Published in final edited form as:

Clin Cancer Res. 2010 December 1; 16(23): 5852–5861. doi:10.1158/1078-0432.CCR-10-1280.

Development of Human Anti-Murine T-cell Receptor Antibodies in Both Responding and Non-responding Patients Enrolled in TCR Gene Therapy Trials

Jeremy L. Davis¹, Marc R. Theoret¹, Zhili Zheng¹, Cor Lamers², Steven A. Rosenberg¹, and Richard A. Morgan¹

¹ Surgery Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD ² Department of Medical Oncology, Erasmus MC, Daniel den Hoed Cancer Center, Rotterdam, The Netherlands

Abstract

Purpose—Immune responses to gene-modified cells are a concern in the field of human gene therapy as they may impede effective treatment. We conducted two clinical trials in which cancer patients were treated with lymphocytes genetically engineered to express murine T cell receptors (mTCR) specific for tumor-associated antigens p53 and gp100.

Experimental Design—Twenty-six patients treated with autologous lymphocytes expressing mTCR had blood and serum samples available for analysis. Patient sera were assayed for development of a humoral immune response. Adoptive cell transfer characteristics were analyzed to identify correlates to immune response.

Results—Six of 26 (23%) patients post-treatment sera exhibited specific binding of human anti-mTCR antibodies to lymphocytes transduced with the mTCR. Antibody development was found in both responding and non-responding patients. Three of these six patients post-treatment sera mediated a 60 – 99% inhibition of mTCR activity as measured by a reduction in antigen-specific IFN- γ release. Detailed analysis of post-treatment serum revealed that antibody binding was beta chain specific in one patient whereas it was alpha chain specific in another.

Conclusions—A subset of patients treated with mTCR engineered T-cells developed antibodies directed to the mTCR variable regions and not to the constant region domains common to all mTCR. Overall, the development of a host immune response was not associated with the level of transduced cell persistence or response to therapy. In summary, patients treated with mTCR can develop an immune response to gene-modified cells in a minority of cases, but this may not affect clinical outcome.

Keywords

Immunity; gene therapy; T-cell receptor

Statement of Translational Relevance

To whom reprint requests should be sent: Richard A. Morgan, PhD, Surgery Branch, National Cancer Institute, National Institutes of Health, 10 Center Drive, Building 10, CRC, Room 3-5940, Bethesda, MD 20892, rmorgan@mail.nih.gov.

Conflict of interests: None

Human gene therapy has application not only in oncology, but also in the treatment of a variety of conditions as diverse as cardiovascular disease and HIV infection. The development of immunity to gene transfer components can be an obstacle to successful gene therapy. Our report describes a subset of patients enrolled in cancer gene therapy trials that developed an immune response to lymphocytes expressing murine T-cell receptors (mTCR). These responses were observed in both responding and non-responding patients suggesting that the development of immunity to mTCR does not preclude effective treatment. Because HLA-A2 transgenic mice can be used to derive mTCR against common tumor antigens such as p53 and CEA, the potential application of mTCR-based cell therapies has broad implications for the treatment of a variety of malignancies.

Introduction

Gene therapy has evolved significantly since the first report two decades ago, which demonstrated the safety and feasibility of human gene transfer (1). At the end of 2009, cancer research accounted for almost 70% of human gene transfer protocols that had been reviewed by the Recombinant DNA Advisory Committee, NIH (2). Our efforts have involved the genetic modification of human lymphocytes used in adoptive cell transfer (ACT) for the treatment of patients with metastatic melanoma. In a series of clinical trials involving 93 patients with metastatic melanoma treated with autologous tumor infiltrating lymphocytes (TIL) following a lymphodepleting regimen, an objective cancer regression rate of 56% was seen. Some patients experienced a clonal repopulation of T cells specific for the melanoma/melanocyte differentiation antigen, MART-1, which suggested that this self-antigen could be a useful target for cancer immunotherapy (3).

To bypass the need to obtain lymphocytes from a tumor specimen, a method was developed to transduce peripheral blood lymphocytes (PBL) with a retrovirus encoding a T cell receptor (TCR) that could recognize the MART-1 tumor-associated antigen. The TCR alpha and beta chains of a MART-1-reactive TIL clone were identified in a patient who demonstrated near complete regression of metastatic melanoma after adoptive cell transfer of TIL (3,4). Autologous PBL were transduced ex vivo with anti-MART-1 TCR genes and reinfused into 15 patients with widely metastatic melanoma. Although the response rate was 13% (2 of 15), less than that achieved with autologous TIL, the method proved that PBL engineered to express TCRs recognizing tumor-associated antigens could mediate the regression of large solid tumors in humans (4). Extensive screening of human T-cell clones that recognized the MART-1₂₇₋₃₅ epitope identified a second more highly reactive human TCR for use in ACT (5,6). In parallel work highly avid murine lymphocytes against gp100₁₅₄₋₁₆₂ and p53₂₆₄₋₂₇₂ epitopes were identified by immunization of human leukocyte antigen-A2 (HLA-A2) transgenic mice, and the genes encoding murine TCRs (mTCR) from those cells were used to transduce human lymphocytes as well (5,7-10).

In recent clinical trials these new high-avidity anti-gp100 and anti-MART-1 TCRs were used to treat patients with metastatic melanoma (6). Following a lymphodepleting chemotherapy regimen, 36 patients received autologous peripheral blood lymphocytes transduced with these TCRs (16 with murine anti-gp100 and 20 patients with human anti-MART-1). Objective tumor regression was seen in 19% and 30% of patients receiving the gp100- and MART-1-specific TCRs, respectively. In a third clinical trial, fourteen patients with a variety of epithelial cancers were treated with transduced cells expressing an anti-p53 murine TCR (M. Theoret, unpublished data) (7). One patient with a salivary gland cancer experienced objective, partial tumor regression and gene-modified cells were detected at one month in all patients tested.

Although gene transfer into human T lymphocytes can be clinically beneficial, host immune responses directed at transgene products, the viral vectors themselves or components of the gene transfer process could be observed in immune-competent patients as well as those with genetic immunodeficiency, chemotherapy-induced immune suppression and HIV infection (11–13). The purpose of the current analysis was to determine the incidence and nature of immune responses to transgenic mTCR engineered lymphocytes in patients enrolled in cancer gene therapy trials. An additional objective was the identification of specific immunogenic determinants, which could influence future use of xenogenic transgenes in human gene therapy trials, and potentially reveal novel factors associated with the development of host immunity.

Materials and Methods

Tumor cell lines and lymphocyte culture

HLA-A2⁺ melanoma cell lines (526, 624.38) and HLA-A2[−] cell lines (888, 938) were generated at the Surgery Branch of the National Cancer Institute (NCI; National Institutes of Health [NIH], Bethesda, MD) as previously described (14). Colon adenocarcinoma H508 (CCL-253), and lung adenocarcinoma H2087 (CRL-5922) were obtained from (American Type Culture Collection, Manassas, VA). Tumor cells were cultured in RPMI (Invitrogen, Rockville, MD) supplemented with 10% fetal bovine serum (FBS; Invitrogen), penicillin/streptomycin (50U/ml) and L-glutamine and maintained in a 37°C incubator at 5% CO₂. Peripheral blood mononuclear cells (PBMC) were collected by leukapheresis from melanoma patients or healthy donors, and cultured as described(5,6).

Production of retroviral supernatant and lymphocyte transduction

MSGV1-based retrovirus encoding the human TCR α and β chains specific for MART-1_{27–35} (MART-1 TCR) and NY-ESO-1_{157–165} (NY-ESO-1 TCR), and murine TCR α and β chains specific for human gp100_{154–162} (gp100 TCR), p53_{264–272} (p53 TCR), MAGE-A3_{112–120} (MAGE-A3 TCR) and CEA_{691–699} (CEA TCR) were developed for clinical use or pre-clinical evaluation and have been described elsewhere (5,6,8,15,16). Four additional retroviral vectors expressing either TCR α or β chains against gp100 and p53 were constructed using similar techniques. Transient retroviral supernatants and lymphocyte transduction was performed as described(17).

Antibody binding and flow cytometry

The presence of post-treatment antibody response to TCRs was assessed by detection of human IgG or IgM binding to TCR-transduced cells. TCR-transduced PBL were used 7–10 days after OKT3 stimulation. Patients' pre-treatment or post-treatment serum (25–50 μ l) was added to 2.5–5 $\times$ 10⁵ cells and incubated on a gentle rocker at 4°C for 1 hour. Cells were washed three times with PBS plus 0.1% human serum albumin then stained with anti-human IgG or anti-human IgM antibody (BD Biosciences, San Jose, CA). Surface staining of cell populations included assessment of transduction efficiency by anti-murine TCR β (BD Biosciences), anti-murine TCR V β 3 (clone KJ25, BD Biosciences), HLA-A2.1 tetramer specific for gp100 TCR, MART-1 TCR and NY-ESO-1 TCR (Beckman Coulter, Fullerton, CA), CD3 (BD Biosciences), or the relevant isotype controls. Immunofluorescence was measured as relative log fluorescence of live cells using a flow cytometer (Becton Coulter).

Serum inhibition assays

Serum inhibition of TCR function was assessed *in vitro* by antigen-specific IFN- γ secretion. Patient serum collected at time points before; during and after adoptive cell transfer was stored at −80°C until use. TCR-transduced allogenic PBL were used 7–10 days following

OKT3 stimulation. Cells were washed with PBS plus 0.1% human serum albumin, serum samples were thawed, and 25 μ l was added to 5×10^5 cells and incubated on a gentle rocker at 4°C for 1 hour. Cells were washed three times then co-cultured (1:1) with appropriate target cells overnight in a 96-well U-bottom plate. Cell-free supernatants were analyzed for IFN- γ levels by ELISA (Pierce Biotechnology, Rockford, IL).

Cell-mediated immune response

To test for the development of a cell-mediated immune response against TCR-transduced lymphocytes, modifications were made to an assay developed elsewhere (C. Lamers, personal communication). Briefly, patients' autologous untransduced lymphocytes and gene-modified (transduced) lymphocytes were expanded to sufficient quantities using the rapid expansion protocol (REP) described in this section. These cells were irradiated (40Gy) prior to use as stimulating cells. Responding cells included pre-treatment PBMC (negative control) and post-treatment PBMC that were suspended in complete medium and AIM-V (1:1 ratio) with OKT3 (30ng/ml) and IL-2 (300IU/ml). Irradiated autologous stimulating cells (1×10^7) were added to responding cells (1×10^6) in 20 ml medium and 200 μ l aliquots added per well of a 96-well U-bottom tissue culture plate. Cells were harvested, counted and re-stimulated with irradiated autologous cells every 7 days for 5 weeks. Following this period of stimulation cells were harvested, counted and placed in complete medium containing IL-2 (50IU/ml) alone for 2 days to eliminate the effects of OKT3 stimulation. Autologous stimulating cells were then labeled with CFSE and 5×10^5 were added to responder cells (1:1) in RPMI medium at 37°C overnight. Autologous untransduced and gp100 TCR transduced cells were incubated with CFSE-labeled antigen-positive tumor cells (624.38) as controls. The next day, cells were washed and stained with antibodies to CD137, CD3 and CD8 (BD Biosciences). Immunofluorescence was measured as relative log fluorescence of live cells gated on CD3-positive, CFSE-negative population using a flow cytometer. The ability of lymphocytes to lyse target cells was measured by ^{51}Cr release as described previously (14).

Results

Cancer gene therapy trials

Fifty-seven patients with metastatic cancer were treated at the Surgery Branch NCI starting in 2006 through 2008 in TCR gene therapy protocols approved by the NCI Institutional Review Board, the NIH Office of Biotechnology Activities, and the Food and Drug Administration. All patients provided informed consent prior to treatment. Forty-three patients with metastatic melanoma were administered autologous PBL expressing either mTCR recognizing an HLA-A*02-restricted epitope of melanoma antigen gp100 (19 patients) or human TCR recognizing an HLA-A*02-restricted epitope of melanoma antigen MART-1 (24 patients). Fourteen patients with a variety of solid tumors were treated with mTCR recognizing an HLA-A*02-restricted epitope of p53 (M. Theoret, unpublished data). The adoptive cell transfer of gene-modified lymphocytes in 36 melanoma patients receiving gp100- and MART-1-specific TCRs resulted in objective tumor regression in a subset of patients and has been reported elsewhere (6). In total, 26 patients treated with murine-derived TCR gene-modified cells (16 gp100 TCR and 10 p53 TCR) had blood and serum samples sufficient for analysis (Table 1). All patients were treated with a course of non-myeloablative, lymphodepleting chemotherapy followed by adoptive cell transfer and infusion of high dose IL-2. Patients were followed for clinical tumor response and peripheral blood samples were assayed for gene-modified cell persistence and biological activity (Table 2).

Serum IgG binding to TCR-transduced lymphocytes

To screen patients for a humoral immune response to mTCR gene-transduced cells, we assayed the patients' sera for antibody binding to gene-modified cells by immunofluorescence. gp100 and p53 TCR-transduced cells were incubated with the pre- and post-treatment sera from patients who received cells transduced with the murine derived gp100 and p53 TCRs. The presence of serum antibody binding to TCR-transduced PBL was then determined by flow cytometric analysis following addition of fluorescently labeled anti-human IgG antibody to the transduced PBL. Pre-treatment serum samples consistently showed background staining of anti-human IgG similar to isotype controls (< 1%). Compared to pre-treatment serum, post-treatment serum demonstrated anti-human IgG binding to TCR-transduced lymphocytes of 18% – 45% in 5 of 16 gp100 patients and 50% in 1 of 10 p53 patients (Figure 1A). In the remaining patients, serum antibody binding was equivalent in pre-treatment and post-treatment samples (data not shown). The time to detection of the post-treatment serum IgG response to the mTCR ranged from 3 to 6 months after cell transfer. Seventeen patients treated with human anti-MART-1 TCR-gene modified cells were similarly screened and none had evidence of post-treatment serum IgG binding to MART-1 TCR-transduced lymphocytes. Post-treatment serum IgM specific for transduced cells was not detected by antibody binding in the 6 patients with a post-treatment IgG response (data not shown). To assess for allo-reactivity of patient sera, post-treatment antibody-positive serum from gp100 mTCR treated patients was tested for specificity by binding to lymphocytes transduced with different mTCR (gp100, p53, MAGE-A3, CEA) or fully human TCR (MART-1, NY-ESO-1), and in repeated experiments antibody binding was only observed in gp100 mTCR transduced cells indicating that the immune response was specific for cells expressing the transgene of interest, and not an allo-response.

Serum inhibition of TCR function in vitro

To determine whether antibody binding to TCR-transduced lymphocytes could impact TCR function, we performed a co-culture of transduced lymphocytes with antigen-specific targets in the presence of patient serum and measured TCR function by IFN- γ release in cell culture supernatant. gp100 and p53 TCR-transduced lymphocytes were incubated with pre- or post-treatment serum prior to co-culture with antigen-positive tumor cells and supernatant was tested for IFN- γ by ELISA at 18–24 hours. Sixteen gp100 TCR patients and 10 p53 TCR patients were screened and three patients (2 gp100, 1 p53 TCR) demonstrated a 60 – 99% reduction of antigen-specific IFN- γ release when transduced lymphocytes were incubated with post-treatment serum (Figure 1B, C). Patient 14 (gp100 TCR) post-treatment serum at up to 10^4 dilution caused > 50% reduction in antigen-specific IFN- γ release by transduced PBL (data not shown). Serum inhibition was reversed when protein G was added to transduced PBL prior to co-culture, providing evidence for IgG-class specific antibody inhibition of TCR-specific function (Figure 2D).

TCR chain specificity of immune response

To determine the specificity of the immune responses detected, patient serum was used in antibody binding and serum inhibition assays utilizing cells expressing mTCRs specific for gp100, p53, MAGE-A3, and CEA as well as a fully human TCR targeting NY-ESO-1. Patients treated with cells transduced with the gp100 TCR (4 & 14) displayed post-treatment serum binding and inhibition of only gp100 TCR-transduced cells (Figure 2A, B). Post-treatment serum from patient 26 (p53 TCR treated) bound to and mediated >90% inhibition of activity of both p53 TCR and CEA TCR-transduced cells (Figure 2A, 3B).

Sequence analysis revealed that the p53 and CEA TCRs utilized the same β chain variable region gene (V β 26.1), which could account for the cross-reactivity of the patient's serum with this second murine TCR. The lack of cross-reactivity with all murine TCRs suggested

that the murine TCR constant region was unlikely the immunogenic determinant. Antibody binding specificity for the TCR α or β chains was further evaluated using constructs that expressed only the TCR α or β chain of the gp100 and p53 TCRs. Lymphocytes were simultaneously transduced with retrovirus to express one of four combinations of α and β chain pairs: 1) gp100 α /gp100 β , 2) gp100 α /p53 β , 3) p53 α /p53 β , 4) p53 α /gp100 β . Using patients 14 & 26 sera, an antibody-binding assay was performed. Consistent with previous experiments, patient 14 exhibited post-treatment IgG binding to lymphocytes expressing both gp100 TCR alpha and beta chains, but not cells transduced with p53 TCR alpha and beta chains (Figure 2C). Likewise, patient 26 had post-treatment serum IgG directed against p53 TCR alpha and beta chain expressing cells, but not cells which co-expressed gp100 alpha and beta chains. Lymphocytes expressing gp100 TCR alpha chain and p53 TCR beta chain elicited strong post-treatment serum IgG binding by both patients' sera, whereas cells that co-expressed p53 TCR alpha and gp100 beta chains generated no antibody binding. The binding of patient sera to these combinations of murine TCR α and β chains suggested that the immune response by patient 14 was gp100 TCR α chain specific, whereas the response by patient 26 to the p53 TCR was specific for the V β 26.1 chain.

Analysis for cellular immune response

To determine whether a cell-mediated immune response developed in these patients, an assay was developed to identify cytotoxic T-cells specific for gene-modified cells. Our methods included cellular expression of CD137 (4-1BB) as indirect evidence of activation as well as direct measurement by 4-hour chromium release assay. In both cases, patient PBMC collected before and after adoptive cell transfer were stimulated in vitro with relevant antigen (TCR-transduced autologous PBL) and a control (untransduced autologous PBL). Following five rounds of repeated antigen-specific stimulation, the resulting post-treatment PBMC was co-cultured with TCR-bearing cells and assayed for CD137 expression. One patient (14) had PBMC collected 4 months after cell transfer that was sufficient for testing. When antigen-stimulated post-treatment PBMC was co-cultured with autologous gp100 TCR-transduced lymphocytes, CD137 expression was not increased compared to negative controls (Supplemental Figure 1). Antigen-stimulated PBMC was also used in a 4-hour chromium release assay in which autologous transduced and untransduced cells were used as targets. There was no evidence of target cell lysis by antigen-stimulated post-treatment PBMC (data not shown). Therefore, a cell-mediated immune response against TCR gene-modified cells was not detected in this patient using peripheral blood obtained 4 months after adoptive cell transfer.

Factors associated with immune response

Factors correlated with development of an immune response were determined by Wilcoxon rank-sum and Fisher's exact tests with two-sided p-values. Treatment data analyzed included the number of transduced cells infused per patient, persistence of transduced cells in the peripheral blood at one month post-treatment, pre-treatment absolute lymphocyte count (ALC), including T-cell and B-cell fraction, and one month post-treatment ALC. The number of transduced cells infused in patients who did not develop a humoral immune response was not significantly different than patients who did develop an immune response ($p=0.6$). Transduced cell persistence in the peripheral blood at one month and objective tumor regression was not different between groups ($p=0.9$ and $p=0.2$, respectively). Pre-treatment ALC was significantly higher in the six patients who developed an immune response compared with those who did not ($p=0.01$). However, pre-treatment T-cell and B-cell fractions of ALC were not significantly different ($p=0.06$ and $p=0.2$, respectively). Similarly, one month post-treatment ALC was not significantly different between groups ($p=0.4$).

Discussion

The potential for host immune responses to gene-modified cells was recognized early in its application in humans. The intensity and type of immune response depends on the type of vector, route of delivery, immunogenicity of the transgene product and level of transgene expression. As such, immune responses reported in clinical trials have been variable with regard to the nature of the response and clinical effect. Host responses to gene therapy encompass innate and adaptive immunity and include humoral and cellular components. The various gene delivery systems employed in pre-clinical and clinical studies have linked immune responses to components of vector production, the transgene product, or the vector itself (11,13,18–21). Moreover, host immunity to any of these elements is relevant to successful human gene therapy trials. Our analysis expands understanding of human cancer gene therapy and the potential impact of host immune responses to gene therapy in general.

Pre-existing immunity to novel or xenogenic transgene products is unlikely in human gene transfer studies. As such, immunity to the xenogenic mTCR was not detected in our patients prior to adoptive cell transfer. Conversely, most individuals exhibit humoral immunity to adenoviruses that can be detected prior to gene therapy, which is one of the disadvantages of in vivo adenoviral vector gene delivery (22). Once a primary immune response is elicited, repeated antigen exposure can provide an immunogenic boost and significantly impact transgene expression or transduced cell persistence as demonstrated by studies in which patients received multiple infusions of gene-modified cells (13,20).

Two patients in our study received more than one infusion of autologous lymphocytes expressing a mTCR; one day apart in patient 20 and eight months apart in patient 27. Although no immune response was detected in these two patients, it is possible that repeated infusion of T-cells expressing mTCR would influence transduced cell persistence and elicit a more robust immune response. Our finding that patients who developed human anti-mTCR antibodies did not cross-react with other mTCRs (except in the case of a shared V β gene), suggest that multiple infusions with distinct mTCRs may be one approach to avoid this possibility in trials requiring multiple treatments.

Similar to mTCR, murine monoclonal antibodies (mAb) used to generate single-chain antibody-based chimeric antigen receptors (CARs) may also elicit inhibitory immune responses. In at least two human studies, patients that received autologous T-cells expressing murine-derived chimeric antigen receptors developed post-treatment antibody responses specific for CAR-transduced cells (23,24). While at first approximation it would seem logical to avoid the use of murine-derived TCRs, the use of murine-derived or chimeric TCRs in the human adoptive immunotherapy may have functional advantages attributed to specific mTCR chain pairing(25–27) and enhanced association of mTCRs with the CD3 complex leading to greater T cell avidity(28). It has been suggested that specific pairing of the introduced TCR chains may lessen the potential of mixed TCR chains (e.g., an endogenous α chain with the introduced β chain) inducing self-reactivity(29). While this remains a theoretical concern, we have not observed any toxicity that could be directly attributed to TCR chain mispairing in over 100 patients that received human TCR gene-engineered T cells (SA Rosenberg, unpublished observation).

The post-treatment immune response to mTCR in six patients described herein developed in the setting of non-myeloablative, lymphodepleting chemotherapy and did not appear to inhibit therapeutic effect in two patients. Our findings strengthen two conclusions relevant to immune responses in human gene therapy: 1) Humoral immune responses can occur in patients who receive a non-myeloablative chemotherapy, and 2) objective clinical tumor regression can occur despite the development of a humoral immune response against a

transgenic T cell receptor. In our patients, peripheral blood lymphocyte counts were nil for approximately 7 days following adoptive cell transfer. However, transduced cells were detectable in the peripheral blood of every patient at one month post-treatment when lymphocyte counts had returned to normal. The exact timing of antigen presentation and generation of the adaptive immune response is not well understood in our patients. It is noteworthy that two of six patients that developed immune responses also experienced objective tumor regression. If immune responses are capable of inhibiting or destroying gene-modified cells in vivo, it is possible that these cells mediated tumor destruction prior to development of the immune response. The concept of therapeutic effect in spite of immune response to gene therapy is also supported by data from patients treated with donor lymphocytes transduced with the thymidine kinase (TK) transgene in which 5 of 6 patients with a complete response to therapy developed a cytotoxic T lymphocyte response against TK-expressing cells (12,30).

Identification of factors correlated with immune response to gene-modified lymphocytes or its sequelae could prove useful in future applications of gene-transfer protocols. Analysis of the number of gene-modified cells infused, cell persistence in peripheral blood, and clinical tumor response was not significantly different between patients who did and did not develop immune responses in this study. For instance, patient 4 was administered a log fewer gp100 TCR gene-transduced cells compared to patient 14. At one month post-treatment, patient 4 had less than 1% TCR-expressing CD8+ T-cells in the peripheral blood compared to almost 20% in patient 14. Yet, both patients developed a robust humoral immune response as detected by serum inhibition and antibody binding studies. Furthermore, absolute lymphocyte recovery following non-myeloablative chemotherapy was not different among patients who developed a humoral immune response versus patients who did not.

In future cancer gene therapy trials, investigators should consider the rapid disappearance of transduced cells as a cautionary sign of immune-mediated clearance. Similarly, the cessation of therapeutic benefit in a previously responding patient also may be considered a possible result of host immunity. Our study was limited in part due to lack of sufficient biological samples for extensive testing as well as the relative infrequent occurrence of immune responses to draw convincing correlations between clinical parameters and immunologic findings. While host immune responses to mTCR occurred in almost 1 in 4 patients in this series, the development of immunity was not associated with the level of transduced cell persistence or response to therapy. Our report expands the understanding of immune responses to human gene therapy by revealing a subset of patients capable of developing immunity to autologous lymphocytes expressing murine T-cell receptors. These results are relevant to the future use of xenogenic gene sequences used in the generation of TCR and chimeric antigen receptors, and enhance awareness of this phenomenon and its sequelae.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Arnold Mixon and Shawn Farid for technical support with FACS analysis; Colin Gross and the TIL laboratory for retrieving blood and serum samples.

References

1. Rosenberg SA, Aebbersold P, Cornetta K, et al. Gene transfer into humans--immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. *N Engl J Med*. 1990;323:570-8. [PubMed: 2381442]

2. Health NIO. Human Gene Transfer Protocols. 2010. [cited 2010 January 15]; Available from: <http://oba.od.nih.gov/oba/rac/PROTOCOL.pdf>
3. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science*. 2002;298:850–4. [PubMed: 12242449]
4. Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science*. 2006;314:126–9. [PubMed: 16946036]
5. Johnson LA, Heemskerk B, Powell DJ Jr, et al. Gene transfer of tumor-reactive TCR confers both high avidity and tumor reactivity to nonreactive peripheral blood mononuclear cells and tumor-infiltrating lymphocytes. *J Immunol*. 2006;177:6548–59. [PubMed: 17056587]
6. Johnson LA, Morgan RA, Dudley ME, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood*. 2009;114:535–46. [PubMed: 19451549]
7. Theoret MR, Cohen CJ, Nahvi AV, et al. Relationship of p53 Overexpression on Cancers and Recognition by anti-p53 TCR Transduced T cells. *Hum Gene Ther*. 2008
8. Cohen CJ, Zheng Z, Bray R, et al. Recognition of fresh human tumor by human peripheral blood lymphocytes transduced with a bicistronic retroviral vector encoding a murine anti-p53 TCR. *J Immunol*. 2005;175:5799–808. [PubMed: 16237072]
9. Theobald M, Biggs J, Dittmer D, Levine AJ, Sherman LA. Targeting p53 as a general tumor antigen. *Proc Natl Acad Sci U S A*. 1995;92:11993–7. [PubMed: 8618830]
10. Yang S, Cohen CJ, Peng PD, et al. Development of optimal bicistronic lentiviral vectors facilitates high-level TCR gene expression and robust tumor cell recognition. *Gene Ther*. 2008;15:1411–23. [PubMed: 18496571]
11. Muul LM, Tuschong LM, Soenen SL, et al. Persistence and expression of the adenosine deaminase gene for 12 years and immune reaction to gene transfer components: long-term results of the first clinical gene therapy trial. *Blood*. 2003;101:2563–9. [PubMed: 12456496]
12. Verzeletti S, Bonini C, Marktel S, et al. Herpes simplex virus thymidine kinase gene transfer for controlled graft-versus-host disease and graft-versus-leukemia: clinical follow-up and improved new vectors. *Hum Gene Ther*. 1998;9:2243–51. [PubMed: 9794208]
13. Riddell SR, Elliott M, Lewinsohn DA, et al. T-cell mediated rejection of gene-modified HIV-specific cytotoxic T lymphocytes in HIV-infected patients. *Nat Med*. 1996;2:216–23. [PubMed: 8574968]
14. Topalian SL, Solomon D, Rosenberg SA. Tumor-specific cytolysis by lymphocytes infiltrating human melanomas. *J Immunol*. 1989;142:3714–25. [PubMed: 2785562]
15. Parkhurst MR, Joo J, Riley JP, et al. Characterization of genetically modified T-cell receptors that recognize the CEA:691–699 peptide in the context of HLA-A2.1 on human colorectal cancer cells. *Clin Cancer Res*. 2009;15:169–80. [PubMed: 19118044]
16. Robbins PF, Li YF, El-Gamil M, et al. Single and dual amino acid substitutions in TCR CDRs can enhance antigen-specific T cell functions. *J Immunol*. 2008;180:6116–31. [PubMed: 18424733]
17. Theoret MR, Cohen CJ, Nahvi AV, et al. Relationship of p53 overexpression on cancers and recognition by anti-p53 T cell receptor-transduced T cells. *Hum Gene Ther*. 2008;19:1219–32. [PubMed: 19848582]
18. Tait DL, Obermiller PS, Hatmaker AR, Redlin-Frazier S, Holt JT. Ovarian cancer BRCA1 gene therapy: Phase I and II trial differences in immune response and vector stability. *Clin Cancer Res*. 1999;5:1708–14. [PubMed: 10430073]
19. Ram Z, Culver KW, Oshiro EM, et al. Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. *Nat Med*. 1997;3:1354–61. [PubMed: 9396605]
20. Tuschong L, Soenen SL, Blaese RM, Candotti F, Muul LM. Immune response to fetal calf serum by two adenosine deaminase-deficient patients after T cell gene therapy. *Hum Gene Ther*. 2002;13:1605–10. [PubMed: 12228015]
21. Manno CS, Pierce GF, Arruda VR, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med*. 2006;12:342–7. [PubMed: 16474400]
22. Chirmule N, Probert K, Magosin S, Qian Y, Qian R, Wilson J. Immune responses to adenovirus and adeno-associated virus in humans. *Gene Ther*. 1999;6:1574–83. [PubMed: 10490767]

23. Kershaw MH, Westwood JA, Parker LL, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin Cancer Res.* 2006;12:6106–15. [PubMed: 17062687]
24. Lamers CH, Sleijfer S, Vulto AG, et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *J Clin Oncol.* 2006;24:e20–2. [PubMed: 16648493]
25. Coccoris M, Straetemans T, Govers C, Lamers C, Sleijfer S, Debets R. T cell receptor (TCR) gene therapy to treat melanoma: lessons from clinical and preclinical studies. *Expert Opin Biol Ther.* 10:547–62. [PubMed: 20146634]
26. Govers C, Sebestyen Z, Coccoris M, Willemsen RA, Debets R. T cell receptor gene therapy: strategies for optimizing transgenic TCR pairing. *Trends Mol Med.* 16:77–87. [PubMed: 20122868]
27. Kieback E, Uckert W. Enhanced T cell receptor gene therapy for cancer. *Expert Opin Biol Ther.* 10:749–62. [PubMed: 20201709]
28. Cohen CJ, Zhao Y, Zheng Z, Rosenberg SA, Morgan RA. Enhanced antitumor activity of murine-human hybrid T-cell receptor (TCR) in human lymphocytes is associated with improved pairing and TCR/CD3 stability. *Cancer Res.* 2006;66:8878–86. [PubMed: 16951205]
29. Bendle GM, Linnemann C, Hooijkaas AI, et al. Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy. *Nat Med.*
30. Traversari C, Marktel S, Magnani Z, et al. The potential immunogenicity of the TK suicide gene does not prevent full clinical benefit associated with the use of TK-transduced donor lymphocytes in HSCT for hematologic malignancies. *Blood.* 2007;109:4708–15. [PubMed: 17327417]

Figure 1. Results of antibody binding and serum inhibition assays. A) Five patients treated with gp100 TCR (3,4,14,16,19) and one patient treated with p53 TCR (26) show post-treatment serum binding of TCR-transduced lymphocytes with which they were treated as detected by anti-human IgG antibody staining. Shaded histogram represents lymphocyte incubation with pre-treatment serum; bold lines represent post-treatment serum incubation. B) TCR-transduced PBL co-cultured with cognate antigen-expressing tumor cell lines in the presence of pre-treatment and post-treatment serum; supernatant interferon-gamma (IFN γ) levels shown (pg/ml). C) Post-treatment serum inhibition results of 16 gp100 TCR patients and 10 p53 TCR patients as a percentage of co-culture IFN γ release relative to pre-treatment serum samples. D) Reversal of post-treatment serum inhibition (patient 14) following pre-incubation in the presence or absence of protein G. (+), with protein G; (-) without protein G.

Figure 2.

Serum inhibition and antibody binding of transduced lymphocytes is specific. A) Antibody binding of TCR-transduced lymphocytes as determined by anti-human IgG antibody staining of lymphocytes following incubation with pre-treatment (shaded) and post-treatment (line) serum. p53 and CEA TCRs share the same β chain variable region gene (V β 26.1). B) Post-treatment serum inhibition of mTCR (gp100, p53, MAGE-A3 and CEA) and one human TCR (NY-ESO-1). Patients 4, 14 (gp100 TCR) and 26 (p53 TCR) had 6 month post-treatment serum incubated with lymphocytes expressing the various TCRs. Results are shown as a percentage of IFN γ release compared to incubation with pre-treatment serum. C) Mismatch of p53 and gp100 TCR alpha and beta chains by independent transduction followed by serum incubation and staining with anti-human IgG antibody.

Table 1

Patient demographics and pre-treatment data

Patient	Age/Gender	Diagnosis	Sites of Disease	Prior Therapy	Pre-treatment cell counts		
					ALC (k/ μ l)	T-cell (%CD3)	B-cell (%CD19)
2	32/M	Melanoma	ln, im, sc	S,C,I	0.644	86	3
3	54/M	Melanoma	sc	S,I	4.046	91	7
4	50/M	Melanoma	ln, gb	S,I	1.678	85	3
5	49/F	Melanoma	ln, lu, sc	S,I	1.817	85	8
6	36/F	Melanoma	ln, bo, li, sp	R,S,C,I	0.672	57	29
7	60/M	Melanoma	ln, bo, li, lu, sc	S,I	1.897	82	8
8	50/F	Melanoma	st, li, lu, int, sc	S,C,I	0.614	72	16
9	25/M	Melanoma	ln, bo, ip	S,I	2.012	nd	nd
11	50/M	Melanoma	br, ln	R,S,C,I	0.584	51	24
12	62/M	Melanoma	lu, ln	S,C,I	1.537	84	5
13	44/F	Melanoma	li, ln	R,S,C,I	1.482	58	33
14	51/F	Melanoma	ln, li, lu, sp	S,I	2.872	77	16
15	33/M	Melanoma	ln	S,I	1.585	85	5
16	41/M	Melanoma	ln	S,I	1.541	91	4
17	52/M	Melanoma	ln	S,C,I	1.057	78	13
19	58/M	Melanoma	ln	S,I	1.706	83	13
20	64/M	Cholangiocarcinoma	li	S	2.534	89	2
21	52/M	Esophageal adenocarcinoma	ln, lu, im	C	1.276	nd	nd
22	33/M	Melanoma	ln, lu, li, sp	R,S,C,I	1.239	81	11
23	42/F	Breast carcinoma	br, ln	R,S,C	0.904	nd	nd
24	47/F	Salivary gland adenocarcinoma	ln, lu	R,S	0.304	nd	nd
25	36/F	Breast carcinoma	li, lu	R,S,C	1.406	76	11
26	39/F	Breast carcinoma	ln, lu	S,C	1.494	86	8
27	57/M	Melanoma	ln, lu, br	S,I	1.131	66	19
28	44/F	Breast carcinoma	ln	R,S,C	0.614	nd	nd
29	32/M	Small bowel adenocarcinoma	li, ip	S,C	1.016	83	11

ALC, absolute lymphocyte count (k/ μ l), normal range 0.71 – 1.87 k/ μ l; T-cell normal range 57.3 – 86.4% CD3+ peripheral blood mononuclear cells by flow cytometry; B-cell normal range 3.5 – 17.1% CD19+ peripheral blood mononuclear cells by flow cytometry; M, male; F, female; R, radiation; S, surgery; C, chemotherapy; I, immunotherapy; br, brain; bo, bone; gb, gallbladder; im, intramuscular; int,

intestine; ip, intraperitoneal; li, liver; ln, lymph node; lu, lung; sc, subcutaneous; sp, spleen; st, stomach; Patients 2 – 19 treated with gp100:154 TCR; patients 20 – 29 treated with p53 TCR; Patient numbers correspond with those reported by Johnson et al, 2009, Patients 1, 10 & 18 lacked sufficient samples required for analysis.

Table 2

Patient treatment and response data

Patient	# Cells× 10 ⁹	Infusion samples					% TCR+ 1 month [†]	Tumor Response [*]	Serum Inhibition	Antibody Binding
		% TCR positive [§]	TCR+ %CD4	TCR+ %CD8	TCR+ %CD8					
2	10	78	36	54			0.9	NR	No	No
3	9.8	87	13	82			2.6	NR	No	Yes
4	4.6	97	11	78			0.7	NR	Yes	Yes
5	9.9	91	21	80			6.6	NR	No	No
6	5.8	77	6	89			1.6	NR	No	No
7	1.8	83	40	56			34	NR	No	No
8	19.4	55	44	53			32	NR	No	No
9	2.3	84	44	56			1.5	NR	No	No
11	68.8	97	5	96			77	CR	No	No
12	46.5	85	20	82			25	NR	No	No
13	110	90	2	97			43	NR	No	No
14	54	92	11	88			19	PR	Yes	Yes
15	94.1	89	16	78			3	NR	No	No
16	39.1	90	18	79			11	PR	No	Yes
17	60	97	5	95			37	NR	No	No
19	55.8	nd	13	88			nd	NR	No	Yes
20	0.8	37	33	59			0.05	NR	No	No
21	1.2	45	40	54			3.96	NR	No	No
22	2.2	42	12	81			0.12	NR	No	No
23	1	55	31	64			4.16	NR	No	No
24	0.5	51	77	15			4.6	PR	No	No
25	2.1	33	29	63			4.72	NR	No	No
26	0.8	52	31	56			4.35	NR	Yes	Yes
27	27.7	85	5	94			1.71	NR	No	No
28	9.2	78	3	94			0.41	NR	No	No
29	3.7	77	40	40			nd	NR	No	No

[§] CD3-positive cell population

Patients 2 – 19 treated with gp100:154 TCR transduced cells; 20 – 29 treated with p53 TCR transduced cells TCR, T-cell receptor; NR, no response; PR, partial response; CR, complete response; nd, no data

[†] CD8-positive cell population

^{*} RECIST criteria