Simultaneous Generation of CD8+ and CD4+ Melanoma-Reactive T Cells by Retroviral-Mediated Transfer of a Single T-Cell Receptor

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Abstract

Adoptive immunotherapy of cancer requires the generation of large numbers of tumor antigen–reactive T cells for transfer into cancer patients. Genes encoding tumor antigen–specific T-cell receptors can be introduced into primary human T cells by retroviral mediated gene transfer as a potential method of providing any patient with a source of autologous tumor-reactive T cells. A T-cell receptor–specific for a class I MHC (HLA-A2)–restricted epitope of the melanoma antigen tyrosinase was isolated from a CD4+ tumor-infiltrating lymphocyte (TIL 1383I) and introduced into normal human peripheral blood lymphocytes by retroviral transduction. T-cell receptor–transduced T cells secreted various cytokines when cocultured with tyrosinase peptide–loaded antigen-presenting cells as well as melanoma cells in an HLA-A2-restricted manner, and could also lyse target cells. Furthermore, T-cell clones isolated from these cultures showed both CD8+ and CD4+ transduced T cells could recognize HLA-A2+ melanoma cells, giving us the possibility of engineering class I MHC–restricted effector and T helper cells against melanoma. The ability to confer class I MHC–restricted tumor cell recognition to CD4+ T cells makes the TIL 1383I TCR an attractive candidate for T-cell receptor gene transfer–based immunotherapy. (Cancer Res 2005; 65(4): 1570-6)

Introduction

Adoptive transfer of T cells with in vitro antitumor activity has been used to treat cancer patients to circumvent difficulties in generating an antitumor immune response in vivo (1). However, isolating and/or expanding sufficient quantities of tumor reactive T cells is a difficult and laborious process for melanoma patients (2, 3) and extremely difficult for patients with other cancers. We recently described an approach to generating large numbers of autologous tumor-reactive T cells by retroviral mediated gene transfer as a potential method of providing any patient with a source of autologous tumor-reactive T cells. We and others have shown that retroviral transfer of TCR genes from tumor-reactive T cells is transferred to normal blood-derived T cells (4). We and others have shown that retroviral-mediated TCR gene transfer can confer tumor antigen (TA)-specific reactivity to the new T cells (4–7). This approach would provide an "off the shelf" reagent capable of engineering a patient’s T cells to recognize their cancer. The key step in this process is to identify TCRs with desirable properties that make them candidates for genetic transfer. We previously reported isolating a tumor-infiltrating lymphocyte (TIL) culture (TIL 1383I) from a melanoma patient that is specific for the melanoma antigen tyrosinase (8). TIL 1383I recognizes an MHC class I (HLA-A2*01) restricted epitope (368-376) of tyrosinase despite being a CD8+ T cell. This violates the standard paradigm of TCR/peptide/MHC recognition whereas CD4+ T cells recognize peptides bound to MHC class II molecules, whereas CD8+ T cells recognize peptides bound to MHC class I molecules. TCRs that can mediate MHC class I–restricted tumor cell recognition in the absence of CD8 have properties that are distinct from TCRs expressed by the majority of T cells found in the normal repertoire in that they violate principles of antigen recognition by CD4+ and CD8+ T cells. In the current study, we describe the transfer of the TIL 1383I TCR to peripheral blood lymphocyte (PBL)–derived normal human T cells. The resulting TCR-transduced T cells recognized antigen-presenting cells loaded with tyrosinase peptide, as well as melanoma cells in an HLA-A2 restricted manner. Furthermore, clones isolated from these cultures showed both CD8+ and CD4+ T cells recognized HLA-A2*/tryosinase+ melanoma cells, giving us the possibility of engineering class I MHC–restricted effector and T helper cells against melanoma.

Materials and Methods

Cell Lines. Tumor cell lines and TILs were established from melanoma patients at the Surgery Branch, National Cancer Institute. Tumor cells, T cells, and Jurkat cells were maintained in RPMI 1640 (Mediatech, Herndon, VA) supplemented with 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA) 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L L-glutamine (Mediatech; cRPMI). 293GP retroviral producer cells were maintained in DMEM supplemented as above (cDMEM). TILs were maintained in X-Vivo 15 (BioWhittaker, Walkersville, MD) supplemented with 10% heat inactivated, pooled human AB serum (Valley Biomedical, Winchester, VA) and 6,000 IU/mL rhIL-2 (Chiron Co., Emeryville, CA).

Retroviral Vector Construction. Full-length cDNAs encoding the TIL 1383I TCR were amplified by PCR using cloning primers designed from genomic sequences of the α and β chain genes as described (9) and ligated into the pCR2.1 cloning vector (Invitrogen Life Technologies). Their sequences were determined using BigDye Terminator Cycle Sequencing kits and analyzed using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). The TIL 1383I TCR genes were subcloned into the SAMEN CMV/SRα retroviral vector (9) to create the A9.3 retrovirus.

Retroviral Production and Transduction. 293GP cells (70-90% confluent) were transiently cotransfected in 10-cm tissue culture dishes with 3 μg A9.3 retroviral plasmid and 3 μg of a plasmid containing the vesicular stomatitis virus envelope gene using LipofectAMINE and PLUS reagents (Invitrogen Life Technologies) as described (9); 10 μl of fresh retroviral supernatants (cRPMI for Jurkat cells or cDMEM for human PBL) were harvested at 48 and 72 hours post-transfection.

PBL from four normal donors (designated B, D, F, and V) were isolated by density gradient centrifugation using Lymphocyte Separation Medium (Mediatech), suspended at 106 cells/mL in fresh retroviral supernatant containing 8 μg/mL polybrene.
Figure 1. The A9.3 retrovirus was created by inserting the TIL 1383i TCR genes into the SAMEN CMV/SRα retroviral vector. The vector is composed of a Moloney murine leukemia virus backbone. The 5′ long-terminal repeat (LTR) has been modified by replacing the R and U segments with a CMV IE promoter enhancer to promote high level transient expression in 239GP producer cells. Other key elements include the 6 packaging signal, splice donor (SD) and splice acceptor (SA), an internal SRα promoter to allow expression of a second gene, TCR β and α chains inserted into unique Sall and XhoI restriction sites respectively and an internal ribosomal entry site (IRES) cassette for expression of a G-418 resistance gene.

(Systems, Minneapolis, MN). 200 IU/mL rhIL-2 was added to PBL. All cells were transduced by spinoculation in a centrifuge at 1,000 × g for 90-minute at 32°C in 24-well tissue culture plates (1 mL per well). Four hours post-spinoculation, 1 mL of fresh medium supplemented as above was added to wells and the cells cultured overnight. This process was repeated once 24 hours later for Jurkat cells or twice at 24 and 48 hours later for primary T cells. Transduced Jurkat cells were selected by the addition of 2.0 mg/mL G-418 (Research Products Intl, Hanover Park, IL) for 7 days then assayed for anti-tyrosinase reactivity. Transduced T cells were selected by addition of G-418 (0.5 mg/mL for donors D and V, 1.0 mg/mL for donors B and F) for 3 to 5 days then expanded by culturing 2.5 × 10^5 cells with 4 × 10^5 irradiated (5,000 rads) allogeneic peripheral blood mononuclear cells in the presence of 30 ng/mL OKT3 and G-418 (concentrations as above) in upright 25-cm² tissue culture flasks. 300 IU/mL rhIL-2 was added day 2. Cultures were replenished with fresh IL-2 and G-418 containing medium on days 5, 8, and 11 and were assayed and frozen for future experiments on day 14.

T Cell Cloning. TIL 1383i TCR transduced T cells were cloned in limiting dilution as described (4) or by single cell sorting of T cells stained with FITC conjugated anti-BV12 (Pierce, Rockford, IL) and PE conjugated anti-CD4 or CD8 (BD PharMingen, San Diego, CA) monoclonal antibodies using a MoFlo HTS cell sorter (DakoCytomation, Carpinteria, CA) at a density of 1 cell/well of 96-well tissue culture plate. Growth positive clones were tested for antigen recognition and reactive clones were expanded in the presence of OKT3 and IL-2 as above (excluding G-418) for later experiments.

Antigen Recognition Assays. Transduced cells were tested for anti-tyrosinase reactivity by coculturing responder cells with stimulator cells in a 1:1 ratio overnight in 200 µL of RPMI in 96-well U-bottomed plates. Stimulator cells included tumor cells or peptide loaded T2 cells. T2 cells were peptide pulsed by incubating 10^6 cells/mL with MART-1 27-35 (AAGIGLVT) or tyrosinase 368-376 (YMDGTMSQV) peptide for 2 hours at 37°C. 5 ng/mL phorbol 12-myristate 13-acetate (Sigma) was added to Jurkat cell cocultures to enhance signaling. The amount of cytokine released [IFN-γ, granulocyte macrophage colony-stimulating factor (GM-CSF), IL-4, tumor necrosis factor-α or IL-2] was measured by ELISA (Pierce; R&D Systems, Minneapolis, MN).

Cell Lysis Assays. Transduced bulk cultures and clones were tested for their ability to lyse peptide loaded T2 cells or melanoma cells. T2 cells were loaded with 2 µg/mL tyrosinase or MART-1 peptide as above. Tumor cells and peptide loaded T2 cells were then labeled for 1 hour with 25Cr (200 µCi) at 37°C. Labeled target cells were then washed thrice with PBS then plated in triplicate wells of a 96-well tissue culture plate in a volume of 100 µL of RPMI per well. Effector cells were added in a volume of 100 µL of RPMI per well at E/T ratios as described in the figure. 1% SDS was added to selected target wells to determine maximum 51Cr release. Plates were incubated 4 hours at 37°C; 50 µL supernatant were harvested and plated onto Lumaplate-96 well scintillation plates (Perkin-Elmer, Boston, MA) and dried overnight. Sample plates were counted the next morning on a TopCount analyzer (Perkin-Elmer). The % specific lysis was determined using the following formula [(Experimental cpm – spontaneous cpm)/(Maximum cpm – spontaneous cpm)] × 100.

Immunofluorescence. Transduced bulk cultures and clones were stained for 30 minutes on ice with the following antibodies. FITC conjugated anti human BV12 antibody (Pierce), FITC labeled isotype control, PE and/or FITC labeled anti human CD8 and CD4 and isotype controls (BD PharMingen). Analysis was done on a FACSscan flow cytometer (BD Biosciences, San Jose, CA).

Results

The A9.3 Retrovirus Functionally Transfers the TIL 1383i TCR to Alternate Effectors. We constructed the A9.3 retrovirus encoding the α (AV4s1) and β (BV12s2) chains of the TIL 1383i TCR (Fig. 1). The functionality of the A9.3 retrovirus was first confirmed by transducing Jurkat cells and assaying them for peptide and/or tumor cell recognition by measuring IL-2 secretion. G-418 resistant cells expressed TCR BV12 as detected by flow cytometry (data not shown) and secreted IL-2 when cocultured with T2 cells loaded with tyrosinase peptide or with HLA-A2/tyrosinase+ melanoma cells (Fig. 2). As expected, transduced cells did not recognize T2 cells loaded with a control peptide (MART-1) or HLA-A2/tyrosinase+ melanoma cells. These results show the A9.3 retrovirus can transfer the TIL 1383i TCR and confer HLA-A2 restricted antigen specificity for tyrosinase to alternate effectors. Furthermore, tumor cell recognition was independent of CD8 since Jurkat cells lack CD8 expression. Therefore, the full specificity and function of the original CD4+ TIL 1383i was conferred by genetic transfer of the TCR.

Transfer of the TIL 1383i TCR to Primary T Cells. The ability to transfer the HLA-A2 anti-tyrosinase reactivity from TIL 1383i to normal human T cells was evaluated by transducing peripheral blood mononuclear cells from four normal donors (designated B, D, F, and V). Activated T cell cultures were transduced with A9.3 retroviral supernatants then cultured and expanded in the presence of G-418 to select for TCR gene–modified cells.

Figure 2. Transduction of Jurkat cells with the A9.3 retrovirus confers HLA-A2-restricted tyrosinase reactivity and melanoma cell recognition. Wild-type Jurkat (10⁶), TIL 1383i TCR Jurkat, or TIL 5 TCR Jurkat were cocultured with 10⁵ T2 cells loaded with 2 µg/mL tyrosinase peptide or 2 µg/mL MART-1 peptide, an irrelevant HLA-A2 restricted melanoma antigen for negative control. 1300 Mel and 1383 Mel are HLA-A2+ melanoma cell lines. 586 Mel and 624-28 Mel are HLA-A2+. TIL 5 Jurkat expresses a transferred MART-1-specific TCR. Columns, mean IL-2 release in triplicate wells. Representative of three independent experiments.

www.aacrjournals.org 1571 Cancer Res 2005; 65: (4). February 15, 2005
Transduced T cell bulk cultures had very low expression of TCR BV12 by flow cytometry (data not shown). Cultures were assayed for reactivity with tyrosinase peptide loaded T2 cells and tumor cell lines in cytokine release assays. Significant cytokine release (defined as >100 pg/mL and twice the specific negative control value) was observed from transduced T cells from all donors when stimulated with tyrosinase loaded T2 cells compared with culture with negative control peptide (Fig. 3A). Three of four transduced cultures (B, D, and F) specifically secreted significant amounts of both GM-CSF and IFN-γ, although only donor B secreted IFN-γ levels comparable to GM-CSF. Transduced donor V cells secreted significant but low levels of GM-CSF but not IFN-γ. No antigen-specific cytokine release was detected from untransduced control donor cells in response to peptide loaded T2 cells (data not shown). Transduced cells from donors B, D, and F secreted 5- to 7-fold more GM-CSF and/or IFN-γ in response to culture with HLA-A2+ melanoma lines (Mel) 1300 and 624 compared with HLA-A2- 624-28 Mel or an HLA-A2+ renal cell carcinoma (RCC) UOK131 which is tyrosinase negative (Fig. 3B). No specific cytokine release was detected from transduced donor V cells in response to tumor cells. IFN-γ secretion by transduced donor D and F cultures was detected by ELISA but below the threshold value of 100 pg/mL in all but one sample (D with 624 MEL), whereas transduced donor B cells secreted both GM-CSF and IFN-γ at significant levels. No specific cytokine secretion was detected from untransduced donor cultures stimulated with

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**Figure 3.** Cytokine secretion by TIL 1383I TCR-transduced bulk T-cell cultures in response to antigen stimulation. Mean GM-CSF (black columns) and IFN-γ (white columns) secretion by 10⁵ transduced T cells in triplicate wells representative of four independent experiments. A, four TCR-transduced bulk T-cell cultures (D, F, V, and B) were cocultured with T2 cells loaded with 2 μg/mL tyrosinase or 2 μg/mL MART-1 control peptide. Transduced T cells secrete significant cytokine (>100 pg/mL and twice the negative control) in response to tyrosinase peptide compared with MART-1. B, 3 of 4 transduced bulk T cell cultures secrete significant cytokine in response to HLA-A2+ melanoma cells. 1300 Mel and 624 Mel are HLA-A2+ melanoma lines, 624-28 is an HLA-A2- melanoma line and RCC UOK131 is an HLA-A2+ renal cell carcinoma (tyrosinase−) for antigen control. C, cytokine release by 25,000 TIL 1383I in response to peptide-pulsed T2 cells and tumor cells for comparison.

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**Figure 4.** Relative Expression of Transferred TCR by transduced T-cell clones. Transduction clones (10⁴) were stained using a monoclonal antibody–specific for the TIL 1383I TCR β chain BV12 (dark line). Clones were also stained with a FITC-conjugated IgG2a isotype control (shaded area). All clones express BV12 with varied levels of expression.
tumor cells (data not shown). Transduction of primary T cells with A9.3 retrovirus therefore resulted in cultures specific for tyrosinase:368-376 and in three of four cases, cultures that recognized HLA-A2+ melanoma cells. Low cytokine release by G-418 resistant donor V cells in response to peptide, in conjunction with no significant cytokine release in response to tumor cells indicate a poor transduction efficiency resulting in a low frequency of cells expressing adequately high levels of transferred TCR.

**CD4+ and CD8+ Transduced T-Cell Clones Recognize Melanoma Cells.** We generated a panel of T-cell clones from transduced donor cultures by limiting dilution or single cell sorting for CD4+/BV12+ or CD8+/BV12+ and further expanded those clones found reactive to antigen. Expanded clones were positive for TCR BV12 expression by flow cytometry (Fig. 4) and were stained for CD4 and CD8 to confirm the coreceptor phenotype of each clone.

We tested clones for sensitivity to antigen by coculture with T2 cells loaded with decreasing concentrations of tyrosinase and the amount of cytokine released measured by ELISA (Fig. 5A). Both CD4+ and CD8+ clones secreted increasing levels of cytokine in response to increasing tyrosinase peptide stimulation. TIL 1383I begins to secrete cytokine when stimulated with T2 cells loaded with 1 to 10 μg/mL tyrosinase, whereas TCR-transduced clones secreted cytokine when stimulated by T2 cells loaded with 10 to 100 μg/mL tyrosinase. GM-CSF was the predominant cytokine released from all clones except CD4+ clone D2F4 (donor D derived), which secreted comparable amounts of IFN-γ.

We tested clones for recognition of a panel of tumor cells and again detected cytokine release from both CD4+ and CD8+ cells in response to HLA-A2+ melanomas 1300 and 624, but not the negative control tumors (624-28 Mel or RCC UOK131; Fig. 5B). Indeed, several clones were highly reactive to HLA-A2+ melanoma cells, secreting ng/mL quantities of IFN-γ and/or GM-CSF. Clones V8B4, V8C4, and V8B11, which secrete GM-CSF in response to tyrosinase peptide and HLA-A2+ melanoma cells, were derived from transduced donor V cells. This shows that although transduction efficiency in donor V was too low for detection of tumor cell recognition in bulk cultures, highly reactive cells could be isolated from the culture. Furthermore, clone D2F4 (donor D) secreted tumor necrosis factor-α (100-500 pg/mL) in specific

Figure 5. Relative avidity of CD4+ and CD8+ T cell clones isolated from TCR transduced bulk cultures. A, GM-CSF (●) and IFN-γ (○) secretion by 5 × 10⁴ transduction clones in response to decreasing concentration of tyrosinase peptide. T-cell clones were cocultured for 24 hours with T2 cells loaded with peptide as indicated. Dots are mean cytokine release from triplicate wells of 5 × 10⁴ T cells. B, CD4+ and CD8+ T cell clones recognize HLA-A2+ melanoma cells (1300 Mel, 624 Mel) but not HLA-A2- melanoma (624-28 Mel) or HLA-A2+ nonmelanoma control (RCC UOK131) as shown by specific secretion of GM-CSF (black columns) and/or IFN-γ (white columns). Columns, mean cytokine release of triplicate wells of 5 × 10⁴ T cells representative of four independent experiments. Cytokine release by 25,000 TIL 1383I for comparison.
response to tyrosinase loaded T2 cells and HLA-A2+ melanoma cells, whereas clones F4D71, and F4E91 (donor F) secreted 100 to 200 pg/mL IL-4 in specific response to both antigen and HLA-A2+ melanoma cells (data not shown).

**Lysis of Melanoma Cells by TCR-Transduced T Cells.** We further tested both transduced bulk cultures and selected clones for the ability to kill target cells in lysis assays. Bulk cultures were cocultured with 51Cr labeled T2 cells preloaded with 2 μg/mL tyrosinase peptide or control MART-1 peptide (Fig. 6A). A lack of sufficient numbers of transduced donor V cells coupled with an inability to obtain additional cells from this donor prevented us from determining the lytic capacity of this culture. Specific lysis between 17% and 50% of tyrosinase loaded T2 cells was observed by transduced donor B, D, and F cells at 100:1 E/T ratio. Bulk cultures were also cultured with 51Cr labeled HLA-A2+ and HLA-A2+ melanoma lines 624 and 624-28, respectively, with specific lysis of the HLA-A2+ melanoma between 8% and 20% at 100:1 E/T ratio (Fig. 6B). Selected CD8+ clones were also tested for CTL capability by coculture with peptide loaded T2 cells and melanoma lines as above (Fig. 6C). Clones V8C4 and V8B11 were both able to specifically lyse tyrosinase loaded T2 cells and HLA-A2+ 624 Mel.

From these data, we concluded that through transduction of primary human T cells with the A9.3 retrovirus encoding the TIL 1383I TCR, we can engineer antigen-specific T-cell responses to melanoma in an HLA-A2 restricted manner. The resulting transduced cultures contain both CD8+ and CD4+ T cells that are highly reactive and can secrete a variety of cytokines, including Th1 and Th2 cytokines, upon interaction with tumor cells and/or kill HLA-A2+ melanoma cells.

**Discussion**

Recent reports of the functional transfer of TCRs specific for various tumor antigens show the interest in TCR gene transfer for adoptive immunotherapy for cancer (4–6, 10, 11). However, numerous factors must be considered when choosing a TCR for gene transfer. Antigen specificity alone may be insufficient criteria for choosing a TCR. Factors such as MHC restriction of the TCR must be considered as this will define the patient pool to be treated. We have chosen HLA-A2 restricted T cells because roughly 50% of melanoma patients in the United States express this HLA allele. Therefore, an HLA-A2 restricted TCR has the potential to treat the largest number of patients.

Avidity of T cells is important to consider since high functional avidity has been shown to correlate with tumor recognition in vitro (12) and tumor regression in vivo (13). Therefore, it is reasonable to conclude that TCRs expressed by high avidity T cells would be superior to those expressed by low avidity T cells. However, Jurkat and normal T cells expressing the TIL 1383I TCR have intermediate to low avidity for antigen yet still efficiently recognize tumor cells representing an exception to this rule. It is difficult to fully characterize the functional avidity of the T-cell clones tested in this study. Transduced clones stained positive for BV12 yet this may not accurately reflect the number of functional transferred TCRs on the cell surface because the introduced V chain may pair with the endogenous V chain and vice versa, diluting the number of functionally paired TCRs. However, our previous work with a TCR–murine T-cell lymphoma showed that the expression level of introduced TCR did correlate with functional avidity (9). In this case the avidity of the clones tested was not widely variable. Most clones secreted >100 pg/mL cytokine at peptide concentrations between 10 and 100 ng/mL. Clones V8C4 and V8B11 however, seemed to have slightly lower avidity with higher level BV12 expression than clone F8B5 which expressed far lower levels of BV12 (Fig. 4) indicating some variability in clonal response to activation mediated through the transferred TCR. The use of high titer stable retroviral producer cell lines, necessary for any clinical application, should increase the infections titer of retrovirus over the transient transfection method employed in this initial analysis. We are also beginning to investigate modifications of the A9.3 retroviral vector in an attempt to increase retroviral production and functional expression of the TIL 1383I TCR with the goal of increasing avidity.

Cancer Res 2005; 65: (4). February 15, 2005 1574 www.aacrjournals.org
Another factor in considering a TCR for gene transfer is the dependence on CD8 for antigen recognition. CD8 enhances the stability of the TCR/pMHC complex and promotes signal transduction through the recruitment of the protein kinase lck to the CD3 complex (14). It has been suggested that CD8-independent antigen recognition by T cells indicates expression of a high affinity TCR (15). However, the vast majority of MHC class I restricted T cells are CD8 dependent and would therefore have to be present in our normal repertoire because they would likely be clonally deleted or rendered immunologically tolerant during T-cell development. In either case, protocols could be developed to remove these cells from the lymphocyte population before transduction. If protocols for expanding Tregs in vitro become more developed the TIL 1383T CR may provide an interesting opportunity to generate Tregs with known antigen specificity for the study of Treg function.

It is worth noting the majority of the T cells expressing the TIL 1383 TCR secreted GM-CSF but not IFN-γ. Of significance was that both the CD4 + and CD8 + T-cell clones from all three donors exhibited cytokine secretion pattern suggesting a feature of the 1383T CR that may influence the phenotype of the T cells. Whereas most investigators favor T cells secreting type I cytokines such as IFN-γ, immune monitoring of clinical trials have generally failed to find a correlation between IFN-γ and clinical responses. Indeed, clones V8C4 and V8B11 failed to secrete IFN-γ in response to tumor cells yet both were able to lyse tumor cell targets. This is consistent with our previous data involving transduction of a MART-1-specific TCR into normal T cells (4). In that study, some transduced clones lysed tumor cells while also secreting cytokine and did not lyse tumor cells. However, a correlation between GM-CSF secretion by adoptively transferred TIL and clinical response has been reported (28). GM-CSF secreting tumor cells have been shown to have enhanced immunogenicity in animal models leading to effective antitumor immunity (29) and tumor regression has been observed in clinical trials of GM-CSF secreting tumor cell vaccines (30, 31). Therefore, it is possible that T cells engineered to express the TIL 1383T CR will not only provide potent antitumor effectors, their secretion of GM-CSF may enhance the endogenous antitumor immunity. In addition, the secretion of the Th2 cytokine IL-4 by some transduced CD4 + clones shows we may be able to generate immune responses beyond the function of the original TIL 1383T which was not shown not to secrete IL-4 in response to antigen stimulation (8).

Because the goal of immunotherapy for cancer is to eliminate malignant cells, we consider the CD8-independent tumor cell recognition exhibited by TIL 1383T CR gene modified T cells to be a critical feature of a cloned TCR. TCRs with this property make it possible to treat patients with MHC class I restricted T helper cells and possibly more potent CTL. Given this TCR was isolated from an MHC class I restricted CD4+ T cell, cells bearing this TCR may represent a class of T cell not usually expected to be present in our normal repertoire because they would likely be clonally deleted or rendered immunologically tolerant during T-cell development.

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