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Immunology

Induction of antigen-specific cytolytic T cells *in situ* in human melanoma by immunization with synthetic peptide-pulsed autologous antigen presenting cells

(cytolytic T-lymphocyte response/melanoma/peptide vaccine/immunotherapy)

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ABSTRACT **Human melanoma cells can process the MAGE-1 gene product and present the processed nonapeptide EADPTGHSY on their major histocompatibility complex class I molecules, HLA-A1, as a determinant for cytolytic T lymphocytes (CTLs). Considering that autologous antigen presenting cells (APCs) pulsed with the synthetic nonapeptide might, therefore, be immunogenic, melanoma patients whose tumor cells express the *MAGE-1* gene and who are HLA-A1$^+$ were immunized with a vaccine made of cultured autologous APCs pulsed with the synthetic nonapeptide. Analyses of the nature of the *in vivo* host immune response to the vaccine revealed that the peptide-pulsed APCs are capable of inducing autologous melanoma-reactive and the nonapeptide-specific CTLs *in situ* at the immunization site and at distant metastatic disease sites.**

The discoveries of genes encoding a number of human melanoma-associated antigens (1–6) and of sequences of antigenic peptides serving as the cytolytic T-lymphocyte (CTL) epitopes on the appropriate major histocompatibility complex (MHC) class I molecules (7–11) have raised considerable interest in peptide-based specific melanoma vaccines. All the CTL-determined melanoma-associated peptide epitopes that have so far been described are, however, "self" peptides (i.e., without any alteration in the sequence in the encoding gene found in the autologous normal cells). The question of whether these self peptide epitopes are immunogenic in humans has, therefore, become a crucial issue. To test the immunogenic potential of a peptide-based melanoma vaccine *in vivo*, we have taken an approach based on the physiologic principles underlying peptide presentation to T cells. This approach involves presenting a relevant CTL-determined peptide on the appropriate MHC-restricting elements of autologous professional antigen presenting cells (APCs). We chose to study the immunogenicity of the MAGE-1 nonapeptide EADPTGHSY (7) *in vivo*, since the *MAGE-1* gene is not expressed in any normal tissues with the exception of certain cells in the testis (1).

We have observed that a population of APCs, obtained by culturing autologous blood-derived monocyte/macrophages in granulocyte–macrophage colony-stimulating factor (GM-CSF), exhibit many of the essential features of authentic APCs (high levels of MHC class I and class II molecules, CD11b antigen, CD54 antigen, B7 molecules, and dendritic morphology expressed by a proportion of the cells). They are also capable of simultaneously providing an additional stimulatory signal(s) toward T-cell activation—analogous to some form of costimulatory, or just another stimulatory, second signal. These cultured APCs can efficiently present the MAGE-1 nonapeptide to the MAGE-1 antigen-specific CTLs (12). More importantly, they seem to be capable of overriding the negative influence of melanoma cells while activating autologous T cells *in vitro* (12). Based on these findings, this study was designed to examine whether the cultured APCs, pulsed with the synthetic peptide EADPTGHSY, could recruit a MAGE-1-specific CTL response *in vivo* at the immunization sites and at distant sites of melanoma deposits in patients with advanced stage IV melanoma, who are HLA-A1$^+$, and whose tumor cells express the *MAGE-1* gene.

MATERIALS AND METHODS

Patients. Patients with advanced metastatic melanoma were studied with informed consent.

Cell Lines. The melanoma cell lines RM-M (HLA-A1, -3; B8, -44) and HS-M (HLA-A1, -28; B14, -57) were established from explants of metastatic melanoma in our laboratory from the two study cases according to procedures described earlier. The melanoma cell line MZ 3.1 (MAGE-1$^+$/HLA-A1$^+$) and the MAGE-1$^-$/HLA-A1$^+$ variant melanoma line MZ 2.2 and the MAGE-1 antigen-specific CTL line MZ 82/30 were gifts of Thierry Boon (Ludwig Institute, Brussels).

APCs. Autologous peripheral blood-derived plastic adherent mononuclear cells cultured in recombinant GM-CSF were used as APCs in this study. Briefly, adherent cells were obtained after a 2-h incubation of the Ficoll/Hypaque gradient-separated mononuclear population on plastic dishes were cultured in AIMS-V medium (GIBCO) in recombinant GM-CSF (1000 units/ml; Immunex). The cells were maintained in continuous culture and were split as needed by harvesting the growing semiadherent or the nonadherent cells by vigorous pipetting.

PCR Amplification. The PCR amplification technique for detection of *MAGE-1* gene expression has been described (12).

Preparation of Peptide-Pulsed APCs as the Vaccine. The vaccine (peptide-pulsed cultured APCs) was prepared with the cultured APCs pulsed with synthetic peptide. The MZ2-E nonapeptide (EADPTGHSY) and the control peptide oligomers (ADPTGHSY) were synthesized using the Applied Biosystems peptide synthesizer (model 431A) using Fast Moc chemistry. The synthetic peptides were purified by reverse-phase chromatography on a Perkin–Elmer BioLc HPLC system with a Waters Nova-Pak C_{18} column and were sequenced

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Abbreviations: CTL, cytolytic T lymphocyte; APC, antigen presenting cell; MHC, major histocompatibility complex; IL-2, interleukin 2; DTH, delayed-type hypersensitivity; TIL, tumor-infiltrating lymphocyte; VIL, vaccine-infiltrating lymphocyte; TNF, tumor necrosis factor.

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on a MilliGen/Biosearch prosequencer (model 6600). The cultured APCs were incubated with the MAGE-1 nonapeptide or with the control peptides (2 μg per 10^6 cells) in 1 ml of AIMS-V medium at 37°C for 2 h after which the APCs were washed three times in phosphate-buffered saline.

Immunization Technique. The immunization protocol consisted of intradermal immunizations with the cultured autologous APCs, which were pulsed with the peptide EADPTGHSY (henceforth referred to as the MAGE-1 nonapeptide) four times at monthly intervals with escalating numbers of cells (5×10^5, 1×10^6, 5×10^6, and 1×10^7). Patients were also simultaneously injected, at separate sites, with APCs alone and with the APCs pulsed with the control octapeptide ADPTGHSY (henceforth referred to as the control peptide). After the first intradermal immunization, a single intravenous injection of 1×10^8 MAGE-1 nonapeptide-pulsed APCs (derived from leukapheresed mononuclear cells) was administered (in 50 ml of saline slowly over 5 min) as a priming dose. The injections were given in 0.1 ml of normal saline close to a peripheral lymph node-bearing region on a rotating basis. Patients were monitored for immediate and delayed toxicities and the injection sites were examined for delayed-type hypersensitivity (DTH) response, if any, at 24 and 48 h. Forty-eight hours after the fourth immunization, the site injected with the MAGE-1 nonapeptide-pulsed APCs and a distant tumor metastatic site, if available, were excised for immunohistological studies.

***In Vitro* Expansion of Infiltrating Lymphocytes.** Tumor-infiltrating lymphocytes (TILs) and vaccine-infiltrating lymphocytes (VILs) were expanded *ex vivo* in recombinant human interleukin 2 (IL-2) (Collaborative Biomedical Products, Bedford, MA; 100 units/ml) from the homogenates mechanically prepared from the excised tissues. The cultures were stimulated every 7–10 days with the respective autologous APCs pulsed with the MAGE-1 nonapeptide. All experiments shown in this paper were performed with the respective TILs and VILs stimulated at least three times and maintained in culture for 4–6 weeks.

Assay for Cytokine Synthesis by the *ex Vivo* Expanded Infiltrating T Cells upon Peptide Presentation. Briefly, 3000 TILs or VILs were stimulated against 5×10^4 tumor cells or APCs pulsed with peptides (2 ng/ml) in individual wells of a 48-well cluster plate (Costar) in 0.5 ml of medium. Fifty-microliter supernatants, harvested after 24 h, were assayed for tumor necrosis factor (TNF) activity in a colorimetric TNF-β bioassay (12) using the WEHI 164 clone 13 (13). Dilutions of recombinant TNF-β (Biosource, Camarillo, CA) were used as TNF-β standard.

Phenotypic Analysis. The immunofluorescence procedure for phenotypic analysis has been published (14). Anti-CD4 antibody and anti-CD8 antibody were purchased from Coulter.

Microcytotoxicity Assay. The ^{51}Cr release microcytotoxicity assay has been described (14).

RESULTS

Three immunocompetent patients (judged by their ability to exhibit a DTH reaction to one or more of the common microbial antigens such as purified protein derivative, monilia, and mumps) with advanced melanoma were studied. Extensive prevaccination immunological evaluation revealed that all study patients had natural killer (NK) cell and lymphokine-activated killer (LAK) cell activities in their blood lymphocytes. Autologous melanoma-specific CTL activity, however, was not observed in the fresh blood lymphocytes, nor could such melanoma-specific CTL activity be generated in the blood lymphocytes by repeated stimulation against the autologous melanoma cells in *in vitro* coculture in the presence of IL-2 (collective data not shown).

The relevant physical, phenotypic, and functional properties of the cultured APCs in general have been described (12). The adherent cells from peripheral blood mononuclear cells grew as partly adherent and partly semiadherent cells in clumps, after an initial lag phase of several days. After several weeks of growth, they grew predominantly as nonadherent cells with a doubling time of 3–5 days. They expressed many of the

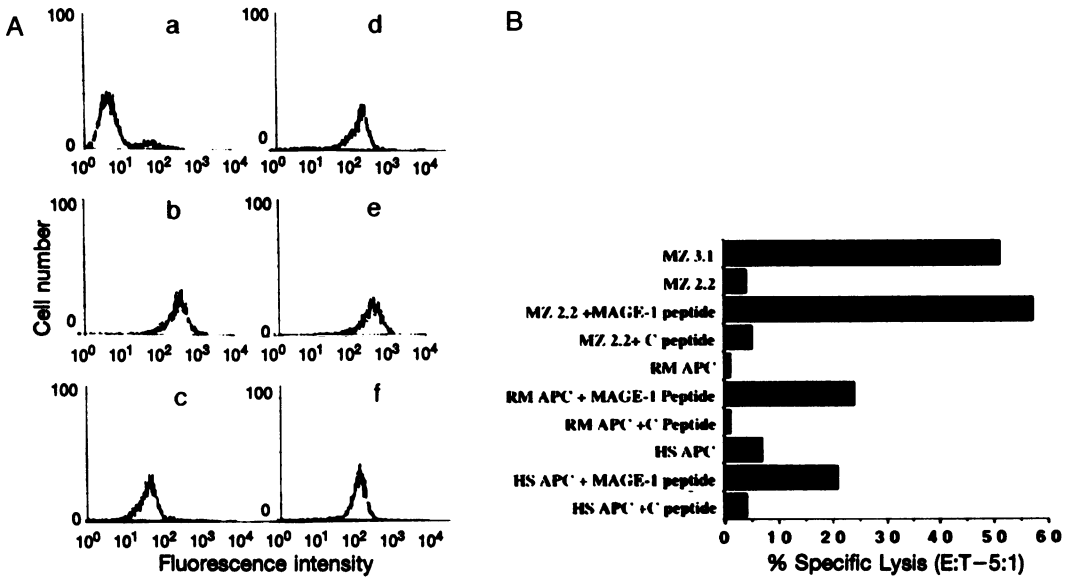

FIG. 1. Phenotypic and functional characteristics of the cultured APCs. (*A*) Phenotype of cultured APCs derived from patient 1. (*a*) PBS control. (*b*) Stained with anti-HLA class I (W6/32) antibody. (*c*) Stained with anti-B7 antibody. (*d*) Stained with anti-CD11b antibody. (*e*) Stained with anti-HLA class II antibody. (*f*) Stained with anti-CD54 (ICAM-1) antibody. (*B*) Representative experiment demonstrating the ability of cultured APCs from the two study patients to present the MAGE-1 nonapeptide to the MAGE-1-specific CTL clone MZ 82/30 (a gift of Thierry Boon). To pulse a target cell with a peptide, ^{51}Cr-labeled target cells (10^3 cells per 100 μl) were incubated in the presence of 2 nM peptide for 30 min at 37°C in 96-well microplates. CTLs were added in an equal vol (100 μl) and then a standard 4-h microcytotoxicity assay was carried out. RM APC and HS APC represent cultured APCs from patients 1 and 2, respectively. MZ 3.1, HLA-A1$^+$/MAGE-1$^+$ positive reference line; MZ 2.2, HLA-A1$^+$/MAGE-1$^-$ variant negative reference line. Both cell lines were gifts of Thierry Boon.

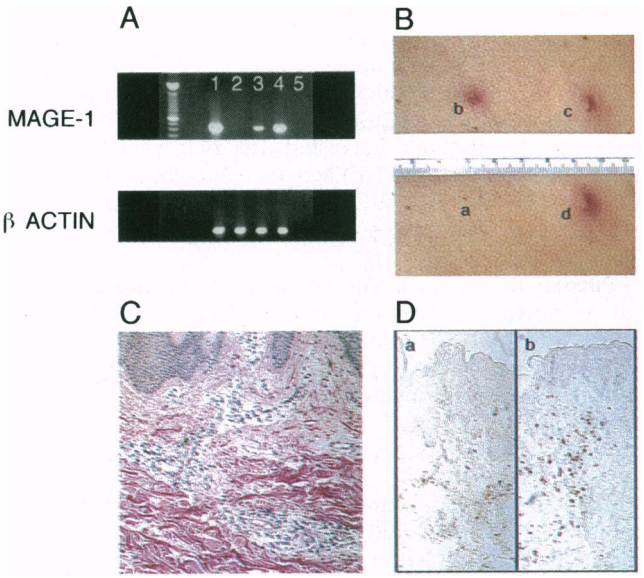

FIG. 2. *In vivo* response to peptide-pulsed APCs. (*A*) Ethidium bromide stain of gel electrophoresis of PCR analysis of MAGE-1 mRNA expression by the respective melanoma cell lines derived from the two patients. Lanes: 1, MZ 3.1, the MAGE-1$^+$ reference melanoma cell line; 2, MZ 2.2, the MAGE-1$^-$ autologous variant line; 3, RM-M melanoma cells (patient 1); 4, HS-M melanoma cells (patient 2); 5, water control. (*B*) Skin reaction 48 h after the fourth immunization in patient 2. (*a*) Saline control. (*b*) APC alone. (*c*) Control peptide-pulsed APC. (*d*) MAGE-1 nonapeptide-pulsed APCs. (*C*) Photomicrograph of the histologic findings of the skin reaction at the fourth vaccine site that was injected with the MAGE-1 peptide-pulsed APCs from patient 2 [shown in *B*(*d*)] demonstrating perivascular infiltration of mononuclear cells and separation of the collagen bundles, both of which are characteristic features of the DTH-type response. (×65.) (*D*) Immunohistological examination of the same specimen showing infiltration with both CD4$^+$ T cells (*a*) and CD8$^+$ T cells (*b*).

phenotypes of cultured dendritic cells [expression of the B7 molecule and high level of MHC class I and II antigens, CD54, CD11b molecules, and no T- and B-cell markers (CD3, CD4, CD8, and CD20)]. Fig. 1*B* shows an experiment demonstrating that these cultured APCs derived from both study patients could present the MAGE-1 nonapeptide to the MZ2-E antigen-specific CTL clone 82/30.

All three patients completed the immunizations without any immediate or late side effects. The injection sites receiving the lower numbers of cells (1×10^5) usually failed to elicit any evidence of inflammation. All patients, however, developed varying degrees of erythema, with or without indurations, at 24 and/or 48 h, to immunizations with the higher cell numbers of APCs (alone, pulsed with the MAGE-1 nonapeptide or with the control peptide). The erythema and the indurations were usually most pronounced at sites injected with the highest number of cells (collective data not shown). In all three patients, although the indurations at the sites injected with the MAGE-1 nonapeptide-pulsed APCs were usually larger, it was not possible to clearly establish any specificity from the skin reactions. The characteristic MAGE-1 gene expression by the melanoma cells derived from the two patients and the nature of the *in vivo* response to the immunizations are shown in Fig. 2. Fig. 2*A* shows the MAGE-1 mRNA expression (analyzed by reverse transcription and the PCR amplification technique) by the melanoma cells of the two study patients. Fig. 2*B* shows an example of the varied degrees of erythema and indurations observed with the last round of immunizations in one of the study patients. Histopathologic examinations of the vaccine site injected with the cultured APCs pulsed with the MAGE-1 nonapeptide showed perivascular infiltrates of lymphocytes and separation of dermal collagen bundles signifying edema, both of which are characteristics of a DTH response (Fig. 2*C*). Immunohistopathological examinations of the same vaccine site revealed evidence of infiltration by both CD4$^+$ and CD8$^+$ T cells without any discernible quantitative or qualitative preponderance of one T-cell subtype or another (Fig. 2*D*). Essentially identical findings were detected with the vaccine (MAGE-1 nonapeptide-pulsed APCs) sites from the other two patients (data not shown). The prevaccination and postvaccination tumors also exhibited varied degrees of lymphocytic infiltrates. When the lymphocytic infiltrations in the pre- and postvaccination specimens were compared, no quantitative or qualitative difference could be clearly established (composite data not shown).

The infiltrating T cells from the minced tissues—VILs and TILs—were expanded *ex vivo* in IL-2 in the presence of the MAGE-1 nonapeptide-pulsed APCs (stimulated every 7–14 days). VILs and TILs (pre- and postvaccination) could be adequately expanded in only two of the three patients for detailed phenotypic and functional characterization. Fig. 3 shows the phenotypic analyses of the infiltrating T cells expanded from the specimens derived from both of these

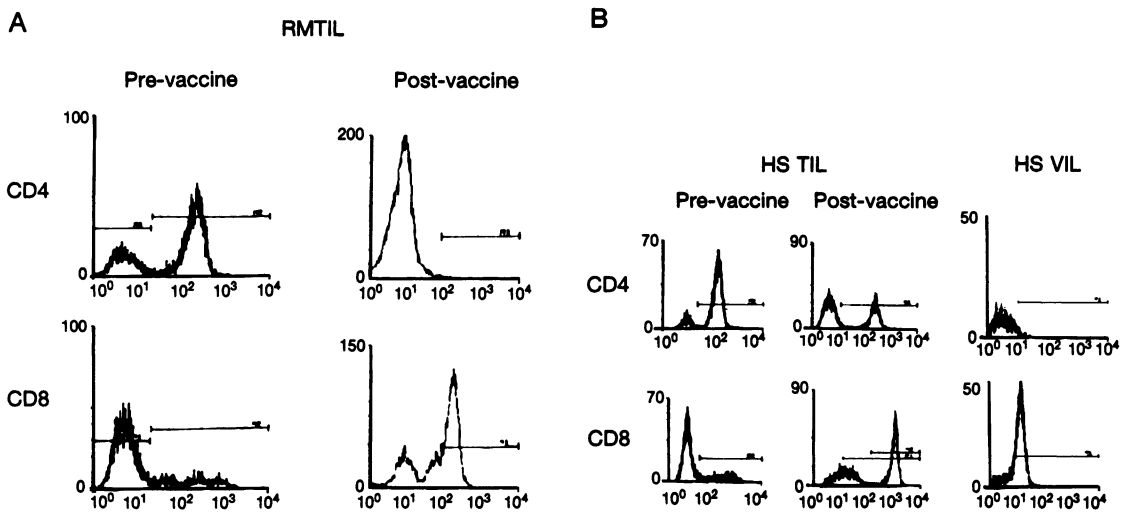

FIG. 3. Phenotype of the expanded *ex vivo* infiltrating lymphocytes from tumor deposits and from the vaccine sites. (*A*) Phenotypic analysis of pre- and postvaccination TILs expanded *ex vivo* from patient 1 (RM). (*B*) Phenotypic analysis of the expanded VILs and TILs derived from patient 2 (HS).

Table 1. Cytolytic activities of the *ex vivo* expanded infiltrating T cells from vaccination site and from metastatic tumor deposits

Target	Patient 1			Patient 2			
	Pre-Rx TILs		Post-Rx TILs	Pre-Rx TILs		Post-Rx TILs	VILs
	Tumor 1	Tumor 2		Tumor 1	Tumor 2		
Autologous melanoma cell	6	5	54 (48, 35)	0	3	53 (30, 20)	26
+ anti-Class I	—	—	20*	—	—	6**	0**
+ anti-Class II	—	—	79	—	—	20	11
MZ 3.1	3	7	34	0	14	32	18
MZ 2.2	10	NT	16	0	26	0	5
MZ 2.2 + MAGE-1 peptide	8	NT	37†	NT	NT	NT	35††
MZ 2.2 + control peptide	10	NT	17	NT	NT	NT	6
K-562	24	10	7	0	30	0	6

Experiments were performed with the respective TILs and VILs stimulated at least three times and maintained in culture for 4–6 weeks. Results are expressed as % lysis at an effector/target cell ratio of 10:1. RM-M, autologous melanoma cell lines from patient 1; HS-M, autologous melanoma line from patient 2 (numbers in parentheses represent % lysis effector/target cell ratios of 5:1 and 2.5:1, respectively). MZ 3.1, HLA-A1$^+$/MAGE-1$^+$ positive reference line; MZ 2.2, HLA-A1$^+$/MAGE-1$^-$ variant negative reference line; K-562, NK cell-sensitive MHC class I$^-$ cell line; NT, not tested; Pre-Rx, prevaccination; Post-Rx, postvaccination. The inhibitory effect of 5 μl of a 1:200 dilution of the anti-MHC class I antibody (culture supernatant of the hybridoma W6.32; a gift of Soldano Ferrone, New York Medical School, Valhalla) on cytolysis, when compared to cytolysis of the autologous melanoma cells in the absence of anti-class I antibody, was significant at $P < 0.01$ (*) and at $P < 0.001$ (**) by Student's t test. Differences in % lysis of the target MZ 2.2 pulsed with the nonapeptide when compared with % lysis of the MZ 2.2 alone or pulsed with the control peptide were significant at $P < 0.01$ (†) and at $P < 0.001$ (††) by Student's t test. The experiment was repeated three times with similar results.

patients. In one case (patient 1), the prevaccination TILs (expanded from a subcutaneous metastatic lesion) showed a predominantly CD4$^+$ phenotype; the postvaccination TILs (expanded from another distant metastatic lesion) in this case were, however, predominantly CD8$^+$ (Fig. 3*A*). In the other case (patient 2), the prevaccination TILs (expanded from a subcutaneous lesion) were also CD4$^+$; the VILs and the postvaccination TILs (from a different subcutaneous metastatic lesion) were predominantly CD8$^+$ (Fig. 3*B*).

The cytolytic properties of the expanded T cells are shown in Table 1. The prevaccination TILs derived from patient 1 exhibited no significant cytolysis against the autologous melanoma cells but they exhibited nonspecific cytolytic function toward allogeneic target cells, especially against the MHC class I$^-$ NK cell-sensitive target cell line K-562. The postvaccination TILs, in contrast, lysed the autologous melanoma cells in a MHC class I-restricted manner and lysed the HLA-A1$^+$/MAGE-1$^+$ reference line MZ 3.1 or the HLA-A1$^+$ but MAGE-1$^-$ variant cells (MZ 2.2) that were pulsed with the MAGE-1 nonapeptide. It should be mentioned that the inhibitory effect of the anti-class I monoclonal antibody on cytolysis by the postimmunization TILs against the RM-M melanoma cells in patient 1 was substantially more profound in other experiments (data not shown). The prevaccination TILs from patient 2 were noncytolytic. The VILs and the postvaccination TILs from this patient, in contrast, lysed the autologous melanoma cells in a MHC class I-restricted manner and exhibited MAGE-1 antigen specificity [i.e., they lysed the HLA-A1$^+$/MAGE-1$^+$ reference target cells (MZ 3.1) and lysed the MAGE-1 peptide-pulsed HLA-A1$^+$/MAGE-1$^-$ variants (MZ 2.2)].

Table 2 shows the ability of the expanded T cells to recognize the MAGE-1 peptide on the appropriate MHC-restricting elements and to synthesize TNF upon recognition. As shown, the prevaccination TILs from both patients synthesized TNF nonspecifically. The postvaccination TILs, however, synthesized TNF only when they were stimulated by the MAGE-1 nonapeptide-pulsed autologous APCs, the autologous melanoma cells, the HLA-A1$^+$/MAGE-1$^+$ reference cell line MZ 3.1, or the MAGE-1 peptide-pulsed MAGE-1$^-$ variant cells (MZ 2.2). In patient 2, the VILs similarly synthesized TNF only when they were specifically stimulated by the HLA-A1$^+$/MAGE-1$^+$ melanoma cells (autologous or allogeneic) or when stimulated by the MAGE-1 nonapeptide-pulsed autologous APCs or when stimulated by the MAGE-1$^-$ variant melanoma cells (MZ 2.2) that were pulsed with the relevant nonapeptide.

DISCUSSION

This study was designed with the hypothesis that in patients bearing melanoma cells that express a CTL-determined anti-

Table 2. Cytokine synthesis by *ex vivo* expanded infiltrating T cells upon peptide presentation

Stimulant	Patient 1		Patient 2		
	Pre-Rx TILs	Post-Rx TILs	Pre-Rx TILs	Post-Rx TILs	VILs
None	70	2	3	0	8
Autologous APCs	62	0	48	0	12
MAGE-1 peptide-pulsed autologous APCs	67	32	50	31	48
Control peptide-pulsed autologous APCs	125	0	49	3	12
Autologous melanoma cells	70	28	4	20	52
MZ 3.1	125	30	2	30	55
MZ 2.2	100	0	0	0	10
MAGE-1 peptide-pulsed MZ 2.2	120	29	NT	23	62
Control peptide-pulsed MZ 2.2	25	0	NT	6	14

TILs or VILs were stimulated in individual wells of a 48-well cluster plate (Costar) in 0.5 ml of medium for 24 h. The supernatants harvested after 24 h were assayed for TNF activity. MZ 3.1, HLA-A1$^+$/MAGE-1$^+$ allogeneic positive reference target line; MZ 2.2, HLA-A1$^+$/MAGE-1$^-$ variant negative reference target line; NT, not tested. The experiment was repeated three times with similar results. Results are expressed as TNF synthesis (pg/ml) by infiltrating T cells. TNF synthesis by the tumor cells or by the cultured APCs alone never exceeded 10 pg/ml. Pre-Rx, prevaccination; Post-Rx, postvaccination.

genic epitope one might be able to break the state of immunologic unresponsiveness through an active specific immunization technique that incorporates a mechanism of delivery of the antigenic as well as the costimulatory second signal simultaneously. Accordingly, we designed the vaccine with autologous professional APCs pulsed with the relevant antigenic peptide. The demonstration of inducibility of the peptide-specific T cells at the site of the immunization as well as at sites of distant metastatic melanoma therefore constituted an important test of the hypothesis. Viewed from this perspective, the results of our study are noteworthy, as they allow a number of conclusions. First, the MAGE-1 nonapeptide when presented on the cultured autologous APCs *in vivo* is immunogenic (i.e., induces a peptide-specific CTL response *in situ*). Second, immunizations with such peptide-pulsed APCs seem to be able to induce peptide-specific CTL responses *in situ* in distant sites of metastatic disease. It is noteworthy that although both patients were simultaneously immunized with APCs pulsed with two peptides (the MAGE-1 nonapeptide and a control octapeptide), the postvaccination TILs derived from the distant metastatic sites recognized only the MAGE-1 nonapeptide and not the control peptide, although both peptides display the binding motifs for HLA-A1 molecules. Admittedly, we did not look for induction of an octapeptide-specific CTL response at the site injected with the octapeptide-pulsed APCs. Nevertheless, the recovery of the relevant nonapeptide-specific CTLs from the vaccine site and from the postvaccination metastatic deposits suggests that this represents true induction of peptide-specific CTLs after immunizations with the same peptide.

It is possible that we might have failed to detect MAGE-1 peptide-specific CTLs in the prevaccination TILs in both patients due to sampling error or due to some other reason. It should, however, be mentioned that more than one metastatic tumor, excised at different times from both patients, was studied for the evidence of specific CTL activities after *ex vivo* expansion under comparable culture conditions (repeated *ex vivo* stimulation by MAGE-1 peptide-pulsed autologous APCs and IL-2) and in none of these expanded prevaccination TILs could we find any evidence of autologous melanoma- and MAGE-1 peptide-specific CTLs (Table 1). Indeed, cytolysis by these preimmunization TILs against the autologous targets never exceeded 10% at as high an effector/target cell ratio of 80:1 (data not shown). Furthermore, TILs expanded from two separate metastatic deposits derived from both patients after immunization (one subcutaneous tumor and a metastatic lung lesion in patient 1, and two different subcutaneous tumors in patient 2) showed autologous melanoma-reactive and MAGE-1 nonapeptide-specific activities (composite data not shown). Third, immunization with synthetic peptide-pulsed cultured APCs is feasible and such immunization seems to be reasonably safe, although additional clinical studies will be needed to establish absolute safety. Finally, CTL precursors capable of recognizing this type of tumor-associated self peptide are not deleted from the T-cell repertoire.

The indiscriminate TNF synthesis by the preimmunization TILs seen in both cases deserves some discussion. We believe that this might have resulted from a polyclonal expansion *in vitro* of mostly $CD4^+$ T cells capable of synthesizing TNF nonspecifically or perhaps from an autologous mixed lymphocyte reaction. In contrast, antigen-specific activation of the postimmunization TILs suggests clonal or oligoclonal expansions of antigen-specific $CD8^+$ T cells, which are activated only upon specific stimulation.

No major therapeutic response was noted. However, an assessment of the potential usefulness of this type of immunization and an evaluation of such recruitment of peptide-specific CTL response *in situ* cannot yet be made. The absence of therapeutic response can be explained by the advanced stage of the disease of our study patients, inadequate dose or duration of immunizations, or insufficient peptide presentation by the melanoma cells *in vivo*. A satisfactory answer to the question of whether this type of immunization might be of therapeutic benefit will have to wait for appropriately designed studies. Meanwhile, the results of our study provide a compelling rationale for vigorous exploration of peptide-based vaccines administered with APCs carrying the right restriction element or with some other form of adjuvant.

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