The possible toxicities resulting from expression of tumor-associated antigens on normal tissues have important implications for the initiation of clinical application of TCR gene transfer for the treatment of cancer.

Prerequisites of antigen-specific T-cell receptors for use in T-cell receptor gene therapy

High-affinity of the transferred T-cell receptor

Since the transferred TCR has to compete for cell-surface expression with the endogenous TCR, and mixed TCR dimers composed of the α-chain of one TCR and the β-chain of the other TCR, gene-transferred TCR need to have a high affinity for their specific peptide/HLA complexes. Due to competition of the different TCR complexes for binding with the CD3 complex the frequencies of the desired TCR at the cell-surface will be lower in TCR-transferred T cells than in the parental T-cell clone.

Target antigens useful for adoptive immunotherapy of leukemia and other tumors include tumor-associated viral antigens, minor histocompatibility antigens, and tumor-associated self-antigens. High avidity T cells directed against tumor-associated viral antigens are relatively straightforward to isolate, as the relevant T-cell repertoire is not affected by tolerance. Likewise, individuals for whom the given minor histocompatibility antigen is non-self are a reliable source of high avidity minor histocompatibility antigen-specific T cells. The affinity of TCR directed against tumor-associated self-antigens is, however, usually relatively low, due to self-tolerance. Several strategies have been developed to generate high affinity TCR directed against self-antigens. Stauss *et al.* used an allogeneic-restricted approach to isolate high avidity T cells specific for the WT1-derived peptide presented in HLA-A2 molecules.[8] These T cells were generated by *in vitro* stimulation of peripheral blood lymphocytes from healthy individuals with peptide-pulsed allogeneic antigen-presenting cells expressing the HLA of interest. Alternatively, we hypothesize that allogeneic-HLA-restricted tumor-associated self-antigen-specific T cells can be isolated from an *in vivo* HLA mismatched transplantation setting. The infused donor T cells have not encountered the allogeneic HLA molecules from the patient during thymic selection, consequently these T cells can exhibit high avidity for self-antigens presented by allogeneic patients' HLA molecules. We have recently identified a high affinity PRAME-specific allogeneic-HLA-restricted T-cell clone during an *in vivo* allogeneic-HLA immune response, potentially useful for TCR gene transfer studies (*unpublished data*). In addition, high-affinity TCR specific for tumor-associated self antigens have been obtained by immunization of HLA transgenic mice with human peptides, leading to murine TCR with specificities for human MDM2 and p53. There are also descriptions of the generation of TCR with different affinities from *in vitro* mutagenesis or *in vitro* selection using phage display. Recently, the group of Blankenstein developed a transgenic mouse expressing the complete human TCR genome as well as human HLA class I molecules. Using different vaccination strategies these mice may induce high affinity TCR complexes directed against tumor-associated self-antigens potentially useful for TCR transfer purposes (*unpublished data*).

Efficiency of T-cell receptor cell surface expression

Besides the affinity of the transferred TCR, the efficiency of cell surface expression of the transferred TCR is also considered to be important for the efficacy of the TCR gene-modified T cells. In a recent study we demonstrated that the TCR cell surface make-up of TCR-transduced T cells is not a random process, but is dependent on the characteristics of both the introduced and the endogenously expressed TCR.[9] Introduced, endogenous and mixed TCR dimers compete for cell-surface expression in favor of the TCR complex with best intrinsic pairing properties. The selection of high affinity TCR with superior pairing properties is, therefore, crucial for successful clinical application of TCR transfer. In addition, selection of those recipient T cells with weak competitor phenotypes is important to increase the efficiency of expression of the transferred TCR.

In addition, TCR cell surface expression and functionality of the TCR gene-modified T cells has been demonstrated to be increased by the introduction of an additional disulfide bond into the constant region of the TCR α

and β chains, or by the use of human-murine hybrid TCR.[10] Finally, a promising strategy to enhance TCR cell surface expression is the use of codon optimized TCRα- and β-chain genes from which mRNA instability motifs and cryptic splice sites are removed.[11]

Safety issues

Insertional mutagenesis

Insertional mutagenesis is a risk factor associated with the genetic modification of hematopoietic stem cells using retroviral vectors. Retroviral gene transfer into mature T cells has, however, been performed on a large scale without apparent evidence of the development of lymphoproliferative disorders.[12] Based on clinical and pre-clinical studies, we conclude that the likelihood of mature T cells transforming into malignant cells due to retroviral integration is low. Furthermore, a recent study in mice demonstrated that, in comparison to hematopoietic progenitor cells, mature T cells are resistant to oncogene transformation induced by retroviral gene transfer.[13]

On-target toxicity mediated by the transferred T-cell receptors

As mentioned earlier several strategies have been developed to isolate high affinity TCR specific for tumor-associated self-antigens. The avidity of these alternatively selected T cells will be higher than that of the previously described tumor-associated self-antigen-specific T cells derived from an autologous setting. We, therefore, postulate that these high affinity T cells may not only recognize tumor cells that over-express the tumor-associated self-antigens, but also non-transformed cells expressing low levels of these self-antigens. Recently, the on-target toxicity of high affinity T cells has been demonstrated in various different systems. Autologous T cells engineered with a high affinity G250-specific chimeric receptor specific for carboxy-anhydrase-IX (CAIX) were adoptively transferred to treat patients with metastatic CAIX$^+$ renal cell carcinoma. Two of the three treated patients, however, experienced serious liver toxicity that was demonstrated to be caused by the G250$^+$ T cells specifically attacking CAIX$^+$ bile duct epithelial cells.[14] Likewise, a clinical study by Rosenberg *et al.*, in which high affinity MART-1-specific human TCR and high affinity gp100-specific murine TCR were used, produced objective cancer regressions,[6] but normal melanocytes in the skin, eye, and ear were destroyed and patients required local steroid administration to treat uveitis and hearing loss, indicating that the TCR gene-modified T cells damaged non-transformed antigen-expressing cells throughout the body.

Together, these studies clearly indicate that the potential usefulness of high affinity tumor-associated self-antigen-specific T cells for immunotherapeutic strategies is conditioned by antigen expression on normal cells, especially on cells essential for human life. To exclude possible toxic side effects the expression pattern of the potential target antigens must be meticulously determined before a clinical study with TCR can be initiated.

Cross-reactive potential of transferred T-cell receptors

Transfer of TCR derived from an individual whose HLA type is not identical to the HLA type of the TCR recipient may cause unexpected cross-reactivity against HLA-peptide complexes expressed by the recipient, since the TCR is not selected on these HLA-peptide complexes. Furthermore, antigen-specific TCR selected by manipulations or strategies bypassing normal positive and negative selection may have a stronger cross-reactive potential, and must be tested in detail against a panel of target cells expressing various different HLA molecules to determine their potential off-target toxicity.

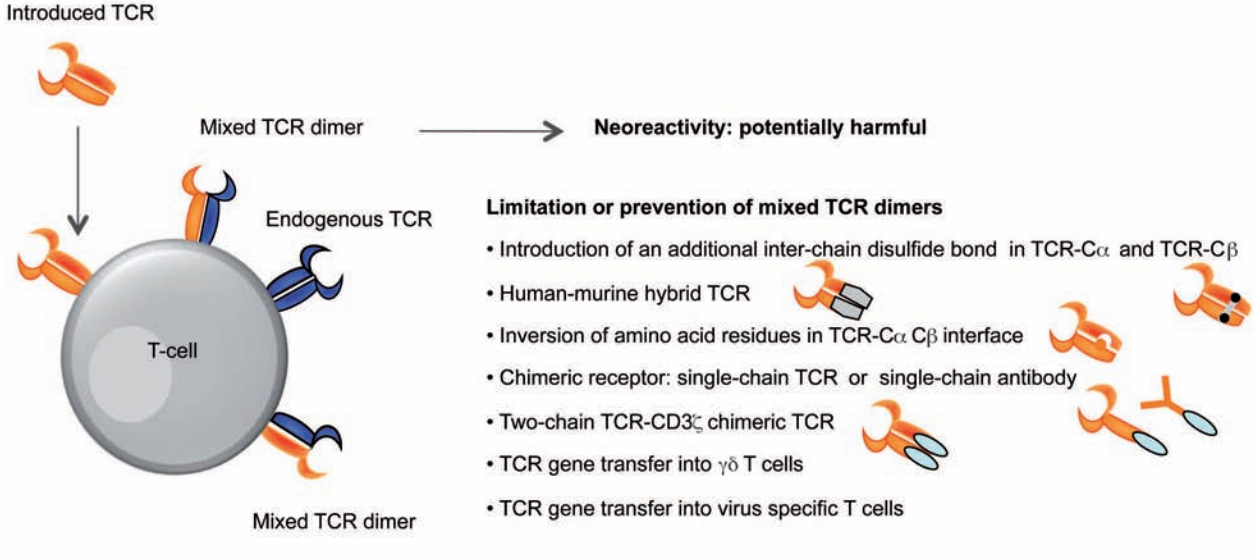

Figure 1. TCR gene therapy can lead to the formation of mixed TCR dimers with potentially harmful neoreactivities. Different strategies have been described to prevent this mis-pairing between TCR α and β chains of the endogenous and introduced TCR.

Mixed T-cell receptor dimers

As previously mentioned the introduced α and β chains can potentially assemble as pairs not only with each other but also with endogenous TCR α and β chains, thereby generating mixed TCR dimers with potentially harmful specificities. We have recently demonstrated that the introduction of TCR resulted in the formation of neoreactive mixed TCR dimers, with HLA class I or II restricted specificities. Most neoreactive mixed TCR dimers had HLA alloreactive activity. However, neoreactive mixed TCR dimers with autoreactive activity were also observed (van Loenen *et al.*, *unpublished data*). In addition, in a recent set of *in vivo* experiments, Schumacher's group observed that mice adoptively transferred with TCR gene-modified polyclonal T cells developed a lethal autoimmune disease. This disease appeared to be induced by conditions that promote expansion of the adoptively transferred T cells, and was shown to be mediated by the activity of mixed TCR dimers.[15] Although in the first clinical trials using adoptive T-cell therapy of TCR gene-modified T cells no toxicity due to mixed TCR dimers was observed, we cannot exclude that in situations of extensive T-cell activation and expansion of TCR-transferred T cells autoimmune pathology could occur.

These results demonstrate a potential risk of TCR gene transfer for clinical application, and underline the importance of searching for techniques to facilitate matched pairing. Several strategies have been explored to promote preferential pairing of the introduced chains to prevent the formation of mixed TCR dimers (see Figure 1). Examples of such strategies are the introduction of an additional inter-chain disulfide bond between the TCR α and β chain constant domains,[16] human-murine hybrid TCR,[10] and inversion of amino acid residues in the constant region of the TCR α and β chains that form the TCR interface.[17] Preliminary data suggest that the introduction of an extra inter-chain disulfide bond reduces the neoreactivity of TCR-transduced T cells (van Loenen *et al.*, *unpublished data*). An alternative approach to prevent the formation of mixed TCR dimers is to use chimeric receptors. Chimeric receptors comprise an extracellular antigen recognition domain of a single-chain antibody[18] or single-chain TCR (composed of VαVβCβ domains)[19] fused to a transmembrane and cytoplasmic signaling domain such as CD3-ζ or FcεRI-γ. Recently, two-chain TCR that encompass total human CD3ζ have been described to induce preferential pairing between the two transferred TCR chains.[20]

Alternative transfer strategies which do not necessarily need modification of the transferred TCR to prevent or reduce the formation of mixed TCR dimers are TCR transfer into alternative recipient T-cell populations. One possibility is transfer of αβ TCR chains into γδ T cells.[21] Since the γδ TCR is unable to form mixed TCR dimers with αβ TCR chains, the formation of mixed dimers can be completely prevented. Human TCR-transferred γδ T cells have been shown to exert high levels of antigen-specific cytotoxicity and cytokine release.[21] In mice we have demonstrated that the TCRαβ gene-modified γδ T cells expanded antigen specifically, produced cytokines upon specific antigen stimulation, were cytolytic and persisted *in vivo*.[22]

Alternatively, TCR gene transfer to virus-specific T cells can reduce the number of different mixed TCR dimers formed. Since anti-viral responses consist of T cells with a restricted TCR repertoire, the variety of different mixed TCR dimers will be limited.[2] Furthermore, there are several other potential reasons why virus-specific T cells could be the ideal recipient T cell for TCR gene transfer purposes. Virus-specific T cells do not induce graft-*versus*-host disease in an allogeneic setting, and exhibit a proper memory and effector phenotype. Since most human individuals have high frequencies of circulating cytomegalovirus (CMV)- and Epstein-Barr virus (EBV)-specific T cells due to latent infections with CMV (~50%) and EBV (~90%), these virus-specific T cells are most useful as recipient T cells.

However, if these strategies to prevent TCR gene transfer are not sufficiently effective the inclusion of a suicide gene or safety switch is warranted. Several possibilities have been described of which at the moment the CD20 molecule has greatest *in vivo* potential, based on the non-immunogenic properties of the molecule and the ability to eliminate the TCR-transduced CD20 co-expressing T cells through the use of the therapeutic anti-CD20 antibody, rituximab.[23]

Clinical implementation for the treatment of leukemia

In clinical studies in which patients with metastatic melanoma were treated with *ex vivo*-expanded tumor-infiltrating lymphocytes, the outcome was shown to be correlated with *in vivo* persistence of the adoptively transferred T cells.[6,7] Based on these results, strategies to influence the persistence of TCR gene-modified T cells have been developed. Depletions of the patient's lymphocyte pool by chemotherapy and irradiation before adoptive T-cell transfer has been used to enhance the *in vivo* expansion of tumor-infiltrating lymphocytes and TCR gene-modified T cells.[6] Although TCR gene-modified T cells persisted *in vivo*, decreased TCR cell surface expression was observed in time. This shutdown of TCR transgene expression was demonstrated not to be due to DNA methylation, and could be reverted by lymphocyte stimulation.[24] *In vitro* we have observed similar changes in TCR transgene expression, which correlated with the activation state of the T cells. TCR gene-modified T cells cultured for several weeks without TCR triggering demonstrated low transgene expression. However, activation of the T cells by triggering antigen specifically via either the introduced or the endogenous TCR, produced a dramatic increase in TCR transgene expression, which correlated with functional activity.[25] Based on these data one could speculate that the use of vaccination strategies in combination with adoptive TCR gene therapy may enhance the anti-tumor efficacy of TCR gene-modified T cells. Conversely, we hypothesize that virus-specific T cells could also have beneficial properties as carriers of the TCR transgene, since due to latent persistence of CMV and EBV *in vivo*, the virus-specific T cells would probably be stimulated by low doses of antigen via the endogenous TCR and, therefore, could exhibit increased TCR transgene expression and prolonged survival *in vivo*. *In vitro* we demonstrated that triggering via the endogenous TCR did not skew the TCR cell surface make-up of T cells toward an unfavorable pattern, an important prerequisite for the use of these T cells in clinical studies.[25]

The first clinical studies using TCR gene-modified T cells showed the feasibility of clinical implementation, and a substantial number of clinical trials in patients with solid tumors and leukemias are planned for the coming years. Most clinical studies will be performed in an autologous setting.

In this issue of the journal, Stauss *et al.*[5] describe the development of a safe and efficient WT1-specific TCR for adoptive immunotherapy. In this translational study WT1-TCR-engineered patient's T cells were able to eliminate autologous leukemia progenitor cells in an *in vivo* model. Based on these results a clinical trial will be initiated in which patients with acute or chronic myeloid leukemia will be treated with optimized WT-1-TCR-transferred autologous peripheral blood mononuclear cells.[5] We aim to start a clinical study using HA-1-TCR-transferred allogeneic T cells in combination with allogeneic stem cell transplantation in which patients with relapsed hematologic malignancies after allogeneic stem cell transplantation who fail to respond to donor lymphocyte infusions will be treated with HA-1-TCR gene-modified virus-specific donor T cells. In addition, patients with refractory hematologic malignancies for whom no other therapies are available will be transplanted with stem cell grafts from HLA-matched donors followed by early administrations of HA-1-TCR-modified virus-specific donor T cells.

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