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Dendritic Cells Infected with a Vaccinia Vector Carrying the Human gp100 Gene Simultaneously Present Multiple Specificities and Elicit High-Affinity T Cells Reactive to Multiple Epitopes and Restricted by HLA-A2 and -A3

Sixun Yang,* David Kittlesen,† Craig L. Slingluff, Jr.,† Carol E. Vervaert,* Hilliard F. Seigler,* and Timothy L. Darrow2*

To investigate the ability of human dendritic cells (DC) to process and present multiple epitopes from the gp100 melanoma tumor-associated Ags (TAA), DC from melanoma patients expressing HLA-A2 and HLA-A3 were pulsed with gp100-derived peptides G9154, G9209, or G9280 or were infected with a vaccinia vector (Vac-Pmel/gp100) containing the gene for gp100 and used to elicit CTL from autologous PBL. CTL were also generated after stimulation of PBL with autologous tumor. CTL induced with autologous tumor stimulation demonstrated HLA-A2-restricted, gp100-specific lysis of autologous and allogeneic tumors and no lysis of HLA-A3-expressing, gp100+ target cells. CTL generated by G9154, G9209, or G9280 peptide-pulsed, DC-lysed, HLA-A2-matched EBV transformed B cells pulsed with the corresponding peptide. CTL generated by Vac-Pmel/gp100-infected DC (DC/Pmel) lysed HLA-A2- or HLA-A3-matched B cell lines pulsed with the HLA-A2-restricted G9154, G9209, or G9280 or with the HLA-A3-restricted G917 peptide derived from gp100. Furthermore, these DC/Pmel-induced CTL demonstrated potent cytoxicity against allologeneic HLA-A2- or HLA-A3-matched gp100+ melanoma cells and autologous tumor. We conclude that DC-expressing TAA present multiple gp100 epitopes in the context of multiple HLA class I-restricting alleles and elicit CTL that recognize multiple gp100-derived peptides in the context of multiple HLA class I alleles. The data suggest that for tumor immunotherapy, genetically modified DC that express an entire TAA may present the full array of possible CTL epitopes in the context of all possible HLA alleles and may be superior to DC pulsed with limited numbers of defined peptides. The Journal of Immunology, 2000, 164: 4204–4211.

Dendritic cells (DC) are highly efficient and specialized APC found in trace numbers in lymphoid and nonlymphoid organs as well as in the circulation (1). These bone marrow-derived immune cells are motile in the body and are specialized for Ag capture and presentation to lymphocytes. Furthermore, they express high levels of MHC class I- and II-encoded gene products, adherence molecules, and costimulatory molecules at the cell surface. These features of DC make them the most potent and efficient of all APC in the immune system. Given these properties, they have attracted great attention as potential cellular adjuvants for tumor vaccines. We and several other groups have demonstrated that DC pulsed with antigenic peptides from tumor-associated Ags (TAA) or tumor lysates can induce specific antitumor responses in both in vitro and in vivo human and murine studies (2–9). Recently, reports have demonstrated the use of DC pulsed with synthetic tumor peptide or tumor lysates in human clinical trials (10, 11). However, the efficacy of peptide-pulsed DC might be limited in vivo. There is concern that the peptide(s) may dissociate from MHC molecules due to low peptide affinity and MHC turnover (12). In some in vitro studies, the CTL generated using peptide-pulsed DC are lytic for peptide-pulsed targets but show a low level of lysis against autologous tumor cells (13). In addition, the strategy also requires knowledge of the restriction element of TAA-derived peptide for any particular HLA allele. In the absence of this information, tumor lysates may be used to load the DC, but autologous tumor adequate for lysis is not always available.

The availability of cloned genes encoding shared melanoma TAA offers a strategy for using TAA gene-modified DC as tumor vaccines. Transduction of DC with an entire TAA gene may allow the DC to process and present TAA peptide(s) in a “physiological” or “natural” manner to T cells. Subsequently, the CTL generated by gene-modified DC may be more likely to recognize the naturally processed antigenic epitopes presented on tumor cell surface. In addition, expression of an entire tumor Ag circumvents the need for prior knowledge of the patient HLA array or the particular TAA-derived peptide epitopes appropriate for the expressed HLA and recognized by autologous TAA-specific T cell precursors.

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Abbreviations used in this paper: DC, dendritic cells; DM, Duke melanoma; TAA, tumor-associated Ags; DC/Pmel, DC infected with vaccinia virus vac-pmel17/gp100 containing the Pmel17/gp100 melanoma TAA; DC/tyr, DC infected with the vaccinia virus vac-tyr containing the tyrosinase melanoma TAA; DC/G917, DC/G9154, DC/G9209, DC/G9280, DC pulsed with the corresponding gp100-derived peptide; Vac-Pmel, vaccinia vector carrying pme17/gp100 gene.

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TAA gene-transduced DC may allow induction of immunity in association with multiple HLA restriction elements and may provide for presentation of multiple peptide-derived antigenic determinants in the context of a single HLA allele. They may also present MHC class II-restricted epitopes to CD4+ T cells, which may be required for long-term maintenance of CTL and induction of memory (14–16).

An immunotherapeutic approach that promotes induction of multiple tumor-specific CTL specificities and HLA restrictions would theoretically provide a superior immune response with greater potential to eliminate growing tumor cells. However, reports describing limited CTL reactivities to melanoma tumor Ags (17, 18), to viral Ags (19, 20), and to histocompatibility Ags (21) suggest that some peptide/MHC complexes generate epitopes that dominate the measurable CTL response, even to the extent that they may actively hinder the evolution of reactivities to other specificities and/or restrictions (19–21). The mechanism of such potential immune interference is currently unclear. However, the persistent and simultaneous presentation of multiple epitopes at physiological concentrations by DC modified to express gene products containing multiple epitopes may allow the induction and development of the broadest possible reactivities (20).

In this study, we have chosen to infect DC derived from patient PBL with a vaccinia vector containing the p100/Pmel17 human melanoma TAA gene gp100 (DC/Pmel) and have evaluated the potential of the DC infected with vaccinia vector carrying pmel17/gp100 gene (Vac-Pmel) to induce peptide-specific, HLA-restricted CTL. The main goals of this study were to assess the ability of DC/Pmel to present multiple TAA-derived peptide Ags in the context of multiple restriction elements and to elicit cytotoxic T cell responses against an array of peptides with multiple restriction elements. We also investigated whether the CTL generated by DC/Pmel would recognize autologous melanoma tumor cells and HLA class I-matched allogeneic melanoma cells.

Materials and Methods

Tumor cell lines

Fresh melanoma tumor cells from involved lymph nodes or metastatic tumors were assigned a Duke melanoma (DM) accession number, minced, isolated in HBSS, washed, and suspended in culture medium consisting of Eagle’s MEM, (Grand Island Biological, Grand Island, NY) supplemented with 10% FCS (HyClone, Logan, UT), antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml amphotericin B, and 50 µg/ml gentamycin), 450 µg/ml 1-glutamine, and 2.5 mg/ml sodium bicarbonate in 75-cm² T flasks (Costar, Cambridge, MA). Established melanoma tumor cell lines grew as monolayer cultures and were passaged by confluency at trypsinization (0.25% trypsin with EDTA). Other cell lines used as targets in cytotoxicity assays included an EBV-transformed B cell line from patient 391 (HLA-A2 and -A3). All lines were periodically tested and were found negative for Mycoplasma.

HLA typing

All tumor cell lines were typed for HLA class I Ags using complement-mediated microcytotoxicity in HLA comprehensive typing trays (Gentrak, Wayne, PA).

Generation of DC from PBL

DC were generated from PBL as described by Romani et al. (22). Briefly, PBL isolated from patient blood were cultured for 2 h in 3 ml AIM-V medium (Life Technologies, Grand Island, NY) at 4 × 10⁶ cells/ml in six-well plates. Nonadherent cells were then gently washed off, and the remaining adherent cells were cultured with 800 U/ml GM-CSF and 500 U/ml IL-4 in AIM-V medium (5 ml/well). After 7 days, the differentiated DC were harvested. The cultured DC were stained and found to be highly positive for HLA class I by W6/32 staining (96%+; mean channel intensity (MCI), 586), for HLA class II by L243 staining (97%+; MCI, 936), and for CD11c (94%; MCI, 491) and were found to be moderately positive for CD80 (48%; MCI, 47), CD83 (37%; MCI, 51), and CD86 (86%; MCI, 117), values consistent with a mixed population of immature and moderately mature DC. Infection with vaccinia vector had a little effect on DC. Our single experiment demonstrated that there were small reductions in expression of class I (17% decrease), CD11c (20% decrease), and CD86 (15% decrease) and no changes in class II, CD80, and CD83 after infection with vaccinia vector.

**Vaccinia vector containing melanoma TAA**

The full-length Pmel17 cDNA was subcloned from pcDNA1 neo (Invitrogen, San Diego, CA) into a modified psc11 vector adjacent to the vaccinia P7.5 early/late promoter using standard RNA methodologies as previously described (23).

**Synthetic peptides**

The peptides used were chosen from the identified HLA class I-restricted epitopes from gp100 and from tyrosinase as reported by others (18, 23–25). The peptides synthesized (Genosys, The Woodlands, TX) included HLA-A2-restricted peptides G3(154 [KTWGQYWQV]) (24), G3(209 [ITDQVPFSV]) (24), and G3(280) [YLEEPGPTVTA] (18) and HLA-A3-restricted G7(17) [ALLAV- DC, IL-2 (5 U/ml; Chiron, Emeryville, CA) was added to the cultures after the third stimulation. CTL lines were also established after culture of PBL with autologous tumor cells in the presence of 5–10 U/ml IL-2 and restimulation every 7–10 days, as previously described (27–29). These T cell lines were designated DTXXX, where XXX denotes the DM number of the autologous tumor cell lines used to stimulate the CTL (e.g., DT342, DT472). CTL lines were also established after stimulation of patient PBL using HLA-matched allogeneic melanomas. These lines were designated by DT followed by the patient initials and the stimulator cells (e.g., DTPW, DC280). CTL activity was measured after three to four cycles of stimulation.

**Cytotoxicity assays**

Cultured CTL were tested for cytotoxicity in a standard 4-h ⁵¹Cr-release assay. Target cells used included peptide Ag-pulsed DC, were labeled with 75 uCi sodium-⁵¹Chromate, and were used as target cells (5000 targets/well) at different E/T ratios. The percentage of specific ⁵¹Cr release was calculated as follows: [(mean experimental cpm – mean spontaneous cpm)/(mean maximum cpm – mean spontaneous cpm)] × 100%, where spontaneous release represents cpm in supernatants from wells containing target cells and medium only, and maximum release represents cpm in supernatants from wells containing target cells in medium with 10% Triton X-100. Spontaneous release was always <20% of maximum release. The SD of triplicate wells was <10%.

**Cold target blocking assay**

CTL (1 × 10⁵) were incubated with 15 × 10⁴, 5 × 10⁴, or 1.5 × 10⁴ unlabeled “cold” targets for 1 h at 37°C before the addition of 5 × 10⁴ s.Cs-labeled “hot” targets. The final effector to “hot” target ratio was 20:1, and the final “cold” target to “hot” target ratios were 30:1, 10:1, and 3:1, respectively. After an additional 4-h incubation, the supernatants were harvested, and specific chromium release was calculated as described above.
Methods.

One to two weeks after the last stimulation, CTL activity was tested against different targets using standard 4-h 51 Cr-release assay. EBV281 is a transformed B cell line and failed to lyse uninfected DC (8% at 10:1) or the gp100/MART-1 targets DM92, DM208, or DM391 but not against HLA-A2/MART-1 melanoma DM441. The data (Fig. 1) show that CTL induced after stimulation with unpulsed DC had a very low level of lytic activity, whereas CTL induced with peptide-pulsed DC or with DC/Pmel were highly lytic for HLA-A2+/gp100+ melanoma cell line DM443.

Results

Autologous Vac-Pmel17/gp100-infected 342DC are recognized and killed by HLA-A2-restricted, gp100-specific DT342 CTL

DT342 was generated from patient 342 PBL by repeated stimulation with autologous melanoma cell line DM342 (HLA-A2+, HLA-A3+, gp100+) in the presence of IL-2 as described previously (27–29). After three cycles of restimulation, DT342 showed high lytic activity against HLA-A2+/gp100+ targets DM13, DM93, DM422, DM443, and DM451 but not against HLA-A3+/gp100+ targets DM92, DM208, or DM391 or against HLA-A2+/gp100+ DM459, demonstrating a pattern of HLA-A2-restricted lysis (Table I). DT342 also failed to lyse the HLA-A2+/gp100–, MART-1+ target DM441, suggesting that this CTL line contains cells with specificity for one or more gp100-derived peptides. We also tested the gp100 peptide specificity of DT342 and found the line lytic for EBV281 target cells pulsed with the Gp209 or the Gp280 peptide but not with the Gb154 peptide. DT342 also failed to lyse C1RA3 cells pulsed with the HLA-A3-restricted Gb17 peptide (Table I). The DT342 CTL line was used to test the lytic sensitivity of Gp280 peptide-pulsed autologous 342DC or Vac-Pmel17/gp100-infected autologous 342DC (DC/Pmel) targets. Tested at a CTL to target ratio of 10:1, DT342 mediated 67% lysis against Vac-Pmel-infected 342DC and 68% lysis against 342DC pulsed with the Gp280 peptide derived from gp100. In the same experiment, DT342 mediated 36% (E:T, 10:1) lysis against HLA-A2-matched gp100+ allogeneic melanoma cell line DM443 and failed to lyse uninfected DC (8% at 10:1) or the gp100+, HLA-A2+ DM459 (5% at 10:1). The data suggest that the Vac-Pmel17/gp100-infected DC can process and present gp100 peptides in the context of HLA-A2.

Vac-Pmel17/gp100-infected DC elicit gp100-specific, HLA-A2-restricted CTL

To determine whether Vac-Pmel17/gp100-infected DC can elicit Ag-specific CTL, PBL from patient 342 (HLA-A2 and HLA-A3) were stimulated with autologous unpulsed DC, DC pulsed with the HLA-A2-restricted peptide Gp280 derived from gp100 (DC/Gp280), or DC/Pmel for three cycles at 7-to 10-day intervals. One to two weeks after the last stimulation, CTL activity by the variously stimulated 342PBL was tested against HLA-A2+, gp100– DM14; against HLA-A2+, gp100+ DM400; against HLA-A2+, gp100+, MART-1+ DM441; or against HLA-A2+, gp100+ DM443. The data (Fig. 1) show that CTL induced after stimulation with unpulsed DC had a very low level of lytic activity, whereas CTL induced with peptide-pulsed DC or with DC/Pmel were highly lytic for HLA-A2+/gp100+ melanoma cell line DM443. In contrast, neither CTL cell line lysed HLA-A2+/gp100–, MART-1+ melanoma DM441, HLA-A2–/gp100+ melanoma DM400, or A2–/gp100+ melanoma DM14.

Table I. HLA-A2 restriction and gp100 specificity of DT342

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a PBL from patient 342 (HLA-A2+, HLA-A3+) were restimulated with autologous melanoma DM342 for three cycles at 7- to 10-day intervals as described in Materials and Methods. One to two weeks after the last stimulation, CTL activity was tested against different targets using standard 4-h 51 Cr-release assay. EBV281 is a transformed B cell line that expresses HLA-A2; C1RA3 expresses HLA-A3. These targets were pulsed with medium or with the indicated HLA class I binding peptide derived from the gp100 melanoma TAA.

FIGURE 1. CTL generated by Vac-Pmel-infected DC are HLA-A2-restricted and gp100-specific. PBL342 (HLA-A2, HLA-A3) were stimulated with autologous DC, DC pulsed with HLA-A2-restricted peptide Gp280 derived from gp100, or DC/Pmel for three cycles at 7-to 10-day intervals. CTL activity was measured 10 days after the last stimulation.
Vac-Pmel-infected DC present multiple epitopes to T cells in the context of HLA-A2

The peptide epitopes presented by DC/Pmel were investigated using HLA-A2-restricted, gp100-derived, peptide-specific CTL lines. The peptide-specific CTL lines were elicited from patient P.W.’s PBL after stimulation with autologous DC pulsed with the HLA-A2-restricted peptides G\(_{9}\)152, G\(_{9}\)209, or G\(_{9}\)280, as described in Materials and Methods. These peptide-specific CTL were then tested for cytotoxicity against peptide-pulsed DC, DC/Pmel, or Vac-Tyrosinase-infected DC (DC/Tyr).

FIGURE 2. gp100 peptide-specific and HLA-A2-restricted CTL recognize Vac-Pmel-infected DC. HLA-A2-restricted, gp100 peptide-specific CTL lines were elicited from patient P.W.’s PBL after stimulation with autologous DC pulsed with the HLA-A2-restricted peptides G\(_{9}\)152, G\(_{9}\)209, or G\(_{9}\)280, as described in Materials and Methods. These peptide-specific CTL were then tested for cytotoxicity against peptide-pulsed DC, DC/Pmel, or Vac-Tyrosinase-infected DC (DC/Tyr).

FIGURE 3. HLA-A2 glycoprotein-modified DC present multiple epitopes to autologous T cells in the context of HLA-A2. CTL were generated from PBL342 by stimulation with Vac-Pmel17/gp100 infected autologous DC. EBV-transformed B cell line EBV391 (HLA-A2\(^*\), HLA-A3\(^*\)) pulsed with or without HLA-A2-restricted gp100 peptide G\(_{9}\)154, G\(_{9}\)209, or G\(_{9}\)280 was used as a target.

DC transduced with the gp100 gene elicit both HLA-A2- and HLA-A3-restricted gp100-specific CTL

We previously reported that HLA-A2 was distinguished as a “dominant” restricting allele that governs autologous melanoma-induced, tumor-specific CTL responses in vitro (30). Specifically, it has been commonly observed that whenever a patient’s genotype includes HLA-A2 and the autologous melanoma tumor cells express HLA-A2, the in vitro-induced autologous melanoma-specific CTL would be restricted by the HLA-A2 allele regardless of the other HLA-A allele expressed (30). To examine the presence of such a limited restriction pattern or “dominant” response after stimulation with gene-modified DC, we tested these DC-induced 342CTL against a number of HLA-A2 or HLA-A3 melanoma targets. The CTL were poorly lytic against the HLA-A2\(^*\), HLA-A3\(^*\) gp100\(^*\), MART-1\(^*\) DM441 and the HLA-A2\(^*\), HLA-A3\(^*\) gp100\(^*\) melanoma DM459 target cells. In contrast, the CTL exhibited a high level of lysis against HLA-A2\(^*\), gp100\(^*\) DM443 and against autologous, gp100\(^*\) DM342 (Table II). DT342/DCPmel also demonstrated a significant level of possibly HLA-A3-restricted lytic activity against the HLA-A3\(^*\), gp100\(^*\) melanoma DM208. These results and those in Fig. 3 demonstrate that both DC/Pmel and DC pulsed with G\(_{9}\)280 elicit strong HLA-A2-restricted, gp100-specific CTL and that the DC/Pmel elicit multiple gp100 peptide specificities with potent lysis directed against autologous tumor cells. The data in Table II also suggest that DT342-induced using Vac-Pmel17-infected autologous DC to present antigenic epitopes to T cells in association with both the HLA-A2 and HLA-A3 gene products and to elicit peptide-specific T cell responses. DT472 CTL induced by Vac-Pmel17/
gp100-infected DC were tested against HLA-A2 and non-A2 melanoma target cells. The CTL demonstrated strong cytolytic activity against autologous melanoma DM472. The CTL also mediated strong lysis against HLA-A2, gp100 + DM443 as well as against HLA-A3+, gp100 + allogeneic melanoma cells DM187 and DM208 (Table II). The CTL did not kill HLA-A2-matched gp100 melanoma cell DM441 or gp100 + HLA-A2 /HLA-A3 melanoma cell DM459 (Table II). These CTL were also tested against HLA-A2+, HLA-A3+ EBV391 target cells loaded with either the HLA-A2-restricted Gp280 peptide or the HLA-A3-restricted Gp17. As seen in Fig. 4, CTL elicited by autologous 472DC/Pmel demonstrate potent lytic activity not only against EBV391 cells pulsed with the gp100-derived, HLA-A2-restricted peptide Gp280 but also against the EBV391 cells pulsed with the HLA-A3-restricted Gp17 peptide. These CTL showed only the control level (unpulsed EBV391) lysis against targets loaded with the HLA-A2-restricted tyrosinase peptide Tyr369.

The HLA-A2 restriction and gp100 specificity was further confirmed by cold target inhibition assays. Specifically, lysis of labeled DM443 target cells (HLA-A2 /gp100) by DT472/DCPmel was completely inhibited by unlabeled (“cold”) HLA-A2 /gp100 DM208 and DM443 targets but not by “cold” HLA-A2 /gp100 target DM441 or “cold” HLA-A2 /gp100 DM59 targets (Fig. 5A). In cold target studies of HLA-A3-restricted recognition by DT472/DCPmel, lysis of DM208 HLA-A3+/gp100 was completely inhibited by unlabeled autologous “cold” target DM208 and substantially, although not completely, inhibited by cold HLA-A3+/gp100 DM187. There was no significant inhibition by HLA-A2+, HLA-A3+/gp100 “cold” DM459 target cells (Fig. 5B). These results (Figs. 4 and 5) are consistent with the concurrent induction of peptide-specific HLA-A2- and HLA-A3-restricted recognition of gp100 + melanomas by 472DC infected with Vac-Pmel17.

**Discussion**

The CTL response to melanoma is characterized by reactivities to multiple peptides recognized in the context of multiple class I-restricting alleles. Work by Kawakami et al. (24) demonstrated the presence of multiple peptide specificities within therapeutic cytotoxic tumor-infiltrating lymphocytes, demonstrating the potential diversity of the in vivo CTL response against melanoma. In other studies, using T cell clones or Ag loss tumor variants, it has been established that multiple specificities exist in CTL lines induced in vitro after stimulation with tumor cells (31–33). Tütting et al. (34), utilizing autologous DC transfected with one or multiple cDNAs encoding melanoma TAA to stimulate PBL from normal donors, utilizing autologous, HLA class I-matched target cells, they also demonstrated novel restrictions for some of the TAA studied, including HLA-A3-restricted recognition of the MART-1/Melan-A melanoma-associated Ags. However, the CTL were not tested against autologous tumor cells.

In this report we have demonstrated that Vac-Pmel-infected DC present TAA to T cells and elicit CTL responses to multiple gp100-derived peptide epitopes in the context of HLA-A2. We also have presented evidence that Vac-Pmel-infected autologous
DC elicit CTL responses to antigenic epitopes from gp100 in association with multiple HLA-A restriction elements, HLA-A2, and HLA-A3. The ability of DC/Pmel to present multiple epitopes to T cells in association with multiple HLA restriction elements was confirmed in three ways. First, DC/Pmel were readily recognized by and were sensitive to lysis by gp100 peptide-specific T cell lines DT342, DTPW/G9 154, DTPW/G9 209, and DTPW/G9 280 (Fig. 2). Second, CTL generated by DC/Pmel were shown to be highly lytic for gp100/Pmel17-derived, HLA-A2- and HLA-A3-restricted peptides pulsed onto HLA-A2- and HLA-A3-matched EBV-B cell lines. Third, the lysis of HLA-A2+/gp100+ or HLA-A3+/gp100+ “hot” targets by HLA-A2+/HLA-A3+ CTL was completely blocked by HLA-A2+/gp100+ or HLA-A3+/gp100+ “cold” targets, respectively, but not by gp100+ non-HLA class I-matched “cold” targets or gp100+ “cold” targets. Perhaps most significant in these studies is the induction of a high level of lytic activity directed against autologous tumor cells after stimulation by the Vac-Pmel17-infected autologous DC.

Our previous work (30) suggested that, in HLA-A2 patients, in vitro induction of autologous melanoma-specific CTL after stimulation with autologous tumor cells yielded potent melanoma-specific CTL with a pattern of restriction limited to the HLA-A2 allele. In the current study, the in vitro-induced CTL from PBL of patients 342 and 472 using stimulation with intact autologous tumor cells failed to display detectable levels of reactivity to HLA-A3-expressing, gp100-positive tumor target cells. Also, we have been unable to detect in either of these T cell lines reactivity to the G9 17, HLA-A3-restricted peptide, even when loading target cells with relatively high (>100 μM) levels of peptide (data not shown). The apparent HLA-A2 restriction and gp100 specificity of DT342 induced by stimulation with autologous tumor cells (Table I) is consistent with our earlier findings.

In distinct contrast, CTL induced in autologous PBL using Vac-Pmel-infected DC from both of these HLA-A2, HLA-A3 patients demonstrate not only HLA-A2-restricted G9 154, G9 209, and G9 280 peptide-specific lysis but HLA-A3-restricted G9 17 peptide-specific lysis as well. Furthermore, the CTL lyse autologous melanoma tumor cells and allogeneic melanomas matched for HLA-A2 and/or HLA-A3.

Work by others (17–21), which recently has been well reviewed (13, 35), has explored and proposed reasons for this phenomenon of limited reactivity or “immunological dominance.” Among the alternative explanations for the phenomenon is the notion that there are limitations on the level of presentation of potentially immunogenic peptides such that only those presented at high enough levels will elicit a measurable CTL response (36). By flow cytometry using mAb BB7.2 to measure the levels of HLA-A2 expression and monoclonal GTPase-activating protein-A3 for HLA-A3, we determined that the DM342 and DM472 tumor cells express equivalent and high levels of both of these MHC gene products (data not shown). Therefore, they may have equivalent capacities to present peptide tumor Ags in the context of HLA-A2 and HLA-A3. However, we have not determined the level of presentation by these tumor cell lines of any of the defined HLA-A2 or HLA-A3 binding peptides derived from the gp100 TAA.

Other results using murine models of antiviral CTL suggest that dominance may be a kinetic phenomenon in which the more rapidly expanding CTL clone results in a feed back to reduce the viral load and subsequent induction/expansion of less vigorously growing CTL clones (19). Such a rapidly growing TAA-specific T cell clone might similarly outstrip other T cell clones, particularly in vitro, to either limit stimulation of lesser clones by remaining tumor cells or by masking the detectability of other reactive T cells. Results published by Busch and Pamer (36) suggested that the stability of the peptide-MHC complex may be higher for immunodominant epitopes. It may be that in the current study, presentation of gp100-derived peptides by Vac-Pmel-infected DC is maintained at higher and/or consistent levels. That, in combination with the effective costimulating capacity of DC (through CD80/CD86), may result in more effective triggering and greater clonal expansion of multiple peptide-reactive T cell precursors. Also, because these responses are derived from PBL from melanoma patients, one may speculate that they represent secondary responses, the primary stimulus being derived from tumor-T cell or T cell-DC tumor interactions that occur in the draining
nodes. Thus, in vitro stimulation by tumor cells (without significant costimulatory function) may only trigger memory or precursor T cells bearing high affinity receptors, whereas Vac-Pmel17-infected, B7-expressing DC are able to trigger and expand memory precursors with either high- or low-affinity receptors, as well as previously unprimed TAA epitope-specific T cells.

An alternative explanation to the broad spectrum of CTL responses by DC/Pmel may exist. The degree of tolerance to different self-TAA epitopes may vary greatly. A low degree of tolerance might be easily broken by nonprofessional APC such as tumor cells, whereas deep tolerance could be only broken by professional APC such as DC. By whatever mechanism(s) peptide specificity and class I restriction are governed during in vitro or in vivo stimulation, the in vitro response to TAA gene-infected autologous DC appears characterized by a broader, more comprehensive antitumor response that more closely represents the breadth of tumor-infiltrating lymphocyte reactivity elicited in vivo, as suggested in the report by Kawakami et al. (24). We have not tested this strategy to elicit primary CTL from normal PBL stimulated by either HLA-A2-, HLA-A3-matched allogeneic tumor cells or autologous Vac-Pmel17-infected DC, although the work by Tuting et al. (34) cited above would suggest that even primary responses might be broader and more vigorous if induced by DC. Regardless of whether these represent primary or secondary responses, our findings indicate that stimulation by DC expressing an entire TAA elicits a more broadly specific in vitro response than that induced by tumor cells alone.

The ability to generate in vitro CTL responses to individual melanoma peptide Ags has been previously demonstrated (37–40). In some of these studies there is frequently reduced or no measurable recognition and lysis of HLA-matched or autologous melanoma target cells expressing endogenous TAA, although there is high peptide-specific lysis observable using peptide-pulsed target cells (38, 39). It has been suggested in those (38, 39) and other (41) studies that the low level of lysis of autologous tumor by peptide-stimulated CTL may be due to induction of low-affinity CTL by peptide-loaded APC. Using a virus peptide in an animal model, Alexander-Miller et al. (41) demonstrated that CTL induction using APC loaded with high levels of peptide resulted in low-affinity CTL. Effective lysis by those CTL required a high concentration of peptide to be loaded onto target cells. CTL induced using APC loaded with lower concentrations of peptide yielded CTL that were able to lyse target cells loaded with even low concentrations of peptide. As demonstrated in the present study, the use of DC expressing entire TAA for induction of CTL appears to allow presentation of immunobiologically relevant/physiological levels of multiple peptides to elicit CTL with multiple specificities for recognition of physiological levels of peptide expressed by native tumor cells. In particular, induction of HLA-A3-restricted reactivity to the G2p17 peptide and to HLA-A3, gp100+ melanomas after stimulation of PBL by Vac-Pmel-infected DC but not reliably by autologous tumor stimulation, points to the utility of the approach to CTL induction reported here. Such an approach for CTL induction may also be useful for identifying relevant but previously unobserved specificities and restrictions. A recent report of activities to multiple MAGE-A1 epitopes after stimulation of normal PBL is consistent with this prediction (42).

In the present study we have not examined the avidities of the CTL populations induced after stimulation with the DC/peptide, DC infected with Vac-Pmel, or autologous tumor cells. Therefore, we cannot comment on the relative avidities of each of the CTL populations. In other studies using peptide-pulsed DC to elicit G2p209-specific CTL, multiple, concurrent CTL cultures from the same patient displayed a wide range of avidities (S. Yang, unpublished data), suggesting that the peptide-specific CTL populations may be very heterogeneous. Further study will be needed to clarify whether the high melanoma lytic activity of CTL generated by DC/Pmel is due to the broad specificity and/or high avidity of the resultant CTL.

Although peptide-based vaccines are easy to administer and are potentially inexpensive, they require characterization of the biochemical properties of each specific epitope/HLA complex and proof of natural processing. CTL generated using peptides derived from a given TAA and selected according to the binding motif for the possible HLA class I restriction elements, however, are not always able to recognize autologous tumor cells, suggesting that such epitopes may not be naturally presented (12). In contrast, the gene product for an entire TAA introduced into DC may be processed and presented to T cells in a natural and immunologically effective form. Thus, the CTL generated by DC expressing TAA are most likely able to recognize TAA determinants naturally processed by tumor cells. This notion is supported by this study in that the CTL elicited by gp100 gene-modified DC are highly cytolytic not only to autologous tumor cells but also to HLA class I-matched allogeneic melanoma.

In the current study, we have not assessed the induction of TAA-specific, HLA class II-restricted CD4 responses. In murine models using DC modified to express an entire human gp100 gene (16, 43) and murine tyrosinase-related protein-2 gene (44), we (16) and others (43, 44) demonstrated that induction of tumor immunity and CTL responses required CD4 T cells that were specific for gp100, indicating that the DC were able to present and elicit responses to class II as well as class I TAA-specific peptides. Because the DC in this study are infected with the Vac-Pmel17 vector encoding the full-length gp100 gene, there is, unlike peptide-pulsed DC, significant potential for processing, selection, and presentation of class II peptides. Just as for HLA class I gene products, the processing and selection of class II peptides would theoretically be limited only by the binding requirements of the HLA class II alleles expressed by these patients.

Induction of CTL using autologous DC engineered to express one or more entire TAA-encoded gene products has the potential to generate lytic T cell populations containing multiple TAA peptide specificities and multiple HLA restrictions. Such broad-spectrum CTL populations represent the best possible instrument with which to eradicate growing tumor cells. Such T cell activation against the large array of potential target Ags displayed by growing tumor cells may reduce the possibility of tumor cell escape from immunotherapy due to the loss and/or mutation of a single HLA allele or TAA. The use of DC expressing entire TAA gene products as immunogens or as stimulator cells in vitro to elicit highly competent tumor-specific CTL for adoptive therapy therefore may present a practical and effective mode for eliciting tumor immunity. TAA-modified autologous DC may also provide a means to elicit and identify novel restrictions and peptide specificities.

References


