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**MINIREVIEW**

# T Lymphocyte Therapy of Cancer

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## Summary

The rationale for the use of T lymphocytes to fight cancer is the immunogenicity of tumor cells. T cells are capable to recognize and finally to kill tumor cells. Adoptive cell transfer therapies provide the opportunity to overcome tolerogenic mechanisms by enabling the selection and activation of highly reactive T cell subpopulations and by manipulation of the host environment into which the T cells are introduced. The aim of this article is to review the possibilities, limitations and recent clinical experience with this novel anticancer treatment, namely with adoptive immunotherapy using antigen-specific T cells.

**Key words**
T lymphocyte • Cancer • Therapy

## Introduction

T lymphocytes play a key role in maintaining antitumor immunity. They therefore provide an important opportunity for the immunotherapy of cancer (Ben-Efraim 1996, Dudley 2000). In adoptive immunotherapy, T lymphocytes with antitumor activity are transferred into a tumor-bearing host. Successful therapy depends on the type of T cells transferred and their effectors functions, the ability of the cells to reach the tumor location, and the ability of the cells to overcome any tolerance or immunosuppression in the host (Cohen *et al.* 2001). Though much of the data are still experimental and many questions remain, several cellular therapies have demonstrated therapeutic benefits. Transfer of T lymphocytes with antitumor activity can be divided into at least two categories: tumor infiltrating lymphocytes and antigen-specific or tumor-specific cytotoxic T lymphocytes. Besides that, there is a strong evidence for graft-versus-tumor effect of allogeneic donor T cells in patients undergoing allogeneic hematopoietic stem cell transplants.

## Tumor infiltrating lymphocytes

Tumor infiltrating lymphocytes (TILs) are lymphocytes



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that have been obtained from tumor tissue by mechanical means and enzymatic digestion of tumor specimens. A single-cell suspension is then cultured for several weeks before TILs can be harvested. TILs cultured in the presence of IL-2 produce cytotoxic effects in mouse sarcoma, melanoma, colon carcinoma, and bladder carcinoma (Dudley 2000). The success of several *in vitro* and *in vivo* studies in animals led researchers to examine the possibilities of TIL therapy in humans. Attempts to obtain TIL cell lines have met with limited success, and when obtained, many lines were not specifically reactive *in vivo* (Kammula and Marincola 1999). However, patients with melanoma and renal cell carcinoma have benefited from TIL therapy, possibly because these tumors are more immunogenic (Weis *et al.* 1992, Rosenberg *et al.* 1994).

In metastatic melanoma, a 34-38 % response rate was achieved and this response was independent of prior chemotherapy. The effect lasted for several months (Rosenberg *et al.* 1994, Kammula and Marincola 1999, Dudley 2000). In renal cell carcinoma, several reports have shown various responses, depending on the treatment protocol used. When TILs were primed with various cytokines *in vivo* and then expanded *in vitro*, an overall response rate was seen in 20 % of patients (Weis *et al.* 1992). The overall response rate increased to 43 % when $CD8^+$ T cells were selectively used from the TIL population (Figlin *et al.* 1997). TILs appear to be a promising therapeutic strategy in some melanoma and renal cell carcinoma patients, but further investigations are needed to optimize the treatment protocols.

## Tumor-specific cytotoxic T lymphocyte therapy

*General strategy*

Studies during the past decade have provided evidence that augmentation of immune effector functions by the infusion of virus-reactive or tumor-reactive T lymphocytes represents a potentially highly specific modality for the treatment of viral diseases or cancer. It has been demonstrated that infusions of donor T lymphocytes to patients with relapsed leukemia after allogeneic hematopoietic stem cell transplantation (SCT) induces remissions in the majority of patients (Peggs and Mackinnon 2001). Since the establishment of methods to isolate genes encoding antigens recognized by cytotoxic T lymphocytes (CTL), many antigens have been identified and characterized (Table 1) for suitability as immunotherapeutic targets. Boon and coworkers pioneered techniques that utilize tumor-reactive CTL clones isolated from cancer patients as the reagent to screen target cells that had been transfected with a cDNA library derived from autologous tumor cells (Van der Bruggen *et al.* 1991). An alternate technique for the identification of tumor antigens called serological analysis of recombinant cDNA expression libraries (SEREX) has been recently described (Sahin *et al.* 1995). It uses serum from cancer patients to detect procaryotically expressed cDNA libraries prepared from tumors.

Antigenic epitopes recognized by tumor-specific T lymphocytes are derived from proteins encoded by tumor-associated viruses, mutated cytosolic proteins, and proteins that exhibit selective expression or overexpression in tumor cells (Van den Eynde and Van den Bruggen 1997, Renkvist *et al.* 2000) (Table 1). Identification of target antigens that are expressed by tumor cells from different individuals enables to develop T cell immunotherapy protocols that could be broadly applied. Patients could be primarily sensitized *in vivo* by antigen immunization. Then, peripheral blood lymphocytes or vaccine-draining lymph nodes could be secondarily sensitized *in vitro* with the same antigen. To improve tumor reactivity, T cell clones with appropriate antigen specificity could be identified. Once isolated from bulk cultures, these clones could be expanded *in vitro* to therapeutic levels and infused into the patient. Several reasons exist to believe that cloned T cells can be highly effective for adoptive immunotherapy: cloned T cells were highly effective in elimination of established tumors in several mouse models and the phenotype of transferred cells can be manipulated by selecting a clone with specific characteristics (Shilyansky *et al.* 1997, Hanson *et al.* 2000).

On the other hand, there are many reasons why *in vitro*-derived tumor-reactive T cells might fail to eradicate tumor cells *in vivo*. For example: 1) the uniformity of antigen expression on tumor cells could influence the efficacy of T cell therapy as the outgrowth of tumor antigen loss variants arise; 2) tumor antigens can be masked by other proteins or lost by subsequent mutations or deletions in tumor variants; 3) tumor cells produce immunosuppressive cytokines such as IL-10, TGF-β or prostaglandins that interfere with activation of T cells; 4) modulation of tumor vasculature results in poor lymphocyte infiltration into the tumor mass; 5) processing and presentation of tumor antigens by antigen

presenting cells is not optimal or costimulation is absent; 6) induction of anergy, apoptosis or elimination of infused tumor-reactive T cells can occur (Jager *et al.* 1997, Musiani *et al.* 1997, Staveley-O´Caroll *et al.* 1998, Theobald *et al.* 1998, Cohen *et al.* 2001).

**Table 1.** Antigens expressed by cancer cells that can be potentially recognized by T lymphocytes (Renkvist et al. 2000, Van den Eynde and Van den Bruggen 1997).

| Tumor antigen group | Name | Cancer type |
|---|---|---|
| *Virus-associated tumor antigens* | HPV E6 and E7 | Cervical carcinoma |
| | EBV LMP-1, EBNA-1 | Hodgkin's disease, nasopharyngeal carcinoma |
| *Product of mutated gene or chromosomal rearrangement* | BCR/ABL | Chronic myeloid leukemia |
| | PML/RARA | Acute promyelocytic leukemia |
| | TEL/AML1 | Precursor B acute lymphoblastic leukemia |
| | | Melanoma |
| | Beta katenin | Melanoma |
| | MUM-1-3, CDK-4 | |
| *Product of overexpressed normal gene* | hTERT[c] | ~90 % of tumors |
| | CEA | Epithelial tumors |
| | Her-2/neu | Breast and other epithelial cancers |
| | WT-1 | Leukemia and epithelial tumors |
| *Tissue-specific differentiation antigens* | Tyrosinase, melan A, gp100, TRP-1, TRP-2 | Melanoma |
| | PSA | Prostate cancer |
| *Embryonic proteins* | MAGE, BAGE, GAGE, NY/ESO-1 | Melanoma and other epithelial tumors |
| *Idiotypic proteins* | Imunoglobulin chains | B non-Hodgkin's lymphoma, multiple myeloma |

*Genetically engineered cells*

The application of efficient gene-transfer techniques to lymphocyte populations might overcome some of the limitations of specific cellular transfer in the therapy of cancer. Approaches to gene therapy of neoplastic disease include, but are not limited to, gene modified tumor cells or antigen presenting cells as vaccines, introduction of wild-type tumor suppressor genes into tumors with mutated nonfunctional tumor suppressor genes or lost genes, introduction of oncogene antisense, and gene-modified effector cells. In this paragraph, we discuss the gene-modified effector cells, the other strategies of gene-transfer techniques extend beyond the scope of this article.

Genetically engineered lymphocytes possess unique functional characteristics, which can be exploited in novel treatment protocols. First, such transfer into humans was performed by Rosenberg *et al.* (1990) demonstrating the feasibility and safety of using retroviral-mediated gene transduction to introduce the gene coding for resistance to neomycin into human TIL before their infusion into patients with metastatic melanoma (Rosenberg *et al.* 1990). The infusion of genetically engineered neomycin-marked T cells has been recently used to assess transfer of immunity, persistence in the peripheral blood, and migration of these cells to lymph nodes or tissues (Rooney *et al.* 1998, Roskrow *et al.* 1998, Walter *et al.* 1995, Riddel and Greenberg 1995, Brodie *et al.* 1999). The ability of lymphocytes to traffic to tumor deposits can be harnessed to deliver therapeutically active molecules to the tumor environment. Hence, such cells can be transfected with cytokine or other genes. Specific changes in the local milieu may augment the host immune response, while avoiding the toxicity associated with high-dose systematic administration of cytokines such as interleukin 2 (IL-2). IL-2 can prolong the lifespan of transferred cells, and TNF-$\alpha$ can mediate the regression of tumors. In

addition to the few examples mentioned above, the construction of chimeric T cell receptors (TCR) has led to direct coupling of the recognition and effector phases of the immune response. Stancovski *et al.* (1993) transfected CTL with the gene encoding a single-chain chimeric TCR gene with specificity for Neu/HER2, a known breast carcinoma-associated antigen. These modified CTL demonstrated specific recognition and lysis of target cells expressing Neu/HER2. Similar examples of chimeric T cell receptor constructs for other neoplasms have been reported (Geraghty and Mule 1998). Genetically modified lymphocytes show great promise for use as therapeutic vehicles in gene therapy of cancer and several ongoing clinical trials are recently testing the safety and efficacy of such approaches.

## Applications

*Viral disease*

In persistent virus infections where viral replication is controlled by specific CTL such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV), adoptive transfer of CTL generated from the original marrow donor to patients immunosuppressed following allogeneic SCT had proved beneficial in reducing the incidence of serious viral disease (Rooney *et al.* 1998, Roskrow *et al.* 1998, Walter *et al.* 1995, Riddel and Greenberg 1995). EBV causes potentially lethal immunoblastic lymphoma in patients receiving T-cell-depleted allogeneic SCT. Donor-derived EBV-specific T lymphocytes were used for prophylaxis of post-transplant immunoblastic lymphoma in 39 children considered to be at high-risk for EBV-induced lymphoma. EBV-specific CTL lines persisted in recipients for as long as 18 weeks and prevented lymphoma development in all patients. In addition, two patients with already established immuno-blastic lymphoma responded fully to EBV-specific CTL infusion (Rooney *et al.* 1998). These encouraging results in transplant recipients suggest that T cell therapy may be applicable to other malignancies that contain EBV genomes, such as nasopharyngeal carcinoma and a subset of Hodgkin's disease. In one study, three patients with multiply relapsed Hodgkin's disease were treated with autologous EBV-specific CTL. The CTL persisted for more than 13 weeks postinfusion and retained their potent antiviral effects *in vivo*, thereby enhancing the patient immune response to EBV (Roskrow *et al.* 1998).

The occurrence of life-threatening CMV disease after an allogeneic SCT is closely correlated with the absence of CMV-specific $CD8^+$ T cell responses. Thus, adoptive transfer of CMV-specific CTL clones isolated from SCT donor can restore protective immunity against CMV. Up to $10^9$ $CD8^+$ CTLs/$m^2$ were infused to 14 patients at risk of posttransplant CMV disease. The therapy did not cause any toxicity, CMV-specific CTLs persisted for more than 12 weeks, and no patient developed CMV disease after therapy (Walter *et al.* 1995). Another evidence demonstrating the potential of adoptive cellular therapy came from HIV positive patients (Riddel and Greenberg 2000, Brodie *et al.* 1999). Brodie e*t al.* (1999) transferred HIV-1-specific CTL to three HIV positive patients demonstrating that the infused CTLs retained lytic function, accumulated adjacent to HIV-infected cells in lymph nodes and transiently reduced the levels of circulating productively infected $CD4^+$ cells. These studies provide direct evidence that virus-specific CTLs mediate strong antiviral activity and indicate that the development of immunotherapeutic approaches to sustain a strong CTL response against target antigens may be useful in other diseases, namely in cancer.

*Cancer*

The discovery of tumor-specific genes that encode tumor antigens recognized by T cells (see Table 1) has provided opportunities for adoptive transfer therapy. CTLs could be senzitized *in vivo* or *in vitro* by antigen immunization. Selection of individual T cell clones with higher degree of antigen specificity and tumor reactivity may further improve the treatment outcome as shown in several preclinical studies (Shilyansky *et al.* 1997, Hanson *et al.* 2000). The ability to select a specific T cell phenotype for adoptive transfer led to the initiation of clinical trials with gp100 peptide-specific T cell clones for treatment of patients with metastatic melanoma. Using defined antigens in mouse models, several reports have shown a correlation between T cell avidity *in vitro* and efficacy of adoptive transfer *in vivo* (Alexander-Miller *et al.* 1996, Dudley *et al.* 2001). Dudley *et al.* (2001) also demonstrated safety and feasibility of cloned T cell transfer even though they lacked clinical effectiveness (one minor response and one mixed response in 13 patients with metastatic melanoma). These data suggested that transfer of different or additional cell types is required for successful therapy. In addition, in contrast to transferred and long-lived virus-specific CTLs (Rooney *et al.* 1998, Roskrow *et al.* 1998, Walter *et al.* 1995, Riddel and Greenberg 2000),

transferred T cells in Dudley's study were undetectable 2 weeks after infusion. The generation of sufficient numbers of CTLs from nonimmunized individuals for adoptive immunotherapy presents additional technical challenges.

## Graft-versus-leukemia T cells and graft-versus-tumor effect

The graft-versus-tumor (GVT) effect seen after allogeneic hematopoietic SCT for human malignancies represents the clearest example of the ability of the human immune system to eradicate cancer. Barnes *et al.* (1956) first suggested the existence of a GVT effect when they noted eradication of leukemia in irradiated mice receiving allogeneic marrow transplant (Barnes *et al.* 1956). The evidence for such an effect in humans came from studies reporting that relapse rates following allogeneic transplantation were markedly less in patients who developed graft-versus-host (GVHD) compared to those who did not (Weiden *et al.* 1979). Subsequent studies demonstrated that the post-transplant relapse rate was higher in patients receiving T cell depleted grafts in an attempt to alleviate GVHD. Donor T cells thus play a major role in GVT effect. Further verification of the GVT effect came from attempts to treat patients for post-transplant leukemic relapse by infusing donor lymphocytes. Sustained complete responses were seen in more than 70 % patients with chronic myeloid leukemia and in some patients with other hematological malignancies (Kolb *et al.* 1995). With increased evidence of the GVT effect and development of methods to better exploit it, clinical research is beginning to focus on allogeneic hematopoietic SCT more as an immunotherapeutic approach (Slavin 2001), rather than solely as a way to rescue patients from high-dose myeloablative therapy.

## Conclusions

Although there is accumulating evidence for the potential of transfer of either polyclonal or antigen-specific T cells to recognize and kill tumor cells, many aspects of the interaction between tumor and immune cells are still not well understood. Ongoing and future basic and clinical research in the field of tumor immunology might enable us to improve the frequently limited efficacy of this cell-based form of therapy

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