Intrathecal Cytotoxic T-Cell Immunotherapy for Metastatic Leptomeningeal Melanoma

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Abstract

A 49-year-old patient with primary, recurrent melanoma on the lower extremity developed metastatic leptomeningeal melanoma that did not respond to treatment with radiation therapy or intrathecal interleukin 2 (IL-2). Disease was characterized by neurological symptoms, including loss of hearing, loss of short-term memory, and gait disturbance. CD8+ CTLs were generated in vitro using autologous dendritic cells pulsed with peptides from the melanoma-associated antigens tyrosinase (145–156), Melan-A/MART-1 (26–35), and gp100/Pmel 17 (209–217). The CTLs exhibited up to 74% specific lysis against peptide-pulsed autologous EBV-transformed B cells, with Melan-A-specific CTLs yielding the greatest lytic activity. CD8+ CTLs possessed a type 1 cytokine profile, expressing tumor necrosis factor α and IFN-γ but not IL-4. Infusions of CTLs were supported with systemic low-dose IL-2 administration. Radiolabeling and computerized gamma imaging were used to monitor the distribution of CTLs up to 48 h after infusion. Intra-arterial delivery via the right carotid artery was followed by redistribution of the CTLs to the lungs, liver, and spleen within 16 h. In contrast, delivery via an indwelling Ommaya reservoir resulted in prolonged retention of CTLs within the brain for at least 48 h after infusion. Marked but transient elevations in tumor necrosis factor α, IFN-γ, and IL-6 in the cerebrospinal fluid were observed within 4 h of CTL infusion. There was no evidence of tumor progression throughout the treatment period, and clinically the patient showed some resolution of neurological symptoms.

Introduction

Although the immunogenic potential of melanoma has been recognized for some time, clinical trials involving the use of tumor-infiltrating lymphocytes or lymphokine-activated killer cells in the treatment of malignant melanoma have met with only modest success (1–6). The reasons for the limited efficacy may include poor definition of the effector cells, failure of the effector cells to recognize defined antigens, and poor localization of the effector cells to the site of the tumor. The recent identification of defined melanoma-associated tumor antigens such as gp100, Melan-A/MART-1, tyrosinase, MAGE-1, and MAGE-3 (7–12), combined with the ability to induce T-cell responses of defined function and phenotype, has led to a strong resurgence of interest in immunotherapy of melanoma. Most notably, the application of DCs3 as powerful inducers of melanoma tumor antigen-specific CD8+ cytotoxic CTL responses has provided investigators with the tools necessary for a targeted approach to the treatment of disease (13–19).

Notwithstanding the major advances in DC-based induction of tumor-antigen-specific T-cell responses in patients with malignant disease, clinical administration of autologous antigen-specific CTLs presents some practical challenges. One of the more important issues centers on election of an appropriate route of delivery for optimal localization and retention of CTLs at the tumor site. The use of radioisotopic cell labeling and subsequent imaging of T-cell localization in the patient affords us the opportunity to tailor the route and method of CTL immunotherapy for optimum delivery to the tumor. Isotopes such as 111In can be used for short-term visualization and tracking of cellular components in real time using standard scanning techniques (20–23).

In this study, we have used autologous DCs pulsed with defined HLA-restricted peptides from the gp100, Melan-A/MART-1, and tyrosinase melanoma antigens to stimulate CD8+ CTLs for immunotherapeutic treatment of a patient with metastatic leptomeningeal melanoma. Antigen-specific CTLs were delivered through catheterization of the internal carotid artery or local delivery through an indwelling intracranial Ommaya reservoir. Radiolabeling and computerized gamma imaging were used to monitor CTL migration and elucidation of the most effective route of delivery. CTL activity in vivo was assessed indirectly through measurement of T-cell and inflammatory cytokine production in the CSF at various time points after treatment.

Materials and Methods

Case History. A 49-year-old woman presented with a melanocytic nevus (Breslow thickness, 0.8 mm), which was removed in February 1995. A local recurrence (Breslow thickness,
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persistent neurological symptoms, and hence from June to November 1999, she was treated under a Food and Drug Administration-approved melanoma antigen-specific CTL immunotherapy protocol (IND #8422), as described in this report.

The patient remained disease free and asymptomatic until December 1998, when she presented with symptoms of dizziness and difficulties with balance and hearing. A MRI scan of the brain showed nodular enhancement along the corpus callosum in the lateral ventricles, two enhancing left parietal lesions, and involvement of both internal auditory canals. CSF cytology was consistent with melanoma. Whole-body scanning did not reveal disease at any other site, and serum chemistries were normal. In January 1999, she was treated with daily intrathecal IL-2 via an Ommaya reservoir receiving a total of 2.7 mg (27 doses) of TriGem (TriGem Pharmaceuticals, Inc., South San Francisco, CA) admixed with QS-21 adjuvant (Biopharmaceuticals, Inc., Worcester, MA).

The patient remained disease free and asymptomatic until December 1999, when she underwent radiation therapy, receiving a total of 3750 cGy in 15 fractions to the whole brain and a total of 1250 cGy in 5 fractions to the ventricles. A repeat MRI in April 1999 revealed disease at any other site, and serum chemistries were normal. In January 1999, she was treated with daily intrathecal IL-2 for in vitro T-cell culture was provided by the Biological Response Modifiers Program, National Cancer Institute, whereas the IL-2 for patient treatment was from Chiron (Emeryville, CA). Prostaglandin E₂ was from Sigma Chemical Co. (St. Louis, MO). The HLA-A1-restricted tyrosinase peptide 145-156, and the HLA-A2-restricted Melan-A/MART-1 peptide 26-35 and gp100 peptide 209-217 (24-27) were synthesized by Genemed Synthesis, Inc. (South San Francisco, CA). PCR primers were synthesized by Life Technologies, Inc.

Reverse Transcription-PCR Analysis. RNA from melanoma cells isolated from the patient’s CSF, and from the MEL-24 cell line (American Type Culture Collection, Rockville, MD) was extracted with TRIzol (Life Technologies, Inc.), according to the manufacturer’s instructions. RNA was reverse-transcribed into cDNA using the SuperScript One-Step RT-PCR System (Life Technologies, Inc.). The Melan-A/MART-1 external primer sequences were 5’-ATG CCA AGA GAA GAT GCT CAC-3’ and 5’-AGC ATG TCT CAG GTG TCT GC-3’, yielding a 384-bp product (28), and the internal primer sequences were 5’-CAC GGC CAC TCT TAC ACC AC-3’ and 5’-AGA GCA TTC GAG ACC ACA GG-3’, yielding a nested product of 252 bp (29). The tyrosinase external primer sequences were 5’-TTG GAG CAT TGT CAG TAG CC-3’ and 5’-AGG CAT TGT GCA TGC TGC TT-3’, yielding a 284-bp product (30, 31), and the internal primer sequences were 5’-GTC TTT ATG CAA TGG AAC GC-3’ and 5’-GCT ATC CCA GAT AGT GGA CT-3’, yielding a nested product of 207 bp (31). The gp100 external primer sequences were 5’-GCT TGG TGT CTC AAG GCA ACT-3’ and 5’-CCT CAG GTA AGT ATG GAC-3’, yielding a 751-bp product (28), and the internal primer sequences were 5’-CAT GAT GCA CCC ATC GGA CT-3’ and 5’-ATA GGT ATC CCA GAA AGA AGT GGA CT-3’, yielding a nested product of 626 bp. PCR products were resolved in 2% agarose gels and visualized with the Gelprint 2000i system (Biophotonics Corp., Ann Arbor, MI).

DCs. DCs were generated as described previously (32), with minor modifications. Briefly, PBMCs were allowed to adhere to six-well culture dishes (5 × 10⁶ PBMCs/well) at 37°C in AIM-V medium. After 2 h, nonadherent cells were removed, and the adherent cells were cultured in AIM-V (3 ml/well) plus

**Fig. 1** MRI analysis prior to induction of adoptive T-cell immunotherapy. MRIs obtained without (A) and with (B) contrast revealed multiple areas of enhancement along the ependymal surfaces of the ventricles and within the brain parenchyma, a small ring enhancing lesion in the left parietal gray/white matter junction measuring <1 cm in size, and bilateral enhancing lesions at the internal acoustic canals (not visible in this plane).
performed essentially as described (34). Briefly, autologous peptide (50 μg/ml, 1 h at 37°C) was subsequently labeled for 1 h with 0.5 μg/ml anti-CD3 monoclonal antibody (OKT3) and EBV-transformed lymphoblastoid cells (LCL) were pulsed with cells were similarly 51Cr labeled. Target cells were plated at 1 × 10^4/well in 96-well round-bottomed plates at a final concentration of 10^6 PBMCs/ml in AIM-V medium plus 100 units/ml IL-2. Two rounds of stimulation in vitro, CD8+ T cells were positively selected with biotin-CD8 Dynabeads (Dynal, Inc., Lake Success, NY), according to the manufacturer’s instructions. Resulting populations were >95% CD8 +, as determined by flow cytometric analysis. To achieve the cell numbers needed for immunotherapy, CD8 + CTL cultures were expanded by stimulation with 10 units/ml IL-2. Responder T cells were restimulated without 50 ng/ml PMA (Sigma) and 500 ng/ml ionomycin (Sigma) in 24-well plates at 37°C. After 3 h of incubation, 10 μg/ml Brefeldin A (Sigma) were added to each well. After 6 h of incubation, the cells were collected, washed, and fixed with 2% paraformaldehyde in PBS for 10 min, washed once more with PBS, and permeabilized with 0.5% saponin (Sigma) and 1% BSA (Sigma) in PBS. Nonactivated and activated CD8+ T cells were then stained with FITC-anti-IFN-γ and phycoerythrin-anti-IL-4 (Becton Dickinson, San Jose, CA) for 30 min at room temperature, washed twice with 0.5% saponin and 1% BSA in PBS, and once with 0.5% BSA in PBS, and fixed a second time with 2% paraformaldehyde in PBS. Fluorescence was recorded with a FACScan (Becton Dickinson), and data were analyzed with WinMDI software (kindly provided by Joe Trotter, Scripps Research Institute, La Jolla, CA).

**Indium Labeling.** CD8+ T cells were labeled with 111In oxine (Amer sham Life Science, Arlington Heights, IL) as described (20). Briefly, 10^8 T cells were spun down and resuspended directly in 1 mL of 111In oxine (approximate volume, 1 ml) and incubated at room temperature for 20 min. Labeling was terminated by the addition of 2 ml of autologous serum, followed by one wash with excess PBS. T cells were then resuspended in 5–10 ml, injection-grade saline plus 2% autologous serum. Incorporated radionuclide was measured with a nuclear medicine dose calibrator prior to infusion. Incorporated activity averaged 750 μCi. Digital gamma camera images after T-cell transfer to the patient were recorded with a Siemens Diacam (Siemens, Chicago, IL) at 1, 16, 24, and 48 h after infusion.

**Cytokine ELISA.** CSF samples collected via the Ommaya reservoir were centrifuged to remove cellular components and then frozen at 20°C. ELISAs for TNF-α, IFN-γ, IL-6, and IL-10 were conducted with ELISA kits (CalTag, Burlingame, CA), according to the manufacturer’s instructions.

**Safety Tests.** In accordance with Food and Drug Administration requirements, lymphocyte cultures were tested for bacterial contamination by Gram’s stain and microbiological culture. Mycoplasma tests were performed by PCR assay (Stratagene, La Jolla, CA), and the presence of endotoxins was assessed by the Limulus amebocyte lysate test by an independent laboratory (ViroMed Biosafety, Camden, NJ). All cultures tested negative for bacterial and *Mycoplasma* contamination, and the levels of endotoxin were less than 0.03125 EU/ml.

**Results**

**Antigen Expression by Melanoma Tumor Cells.** Numerous studies have shown that the melanoma differentiation antigens tyrosinase, gp100, and Melan-A/MART-1 are frequently expressed in metastatic lesions (8–10). Accordingly, we tested for expression of these antigens in tumor cells recovered from the patient’s CSF. Melanoma cells were found in very low numbers (<100/ml) in CSF drawn via the Ommaya reservoir, and thus the cellular fractions from several CSF samples were pooled, and the RNA was extracted and subject to nested set PCR. The outer primers gave PCR products for gp100, tyrosinase, and Melan-A from RNA extracted from the MEL-24 melanoma cell line (Fig. 2A, Lanes 1, 5, and 9). However, an initial round of PCR using the outer primers failed to detect tyrosinase or gp100 expression and revealed only faint expression of Melan-A (Fig. 2A, Lanes 2, 6, and 8) in the patient-derived RNA. In contrast, a second round of PCR using nested primers resulted in amplification of products for all three antigens from the patient-derived RNA sample (Fig. 2B, Lanes 2, 5, and 8).

**Generation of Melanoma Antigen-specific CD8+ CTLs.** The patient’s class I HLA type (A1, A2, B7, B62) included both the A1 and A2 alleles, which afforded us a wide choice of potential T-cell epitope targets within the gp100, tyrosinase, and Melan-A/MART-1 antigens found to be expressed in tumor cells from the patient’s CSF. Melanoma cells were found in very low numbers (<100/ml) in CSF drawn via the Ommaya reservoir, and thus the cellular fractions from several CSF samples were pooled, and the RNA was extracted and subject to nested set PCR. The outer primers gave PCR products for gp100, tyrosinase, and Melan-A from RNA extracted from the MEL-24 melanoma cell line (Fig. 2A, Lanes 1, 5, and 9). However, an initial round of PCR using the outer primers failed to detect tyrosinase or gp100 expression and revealed only faint expression of Melan-A (Fig. 2A, Lanes 2, 6, and 8) in the patient-derived RNA. In contrast, a second round of PCR using nested primers resulted in amplification of products for all three antigens from the patient-derived RNA sample (Fig. 2B, Lanes 2, 5, and 8).
specific for the gp100 peptide were also effective against peptide-pulsed autologous LCLs (55% lysis at an E:T ratio of 20:1; Fig. 3B). The Melan-A/MART-1-stimulated CTLs did not lyse control LCLs or NK-sensitive K562 target cells to a significant level, but the gp100 peptide-stimulated CTLs did show a high level of background lysis against control LCLs. The lowest level of specific cytotoxicity was observed with the tyrosinase peptide-stimulated CD8+ CTLs, which lysed both peptide-pulsed and control LCLs (Fig. 3B). The Melan-A/MART-1 peptide 26–35 (A), gp100 peptide 209–217 (B), or tyrosinase peptide 145–156 (C) were tested in standard 6-h 51Cr-release assays performed against autologous LCLs pulsed with melanoma antigen peptide (●), LCLs pulsed with the irrelevant peptide (○), or NK-sensitive K562 cells (□). Assays were conducted in triplicate wells. Results are expressed as the percentage of specific lysis at the indicated E: T ratios and are representative of at least three individual experiments.

Cytokine Expression by CD8+ CTLs. In view of the documented importance of type 1 cytokines in T-cell-mediated tumor regression in vivo (35, 36), we evaluated peptide-specific CD8+ T cells for cytokine expression. Flow cytometric analysis of intracellular IFN-γ and IL-4 expression by peptide-specific CD8+ T cells revealed a strong bias in favor of an IFN-γ+/IL-4- type 1 cytokine profile in all cases (Fig. 4). Intermediate IFN-γ+/IL-4+ CD8+ T cells constituted a small minority (typically <5%), and type 2 IFNγ−/IL-4+ CD8+ T cells were also infrequent, although almost 10% of the gp100 peptide-specific CD8+ T cells exhibited this phenotype (Fig. 4D).

Adoptive Transfer of Melanoma Antigen Peptide-specific CD8+ CTLs. A summary of the treatment regimen for T-cell immunotherapy, including the dates of T-cell transfer, the route of delivery, and the number of T cells transferred, is provided in Table 1. For the first treatment, T cells were given systemically, through catheterization of the right carotid artery for suborbital delivery. The CD8+ CTLs used for the initial intra-arterial transfer (1 × 10⁸ T cells) were produced by stimulation with DCs loaded with a mixture of the three melanoma antigen peptide epitopes described above, and thus it is not possible to ascertain the numbers of T cells specific for any given peptide. For subsequent intrathecal treatments via the Ommaya reservoir, peptide-specific CD8+ CTLs were prepared in separate cultures stimulated with DCs pulsed with the respective peptides, and the T cells were pooled at the time of delivery. For the first intrathecal treatment, 8.33 × 10⁷ Melan-A-specific T cells and 2.22 × 10⁷ tyrosinase-specific T cells were delivered, giving a total of 1.055 × 10⁸ CTLs. On the second intrathecal treatment, the dose was increased to 2.3 × 10⁸, of which 8.5 × 10⁷ T cells were specific for gp100, 1.1 × 10⁷ were Melan-A specific, and 3.5 × 10⁷ were tyrosinase specific. For the final intrathecal treatment, the dose was further escalated to 1.8 × 10⁹ T cells, of which 1.05 × 10⁸ were gp100 specific, 1.47 × 10⁸ were Melan-A specific, and 1.5 × 10⁷ were tyrosinase specific. In this last case, a large proportion (>90%) of the tyrosinase peptide-stimulated T cells were CD4−, which we elected to transfer, rather than purify the relatively small number of CD8+ T cells in the culture. In all other cases, purified CD8+ T cells were used for immunotherapy.

Concurrent with the adoptive T-cell therapy, systemic IL-2 was administered in de-escalating doses for in vivo T-cell support (Table 1). The decrescendo IL-2 regimen (30 × 10⁶ units to 44 × 10⁶ units) was administered by continuous i.v. infusion over 30–96 h. The 96-h schedule was associated with hypotension and hypoxemia. In contrast, the 30-h schedule was well tolerated and was adopted for the last three rounds of intrathecal T-cell immunotherapy.

In Vivo Localization of Transferred T Cells. For the first transfer via the right carotid artery, 1 × 10⁸ T cells were labeled, and the cellular recirculation patterns were monitored by digital camera imaging at 1, 16, 24, and 48 h after infusion (Fig. 5A). An initially high level of activity was observed within the right hemisphere of the head, although a large number of T cells had also localized in the lungs, and to a lesser extent in other areas.
Fig. 4 Two-color flow cytometric analysis of intracellular IFN-γ and IL-4 expression by CD8⁺ T cells specific for gp100 peptide 209–217 (A and D), Melan-A/MART-1 peptide 26–35 (B and E), and tyrosinase peptide 145–156 (C and F). T cells were unstimulated (A–C) or activated with PMA and ionomycin (D–F).

Table 1  Treatment regimen for infusion of autologous peptide-specific CTLs

<table>
<thead>
<tr>
<th>Date of in vitro T-cell stimulation</th>
<th>Date of T-cell infusion</th>
<th>Route of administration</th>
<th>No. of cells infused</th>
<th>Amount of IL-2 administered</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/20/99</td>
<td>6/9/99</td>
<td>Intrathecal</td>
<td>2.5 × 10⁸</td>
<td>44 × 10⁶ units/96 h*</td>
</tr>
<tr>
<td>6/10/99</td>
<td>6/23/99</td>
<td>Intrathecal</td>
<td>1.0 × 10⁸</td>
<td>30 × 10⁶ units/30 h</td>
</tr>
<tr>
<td>9/2/99</td>
<td>9/16/99</td>
<td>Intrathecal</td>
<td>2.3 × 10⁸</td>
<td>30 × 10⁶ units/30 h</td>
</tr>
<tr>
<td>10/27/99</td>
<td>11/10/99</td>
<td>Intrathecal</td>
<td>1.8 × 10⁹</td>
<td>30 × 10⁶ units/30 h</td>
</tr>
</tbody>
</table>

* The IL-2 was initially administered as 22 × 10⁶ units over the initial 24 h, 11 × 10⁶ units over the subsequent 24 h, and followed by 11 × 10⁶ units over the final 48 h for a total of 44 × 10⁶ units of IL-2. Because of the associated hypotension and hypoxemia, the subsequent dosages were altered such that 12 × 10⁶ units of IL-2 was given over the first 6 h, followed by 12 × 10⁶ units over the next 12 h, followed by 6 × 10⁶ units over the final 12 h, for a total of 30 × 10⁶ units IL-2.

In Vivo Cytokine Expression. To assess activity of transferred T cells, the patient’s CSF was tapped via the Ommaya reservoir at the time of treatment and at 4, 24, 48, and 114 h after treatment. ELISA quantification revealed a marked elevation in IFN-γ and TNF-α levels at 4 h after transfer, followed by a rapid return to baseline levels within 24 h for a total of 44 × 10⁶ units of IL-2. Because of the associated hypotension and hypoxemia, the subsequent dosages were altered such that 12 × 10⁶ units of IL-2 was given over the first 6 h, followed by 12 × 10⁶ units over the next 12 h, followed by 6 × 10⁶ units over the final 12 h, for a total of 30 × 10⁶ units IL-2.

Discussion

Immunotherapeutic treatment of malignant disease involving the brain presents a unique set of challenges. Of the approaches described, the majority have involved administration of intrathecal IL-2, either alone or in combination with lymphokine-activated killer cells (37–41). Response to treatment of multidrug-resistant intracranial metastatic melanoma with a combination of systemic IL-2, IFN-α, and 5-fluorouracil has also been documented (42). Of particular interest in the context of the present study, intrathecal injection of autologous CTLs via an Ommaya reservoir has been shown to induce regression extent in the liver and spleen. By 16 h after infusion, nearly all detectable radiolabel was resident in the liver and spleen. In contrast, intrathecal infusion of [11]In-labeled T cells via an indwelling Ommaya reservoir resulted in prolonged retention of labeled cells within the cranium, with a low level of migration along the spinal column (Fig. 5B). The smaller spot above the main area of cellular accumulation most probably represents signal from the Ommaya reservoir, which was located on the right side of the cranium. There was minimal systemic recirculation, with very few cells localized in the lungs, liver, or spleen at any time point tested. In the light of these results, all subsequent treatments were by the intrathecal route.
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Fig. 5 Computerized gamma imaging of 111In-labeled melanoma antigen-specific CD8\(^+\) T cells adoptively transferred by intra-arterial infusion via the right carotid artery (A) or intrathecal infusion via an indwelling Ommaya reservoir (B). Images were obtained at 1, 16, 24, and 48 h after infusion.

Fig. 6 Evaluation of CSF cytokine levels after intrathecal C-8\(^+\) T cell infusion. ELISAs for TNF-\(\alpha\) (A), IL-6 (B), IFN-\(\gamma\) (C), and IL-10 (D) were performed on CSF samples drawn via the Ommaya reservoir immediately preceding treatment (0 h) and at 4, 24, 48, and 114 h after intrathecal administration of autologous melanoma antigen peptide-specific CD8\(^+\) T cells. Assays were performed in duplicate, and the results are expressed as the mean cytokine titer (pg/ml). Results are representative of at least two individual experiments.

of end-stage malignant glioma with minimal treatment-associated toxicity (43), thus illustrating the safety and potential efficacy of intrathecal CD8\(^+\) T-cell immunotherapy for the treatment of malignant disease with CNS involvement.

In this report, we describe the application of melanoma antigen-targeted CD8\(^+\) CTLs for intrathecal immunotherapy of advanced metastatic leptomeningeal melanoma. This approach was adopted because computerized gamma imaging of 111In-labeled CTLs showed that systemic administration of T cells, even when locally delivered via catheterization of the carotid artery, failed to result in accumulation of T cells at the site of disease. T cells rapidly washed out of the cranium and were almost totally resident in the liver and spleen within 24 h of transfer. From these observations, we conclude that the blood-brain barrier represents an insurmountable obstacle to cellular immunotherapy of metastatic disease within the brain. To circumvent this problem, we took advantage of an indwelling Ommaya reservoir that had been placed for prior intrathecal IL-2 therapy. Gamma imaging showed that intrathecal transfer of CD8\(^+\) CTLs resulted in extended cranial localization, at least through the 48-h monitoring period, with minimal wash-out to systemic sinks for lymphocyte accumulation. However, the extent to which the T cells localized to tumor cannot accurately be determined from our imaging studies. Similarly, because control studies could not be done, it is unclear whether antigen nonspecific T cells would have shown equally good retention within the CNS. Intrathecal transfer of CD8\(^+\) T cells was well tolerated by the patient, even at the highest dose administered (>10\(^9\) T cells).

Evidence for in vivo T-cell activation was provided by detection of a rapid onset peak in cytokine levels, in particular IFN-\(\gamma\), in the patient’s CSF within 4 h of intrathecal CD8\(^+\) T-cell transfer. TNF-\(\alpha\) production in the CSF may also be a direct result of CD8\(^+\) T-cell activation in vivo, although it should be noted that TNF-\(\alpha\) can be secreted by many other cell types, particularly resident monocytes and macrophages. More prolonged detection of IL-6 in the CSF up to 48 h after treatment may also be indicative of downstream inflammatory processes. The rapid decline in IFN-\(\gamma\) and TNF-\(\alpha\), which are not detectable later than 24 h after transfer, is unlikely to be a reflection of loss
of T-cell viability, because the indium scans clearly indicate prolonged residence of viable cells.

One of the more intriguing observations was the detection of very high levels of IL-10, both in the CSF and in the patient’s serum, both prior to T-cell immunotherapy and throughout the posttreatment monitoring period (up to 114 h). IL-10 is widely regarded as an immunosuppressive cytokine (44) and can also down-regulate the transporter associated antigen processing (45) and inhibit HLA class I and class II expression (46, 47). IL-10 may thus play a key role in tumor evasion of T-cell immune-surveillance and may also represent a barrier to T-cell immunotherapy. In contrast, however, IL-10 has also been reported to increase cytotoxic potential in cervical tumor-specific CD8+ CTLs (48) and may thus enhance cytotoxic effector function in vivo. In this case, the impact of IL-10 on intrathecal CD8+ T cell immunotherapy is open to conjecture.

For almost 2 years (April 1997 to March 1999), the patient was also treated with the TriGem vaccine, an anti-idiotypic antibody, targeting the disialoganglioside GD2. Preliminary results from the TriGem vaccine trial (n = 47) in advanced melanoma were reported recently (49) and revealed minimal clinical responses. However, it is conceivable that this vaccine influenced the biology of the patient’s disease, given that at date (June 2000), she has no evidence of recurrent melanoma outside the CNS.

Clinically, the patient showed a significant improvement in neurological symptoms and general performance status throughout the treatment period. Radiological examination has indicated stabilization of the leptomeningeval lesions, coincident with intrathecal T-cell immunotherapy (June through November, 1999), and up to the present time (June 2000). However, some progression of the lesion in the left parietal region has been observed since the last treatment, suggesting that this site is less amenable to CTL immunotherapy. Collectively, we conclude that intrathecal CD8+ CTL immunotherapy may be a viable and well-tolerated approach to treatment of metastatic leptomeningeval melanoma but may be less effective against parenchymal CNS disease. Our results provide some evidence for in vivo T-cell activation after transfer and further suggest that some therapeutic benefit may accrue from this novel treatment strategy.

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References


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