Interleukin-7-Dependent Expansion and Persistence of Melanoma-Specific T Cells in Lymphodepleted Mice Lead to Tumor Regression and Editing

Li-Xin Wang,1,3,4 Rui Li,1,3 Guojun Yang,1,3 May Lim,1,3 Aisling O’Hara,1,3 Yiwei Chu,1,3,5 Bernard A. Fox,2,3 Nicholas P. Restifo,6 Walter J. Urba,3 and Hong-Ming Hu1,3

1Laboratory of Cancer Immunobiology; 2Laboratory of Molecular and Tumor Immunology; 3Robert W. Franz Cancer Center, Earle A. Chiles Research Institute, Providence Portland Medical Center, Portland, Oregon; 4Department of Microbiology and Immunology, School of Medicine, Southeast University, Nanjing, Jiangsu; 5Department of Immunology, Shanghai Medical School of Fudan University, Shanghai, China; and 6National Cancer Institute, NIH, Bethesda, Maryland

Abstract

Active-specific immunotherapy with dendritic cells loaded with peptide derived from the melanoma antigen, gp100, failed to mediate regression of established B16F10 melanoma in normal mice. Dendritic cell vaccination induced activation and subsequent deletion of adoptively transferred naive CD8+ T-cell receptor transgenic (pmel-1) T cells specific for gp100 in normal mice. In lymphodepleted mice, dendritic cell vaccination produced greater T-cell expansion, long-term persistence of memory T cells, and tumor regression. Most tumors that persisted in the presence of functional memory T cells had either lost or exhibited reduced expression of MHC class I or gp100 proteins. In contrast to other naive T cells, pmel-1 T cells adoptively transferred to lymphodepleted mice exhibited faster proliferation and a more differentiated phenotype after exposure to peptide-pulsed dendritic cells. Proliferation and persistence of pmel-1 T cells was highly dependent on interleukin-7 (IL-7) in irradiated mice, and IL-15 when IL-7 was neutralized, two critical homeostatic cytokines produced in response to the irradiation-induced lymphodepletion. (Cancer Res 2005; 65(22): 10569-77)

Introduction

One of the most promising approaches to immunotherapy for cancer is based on the recognition of antigen-presenting cells by T cells. The identification of a large number of well-defined tumor-associated antigens offers an unprecedented opportunity for cancer immunotherapy. The major goal of active-specific immunotherapy is to generate tumor-specific T cells in tumor-bearing hosts. These T cells need to be functional, exhibit high avidity, and their numbers must reach levels above the threshold required to mediate regression of established tumors. Generation of memory cells to prevent tumor recurrence is also desirable. Thus far, vaccine strategies, including dendritic cell–based vaccines, have not been effective against nonhematopoietic tumors; the rate of tumor regression has been <10% (1). The primary obstacle to the success of active-specific immunotherapy has been the inability of the current vaccines to elicit a strong and persistent immune response to tumor antigens, which for most tumors, particularly melanoma, are primarily self-antigens (2). When naive T cells are transferred into lymphodepleted mice (rendered lymphopenic by genetic manipulation, sublethal irradiation, or chemotherapy), they undergo “lymphopenia-driven homeostatic proliferation” in the absence of foreign antigens or inflammatory signals. However, if T cells are exposed to foreign antigens during homeostatic proliferation, a dramatic expansion of antigen-specific T cells was observed in thymic-deficient mice after bone marrow transplantation and adoptive transfer of a small number of antigen-specific T-cell receptor (TCR) transgenic T cells (3). The therapeutic benefit of homeostatic proliferation–induced expansion of T cells against tumor antigens was subsequently shown in an animal tumor model (4). We and others have shown that tumor rejection was enhanced by presentation of tumor antigens during homeostatic proliferation in lymphodepleted mice (5–8). Conversely, homeostatic proliferation was shown to be an obstacle to the induction of transplantation tolerance and a key factor in the development of autoimmune diabetes in nonobese diabetic mice (9, 10). The importance of lymphodepletion for the expansion and persistence of tumor-reactive T cells and in the success of adoptive immunotherapy was highlighted by the recent demonstration that >50% of melanoma patients experienced tumor regression after they were reconstituted with a large number of activated tumor reactive lymphocytes after chemotherapy-induced lymphodepletion (11–13). A significant correlation between clinical response and the degree of persistence of transferred T cells in blood was observed (14). These studies strongly indicated that the threshold level of circulating antitumor T cells necessary to mediate tumor regression might be high.

Using a melanoma antigen gp100-specific TCR transgenic T-cell transfer model (15), our current study is aimed to determine whether vaccination during lymphopenia would drive a rapid and preferential expansion of naive tumor-specific CD8+ T cells and whether the lymphopenic conditions would allow a large fraction of the activated T cells to survive, persist, and mediate tumor regression. The second objective was to gain mechanistic insights into the enhanced expansion and persistence of tumor-specific CD8+ memory T cells in the reconstituted lymphodepleted mice.

Materials and Methods

Mice and tumor cell lines. Female C57BL/6(B6) mice and congenic C57BL/6-Ly5.1 mice were purchased from the Charles River Laboratories, Inc. (National Cancer Institute-Frederick, Frederick, MD). Pmel-1 transgenic mice express a TCR specific for an H-2Dd-restricted CD8+ T cells epitope from the marine melanoma tumor antigen gp10025-33 (EGSRNQDWL) or human gp100a23 (KVPRNQDWL; ref. 11). Pmel-1 transgenic mice on a C57BL/6 background were bred with green fluorescent protein (GFP) transgenic mice

Requests for reprints: Hong-Ming Hu, Laboratory of Cancer Immunobiology, Providence Portland Medical Center, Robert W. Franz Cancer Center, Earle A. Chiles Research Institute, 4805 Northeast Glisan Street, Portland, OR 97213. Phone: 503-215-6531; Fax: 503-215-6841; E-mail: hhu@providence.org. ©2005 American Association for Cancer Research.

doi:10.1158/0008-5472.CAN-05-2117
C57BL/6 mice were inoculated s.c. with 2 × 10^6 B16-F10 melanoma cells and irradiated 5 days later (500 rad). On day 6, one million splenocytes from irradiated C57BL/6 (CD45.2^+) mice were adoptively transferred into tumor-bearing mice (n = 10 per group). Adoptive transfer was followed immediately by i.s. vaccination with 1 × 10^6 dendritic cells pulsed with hgp-9 peptide or a control peptide. In some experiments, one additional dendritic cell vaccination was administrated at 2-week intervals. Growth of s.c. tumor was monitored thrice a week by measurement of two perpendicular diameters using a digital caliper. Mice were sacrificed when one diameter exceeded 15 mm. All experiments were carried out in a blinded and randomized fashion. In some experiments, IL-7 was blocked by the injection of mice with 1 mg purified monoclonal anti-IL-7 antibody (M2).

**Flow cytometry analysis and sorting.** Single-cell suspensions prepared from blood, spleen, bone marrow, or lung were stained with APC-labeled anti-CD8 and phycoerythrin (PE)-labeled anti-CD45.1, CD43, CD44, CD62L, or CD25 antibodies (eBioscience, San Diego, CA). APC-labeled hgp-9/H-2Db MHC tetramer was used to stain peptide-specific cells (obtained from NIH tetramer core facility). Pmel-1 transgenic T cells were gated on GFP^+^/CD8^+^/CD45.1^+^ population was further divided into two populations; GFP^+^CD8^+^CD45.1^+^ population was taken as congenic adoptively transferred T cells, GFP^+^CD8^+^CD45.1^+^ was taken as the host T cells that were regenerated after irradiation. At least 20,000 live cell events gated by scatter plots were analyzed for each sample. CD4/CD62L staining was further gated on CD4^+^ or CD8^+^ populations. For cell division analysis and in vivo CTL assay, spleen cells were labeled with far-red tracer DDAO-SE (Molecular Probes, Eugene, OR) according to the suggested protocol. For bromodeoxyuridine (BrdUrd) incorporation assay, mice were injected i.p. with 0.8 mg BrdUrd per mouse 1 day before the collection of blood and spleens. BrdUrd^+^/peml-1 T cells were determined by flow cytometry with anti-BrdUrd antibody conjugated with APC (BD Biosciences, San Jose, CA) after staining with PE-anti-CD8. Flow cytometric analysis was done with the FACSCalibur and CellQuest software (Becton Dickinson, Mountain View, CA).
**Results**

Antigen-driven proliferation of transgenic pmel-1 T cells and homeostatic-driven proliferation of polyclonal naive T cells exhibited different kinetics. Previously, we showed the selective expansion of melanoma-specific CD4\(^+\) and CD8\(^+\) T cells in lymphopenic mice after adoptive transfer of polyclonal naive T cells and vaccination with irradiated tumor cells (7, 8). In this study, naive CD8\(^+\) T cells from pmel-1 TCR transgenic mice were used to track the fate of tumor antigen-specific T cells in normal and lymphodepleted mice after vaccination. The majority of T cells (pmel-1 T cells) in these transgenic mice are naive T cells with TCR specific for a MHC I–restricted epitope derived from the human or mouse melanoma antigen, gp100/pmel-1 (15). One million pmel-1 T cells (Ly5.2/CD45.2) were adoptively transferred to normal and irradiated mice together with 10 million congenic spleen cells (Ly5.1/CD45.1) as the filler cells. This ratio of pmel-1 and naive spleen cells was used because it allowed the largest expansion of pmel-1 T cells and rapid reconstitution of the polyclonal repertoire of naive T cells (data not shown). Mice were then immunized with dendritic cells loaded with human gp100 peptide (hgp-9). Dendritic cells loaded with hgp-9 but not the control peptide (data not shown) induced expansion of pmel-1 T cells in both normal and lymphodepleted hosts, which peaked at day 7 (Fig. 1A and B). There was an average of 10 pmel-1 T cells/µL of blood in normal mice, which represented 5% of the CD8\(^+\) T cells, whereas an average of 70 pmel-1 T cells/µL, which represented 50% of all CD8\(^+\) T cells were in the blood from lymphodepleted mice. Following peak expansion on day 7, pmel-1 T cells decreased rapidly in normal hosts and were undetectable 6 weeks after vaccination. In contrast, in vaccinated lymphodepleted hosts, pmel T cells persisted at high levels for up to 12 weeks after vaccination. The decline in the percentage of pmel-1 T cells was primarily due to the delayed expansion of donor filler non-pmel T cells and the recovery of host T cells. However, the absolute number of pmel-1 cells did not change significantly in 12 weeks, and there might have been a slight increase from weeks 3 to 6 after vaccination (50-70 cells/µL of blood; Fig. 1B). At 2 weeks after vaccination, there was a significantly higher percentage and absolute number of pmel-1 T cells in the blood, spleen, bone marrow, and lung from lymphodepleted hosts compared with normal hosts (Fig. 1C). Lymphopenia-driven proliferation of self-reactive T cells could induce development of autoimmunity. We observed more severe depigmentation in irradiated mice that received pmel-1 and dendritic cell vaccinations than in similarly treated normal mice (Fig. 1D).

To gain insight into the profound difference in the pmel-1 T-cell expansion and persistence, we used the BrdUrd incorporation assay to determine the possible differences in pmel-1 T-cell cycling and the vital dye dilution assay to compare the kinetics of antigen-driven versus homeostatic proliferation–driven T-cell proliferation. BrdUrd was injected into normal or lymphodepleted mice 7 days after dendritic cell vaccination. Eight hours later, >20% of the pmel-1 T cells in the spleen and blood from lymphodepleted mice, versus <10% of the pmel-1 T cells from normal hosts, were BrdUrd\(^+\) (Fig. 2A). The DDAO-SE dilution assay was used to track pmel-1 T-cell division in lymphodepleted mice after vaccination with cognate hgp-9 peptide or control gp33 peptide. The pmel-1 T cells (GFP\(^+\)CD8\(^+\)) completely lost DDAO-SE label 7 days after transfer into mice vaccinated with hgp-9-loaded dendritic cells (antigen-driven proliferation of T cells; Fig. 2B, top), whereas congenic T cells (GFP\(^+\)CD8\(^+\)) and pmel-1 T cells in mice vaccinated with gp33 peptide (homeostatic proliferation–driven proliferation of T cells; Fig. 2B, bottom) lost their DDAO-SE 14 days after transfer.

Transgenic pmel-1 T cells and polyclonal naive T cells differ in the expression of memory T-cell markers after their activation and expansion in lymphodepleted mice. Naive T cells proliferate upon adoptive transfer to lymphopenic hosts and begin to express many of the surface markers of memory T cells. These memory-like T cells are unable to revert to naive T-cell function.

**Figure 2.** Kinetics of antigen-driven and homeostatic proliferation–driven proliferation of T cells in lymphodepleted mice. A, at the peak of their response, a higher percentage of pmel-1 T cells incorporated BrdUrd in lymphodepleted mice than in normal mice after vaccination. BrdUrd was administered to normal and lymphodepleted mice 7 days after adoptive transfer and vaccination. The percentage of pmel-1 transgenic T cells in both blood and spleen that had incorporated BrdUrd was determined by intracellular staining with APC-conjugated anti-BrdUrd antibody (BD PharMingen, San Diego, CA), and flow cytometry analysis was done according to the manufacturer’s protocol. B, pmel-1 transgenic T cells from irradiated mice lost completely DDAO-SE labeling 1 week after vaccination with dendritic cells (DC) loaded with cognate peptide hgp-9. It took 2 weeks to lose DDAO-SE labeling when mice were vaccinated with dendritic cells loaded with control peptide gp33. Congenic nontransgenic T cells also needed at least 2 weeks to dilute all of their DDAO-SE labeling when transferred into irradiated vaccinated with either hgp-9 or gp33 peptide-loaded dendritic cells. Before adoptive transfer, pmel-1 and congenic spleen cells were labeled with 10 μm/L DDAO-SE and adaptively transferred into mice shortly after irradiation. One group of mice was vaccinated with hgp-9–loaded dendritic cells, whereas the other group of mice was vaccinated with gp33–loaded dendritic cells. Mice were sacrificed at indicated time points, and spleen cells were analyzed by flow cytometry.
phenotype but do partially fill the niche of memory T cells (16, 17). The differentiation of memory T cells was coupled to the extent of cell division (18). After exposure to antigen, naive T cells undergo multiple rounds of division and differentiate into true memory T cells with acquisition of appropriate surface markers (e.g., CD44hi, CD43hi, and CD62Llow). In contrast, non–antigen-specific T cells driven by homeostatic proliferation undergo fewer rounds of division and became memory-like T cells with increased expression of CD44 and CD43 but do not down-regulate CD62L expression. This prompted us to examine the phenotype of pmel-1 T cells and congenic T cells under different condition of adoptive transfer. At the peak of proliferation, pmel-1 T cells in the normal hosts expressed markers typical of effector T cells (CD44hi, CD43hi, and CD62Llow). In the same mice, the phenotype of non-pmel-1 congenic T cells resembled naive T cells (CD43low, CD44low/hi, and CD62Lhi; Fig. 3A). In lymphodepleted mice, pmel-1 and non-pmel-1 congenic T cells not only differed in their kinetics of expansion but also exhibited different patterns of memory marker expression at the peak of proliferation (Fig. 3B). Although peripheral blood pmel-1 T cells from vaccinated lymphodepleted mice exhibited a phenotype similar to cells from vaccinated normal hosts, they seemed more activated as indicated by the higher mean channel fluorescence intensity of CD43 and CD44 and the complete down-regulation of CD62L (Fig. 3A and B). As expected, the homeostatic proliferation–driven expansion of non-pmel-1 T cells resulted in intermediate levels of CD43 and CD44 but minimal down-regulation of CD62L. Pmel-1 T cells in vaccinated lymphodepleted mice gradually regained expression of CD62L; ~50% of pmel-1 T cells expressed high levels of CD62L at day 19 after vaccination. In contrast, CD62L expression on non-pmel-1 T cells exhibited very little change over the whole period (data not shown). Thus, antigen-driven memory T cells and homeostatic proliferation–driven memory-like T cells exhibit different kinetics of response and expression of memory markers.

Interleukin-7 production following irradiation prevented the contraction of activated pmel-1 T cells. IL-7 and IL-15 seem critical for the generation and survival of memory CD8+ T cells and maintenance of the basal turnover of memory T cells, respectively (19–21). We posited that the differential expression of receptors for IL-7, IL-2, or IL-15 might explain the improved survival of pmel-1 T cells in lymphodepleted mice. Therefore, the expression of CD127 (IL-7Rα), CD25 (IL-2Rα), and CD122 (IL-2 and IL-15Rα) by pmel-1 T cells at the peak of their response in normal and lymphodepleted mice was compared by flow cytometry analysis (Fig. 3C and D). CD25 expression was associated with early T-cell activation; neither pmel-1 nor non-pmel-1 T cells from normal hosts expressed CD25; low but significant levels of CD25 expression was found on both pmel-1 and non-pmel-1 T cells from lymphodepleted mice. Pmel-1 T cells from both normal and lymphodepleted mice exhibited similar and uniform CD122 and CD127 expression. CD127 expression of non-pmel-1 T cells from either normal and lymphodepleted mice did not change. CD122

CD122low non-pmel-1 T cells seemed to comprise two subsets cells with a CD122low and CD122hi phenotype. Compared with the normal host, the CD122hi subset was increased in lymphodepleted versus normal mice. Because both proliferation and survival of CD122hi subset CD8+ memory T cells depended on IL-15 (22), it is likely reflecting increased levels of IL-15 in irradiated mice. After the
peak of the immune response, the pmel-1 T cells continued to differentiate into mature memory T cells and eventually consisted of a mixture of roughly equivalent numbers of central memory (CD62Lhi) and effector memory (CD62Llow) T cells, whereas most (80%) non-pmel-1 congenic T cells remained CD62Lhi phenotype (data not shown).

Although pmel-1 T cells from lymphodepleted hosts underwent more divisions and acquired a more mature phenotype, their persistence could not be attributed to differences in IL-7 or IL-15 receptor expression. Alternatively, the persistence of activated pmel-1 T cells could be due to the higher level of cytokines, such as IL-7 and IL-15, critical for the maintenance of CD8+ memory T cells in lymphodepleted mice. Increased levels could be the direct result of irradiation to decreased consumption after T-cell depletion (23). To determine whether IL-7 and IL-15 were involved in the expansion and persistence of pmel-1 T cells in vaccinated normal and lymphodepleted mice, anti-IL-7 antibody was used to neutralize IL-7 in normal and IL-15 knockout (IL-15−/−) mice. Because naive congenic T cells were also transferred into lymphodepleted mice, both antigen-driven proliferation of pmel-1 T cells and homeostatic proliferation–driven proliferation of naive congenic donor T cells could be observed. In IL-15−/− mice, the number of pmel-1 T cells found in the blood from lymphodepleted mice was slightly reduced (36 versus 46 per µL of blood) at week 1 but was not significant (P = 0.0512, paired t test). Moreover, there was no difference in the number of pmel-1 T cells from either normal or IL-15−/− mice at weeks 2, 3, or 4 after vaccination (Fig. 4A). However, administration of IL-7 antibody greatly reduced the number of pmel-1 T cells in both blood at the peak (1 week) and contraction phases (2–4 weeks) of T-cell expansion compared with wild-type (wt) mice (P = 0.0023 at day 7 compared with wt control, paired t test). Elimination of both IL-7 and IL-15 by administration of IL-7 antibody to IL-15−/− mice resulted in an even greater reduction in the expansion of pmel-1 T cells to the levels found in normal hosts (P < 0.0001 compared with wt control at day 7; Fig. 4A). The significant role of IL-15 was only evident when IL-7 was also neutralized in IL-15-deficient mice. These results are consistent with an earlier study that showed IL-15-independent generation of memory CTL in IL-7 transgenic mice (24). Polyclonal naive T cells of donor origin were also proliferating in lymphodepleted mice. Homeostatic proliferation of non-pmel-1 congenic donor T cells in lymphodepleted mice was significantly reduced by elimination of either IL-7 (P = 0.020) or IL-15 (P = 0.0032) and dramatically blocked by elimination of both IL-7 and IL-15 (P = 0.0027 at day 21 compared with wt control; Fig. 4B).

In contrast, the peak proliferation of antigen-driven pmel-1 T cells in normal host was IL-7 and IL-15 independent. Neutralization of IL-7 changed neither the peak expansion nor subsequent contraction of pmel-1 T cells, but surprisingly, pmel-1 T cells exhibited a greater expansion in IL-15−/− mice after vaccination than in wt mice (Fig. 4C). IL-7 neutralization did abrogate the effect of IL-15 deficiency, suggesting that the heightened pmel-1 expansion in IL-15−/− mice required IL-7. In hindsight, this would be predicted because mice deficient in IL-15 or lacking the IL-15Ra are markedly lymphopenic; they would be particularly deficient in IL-15-dependent CD8+ T cells with memory phenotype (22, 23, 25). This would support the idea that competition of IL-7 from the pre-existing IL-7-dependent expansion and persistence of pmel-1 T cells in lymphodepleted mice is largely IL-7 dependent and IL-15 played only a minor role during the peak of expansion. In contrast, homeostatic proliferation–driven proliferation and survival of polyclonal donor T cells depended on both IL-7 and IL-15.

**Functional characterization of pmel-1 memory T cells from lymphodepleted mice.** The function of pmel-1 T cells activated in lymphodepleted mice was measured using the ex vivo IFN-γ intracellular staining and in vivo CTL assays. At day 7 after vaccination, >70% of pmel-1 T cells (GFP+ and CD8+) produced IFN-γ after direct stimulation with hgp-9 peptide but not with control peptide (data not shown; Fig. 5A). IFN-γ-producing, hgp-9-specific non-pmel-1 T cells (GFP+ and CD8+) also expanded in lymphodepleted mice. Approximately 17% of CD8+ non-pmel-1 T cells from lymphodepleted mice recognized hgp-9 at the peak of expansion compared with only 2% of CD8+ T cells from normal mice.
Regression of established tumors in vaccinated lymphodepleted mice following adoptive transfer of pmel-1 T cells.

Six days after s.c. inoculation of live B16F10 melanoma, tumor-bearing mice were treated with pmel-1 T cells and dendritic cell vaccination with and without irradiation. Because a single vaccination caused only a temporary halt in tumor growth, dendritic cell vaccination was given twice at 2 weeks apart to boost pmel-1 T cells (Fig. 6A). Treatment of normal mice with pmel-1 T cells and dendritic cell vaccination only slightly delayed tumor growth. The tumors in most vaccinated lymphodepleted mice underwent transient regression at the peak of the primary pmel-1 expansion, but tumors recurred rapidly despite the rapid expansion of pmel-1 T cells that occurred after the booster vaccination on day 20. Nonetheless, vaccinated lymphodepleted mice had a significantly longer survival (median survival of 62 days), and 20% of mice survived tumor free for >80 days, whereas vaccinated normal mice had a median survival of 31 days with no long-term survivors (Fig. 6B).

Tumors that recurred after the initial regression might have escaped via tumor immunoediting mediated by memory pmel-1 T cells induced in lymphodepleted mice; they were resected on multiple days (26). Tumors were resected on day 30 from mice that had received irradiation alone, normal, or irradiated mice that received pmel-1 T-cell transfer and dendritic cell vaccinations at days 30 to 85 (Table 1). Tumor cells were dissociated and cultured for >2 weeks to remove contamination by nontumor cells and then were assessed by flow cytometry for the expression of MHC class I antigens. Cultured B16F10 tumor cells were included as a control. IFN-γ production by T cells was used to determine whether tumor cells could process and present the tumor-derived gp100 peptide. Three tumors each from the irradiated but not vaccinated and vaccinated normal groups and six tumors from vaccinated lymphodepleted mice were analyzed. The expression of the MHC class I molecule, H-2D^b, as indicated by the intensity of mean channel fluorescence, varied significantly among the different tumor cell lines (Table 1). Every tumor cell line derived from irradiated mice or vaccinated normal mice expressed surprisingly high levels of D^b and gp100. In most cases, they had high levels of Db and gp100. In most cases, they had mean channel fluorescence, varied significantly among the different tumor cell lines (Table 1). Every tumor cell line derived from irradiated mice or vaccinated normal mice expressed surprisingly high levels of D^b and gp100. In most cases, they had...
more class I expression. They all stimulated the production of IFN-γ by activated pmel-1 T cells. In contrast, five of six tumor cell lines derived from vaccinated irradiated mice exhibited a markedly diminished ability to stimulate pmel-1 T cells. Three tumor cell lines (#438, #439, and #442) completely failed to stimulate IFN-γ production; two (#438 and #442) were amelanotic and did not express gp100, and the other (#439) had no Db expression. When cells from #439 or #442 cell lines were used to establish day 6 tumors, treatment with pmel-1 cells and vaccination were ineffective, even in lymphodepleted mice (Fig. 6C-D). Western blot analysis with anti-tyrosinase antibody showed that each of these tumor cells (#438, #439, and #442) continued to express tyrosinase (Fig. 6E). These results confirm that tumors may escape both naturally occurring and therapeutically induced immune responses via immunoeediting mechanisms.

Discussion

By using pmel-1 T cells from gp100-specific TCR transgenic mice, we have shown that tumor-specific CD8+ T cells had very different fates in normal and lymphodepleted mice after antigen-driven proliferation. Dendritic cells pulsed with peptide could drive IL-7- and IL-15-independent expansion of pmel-1 T cells in normal hosts, pmel-1 transgenic T cells were subsequently deleted in normal mice, whereas in lymphodepleted mice, they underwent a dramatic IL-7 and IL-15 dependent expansion and persisted at very high levels for long periods. This heightened immune response in lymphodepleted hosts was associated with tumor regression and prolonged survival of mice bearing well-established tumors. IL-15 only played a minor role for the expansion but not survival of pmel-1 T cells in lymphodepleted mice. Finally, we also provide direct evidence that an active immunoeediting process could lead to tumor evasion in the face of a large and persistent population of memory CD8+ T cells specific for a single peptide.

We propose that the preferential expansion and long-term persistence of antigen-specific T cells during homeostatic proliferation is primarily due to the faster kinetics of antigen-driven proliferation of antigen-specific T cells in lymphodepleted mice, which provided antigen-specific T cells easier access to IL-7 and IL-15. This explanation is consistent with recent publications, which reported that both homeostatic proliferation of naive T cells and survival of memory CD8+ T cells require IL-7 and IL-15 (27). IL-7 is responsible primarily for the survival of naive or a subset of activated T cells that lead to generation of memory T cells, whereas IL-15 maintains the antigen-independent basal cycling and renewal of memory T cells (19–21). Previous studies showed that homeostatic proliferation–driven proliferation and survival of naive CD8 T cells depends on self-antigen
Another mechanism that may contribute to enhance antigen-driven expansion is the diminished number of T<sub>reg</sub> cells after cytokine when IL-7 is limited. Our results clearly showed that during the early burst proliferation in vaccinated lymphodepleted mice pmel-1 T cells was nearly identical in mice whether or not they received adoptive transfer and vaccinations were resected at the indicated days after tumor injection. Tumors were digested with triple enzyme solution and kept in culture. Tumor cell lines were developed ~2 weeks after <i>in vitro</i> culture to remove nontumor cells. The expression of MHC I H-2Db molecules on tumor cell surface was determined by flow cytometry analysis with PE-labeled anti-D<sup>b</sup> antibody. The expression of gp100 was determined by intracellular staining with anti-gp100 antibody (HMB-45) and PE-conjugated secondary antibody. The ability of tumor cells to stimulate the IFN-γ production by activated pmel-1 T cells was determined by ELISA. Abbreviation: MFI, mean channel florescence intensity.

Table 1. Characteristics of tumor escape variants

<table>
<thead>
<tr>
<th>Source of F10 Mouse ear tag number</th>
<th>Day of tumor resections</th>
<th>H-2Db expression (MFI)</th>
<th>gp100 expression (MFI)</th>
<th>IFN-γ production (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated normal mice #228</td>
<td>32</td>
<td>3.08</td>
<td>8.58</td>
<td>141.16</td>
</tr>
<tr>
<td>#229</td>
<td>31</td>
<td>119.08</td>
<td>4.00</td>
<td>223.18</td>
</tr>
<tr>
<td>#232</td>
<td>31</td>
<td>10.30</td>
<td>4.08</td>
<td>145.83</td>
</tr>
<tr>
<td>#212</td>
<td>31</td>
<td>5.57</td>
<td>4.24</td>
<td>95.74</td>
</tr>
<tr>
<td>#213</td>
<td>31</td>
<td>5.28</td>
<td>5.22</td>
<td>311.94</td>
</tr>
<tr>
<td>#219</td>
<td>32</td>
<td>8.65</td>
<td>11.19</td>
<td>300.52</td>
</tr>
<tr>
<td>#436</td>
<td>65</td>
<td>9.39</td>
<td>12.10</td>
<td>288.0</td>
</tr>
<tr>
<td>#437</td>
<td>55</td>
<td>44.63</td>
<td>1.48</td>
<td>106.12</td>
</tr>
<tr>
<td>#441</td>
<td>58</td>
<td>33.03</td>
<td>18.25</td>
<td>37.86</td>
</tr>
<tr>
<td>#438</td>
<td>65</td>
<td>50.52</td>
<td>5.54</td>
<td>23.33</td>
</tr>
<tr>
<td>#439</td>
<td>85</td>
<td>5.78</td>
<td>0</td>
<td>&lt;10</td>
</tr>
<tr>
<td>#442</td>
<td>65</td>
<td>116.43</td>
<td>&lt;10</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: C57B6 mice were injected with 2 × 10<sup>6</sup> F10 tumor cells s.c. and irradiated at day 5. Adoptive transfer and dendritic cell vaccination were done 6 and 20 d after tumor injection. Tumors that developed in vaccinated normal mice, irradiated mice without vaccination, and irradiated mice that received adoptive transfer and vaccinations were resected at the indicated days after tumor injection. Tumors were digested with triple enzyme solution and kept in culture. Tumor cell lines were developed ~2 weeks after <i>in vitro</i> culture to remove nontumor cells. The expression of MHC I H-2Db molecules on tumor cell surface was determined by flow cytometry analysis with PE-labeled anti-D<sup>b</sup> antibody. The expression of gp100 was determined by intracellular staining with anti-gp100 antibody (HMB-45) and PE-conjugated secondary antibody. The ability of tumor cells to stimulate the IFN-γ production by activated pmel-1 T cells was determined by ELISA. Abbreviation: MFI, mean channel florescence intensity.

Another mechanism that may contribute to enhance antigen-driven expansion is the diminished number of T<sub>reg</sub> cells after lymphodepletion. Many publications have shown the regulatory role of CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cells in suppressing immune responses to various antigens, including tumor-associated antigens in normal T-replete mice (30). The ability of CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cells to downregulate homeostatic proliferation–driven proliferation of polyclonal memory CD8<sup>+</sup> T cells in lymphodepleted mice (31) and antigen-driven proliferation of pmel-1 T cells in RAG<sup>−/−</sup> mice has been shown (32). Interestingly, our preliminary data suggested that depletion of preexisting memory T cells (using CD122 antibody) or T<sub>reg</sub> cells (using CD25 antibody) from the congenic filler cells before the adoptive transfer could decrease the pmel-1 contraction and promote the survival and persistence of activated pmel-1 T cells. Thus, one must not discount the contribution of simple competition among different subset of T cells, which may well be responsible for many of the regulatory phenomenon observed in lymphodepleted hosts (33).

Using the B16 melanoma and pmel-1 transgenic model, Lou et al. showed that dendritic cell–based vaccines greatly improved the efficacy and survival of adoptively transferred, <i>in vitro</i> activated, pmel-1T cells in normal or irradiated hosts (34). Their study focused on the ability of peptide-pulsed dendritic cells to increase the antitumor activity of adoptively transferred activated effector T cells with concurrent administration of IL-2. In contrast to our observations, they noted that <i>in vivo</i> proliferation of pmel-1 T cells was nearly identical in mice whether or not they were irradiated. The difference may be explained by the absence of exogenous IL-2 administration and the small number of naive T cells that were transferred in our study, whereas high levels of exogenous IL-2 were administered after adoptive transfer of a relatively large number of <i>in vitro</i> activated T cells in their study. Studies are under way to determine whether provision of exogenous IL-2, IL-7, IL-15, and/or IL-21 would further improve the efficacy of our treatment strategies as suggested by recent studies (35, 36).

The successful evasion of the immune system by tumor cells via a process now called immunoediting has been documented by others (37). Despite low levels of MHC class I expression, B16F10 melanoma cells could stimulate pmel-1 T cells and were killed specifically following activation. Following treatment with our optimal regimen, ~20% of mice were rendered tumor free and the remainder of mice generally experienced an initial tumor regression followed by tumor cell recurrence and continued disease progression. Examination of MHC class I and gp100 expression on recurrent tumors revealed that at least half of the tumors that escaped elimination despite the presence of large numbers of functional pmel-1 T cells did so via an immunoediting...
process (26). Using PIA-specific TCR transgenic T cells, Bai et al. recently documented similar tumor escape mechanisms (38). In addition to the loss of MHC or PIA expression, they also identified mutations in PIA that resulted in diminished T-cell recognition. Additional escape mechanisms must be operational, because half of the recurrent tumors continued to express MHC class I and gp100 and were still able to stimulate pmel-1 T cells. One possible mechanism is functional silencing of pmel-1 T cells in tumor sites (39, 40).

In summary, we have established a mechanism for the enhanced expansion and persistence of antitumor T cells in lymphodepleted mice. More importantly, regardless of their phenotype or stages of differentiation, only a small number of antigen-specific T cells were required for tumor regression in vaccinated lymphodepleted mice. Our current studies provide a strong experimental basis for novel clinical trials to determine whether vaccine-induced expansion and persistence of tumor-specific effector/memory T cells in lymphodepleted cancer patients would enhance tumor regression and improve survival.

Acknowledgments

Received 6/16/2005; revised 8/15/2005; accepted 8/31/2005.

Grant support: Providence Portland Medical Foundation, Murdock Trust, American Cancer Society grant LIB-106810, and National Cancer Institute/NIH/Department of Health and Human Services grant R01-CA107243.

I thank Drs. Suyu Shu, Edward Walker, and Andrew D. Weinberg for their advice; Drs. Peter Doherty (Earle A. Chiles Research Institute, Portland, OR) for providing us the anti-IL-7 antibody; Dan Haley for his excellent technical support; Mary Healy and Marlene Riske for their administrative support; Trish Raune for her excellent animal husbandry; and NIH MHC tetramer core facility for the hgp-9/Db tetramers.

References


Received 6/16/2005; revised 8/15/2005; accepted 8/31/2005.

Grant support: Providence Portland Medical Foundation, Murdock Trust, American Cancer Society grant LIB-106810, and National Cancer Institute/NIH/Department of Health and Human Services grant R01-CA107243.

I thank Drs. Suyu Shu, Edward Walker, and Andrew D. Weinberg for their advice; Drs. Peter Doherty (Earle A. Chiles Research Institute, Portland, OR) for providing us the anti-IL-7 antibody; Dan Haley for his excellent technical support; Mary Healy and Marlene Riske for their administrative support; Trish Raune for her excellent animal husbandry; and NIH MHC tetramer core facility for the hgp-9/Db tetramers.
Interleukin-7-Dependent Expansion and Persistence of Melanoma-Specific T Cells in Lymphodepleted Mice Lead to Tumor Regression and Editing

Li-Xin Wang, Rui Li, Guojun Yang, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/22/10569

Cited articles
This article cites 39 articles, 21 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/22/10569.full.html#ref-list-1

Citing articles
This article has been cited by 18 HighWire-hosted articles. Access the articles at:
/content/65/22/10569.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.