Mannose Receptor Targeting of Tumor Antigen pmel17 to Human Dendritic Cells Directs Anti-Melanoma T Cell Responses via Multiple HLA Molecules

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Mannose Receptor Targeting of Tumor Antigen pmel17 to Human Dendritic Cells Directs Anti-Melanoma T Cell Responses via Multiple HLA Molecules


Targeting recycling endocytic receptors with specific Abs provides a means for introducing a variety of tumor-associated Ags into human dendritic cells (DCs), culminating in their efficient presentation to T cells. We have generated a human mAb (B11) against the mannose receptor that is rapidly internalized by DCs through receptor-mediated endocytosis. By genetically linking the melanoma Ag, pmel17, to Ab B11, we obtained the fully human fusion protein, B11-pmel17. Treatment of DCs with B11-pmel17 resulted in the presentation of pmel17 in the context of HLA class I and II molecules. Thus, potent pmel17-specific T cells were cytotoxic toward gp100+ HLA-matched melanoma targets, but not HLA-mismatched melanoma or gp100− nonmelanoma tumor lines. Importantly, competitive inhibition of lysis of an otherwise susceptible melanoma cell line by cold targets pulsed with known gp100 CD8+ T cell epitopes as well as a dose-dependent proliferative response to Th epitopes demonstrates that DCs can process targeted Ag for activation of cytotoxic as well as helper arms of the immune response. Thus, specific targeting of soluble exogenous tumor Ag to the DC mannose receptor directly contributes to the generation of multiple HLA-restricted Ag-specific T cell responses. The Journal of Immunology, 2004, 172: 2845–2852.

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Materials and Methods

Cells and reagents

Human PBMCs were isolated from normal donor heparinized Leukopaks (Biological Specialty Corp., Colmar, PA) using a standard density gradient centrifugation method (Lymphocyte Separation Medium; ICN Biomedical, Irvine, CA). HLA information was obtained from available records. Two donor samples described in this study were HLA-A2, 31 B13, 35, DR2 (donor 1) and HLA-A1, 30 B7, 13, DR7 (donor 2). All materials were handled aseptically according to standard guidelines.

Human dendritic cells (DCs, culminating in their efficient presentation to T cells. We have generated a human mAb (B11) against the mannose receptor that is rapidly internalized by DCs through receptor-mediated endocytosis. By genetically linking the melanoma Ag, pmel17, to Ab B11, we obtained the fully human fusion protein, B11-pmel17. Treatment of DCs with B11-pmel17 resulted in the presentation of pmel17 in the context of HLA class I and II molecules. Thus, potent pmel17-specific T cells were cytotoxic toward gp100+ HLA-matched melanoma targets, but not HLA-mismatched melanoma or gp100− nonmelanoma tumor lines. Importantly, competitive inhibition of lysis of an otherwise susceptible melanoma cell line by cold targets pulsed with known gp100 CD8+ T cell epitopes as well as a dose-dependent proliferative response to Th epitopes demonstrates that DCs can process targeted Ag for activation of cytotoxic as well as helper arms of the immune response. Thus, specific targeting of soluble exogenous tumor Ag to the DC mannose receptor directly contributes to the generation of multiple HLA-restricted Ag-specific T cell responses. The Journal of Immunology, 2004, 172: 2845–2852.

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Development of human mAb specific for MR

Transgenic HuMAb mice, strain HC2/KCo7, with four distinct genetic modifications were used for immunizations (30). These transgenic mice contain a human Ig gene miniloci that encodes unarranged human H (µ)
and γ) and κ L chain Ig sequences together with targeted mutations that inactivate the endogenous μ- and κ-chain loci. Accordingly, the mice exhibit no expression of mouse IgM or κ, and in response to immunization, the introduced human H and L chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ mAbs. Immature human DCs (1–5 × 10^6) were used to immunize mice in CFA. Single-cell suspensions of splenic lymphocytes from immunized animals were fused with the murine myeloma cell line P3X63Ag.653 (American Type Culture Collection) in the presence of polyethylene glycol. Hybridosomes were selected by the addition of HAT 24 h after fusion. Human IgGe-producing hybridosomes were screened by flow cytometry for binding to DCs. Ab specificity was determined by immunoprecipitation and sequencing. Preclreated supernatant was incubated overnight with B11 mAb bound to anti-human IgG-agarose. After washing, the bound proteins were removed from the agarose by boiling in SDS-PAGE sample loading buffer. Samples were applied to precast 4–20% gels (Bio-Rad, Hercules, CA) and separated under nondenaturing conditions. The proteins were transferred to a polyvinylidene difluoride membrane and stained with Coomassie Blue. The band corresponding to the B11 Ag was N-terminal microsequenced by Edman N-terminal protein sequencing (Commonwealth Biotechnologies, Richmond, VA). The N-terminal 20 aa, LLDTRQFLIYLEDTKRCVDA, were found to share 100% identity with the introduced human H and L chain transgenes undergoing class switching in immature monocyte-derived DCs. Normal rabbit serum was included as a negative control, and all samples were analyzed by flow cytometry.

**Internalization assays**

To investigate the mechanism of internalization, DCs were treated on ice with B11-FITC (20 ng/ml) or mannansylated BSA-FITC (400 ng/ml; Sigma-Aldrich) for 30 min in AIMV medium plus 3 μg/ml human γ-globulin (Sigma-Aldrich) and 1% BSA (Sigma-Aldrich) with or without 400 mM sucrose. Cells were then warmed to 37°C for 20 min. After treatment, cells were washed twice with ice-cold PBS containing 1% BSA and 0.05% sodium azide (Fisher Scientific, Fairlawn, NJ) and fixed in ice-cold 1% formaldehyde (Polysciences, Warrington, PA) in PBS overnight. Cells were then washed twice with ice-cold PBS containing 1% BSA and 0.05% sodium azide and prepared for imaging. Fixed cells were pelleted and imaged using an MRC-1024 confocal scanning laser microscope system and LaserSharp software version 3.2 (Bio-Rad). 605DF32, 522DF32, and 680DF32 bandpass filters were used for photomultiplier tubes 1, 2, and 3, respectively. Laser power was set at 3% on all lines (488, 568, and 647 nm wavelengths). All cells were imaged using a 63X/1.4NA PlanApo objective with oil and an iris setting of 1.5 for all photomultiplier tubes used in a given experiment. The section thickness is estimated to be at the optimal axial resolution for this system (~0.5 μm). Image acquisition settings included the Kalman filtered mean of three slow scan accumulations with all laser lines. Images were captured as a single section from the center plane of cells and are representative of 30 fields captured/slide.

**Generation of B lymphoblastoid cell lines (B-LCL)**

Autologous B cell lines were prepared as previously described (32). Briefly, 2.5–3 × 10^6/ml PBMCs were infected by incubation with B95.8 supernatants (American Type Culture Collection) for 90 min at 37°C, followed by addition of 5 ml of RP-15 medium (RPMI 1640 supplemented with 20 mM HEPES, 2 mM L-glutamine, 1% sodium pyruvate, 15% FBS, 500 U/ml of either B11-F(ab’2) or mannosylated BSA-FITC (400 ng/ml; Sigma-Aldrich) for 30 min at 4°C. B11-F(ab’2) was used as a negative control, and all samples were analyzed by flow cytometry.

**T cell stimulation and expansion**

Nonadherent PBMC (PB) were used as a source of T cells from frozen stocks and stimulated with autologous Ag-targeted immature DC every week for 4–5 wk. Briefly, 1.2 × 10^6/ml DC were exposed to Ag, B11-pmel17 (20 μg) in 1.0 ml of AIM-V serum-free medium for 45 min and allowed to mature with CD40 ligand (CD40L, PeproTech, Rocky Hill, NJ; 20 ng/ml) with 5% human AB serum (American Red Cross, Salt Lake City, UT) for 24 h. PBMCs (2 × 10^7/ml) were cocultured with Ag-loaded DC in 24-well culture plates (B-D Biosciences, San Jose, CA) in the presence of IL-7 (10 ng/ml, day 0), followed by addition of other cytokines, IL-10 (10 ng/ml) on day 1 and IL-2 (20 U/ml) on day 2. The T cell:DC ratio was maintained at 20 throughout the course of stimulations. IL-2 was added every 3–4 days, whereas restimulations were performed on days 8, 16, and 24 as before, except that the Ag dose was cut by half compared with that used in the preceding stimulation (10.0, 5.0, and 2.5 μg/ml, respectively).
T cells were maintained as bulk cultures (containing both CD4+ and CD8+ T cells) unless otherwise indicated. Effector T cells (5 × 10^5 to 1 × 10^6/flask) were expanded in T25 flasks (Corning Glass, Corning, NY) on allogeneic mitomycin C-treated PBMC feeder layers (2.5 × 10^5/ml) pooled from three donors with added anti-CD3 Ab (25 ng/ml) and IL-2 at a dose of 20 U/ml. Medium was changed on days 5 and 8 by first removing half the spent medium and replacing with fresh medium containing 50 U/ml IL-2. T cells were harvested and assayed between days 10–12 or were cryopreserved for later use (32).

**Cytotoxicity assays**

T cells generated with B11-pmel17-stimulated DCs were tested for reactivity against various HLA-A2–labeled targets. The targets included autologous B-LCL or TAP-deficient T2 (HLA-A2.1+) and a panel of HLA-matched/mismatched melanoma and nonmelanoma tumor cell lines. Cytotoxicity was determined in a standard 4-h chromium release assay. MHC class I and II restriction was ascertained by assessing the reactivity in the presence of targets preincubated with HLA-specific antisera. All assays were performed in triplicate. Data shown are from a representative experiment of two or three independent experiments performed. The percent specific killing of targets was calculated from the formula: percent specific lysis = ([experimental release cpm − spontaneous release cpm]/[maximal release cpm − spontaneous release cpm]) × 100. Experimental release is the radioactivity released by CTL in the presence of 51Cr-labeled targets, and spontaneous and maximal release correspond to radioactivity in wells containing no added CTL, i.e., 51Cr-labeled targets in medium and 2% Nonidet P-40 (Igepal CA630; Sigma-Aldrich)-containing medium, respectively. Radioactivity was counted using a gamma counter (Wizard 1470; Wallac, PerkinElmer, Shelton, CT).

**Cold target inhibition assay**

T cells from donor 1 (HLA-A2+), previously stimulated with DC-B11-pmel17, were tested for reactivity against 51Cr-labeled (hot) SK-Mel 19 melanoma cells (HLA-A2+) in the presence or the absence of unlabeled (cold) HLA-A2–T2 cells with or without peptides. For inhibition experiments, several gp100 peptides known to bind HLA-A2 were loaded on T2 cells (10 μg/ml for 3.0 × 10^3/ml cells in AIM-V medium) in the presence of β2-microglobulin (β2m; 3.0 μg/ml) for 2 h at room temperature. Peptide-bound T2 cells were washed once in PBS and centrifuged to remove unbound peptide. A 10-fold excess of peptide-loaded or unloaded T2 cells was then added to CTL, followed by addition of labeled SK-Mel 19 targets. The E:T cell ratio was maintained at 40, and the cold:hot target ratio was maintained at 10. T2 cells pulsed with irrelevant HLA-A2 binding peptide (HBVcore18-27) served as controls. Percent inhibition of specific lysis = 1 − [specific lysis in the presence of cold targets]/(specific lysis in the absence of cold targets) × 100.

**Proliferation assays**

T cells generated from B11-pmel17-treated DCs (5 × 10^5) were cocultured with autologous DC (5 × 10^3) not pulsed or pulsed with gp100 peptides (74–89 and 576–590) for 3 days at 37°C in a final volume of 0.2 ml of RP-10 medium. On day 3, cultures were pulsed with [3H]thymidine (1 μCi/well; NEN-PerkinElmer, Boston, MA) for the last 8 h. Cells were then harvested onto filters with a Cell Harvester (Wallac, Shelton, CT) and washed three times with water, followed by a final wash in ethanol. Filters were air-dried and loaded with 20 μl of OptiPhase SuperMix scintillant/well (PerkinElmer, Turku, Finland). Filter-bound radioactivity was counted using a beta scintillation counter (I450 MicroBeta Jet; Wallac PerkinElmer, Downers Grove, IL). MHC restriction was conducted by addition of MHC class I– or II–specific Ab (20 μg/ml) to DC before incubation with T cells.

**Results**

**Characterization of anti-MR mAb, B11**

The expression of MR is primarily restricted to tissue macrophages, myeloid DCs, and hepatic endothelial cells (33–36), and as such makes an attractive candidate for targeting Ags to APCs. We generated a human anti-MR mAb, B11, by immunization of human Ig-expressing mice with immature human DCs, followed by standard hybridoma methodology. The specificity of the B11 mAb was investigated by immunoprecipitation experiments with lysates prepared from DCs. Electrophoresis identified a single product band at ~180 kDa (data not shown) with the N-terminal amino acids LLDTRQFLIYLEDTKRCVDA, which share 100% identity with the N-terminal sequence of the human macrophage MR. Interestingly, B11 Ab did not block binding of mannosylated BSA to DCs (data not shown), a property that has been ascribed to other anti-MR Abs (36).

MR-mediated internalization by DCs occurs through macropinocytosis or receptor-mediated endocytosis (37). Macropinocytosis is the predominant mechanism of endocytosis for the MR ligands, dextran, HRP, and mannosylated BSA (22). To determine the mechanism of B11 internalization, experiments were performed under hypertonic conditions that disrupt clathrin-dependent receptor-mediated endocytosis (38). Immature DCs were incubated on ice with or without 400 mM sucrose for 30 min in the presence of either B11 mAb or mannosylated BSA. Cells were then warmed to

**FIGURE 1.** Inhibition of clathrin-mediated internalization. Immature DCs were incubated on ice for 30 min in the presence of B11-FITC (A–C) or mannosylated BSA-FITC (D and E). Cells were then warmed to 37°C and incubated for 20 min in the presence (C and F) or the absence (B and E) of 400 mM sucrose. Cells were then washed, fixed, and analyzed by confocal microscopy.

\[ t = 0 \text{ min.} \quad t = 20 \text{ min.} \quad t = 20 \text{ min. Sucrose} \]
37°C and allowed to internalize for 20 min. After being washed and fixed, cells were analyzed by confocal microscopy. When B11 was bound to the MR, its uptake was inhibited by hypertonic shock, indicating that its mechanism of internalization was through clathrin coated-pits (Fig. 1). As expected, the uptake of mannosylated BSA was not inhibited by hypertonic shock, indicating that its mechanism of internalization was not dependent on clathrin coated-pit formation. Even at a 20-fold higher concentration relative to B11, the surface staining by mannosylated BSA-FITC was relatively weak. Subsequent studies revealed that internalized mannosylated BSA-FITC colocalized with nonspecific, fluid phase tracers, whereas vesicles containing internalized B11 excluded the nonspecific tracer (data not shown). In contrast to B11-FITC, the uptake of both mannosylated BSA-FITC and the fluid phase tracer was largely blocked by pretreatment with the phosphatidylinositol 3-kinase inhibitor, wortmannin (data not shown). These results show the rapid uptake of the B11 occurs by a distinct mechanism relative to the uptake of mannosylated BSA.

Biochemical characterization of B11-pmel17

Having demonstrated efficient DC binding and internalization of the B11 mAb, we designed a tumor Ag containing fusion protein for investigation of Ag-specific presentation to T cells. Pmel17 is a melanocyte-specific protein and a splice variant of gp100 that encodes all of gp100 plus an additional seven amino acids (39). The selective expression profile of pmel17/gp100 has made this protein an attractive target for active immunotherapy strategies for the treatment of melanoma (reviewed in Refs. 40 and 41). Despite the fact that only the ectodomain of pmel17 was included in the fusion protein, most B11-pmel17 produced by CHO transfectants remained cell-associated. B11-pmel17 purified from cell lysates was characterized by SDS-PAGE and Western blot analysis (Fig. 2), which revealed a fully assembled Ab fusion protein of the expected size.

Specific binding of B11-pmel17 to immature DCs

The B11-pmel17 fusion protein also retained the functional properties of B11 Ab, as demonstrated by its ability to bind monocyte-derived immature DCs. Furthermore, the pmel17 component of B11-pmel17 does not significantly contribute to its recognition of these cells, because the B11 F(ab’)2, which lacks both the Fc and pmel17 regions, is nevertheless able to completely abrogate binding of B11-pmel17 to immature human DCs (Fig. 3). The fact that sMR is also able to abrogate B11-pmel17 binding indicates that B11-pmel17 interacts with MR present on the cell surface of immature DCs.

Induction of an MHC class II-dependent, pmel17-directed Th response

An autologous in vitro culture system incorporating PBL and monocyte-derived DCs was established to investigate the ability of B11-pmel17 to enhance cellular immune responses from two normal donors. Briefly, PBL were stimulated as bulk culture with CD40L-matured, B11-pmel17-loaded DCs. To define the MHC class II-dependent responses, T cells were induced to proliferate in response to specific antigenic stimulation by Ag pmel17-bearing mature DCs. As shown in Fig. 4, T cells were markedly stimulated only in the presence of autologous DCs loaded with sensitizing Ag.
B11-pmel17 compared with DC loaded with the vehicle B11 alone or unloaded DC; the specific stimulation was 5- to 6-fold greater than control stimulation. These results also reflect the fact that the response is primarily directed toward the Ag, pmel17, rather than toward the B11 component of the fusion protein. These responses were fairly reproducible in the two donors tested (donor 1, DR2+; donor 2, DR7+). The most significant result pertaining to the HLA class II-dependent, pmel17-specific response is the observation that anti-HLA-DR-specific Ab (L243), but not anti-HLA-A, -B, or -C-specific Ab (W6/32), was effective in blocking the proliferation, as reflected in reduced uptake of the radioactive tracer.

**Synthetic gp100 epitopes can mimic the Th response initiated with pmel17 protein**

Taking this analysis a step further, we were curious to determine whether exogenously added helper peptides derived from pmel17/gp100–gp100576–590 and gp10026–41 would be sufficient to trigger a similar proliferative response using T cells derived from HLA-DR7+ donor. Autologous B-LCLs generated from this donor were used, with or without the added peptides, as stimulators. Thus, bulk T cells from DC-pmel17 stimulations were cocultured with synthetic gp100576–590 and gp10026–41 peptide-loaded B-LCL (at a ratio of 10:1) for 72 h and pulse-chased with tritiated thymidine for the last 8 h. As depicted in Fig. 5, robust proliferative responses were noted for both helper peptides over a range of concentrations tested. These results suggest that the proliferative response is similar in magnitude to that observed with DC-pmel17-induced stimulation (see Fig. 4) and, therefore, is likely to be directed against preprocessed helper peptides being presented by an alternate APC, i.e., a B-LCL. Similar responses were not obtained with T cells derived from the HLA-DR7-negative donor, as expected (data not shown). These results clearly demonstrate that DC targeting of pmel17 tumor Ag using B11-pmel17 fusion protein can result in the stimulation of an HLA class II-dependent Ag directed Th function.

**B11-pmel17-treated DCs can direct the development of anti-melanoma cytolytic T cells**

A requirement for pmel17/gp100-specific CTL recognition of melanomas is that these tumor cells also process and present pmel17/gp100 peptides in association with matched class I molecules. We examined the ability of pmel17/gp100-specific T cells to recognize a number of cancer cell lines. As shown in Fig. 6, T cells stimulated with DC-B11-pmel17 recognize HLA-compatible SK-Mel 31 melanoma targets, but not the completely HLA-matched autologous B-LCL. Importantly, we show that the anti-melanoma response is significantly blocked only in the presence of HLA class I-specific Ab, but not with a class II-specific Ab. To assess the broad specificity of the pmel17-specific CTL, a panel of nine different HLA class I-matched human melanoma targets was tested (Table I), T cells of at least one donor (HLA-A2+) were lytic on a majority of the melanomas tested, albeit to different degrees, whereas T cells from the second donor (HLA-A1+, -DR7+) were not lytic on most targets tested, except one (SK-Mel 31). As the control targets, which included autologous B-LCLs, T24 bladder carcinoma cells, and SK-Br-3 breast carcinoma cells, were not lysed by anti-pmel17 CTL, the T cell response observed was highly specific to an epitope derived from pmel17/gp100 melanoma Ag presented in the context of class I MHC molecules.

**Competitive inhibition with peptides reveals multiprofile specificity of CTLs**

Previously, several T cell epitopes derived from gp100 have been reported with some currently undergoing clinical evaluation (27, 40, 41). In this regard, the modified synthetic peptide, pmel17/gp100209–217 (209–219: PVTA, KTWGQYWQV, VLYRYGSFSV, and LLDGTATLRL) has been reported to bind HLA-A2 better than the native Ag (ITDQVPFSV). It was therefore interesting to understand whether processing of B11-pmel17 by DCs indeed results in recognition by T cells with specificity for the modified peptide. As depicted in Fig. 7, potent killing was observed with peptide-pulsed B-LCL compared with no peptide B-LCL targets, indicative of an Ag-specific class I-dependent sensitization of CTL. Using a peptide-pulsed cold target inhibition assay, we have examined four HLA-A2-binding gp100 T cell epitopes (YLEGPVTA, KTWGGQYWQV, VLYRGGPSGV, and LLDCATLRL) in addition to gp100209–219 (IMDQVPFSV) for recognition by pmel17-specific CTL. Thus, only peptides pulsed on cold T2 cells (TAP-deficient), but not T2 alone, were able to inhibit the lysis of labeled HLA-A2+ SK-Mel 19 targets by HLA-A2+ pmel17 CTL (Table II). Furthermore, T2 pulsed with HLA-A2-binding control peptide (HBV core18–27: FLPSDFCSFV) was significantly less effective at blocking CTL activity than gp100 peptides. These results demonstrate that DCs are able to process internalized B11-pmel17

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**FIGURE 5.** B11-pmel17-sensitized T cells proliferate in response to defined Th epitopes derived from gp100. Autologous B-LCLs (donor 2, HLA-DR7+) were loaded with a range of peptide concentrations for 2 h at 26°C, washed, and incubated with CTL for 3 days at 37°C. Cultures were pulsed with tritiated thymidine for the last 8 h and counted. | gp10026–41 (GPTLGANASEFSIALV; □), gp100576–590 (SLAVSTQILMPQGE) (28). Shown is a representative experiment of two independent experiments performed in sextuplicate.

**FIGURE 6.** Cytolytic effector T cells specific for pmel17 can mount anti-melanoma responses. SK-Mel-31 melanoma cells (targets (T), ■) were pretreated with IFN-γ for 48 h and labeled with 51Cr. Labeled target cells were then added to CTL (effectors (E)) titrated to give different E:T cell ratios, and incubated at 37°C for 4 h. Supernatants were harvested for gamma counting. Autologous B-LCLs served as the HLA-compatible control target (□). For Ab-blocking experiments, SK-Mel 31 cells were pre-incubated with anti-class I (W6/32; ■), or anti-class II (L243; ○) Ab (20 μg/ml each) at room temperature for 30 min, washed once, and added to CTL. Targets and effector T cells share HLA-A31 (donor 1). Shown is a representative experiment of three independent experiments performed in triplicate with similar results.
fused protein and present multiple pmel17-derived CD8 T cell epitopes associated with HLA-A2.

Discussion

Evidence has accumulated in recent years to suggest that DCs are central to the generation of T cell responses. They can be customized in vitro and ex vivo to meet different goals of harnessing the immune repertoire. Notwithstanding, however, is the fact that DCs can also capture proteins nonspecifically for eventual presentation to T cells (42). The rationale behind specific targeting of Ags, therefore, appears to be centered on the relative efficiency of Ag processing and presentation in terms of both qualitative (i.e., efficient access to class I pathway) as well as quantitative (i.e., efficiency of MHC-peptide complex formation) responses (43). Recently, work from Steinman’s laboratory (15) has shown that Ab-targeting Ag to another member of the C-type lectin family, DEC-205, results in class I-restricted Ag-specific responses (44). Consistently, their study makes a clear distinction between targeting of Ags in the absence of adjuvant leading to peripheral tolerance and targeting with an adjuvant, shifting the response toward Th1 immunity (44).

In this study we show that selective targeting of Ag and uptake by the MR present on DCs can elicit cellular immunity in a manner consistent with a directed Ag-specific response restricted by HLA class I and II molecules. Confocal microscopy studies have further shown that immature DCs readily internalize B11-pmel17 by receptor-mediated endocytosis, in contrast to the macropinocytic uptake of mannosylated BSA. Interestingly, B11 was found to localize within MHC class I-containing vesicles after uptake, yet no colocalization of B11-pmel17 with MHC class II-enriched compartments has been observed to date (J. E. Connolly, unpublished observations). Bypassing the conventional pathways for Ag processing and presentation has been generally accomplished using synthetic peptides exogenously added to single MHC allele-expressing reporter cell lines (e.g., Tap-deficient T2 cells) or autologous B-LCL, although this is limited to the availability of well-defined HLA-binding peptides. Therefore, in terms of defining an MHC class I or class II-dependent responses beyond Ab-mediated blocking, we have obtained productive T cell responses to synthetic epitopes, previously confirmed by others, although the responses were initiated using the soluble form of the Ag pmel17.

The recognition of HLA-compatible melanoma cell lines by CTL generated to DC-targeted pmel17 Ag represents the ultimate demonstration of an Ag-directed anti-melanoma response. Although HLA-A2 appears to be the major allele contributing to this response, other alleles (-A31, -B13, and -B35) also may be involved in Ag presentation, because the pmel17 CTL also recognizes pmel17/gp100 targets that lack HLA-A2. Thus, a favorable lytic response appears to be directly related to Ag recognition in the context of a cognate HLA allele present on the targets, as evidenced by lysis of HLA-matched, but not HLA-mismatched, targets. Consistent with this paradigm, SK-Mel-28, although gp100+, is HLA mismatched and therefore ignored as a CTL target. The lytic activity of our CTL lines on multiple targets further suggests that DCs targeted with tumor Ag fused to anti-MR Ab are capable of activating T cells with specificity for multiple Ags associated with diverse HLA-restricting elements contributing to the

Table I. Induction of a broad anti-melanoma CTL response by stimulation with pmel17 targeted to DC mannose receptor

<table>
<thead>
<tr>
<th>Targeta</th>
<th>HLA Class I Typing A and B Locus</th>
<th>CTL Activity % Specific Lysisb</th>
<th>Possible Restriction Elementc</th>
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<td>Partial HLA class I match</td>
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</tr>
<tr>
<td>10. SK-Br-3 (breast carcinoma)</td>
<td>A3, 11 B18, 40</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>11. T-24 (bladder carcinoma)</td>
<td>A1, 3 B18, 35</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>12. Autologous B-LCL</td>
<td>A2, 31 B13, 54</td>
<td>5.4</td>
<td></td>
</tr>
</tbody>
</table>

a T cells from an HLA-A2+ donor were stimulated at 8- to 9-day intervals by autologous DC loaded with B11-pmel17. After 4-5 wk, effector T cells were tested for activity in a 4-h chromium release assay.

b Targets were pretreated with 100 U/ml IFN-γ for 48 h.

c E:T cell ratio = 40.

d Restriction element is based on matching HLA alleles of the donor (see B-LCL above) with that of target cells.
Table II. Competitive inhibition of SK-Mel 19 lysis by gp100 peptide-pulsed cold targets reveals multipletsope presentation by B11-pmel17-targeted DC.

<table>
<thead>
<tr>
<th>Cold Targets Added</th>
<th>Inhibition of Specific Lysis (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
</tr>
<tr>
<td>T2 only</td>
<td>15 ± 0.55</td>
</tr>
<tr>
<td>gp100 peptides</td>
<td></td>
</tr>
<tr>
<td>T2 + YLEPGPVT</td>
<td>56 ± 2.90</td>
</tr>
<tr>
<td>T2 + IMDQVVPFVS (209-2M)</td>
<td>70 ± 1.22</td>
</tr>
<tr>
<td>T2 + KTQQGWQV</td>
<td>69 ± 1.88</td>
</tr>
<tr>
<td>T2 + VLYRYSFSV</td>
<td>84 ± 0.66</td>
</tr>
<tr>
<td>T2 + LLGATRLR</td>
<td>82 ± 1.66</td>
</tr>
<tr>
<td>Control peptide</td>
<td></td>
</tr>
<tr>
<td>T2 + FLPSDCFPSV</td>
<td>28 ± 9.50</td>
</tr>
</tbody>
</table>

* Cold target inhibition of SK-Mel 19 lysis. T cells from normal HLA-A2+ donor previously sensitized with autologous DC-loaded B11-pmel17 were tested for lytic activity against HLA-A2+ 32P-labeled (hot) SK-Mel 19 targets (E:T cell ratio = 40) in the presence or the absence of unlabeled (cold) targets (TAP-deficient HLA-A2+ T2 cells) pulsed with HLA-A2-restricted gp100 synthetic peptides (10 µM) and β2m (3.0 µg/mL). Cold-hot target ratio = 10.

* Specific lysis of SK-Mel 19 target cells in the absence of cold targets was 27%.

* Peptide modified at position 6; Y → C from the parental sequence of HBV (p23: FLPSDCFPSV).

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References


