Active Immunotherapy with Ultraviolet B-irradiated Autologous Whole Melanoma Cells plus DETOX in Patients with Metastatic Melanoma

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ABSTRACT

Our objective was to determine the clinical activity, toxicity, and immunological effects of active immunotherapy using UVB-irradiated (UVR) autologous tumor (AT) cells plus adjuvant DETOX in metastatic melanoma patients. Eligibility included nonanergic patients fully recovered after resection of 5 or more grams of metastatic melanoma. Treatment consisted of intradermal injections of 10^6 UVR-AT plus 0.25 ml of DETOX every 2 weeks × 6, then monthly. Peripheral blood mononuclear cells (PBMCs) were harvested for cytotoxicity assays, and skin testing was performed for delayed-type hypersensitivity (DTH) determinations before the first, fourth, seventh, and subsequent treatments. Forty-two patients were treated, 18 in the adjuvant setting and 24 with measurable disease. Among the latter group, there were two durable responses in soft-tissue sites and in a bone metastasis. Treatment was well tolerated. Thirty-five patients were assessable for immunological parameters; 10 of these patients, including the 2 responders, demonstrated early induction of PBMC cytotoxicity against AT cells that persisted up to 10 months on treatment before falling to background levels. In five of seven patients, the fall-off heralded progressive disease. Late induction of a weak DTH reaction to AT cells was observed in eight patients. Active immunotherapy with UVR-AT + DETOX had modest but definite clinical activity in advanced melanoma. The induction of both PBMC cytotoxicity and DTH reactivity to AT cells supported a specific systemic immune effect of treatment, although the former more closely followed disease course in this study.

INTRODUCTION

Malignant cells must elude any host immune surveillance to grow and overcome the host. Hewitt et al. (1) have shown that spontaneous tumors in aging mice do not induce a detectable host immune response. Nevertheless, the hypothesis that those same cells that escape immune surveillance in vivo might be manipulated in vitro to become more immunogenic is a basis for tumor immunotherapy. Methylcholanthrene and UVB-induced tumors, when excised and reimplanted in mice, have resulted in varying degrees of specific immune protection (2–5). In patients, tumor burden, immunoselection (6), tolerance induction (7–10), and biophysical barriers (11) all present challenges to developing effective antitumor immunotherapy programs.

Efforts to circumvent these obstacles in the 1990s are focused on identifying specific factors that either drive or inhibit antitumor immunity (12). However, while the relative importance of these factors continues to evolve almost on a daily basis, crude vaccine preparations from the patients' tumors continue to have a role in providing a plethora of characterized and as yet unidentified targets. These vaccines have resulted in objective response rates under 20%, including responses in visceral organ metastases consisting of billions of tumor cells (13–15). Such clinical activity supports the continued development of active immunotherapy in the multidisciplinary anticancer armamentarium. Viable autologous whole tumor cells tested in melanoma vaccine preparations have some advantages: (a) the relative safety of intradermal injection of autologous rather than allogeneic material; (b) the potential for inducing as yet uncharacterized immune responses to altered self-antigens in common with the patient’s tumor; and (c) the diminution of some artifacts of vaccine preparation. Disadvantages include: (a) the difficulty in obtaining and preparing individualized vaccines; (b) the limited source material; and (c) the inability to control for intervaccine variation important in clinical trials.

At M. D. Anderson Cancer Center, Hostetler et al. (16–18) demonstrated that UVR3 (280–320 nm) of the weakly immu-
nogenic melanoma cell line K1735P (C3H/HeN; MTV−) resulted in the generation of a high frequency of highly immunogenic AVs. Immunizing syngeneic mice with AV clones induced protective immunity in vivo challenge assays against the immunizing AV clone and the untreated parental tumor line but not against unrelated tumors (16–18). The parental tumor did not induce such protective immunity. These AV clones grew in immunosuppressed mice but not in immunocompetent mice, and they induced cytoxic/helper T-lymphocyte activity that cross-reacted with parental tumor cells (18).

Based on these data, it was hypothesized that the polyclonal population of UVR-parental cells might provide a multitude of immunogenic AVs for active immunotherapy without requiring individual AV clones to be isolated. Umezu et al. (19) developed conditions for UVR of human melanoma and renal cell carcinoma cells to optimize inducing cytoxic activity of PBMCs against irradiated- and nonirradiated parental cells. Using in vivo immunization/challenge and DTH experiments, Pride et al. (20, 21) demonstrated that UVR of the K1735-M2 cell line greatly increased its immunogenicity. Pride et al. (21) also found that UVR not only up-regulated expression of MHC class I and II molecules but also induced expression of murine B7.1 on K1735-M2 melanoma.

Mycobacterial preparations are useful adjuvants in active immunotherapy (22). In melanoma, Bacillus Calmette-Guérin induces responses in up to 90% of locally injected and 15% of adjacent lesions but has had no effect on survival in Phase III trials and can cause disseminated infection. DETOX (Ribi ImmunoChem Research, Inc., Hamilton, MT) is a novel adjuvant available in a lyophilized oil droplet emulsion consisting of both cell wall cytoskeleton from Mycobacterium phlei and monophosphoryl lipid A from Salmonella minnesota R595. DETOX can augment T cell and humoral immunity. In the murine experiments by Pride et al. (20), DETOX was effective in significantly augmenting immunoprotection and DTH. In patients, DETOX has been used only as an adjuvant, and dosing has been empiric because its effects have been more host than dose related (23, 24).

We undertook the present study to determine the clinical activity and toxicity of active immunotherapy with UVR-AT cells + DETOX in patients with metastatic melanoma. We used serial monitoring of PBMC cytotoxicity and DTH reactions against AT cells with controls to detect and track any evidence of a specific systemic immune response to local intradermal therapy during the course of vaccine treatment.

PATIENTS AND METHODS

Patients. Patients >15 years with metastatic melanoma pathologically confirmed at M. D. Anderson Cancer Center and from whom more than 5 g of nonneoplastic tumor had been resected as a part of routine management were candidates for treatment. Patients were enrolled only after full postoperative recovery and with a Karnofsky performance status >70%; life expectancy >3 months; DTH reaction positive to foreign antigens (see below); results of complete blood counts, serum chemistry studies, immunoglobulins (IgG, IgA, and IgM), complement (C3, C4, and CH50), and T-cell subsets in the clinically normal range; and signed informed consent forms. Patients were required to avoid immunomodulatory agents (e.g., corticosteroids, nonsteroid anti-inflammatory agents, antibiotics, high doses of vitamins, and concurrent specific antitumor therapy). All sites of clinically detectable disease were objectively evaluated at baseline using computed tomography, magnetic resonance imaging, sonography, photographs, and, for s.c. disease, multiple investigator assessment.

Vaccine Preparation. Fresh tumor was collected at the time of surgery from the frozen section laboratory and fragmented by slicing. Aliquots of 106 cells each were frozen for in vitro assays. To maximize yield of viable tumor cells for vaccine preparation, the bulk of tumor was dissociated using collagenase type I (2 mg/ml) and type IV DNase (0.4 mg/ml; Sigma Chemical Co., St. Louis, MO; Ref. 25). These enzymes can alter the immunogenicity of the resulting cell preparation (26–29). In control flow cytometry experiments, Itoh et al. (26) did not find enough significant alteration to obviate the use of the enzymes; furthermore, incubation of the tumor cells for 24 h before vaccine administration, as described below, resulted in reexpression of specific suppressed antigens (26, 29).

The dissociated cells were washed in sterile HBSS and resuspended in equal volumes of HBSS and chilled 10% DMSO + 4% human serum albumin. Aliquots containing 1.5–2 × 107 viable tumor cells were stored under liquid nitrogen. For some specimens, an aliquot of fresh tumor was also put into culture to establish a tumor cell line.

Twenty-four h before patient arrival, an aliquot of tumor cells was thawed in a 37°C water bath, washed twice, resuspended in 5 ml of PBS in a 10-cm Petri dish, and exposed to UVR (2.2 J/m2/s) for 30 s using an FS40 sunlamp (Westinghouse Electric Corp., Bloomfield, NJ; Refs. 18 and 19). Incubation in macrophage-serum-free medium (Life Technologies, Inc.) for 24 h resulted in >80% cell viability. Cell suspensions were checked for bacterial contamination and for Mycoplasma contamination using the Gene-Probe rapid detection system (Gene Probe, Inc., San Diego, CA). After incubation, cells were detached by scraper, washed, resuspended in 0.75 ml of PBS, and X-irradiated (20,000 cGy) to prevent propagation in vivo and insure sterility (30, 31). Concurrently, DETOX (each vial stored at 2–7°C consisted of 500 µg of cell wall cytoskeleton, 50 µg of monophosphoryl lipid A, 2% squalene, and 0.4% Tween 80 in 2X normal saline) was reconstituted by repeated aspiration with 0.5 ml of sterile water. In a total volume of 1 ml, approximately 107 viable replication-deficient UVR autologous melanoma cells in PBS were mixed with 0.25 ml of DETOX just before each treatment (24).

Treatment Plan. Active immunotherapy was administered at M. D. Anderson Cancer Center by intradermal injection into the anteromedial deltoid and femoral regions near intact lymph node basins, every 2 weeks × 6 and then every 4–6 weeks. Treatment ended with progression of disease (25% growth) or exhausted supply of vaccine material. Response was assessed initially at 6 weeks and then at 8–12-week intervals during long-term follow-up. Toxicity was graded according to National Cancer Institute guidelines.

Cytotoxicity Assays. For uniformity, all cytotoxicity assays were performed by a single investigator (D. K.) throughout the study. At baseline and before the fourth, seventh, and subsequent vaccinations, PBMCs were isolated by Ficoll-Paque
Table I

Pretreatment patient and tumor characteristics

<table>
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<th></th>
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<th>% of total</th>
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<tr>
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<td>80%</td>
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<td>Normal serum level of LDH &amp;</td>
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<tr>
<td>Normal serum level of albumin</td>
<td>40</td>
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- 3'CD, LDH, lactate dehydrogenase; AJCC. American Joint Committee on Cancer.

gradient (Pharmacia AB, Uppsal, Sweden) from 30 ml of fresh heparinized peripheral blood at room temperature (22). These PBMCs were assayed fresh and were also cryopreserved until sufficient specimens could be obtained over 2 or more months of treatment to perform a single experiment, thereby minimizing interassay variability. Four-h 3'Cr-release assays were used to detect cytotoxic activity, as described previously (32). Cryopreserved, uncultured autologous and allogeneic melanoma cells, renal cell carcinoma cells, cultured tumor cell lines, and natural killer-sensitive tumor line K562 were used as the targets in these assays. Frozen aliquots of uncultured tumor cells were thawed on the day of the assay; adherent cell lines were trypsinized. Target cells were labeled with 50 p.Ci/million cells of Na25tCrO4 (Amersham Corp., Arlington Heights, IL) at 37°C for 1 h. After washing twice, labeled cell suspensions were passed through a cotton column to remove cell aggregates and debris. Labeled target cells at a concentration of 5000 cells/well in triplicate were added to effector lymphocytes at each of three E:T ratios, 40:1, 20:1, and 10:1, in 96-well, round-bottomed plates. The plates were incubated for 4 h at 37°C, after which 100 μl of supernatant/well was harvested, and radioactivity was measured in a gamma counter. In all experiments performed, the spontaneous release did not exceed 20% of the maximum release. Cytotoxicity assays were also performed with adherent melanoma cell targets in 96-well, flat-bottomed plates. The percentage of specific lysis was calculated as: 

\[
\frac{(X_a - x_s)}{(X_{max} - x_s)} \times 100, 
\]

where \(x_s\) is the mean 3'Cr release at a specified E:T ratio; \(x_{max}\) is the mean spontaneous 3'Cr release (no effector cells); \(x_{max}\) is the maximum 3'Cr release by target cells treated with 0.1 N HCl. Positive cytotoxicity as a result of treatment was defined as >10% specific lysis and at least 2-fold higher specific lysis than at baseline.

**Antibody-blocking Experiments.** To confirm and characterize any positive cytoxicity results detected, blocking experiments were performed using antibodies to CD3, CD4, CD8, DR, DP, DQ (Becton Dickinson, Sunnyvale, CA), and class I MHC (W6/32; DAKO, Copenhagen, Denmark). Monoclonal antibodies were mixed with 3'Cr-labeled AT targets in a volume of 50 μl each for 1 h at 4°C to try to abrogate lysis. Effector cells were added at a 40:1 E:T ratio, and cytotoxicity was measured as described above. Controls were murine IgG and medium alone.

**DTH Assays.** Intradermal skin testing on the volar surface of the forearm was performed at baseline against: foreign recall antigens (Merieux Skintest Multitest Device: tetanus, diphtheria, strep, tuberculosis, Candida, trichophyton, and proteins); 10^5 X-irradiated AT, and 10^5 autologous PBMCs (negative control). AT and PBMC skin tests were repeated before the fourth, seventh, and subsequent vaccinations. A positive DTH reaction consisted of induration over 2 mm in diameter at 48 h. Hypoergy to foreign antigens using the Merieux skin-test device was defined as two or fewer positive DTH reactions or sum of induration diameters under 1 cm. In this study, patients were not asked to undergo punch biopsy of skin test sites to detect subclinical evidence of a DTH reaction.

**Statistical Considerations.** Complete or partial response required disappearance or >50% decrease, respectively, in the sum of the products of largest diameters of all indicator lesions lasting at least 1 month. Stable disease
**RESULTS**

Between September 1992 and May 1995, 42 patients received a median of eight treatments (range, 3–21) with UVR-autologous melanoma cells plus DETOX. Pretreatment characteristics are summarized in Table I. All patients were assessable for clinical response and toxicity and 35 for biological response. Seven patients had disease progression before completing the first six treatments.

**Clinical Responses.** Among 24 patients with indicator lesions, two had major responses and six showed stabilization in soft-tissue metastases. One 39-year-old woman treated previously with chemo/biotherapy underwent resection of painful s.c. nodules for vaccine preparation. Her indicator lesions consisted of nine s.c. nodules, each 1 cm or smaller, and a large sympto-

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Fig. 1  Clinical responses to active immunotherapy using autologous UVR-melanoma cells + DETOX. A, partial response in bone 6 months from the start of treatment; this patient also had a complete response in s.c. nodules. B, single view of some of several dozen in-transit lower-extremity metastases which, after an initial flare at 10 weeks, disappeared over a period of 17 months from the start of treatment; surgery to harvest more cells for vaccine preparation resulted in this patient’s formal response being characterized as mixed to vaccine and complete to vaccine plus surgery.
tomatic lytic bone metastasis that had progressed radiographically 1 year after radiation therapy. Four days after her first treatment, she noted soreness at all tumor sites. Three skin nodules initially enlarged by less than 20% with minimal inflammatory changes, three remained stable, and three regressed; however, by the seventh treatment (4 months), all s.c. lesions had completely regressed as had her bone pain, with subsequent confirmation of major radiological response (Fig. 1A). Nine months after her last treatment, she developed new s.c. nodules and recurrence in bone (time to progression, 21 months). From a negative baseline, her unstimulated PBMCs demonstrated persistently positive cytotoxicity against AT cells (Fig. 2A). She had an unusually large DTH reaction (4 cm with 10 cm of surrounding inflammation) at the first vaccine intradermal injection site, smaller with subsequent treatments. No DTH reaction to the 10^3 AT control was detected during 1 year of treatment.

The second responder was a 63-year-old man with extensive, progressing, lower-extremity, in-transit nodules, despite regional and systemic chemo/biotherapy and radiation therapy. Resected symptomatic lesions were used for vaccine preparation. Innumerable in-transit nodules, 0.5–2 cm in caliber, were used as indicator lesions. By 10 weeks, most lesions had flattened or remained stable; however, a minority appeared more prominent, although by <20%. Because of early resection of these lesions to make more vaccine, this was strictly a “mixed” response. However, after 19 treatments for >20 months, together with surgery, he ultimately achieved a complete response lasting 5 months (Fig. 1B). From a negative baseline and extending through the 16th treatment, his unstimulated PBMCs demonstrated cytotoxicity against AT cells (Fig. 2B). Fall-off in cytotoxicity coincided with progressive soft-tissue disease. He consistently had a large DTH reaction (3 cm) to vaccine but never to the AT control.

**Survival.** The median survival of the stage IV patients was 16 months from start of treatment, 20 months from initial diagnosis of stage IV disease. Review of pretreatment prognostic factors revealed a high frequency of favorable factors (normal serum levels of lactate dehydrogenase; Refs. 33 and 34), metastases in soft tissue sites alone, or metastases in one visceral organ such as lung (33), in this study.

**Toxicity.** Grade I toxicities were limited to local irritation at the vaccine injection site and in 10% of patients, with malaise lasting 24–48 h. There was no incidence of skin ulceration or other grades II-IV toxicities. Palpable residual granulomas at the injection sites measuring <2 cm became less apparent over weeks to months. In one-half of the patients, routine evaluation by an ophthalmologist yielded no abnormalities attributable to treatment. Five of 15 patients (33%) purified protein derivative negative at baseline converted to purified protein derivative positive (asymptomatic) using the Merieux skin-test device, possibly a result of repeated exposure to DETOX (or to the anergy panel).

**Detection of PBMC Cytotoxicity against Autologous Tumor.** During the course of treatment, 10 of 35 assessable patients had an early rise in mean PBMC cytotoxicity against AT cells within 6 weeks, all from a negative baseline of under 10% specific lysis (Table 2). Lytic activity remained detectable up to 10 months, later falling back to background levels in 7 of the 10 patients. In five patients, the fall-off coincided with progression of disease within 3 months. For the three patients without fall-off, autologous material was insufficient to continue long-term treatment and monitoring.

Table 3 illustrates the individual spectrum of specificity for PBMC cytotoxicity. The selective rather than general positivity in the cytotoxicity assay results against a panel of allogeneic tumor cells, and K562 does not support a non-specific effect of vaccine preparation, such as the use of collagenase. In four patients, the level of lysis of K562 cells was minimal, indicating that the cytotoxicity was not mediated by natural killer cells. Table 4 shows that lysis could be abrogated by anti-CD3, anti-class I, and anti-CD8 but not by anti-class II and anti-CD4 antibodies, indicating that the observed cytolytic activity was restricted by class I and was mediated by CD8+ T cells.

Univariate analysis indicated that patients with stage IV disease and positive cytotoxicity had both a prolonged median time to progression and overall survival duration compared with those with negative cytotoxicity (Table 5 and Fig. 3). Although time bias was minimized because PBMC cytotoxicity was always detected early, the positive cytotoxicity group also had a lower disease burden, confounding the results of the analysis.

**DTH.** Among 42 nonanergic patients, 21 (50%) were hyporesponsive to foreign recall antigens at baseline, a finding that did not correlate with any pre- or posttreatment factors. Baseline hyporesponsiveness was evident in 4 of the 10 patients with positive PBMC cytotoxicity results (Table 5).

Among the 35 assessable patients, 8 (23%) developed a late DTH reaction to 10^5 AT cells (and no reaction to PBMC control); indurations under 1 cm first appeared after a median of 8 courses (4–15 courses). Neither responder and only three patients with positive PBMC cytotoxicity also had a DTH reaction.
Ten patients with specific lysis >10% and rising over 2× baseline during the course of treatment

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Table 3: Specificity of positive PBMC cytotoxicity in four patients

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<thead>
<tr>
<th>Patient no.</th>
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<th>Allogeneic melanoma</th>
<th>Renal cell carcinoma</th>
<th>NK*-sensitive K562</th>
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<td>11</td>
<td>+</td>
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a) Vaccine number.  
b) Months.

**DISCUSSION**

Among 24 patients with indicator lesions, intradermal active immunotherapy with UVR-AT + DETOX resulted in two major responses in soft-tissue sites and in a bone metastasis, supporting clinically meaningful systemic activity, albeit in <20% of patients with 95% confidence. This is no different from the results reported in other vaccine trials and does not support using UVR alone to enhance the clinical activity of autologous whole-cell vaccines plus adjuvant. As in other such trials, the overall survival data were encouraging but likely reflect selection bias. The present American Joint Committee on Cancer criteria for stage IV disease only distinguish between soft-tissue and visceral metastases in patients having a median overall survival of 8 months. Newer, more effective stratification parameters for clinical trials using pretreatment serum levels of lactate dehydrogenase and albumin (33-34) and the presence of visceral disease in zero-to-one organ versus more-than-one organ (33) have identified prognostic groups with median survivals of over >12 months versus <6 months. Until a regimen with a high response rate is found, stratification might help determine which active immunotherapy trials warrant confirmation of “encouraging” survival data in a Phase III trial setting.

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reaction to AT cells. Skin testing using UVR-AT was not performed. Contaminants in preparation could have resulted in a high frequency of false-positive DTH reactions to both AT and PBMC control skin tests; this was not observed. No tests, such as punch biopsy, were done to evaluate for false-negative results.

The size of the DTH induration to the vaccine itself (UVR-AT + DETOX), measuring primarily response to adjuvant, was above the average and median values in 7 of the 10 patients with positive cytotoxicity, including the two responders and the three patients with a DTH reaction to AT. No consistent trend in the size of the DTH reaction to vaccine was observed in individuals or in population groups over time. Biopsy and staining of a 1-month-old indurated injection site in one patient revealed heavy infiltration by histiocytes and T-cells but not by B cells (data not shown).
Table 5  Characteristics of patients with positive and negative detection of PBMC cytotoxicity against autologous tumor

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<td>6</td>
<td>42</td>
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</table>

* Percentage of cytotoxicity.

Table 4  Antibody inhibition of PBMC cytotoxicity against autologous melanoma

<table>
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<th>Patient no.</th>
<th>Media alone</th>
<th>IgG</th>
<th>anti-CD3</th>
<th>anti-CD8</th>
<th>anti-class I</th>
<th>anti-CD4</th>
<th>anti-DR</th>
<th>anti-DP</th>
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With regard to laboratory tests that might also guide such determination, none to-date has proven reliable, in part because of the dearth of correlatable clinical responses and in part because of the inability to demonstrate reproducible dose-response relationships in a heterogeneous patient population. Posttreatment laboratory results cannot be stratified prospectively in Phase I or II studies because of time bias and other factors that complicate any analysis of such data (35).

The serial detection of PBMC cytotoxicity against AT in 10 of 35 assessable patients (Tables 2 and 5) supported induction of a specific systemic immune response to intradermal treatment, a primary end point of this study. There are no data to permit a comparison of the frequency of induction of positive cytotoxicity with that using a non-UVR-AT + DETOX vaccine because no patient received such treatment in this pilot study. Among the stage IV patients, almost uniformly with favorable clinical pretreatment prognostic features, detection of PBMC cytotoxicity against AT cells correlated significantly with survival ($P = 0.009$; Fig. 3). Different patterns of specificity were observed, suggesting that the positive results were not likely to be an artifact of the assays themselves (Table 3). Also, MHC class I-restricted CD8+ T cells and not natural killer cells were the primary mediators of this activity in the patients tested (Table 4). The fall-off of detection of PBMC cytotoxicity concurrent with tumor progression in some patients suggested induction of tolerance. It must be noted, however, that the peripheral blood is only one pharmacological compartment (36), and this study did not account for efficient trafficking of cytotoxic T cells to metastatic deposits and any resulting immunoselection (37).

The induction of a DTH reaction to AT cells was not observed in clinical responders. Subclinical reactivity could have been evaluated further by punch biopsy of skin test sites; this would require an informed consent from the patients. The prolonged time to first clinically apparent DTH reaction to AT cells (median of 8 courses) introduced a substantial “time bias” precluding developing this test for prognostic purposes (35). Among all DTH assays performed, only above average DTH reaction to vaccine, largely a response to the adjuvant DETOX, correlated favorably with the clinical course of disease. The study was too small to evaluate the effect of concurrent detection of both PBMC cytotoxicity and DTH reactivity to AT cells, observed in only three patients.

Demonstration of UVR-induced expression of B7.1 on human melanoma, as reported recently on murine melanoma (21) as well as on CT-26 (colon carcinoma) and HCA (hepatocellular carcinoma) cell lines,* could support using UVR to study the antigen-presenting capability of tumor cells without resorting to gene transfection. Besides the ongoing character-

* M. Pride, unpublished data.
ization of the positive cellular immune responses as presented above and in cytokine elaboration experiments, future efforts will be geared to testing cytokines that drive Th1 immune responses (25) or that recruit dendritic cells or other professional antigen-presenting cells to the vaccine injection site (15).

Suto and Srivastava (38) has been able to extract from autologous tumor cells heat shock protein-96, a chaperon that complexes with the cells’ unique repertoire of soluble immunogenic peptides (HSPPC-96). He has shown that HSPPC-96 can elicit antigen-specific CTLs. Treatment with HSPPC-96 could provide an array of autologous tumor-specific peptide targets for CTL activation without the need to characterize each peptide or to exclude patients based on human leukocyte antigen phenotype. This may prove to be a far more exploitable and versatile strategy for immunotherapy than trying to convert an autologous tumor cell that has already eluded host immune surveillance into a better antigen-presenting cell. Autologous tumor HSPPC-96 could be used in the future to prime dendritic cells activated or expanded in vitro with cytokines such as granulocyte/macrophage colony-stimulating factor, interleukin 4, or flt3 ligand.

No active immunotherapy program will be optimized, however, until methods are developed in individual patients to circumvent immunoselection (6), local secretion of tolerizing molecules by tumors (7–10), and biophysical barriers that make metastases sanctuary sites from the immune system (11).

ACKNOWLEDGMENTS

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Active immunotherapy with ultraviolet B-irradiated autologous whole melanoma cells plus DETOX in patients with metastatic melanoma.

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