Cancer Therapy: Clinical

Development of Human Anti-Murine T-Cell Receptor Antibodies in Both Responding and Nonresponding Patients Enrolled in TCR Gene Therapy Trials

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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 2 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 the development of a humoral immune response. Adoptive cell transfer characteristics were analyzed to identify correlates to immune response.

Results: Six of 26 (23%) patients’ posttreatment sera exhibited specific binding of human anti-mTCR antibodies to lymphocytes transduced with the mTCR. Antibody development was found in both responding and nonresponding patients. The posttreatment sera of 3 of these 6 patients mediated a 60% to 99% inhibition of mTCR activity as measured by a reduction in antigen-specific interferon-γ release. Detailed analysis of posttreatment serum revealed that antibody binding was β-chain specific in 1 patient whereas it was α-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.

Gene therapy has evolved significantly since the first report 2 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, 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 with a retrovirus encoding a T-cell receptor (TCR) that could recognize the MART-1 tumor-associated antigen. The TCR α- and β-chains of a MART-1-reactive TIL clone were identified in a patient who demonstrated near complete regression of metastatic melanoma after ACT of TIL (3, 4). Autologous PBL were transduced ex vivo with anti-MART-1 TCR genes and reinjected 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 peripheral blood lymphocytes (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 gp100\textsubscript{154–162} and p53\textsubscript{264–272} epitopes were identified by immunization of
highly avid murine lymphocytes against gp100\textsubscript{154–162} and
cytes 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
PBLs 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, respect-
ively. In a third clinical trial, 14 patients with a variety of
epithelial cancers were treated with transduced cells expres-
sing an anti-murine TCR against common tumor antigens, such as
p53 and carcinoembryonic antigen, the potential applica-
tion of mTCR-based cell therapies has broad implications
for the treatment of a variety of malignancies.

**Translational Relevance**

Human gene therapy has application not only in oncolgy, 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 nonresponding patients, suggesting that the development of immunity to mTCR does not preclude effective treatment. Because human leukocyte antigen-A2 transgenic mice can be used to derive mTCR against common tumor antigens, such as p53 and carcinoembryonic antigen, the potential application of mTCR-based cell therapies has broad implications for the treatment of a variety of malignancies.

Materials and Methods

**Tumor cell lines and lymphocyte culture**

HLA-A2\textsuperscript{+} melanoma cell lines (526, 624.38) and HLA-A2\textsuperscript{+} cell lines (888, 938) were generated at the Surgery Branch of the National Cancer Institute [NCI; National Institutes of Health (NIH)] as previously described (14). Colon adenocarcinoma H508 (CCL-253) and lung adeno-
carcinoma H2087 (CRL-5922) were obtained from American
Type Culture Collection. Tumor cells were cultured in
RPMI (Invitrogen) supplemented with 10% FBS (Invitro-
gen), penicillin/streptomycin (50 U/mL), and l-glutamine
and maintained in a 37\textdegree C incubator at 5% CO\textsubscript{2}. Peripheral
blood mononuclear cells (PBMC) were collected by leu-
kapheresis from melanoma patients or healthy donors and
cultured as described (5, 6).

**Production of retroviral supernatant and lymphocyte transduction**

MSGV1-based retroviruses encoding the human TCR\textalpha- and \textbeta-chains specific for MART-1\textsubscript{27–35} (MART-1 TCR) and NY-
ESO-1\textsubscript{157–165} (NY-ESO-1 TCR), and murine TCR\textalpha- and
\textbeta-chains specific for human gp100\textsubscript{154–162} (gp100 TCR),
p53\textsubscript{264–272} (p53 TCR), MAGE-A3\textsubscript{112–120} (MAGE-A3 TCR),
and CEA\textsubscript{691–699} [CEA (carcinoembryonic antigen) TCR]
were developed for clinical use or preclinical evaluation and
have been described elsewhere (5, 6, 8, 15, 16). Four
additional retroviral vectors expressing either TCR\textalpha- or
\textbeta-chains against gp100 and p53 were constructed using
similar techniques. Transduction of transient retroviral
supernatants and lymphocytes was performed as described
(17).

**Antibody binding and flow cytometry**

The presence of posttreatment antibody response to TCRs
was assessed by detection of human IgG or IgM binding to
epithelial cancers treated with transduced cells expres-
sing an anti-p53 murine TCR (7). One patient with a
salivary gland cancer experienced objective, partial tumor
regression and gene-modified cells were detected at
1 month in all patients tested (M. Theoret, unpublished data).

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.

**Serum inhibition assays**

Serum inhibition of TCR function was assessed in vitro
by antigen-specific interferon-\gamma (IFN-\gamma) secretion. Patient
serum collected at time points before, during, and after ACT
Cancer gene therapy trials

Fifty-seven patients with metastatic cancer were treated at the Surgery Branch of 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 PBLs expressing either mTCR 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 ACT 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 nonmyeloablative, lymphodepleting chemotherapy followed by ACT 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. The gp100 and p53 TCR-transduced cells were incubated with the pre- and posttreatment sera from patients who received cells transduced with the murine derived gp100 and p53 TCRs. The presence of serum antibody binding to TCR-transduced PBLs was then determined by flow cytometric analysis following addition of fluorescently labeled anti-human IgG antibody to the transduced PBLs. Pretreatment serum samples consistently showed background staining of anti-human IgG similar to isotype controls (<1%). Compared with pretreatment serum, posttreatment serum demonstrated anti-human IgG binding to TCR-transduced lymphocytes of 18% to 45% in 5 of 16 gp100 patients and 50% in 1 of 10 p53 patients (Fig. 1A). In the remaining patients, serum antibody binding was equivalent in pretreatment and posttreatment samples (data not shown). The time to detection of the posttreatment 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 posttreatment serum IgG binding to MART-1 TCR-transduced lymphocytes. Posttreatment serum IgM specific for transduced cells was not detected by antibody binding in the 6 patients with a posttreatment IgG response (data not shown). To assess for alloreactivity of patient sera, posttreatment 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 alloresponse.

Serum inhibition of TCR function in vitro

To determine whether antibody binding to TCR-transduced lymphocytes could impact TCR function, we
performed a coculture 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. The gp100 and p53 TCR-transduced lymphocytes were incubated with pre- or posttreatment serum prior to coculture with antigen-positive tumor cells and supernatant was tested for IFN-γ by ELISA at 18 to 24 hours. Sixteen gp100 TCR patients and 10 p53 TCR patients were screened and 3 patients (2 gp100, 1 p53 TCR) demonstrated a 60% to 99% reduction of antigen-specific IFN-γ release when transduced lymphocytes were incubated with posttreatment serum (Fig. 1B and C). In patient 14 (gp100 TCR), posttreatment serum at up to 10^4 dilution caused a greater than 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 coculture, providing evidence for IgG-class–specific antibody inhibition of TCR-specific function (Fig. 1D).

### 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 (patients 4 and 14) displayed posttreatment serum binding and inhibition of only

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### Table 1. Patient demographics and pretreatment data

<table>
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<tr>
<th>Patient</th>
<th>Age/gender</th>
<th>Diagnosis</th>
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<th>Prior therapy</th>
<th>Pretreatment cell counts</th>
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<td></td>
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<td>Small-bowel adenocarcinoma</td>
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<td>S, C</td>
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NOTE: ALC, normal range: 0.71–1.87 k/μL; T cell, normal range: 57.3%–86.4% CD3+ PBMC by flow cytometry; B cell, normal range: 3.5%–17.1% CD19+ PBMC by flow cytometry. 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. (ref. 6); patients 1, 10, and 18 lacked sufficient samples required for analysis. Abbreviations: ALC, absolute lymphocyte count; 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.
gp100 TCR-transduced cells (Fig. 2A and B). Posttreatment serum from patient 26 (p53 TCR treated) bound to and mediated more than 90% inhibition of activity of both p53 TCR and CEA TCR-transduced cells (Fig. 2A and 2B).

Sequence analysis revealed that the p53 and CEA TCRs utilized the same \( \beta \)-chain variable region gene (\( V_\beta 26.1 \)), 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 \( \alpha \)- or \( \beta \)-chains was further evaluated using constructs that expressed only the TCR \( \alpha \)- or \( \beta \)-chain of the gp100 and p53 TCRs. Lymphocytes were simultaneously transduced with retrovirus to express 1 of 4 combinations of \( \alpha \)- and \( \beta \)-chain pairs: (1) gp100\( \alpha \)/gp100\( \beta \), (2) gp100\( \alpha \)/p53\( \beta \), (3) p53\( \alpha \)/p53\( \beta \), and (4) p53\( \alpha \)/gp100\( \beta \). Using sera of patients 14 and 26, an antibody-binding assay was performed (Fig. 2C). Consistent with previous experiments, patient 14 exhibited posttreatment IgG binding to lymphocytes expressing both gp100 TCR \( \alpha \)- and \( \beta \)-chains, but not cells transduced with p53 TCR \( \alpha \)- and \( \beta \)-chains (Fig. 2C). Likewise, patient 26 had posttreatment serum IgG directed against p53 TCR \( \alpha \)- and \( \beta \)-chain–expressing cells, but not cells which coexpressed gp100 \( \alpha \)- and gp100 \( \beta \)-chains. Lymphocytes expressing gp100 TCR \( \alpha \)-chain and p53 TCR \( \beta \)-chain elicited strong posttreatment serum IgG binding by both patients’ sera, whereas cells that coexpressed p53 TCR \( \alpha \)- and gp100 \( \beta \)-chains generated no antibody binding. The binding of patient sera to these combinations of murine TCR \( \alpha \)- and \( \beta \)-chains suggested that the immune response by patient 14 was gp100 TCR \( \alpha \)-chain specific, whereas the response by patient 26 to the p53 TCR was specific for the \( V_\beta 26.1 \) chain.

### Table 2. Patient treatment and response data

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<th>Patient</th>
<th>Cells ( \times 10^9 )</th>
<th>%TCR positive(^a)</th>
<th>TCR + %CD4</th>
<th>TCR + %CD8</th>
<th>%TCR + 1 month(^b)</th>
<th>Tumor response(^c)</th>
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NOTE: Patients 2–19 treated with gp100:154 TCR-transduced cells; 20–29 treated with p53 TCR-transduced cells.

Abbreviations: TCR, T-cell receptor; NR, no response; PR, partial response; CR, complete response; nd, no data.

\(^a\)CD3-positive cell population.

\(^b\)CD8-positive cell population.

\(^c\)RECIST criteria.
Fig. 1. Results of antibody-binding and serum inhibition assays. A, 5 patients treated with gp100 TCR (3, 4, 14, 16, 19) and 1 patient treated with p53 TCR (26) show posttreatment 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 pretreatment serum; bold lines represent posttreatment serum incubation. B, TCR-transduced PBL cocultured with cognate antigen-expressing tumor cell lines in the presence of pretreatment and posttreatment serum; supernatant IFN-γ levels shown (pg/mL). C, posttreatment serum inhibition results of 16 gp100 TCR patients and 10 p53 TCR patients as a percentage of coculture IFN-γ release relative to pretreatment serum samples. >, greater than 120%. D, reversal of posttreatment serum inhibition (patient 14) following preincubation in the presence or absence of protein G. (+), with protein G; (-) without protein G.
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 pretreatment (shaded) and posttreatment (line) serum. p53 and CEA TCRs share the same β-chain variable region gene (Vβ 26.1). B, posttreatment serum inhibition of mTCR (gp100, p53, MAGE-A3, and CEA) and 1 human TCR (NY-ESO-1). Patients 4, 14 (gp100 TCR), and 26 (p53 TCR) had 6-month posttreatment serum incubated with lymphocytes expressing the various TCRs. Results are shown as a percentage of IFN-γ release compared to incubation with pretreatment serum. >, greater than 120%. C, mismatch of p53 and gp100 TCR α- and β-chains by independent transduction followed by serum incubation and staining with anti-human IgG antibody.
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 ACT were stimulated in vitro with relevant antigen (TCR-transduced autologous PBL) and a control (untransduced autologous PBL). Following 5 rounds of repeated antigen-specific stimulation, the resulting posttreatment PBMC was cocultured 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 posttreatment PBMC was cocultured with autologous gp100 TCR-transduced lymphocytes, CD137 expression was not increased compared with negative controls (Supplementary Fig. S1). 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 posttreatment 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 ACT.

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 2-sided P-values. Treatment data analyzed included the number of transduced cells infused per patient, persistence of transduced cells in the peripheral blood at 1 month posttreatment, pretreatment absolute lymphocyte count (ALC), including T-cell and B-cell fraction, and 1 month posttreatment 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 1 month and objective tumor regression were not different among groups (P = 0.9 and P = 0.2, respectively). Pretreatment ALC was significantly higher in the 6 patients who developed an immune response compared with those who did not (P = 0.01). However, pretreatment T-cell and B-cell fractions of ALC were not significantly different (P = 0.06 and P = 0.2, respectively). Similarly, 1 month posttreatment ALC was not significantly different among 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 preclinical 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.

Preexisting 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 ACT. Conversely, most individuals exhibit humoral immunity to adenoviruses that can be detected prior to gene therapy, which is 1 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 1 infusion of autologous lymphocytes expressing an mTCR; 1 day apart in patient 20 and 8 months apart in patient 27. Although no immune response was detected in these 2 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 Vβ gene), suggest that multiple infusions with distinct mTCRs may be 1 approach to avoid this possibility in trials requiring multiple treatments.

Similar to mTCR, murine monoclonal antibodies used to generate single-chain antibody-based chimeric antigen receptors (CARs) may also elicit inhibitory immune responses. In at least 2 human studies, patients that received autologous T cells expressing murine-derived chimeric antigen receptors developed posttreatment antibody responses specific for CAR-transduced cells (23, 24). Although 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). Although 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 posttreatment immune response to mTCR in 6 patients described herein developed in the setting of nonmyeloablative, lymphodepleting chemotherapy and did not appear to inhibit therapeutic effect in 2 patients. Our findings strengthen 2 conclusions relevant to immune responses in human gene therapy: (1) Humoral immune responses can occur in patients who receive a nonmyeloablative 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, PBL counts were nil for approximately 7 days following ACT. However, transduced cells were detectable in the peripheral blood of every patient at 1 month posttreatment 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 2 of the 6 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 (1K) 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 among 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 with patient 14. At 1 month posttreatment, patient 4 had less than 1% TCR-expressing CD8+ T cells in the peripheral blood compared with 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 nonmyeloablative 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 relatively infrequent occurrence of immune responses to draw convincing correlations between clinical parameters and immunologic findings. Although 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 mTCR. 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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