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Vaccine-Stimulated, Adoptively Transferred CD8\(^+\) T Cells Traffic Indiscriminately and Ubiquitously while Mediating Specific Tumor Destruction\(^1\)

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It has been suggested that antitumor T cells specifically traffic to the tumor site, where they effect tumor destruction. To test whether tumor-reactive CD8\(^+\) T cells specifically home to tumor, we assessed the trafficking of gp100-specific pmel-1 cells to large, vascularized tumors that express or do not express the target Ag. Activation of tumor-specific CD8\(^+\) pmel-1 T cells with IL-2 and vaccination with an altered peptide ligand caused regression of gp100-positive tumors (B16), but not gp100-negative tumors (methylcholanthrene 205), implanted on opposing flanks of the same mouse. Surprisingly, we found approximately equal and very large numbers of pmel-1 T cells (>25% of all lymphocytes) infiltrating both Ag-positive and Ag-negative tumors. We also found evidence of massive infiltration and proliferation of activated antitumor pmel-1 cells in a variety of peripheral tissues, including lymph nodes, liver, spleen, and lungs, but not peripheral blood. Most importantly, evidence for T cell function, as measured by production of IFN-γ, release of perforin, and activation of caspase-3 in target cells, was confined to Ag-expressing tumor. We thus conclude that CD8\(^+\) T cell-mediated destruction of tumor is the result of specific T cell triggering at the tumor site. The ability to induce ubiquitous homing and specific tumor destruction may be important in the case of noninflammatory metastatic tumor foci. The Journal of Immunology, 2004, 173: 7209–7216.

Eliciting the infiltration of tumor sites with large numbers of tumor-reactive T cells capable of destroying tumor remains an elusive goal (1). Tumor-reactive T cell migration to the tumor may be a requirement for the regression of bulky disease (2, 3), but the precise mode of lymphocyte migration into peripheral tissue remains unclear. Some workers have reported that CD8\(^+\) T cells migrate in an Ag-specific manner (4). α\(β\) integrin is reportedly involved in T cell homing to the gut (5), but it has been difficult to show such specificity for homing to other peripheral tissues (6, 7). The homing of Ag-experienced lymphocytes may initially be nonspecific, because they forage for their cognate Ag. When T cells come into contact with their specific Ag, they may be retained in Ag-containing tissue (8).

Adoptive transfer of activated T cells, followed by specific stimulation, can result in in vivo destruction of tumor in mice and humans (9, 10), but how these cells infiltrate tumor tissue remains unknown. We have previously analyzed the trafficking of antitumor T cells in patients with melanoma using \(^{111}\text{In}\)-labeled lymphocytes and whole-body gamma camera imaging (2). We found \(^{111}\text{In}\) in liver, spleen, and lungs as well as in metastatic deposits, but it could not be determined whether live \(^{111}\text{In}\)-labeled T cells specifically trafficked to these locations, or the \(^{111}\text{In}\) nuclide had lodged at these locations as a result of the death, phagocytosis, or target specificity of the adoptively transferred T cells (2).

To experimentally examine T cell trafficking into tumors, we used a recently described murine model, termed pmel-1, which expresses a transgenic α\(β\) CD8\(^+\) TCR and is reactive against the melanoma/melanocyte Ag, gp100\(_{25,33}\). Adoptive transfer of these cells along with a vaccine encoding the altered peptide ligand and administration of exogenous IL-2 result in the regression of established s.c. B16 melanoma in C57BL/6n mice (9). We examined the role of T cell activation in tumor infiltration and determined the kinetics and specificity of T cell trafficking into tumor that expressed or did not express the target Ag.

Materials and Methods

Mice and tumor lines

Pmel-1 TCR, pmel-1/GFP\(^-\), pmel-1/Thy1.1\(^-\) transgenic mice (9) and C57BL/6n mice were bred and housed at the National Institutes of Health according to the guidelines of the animal care and use committee at National Institutes of Health. Pmel-1/Thy1.1\(^-\) transgenic mice were deposited at The Jackson Laboratory (strain 5023; Bar Harbor, ME). Transgenes were confirmed by PCR analysis for the pmel-1 TCR α- and β-chains, at 100 pM of each of the following: TCR α sense, 5′-GGT CCT GTG CTC CCA GTT TAA T-3′; TCR α antisense, 5′-CTG CTT AAC ATG TCC CTC ATG T-3′; TCR β sense, 5′-CTG GCC AGT GTG TCT TCT CC-3′; TCR β antisense, 5′-ACC ATG GTC ATC CAA CAC AG-3′; and gold Tag (Applied Biosystems, Branchburg, NJ), with 1X PCR buffer, 3 mM MgCl\(_2\), and 10 mM dNTPs. PCR was performed at 94°C for 13 min, then (94°C for 1 min, 61°C for 1 min, 72°C for 1.5 min) for 35 cycles and at

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72°C for 15 min. Both (H-2b) B16, a gp100− spontaneous murine melanoma, and methylcholanthrene 205 (MCA-205)4 were obtained from the National Cancer Institute tumor repository. Tumors were maintained in complete medium (CM) comprised of RPMI 1640 with 10% heat-inactivated FBS (Biofluids, Rockville, MD).

RNA preparation and RT-PCR
B16 and MCA-205 tumors were lysed, and RNA was isolated using the RNAsesy Mini kit (Qiagen, Valencia, CA). cDNA was obtained using random hexamer primers and the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies, Carlsbad, CA). The quantities of gp100 and β-actin mRNAs were evaluated by PCR primers using intron-spanning primers (11, 12) at 25, 30, and 35 cycles and digital capture.

In vitro activation and cytokine release
Pmel-1 splenocytes from transgenic mice were cultured for 1 wk as previously described (9). For cytokine release assays, 1 × 10^7 T cells were cocultured in CM with 1 × 10^5 B16, MCA-205, or C57BL/6n splenocytes that were pulsed with or without 1 μM human gp100(25–33) peptide and washed twice. Supernatants were collected after 24 h and tested using a mouse IFN ELISA kit (Endogen, Rockford, IL).

Adoptive cell transfer, vaccination, and cytokine administration
Pmel-1 transgensics or C57BL/6n mice at 6–12 wk of age were implanted s.c. with 2–5 × 10^5 B16 melanoma cells and/or 2–5 × 10^5 MCA-205 colon carcinoma on opposing flanks and in vivo stimulated as described previously (9). Briefly, at the time of T cell transfer, mice (n = 5 for each group) were injected i.v. with 2 × 10^7 PFU of recombinant fowlpox encoding human gp100 and injected i.p. with 6 × 10^6 IU of recombinant IL-2 in PBS (Chiron, Emeryville, CA) twice daily for a total of five or six doses. To induce lymphopenia, C57BL/6n tumor-bearing mice were sublethally irradiated with 5 Gy before adoptive transfer of in vitro-activated pme-l-1 splenocytes (1–2 × 10^8 CD8+/H9262+ T cells) and in vivo stimulation (9). Mice were randomized, and tumors were monitored in a blinded fashion using digital calipers. The products of the perpendicular diameters are presented as the mean ± SEM.

Organ collection and cytfluorometric analysis of adoptively transferred cells
On the days indicated, mice were bled by tail vein into heparin-containing microcentrifuge tubes, killed, and perfused with heparin in HBSS or PBS (1 U/ml; injection of 10–15 ml in the left ventricle of the heart with rupture of the right ventricle), and their organs were harvested, homogenized, and stained extracellularly for cytokines. Their organs were harvested, homogenized, and stained extracellularly for cytokines. They were permeabilized using the Cytofix/Cytoperm kit (BD Biosciences), and stained with allophycocyanin-conjugated anti-IFN-γ mAbs (XM1G2.1).

BrdU incorporation
Four days after treatment, mice were injected i.p. with 1–1.5 mg of BrdU (Sigma-Aldrich, St. Louis, MO) for 2 h, then bled, killed, and perfused. Their organs were harvested, homogenized, and stained extracellularly for pme-l-1. Cells were permeabilized using Cytofix/Cytoperm (BD Biosciences), treated with DNase I (Sigma-Aldrich) for 1 h at 37°C, then stained with FITC-, PE-, or allophycocyanin-conjugated anti-BrdU (BD Biosciences), and analyzed using FACS.

Confocal microscopy
Organs were harvested, imbedded in optimal cutting temperature compound (4583; Sakura Finetek, Torrance, CA), frozen at −80°C, and sectioned using a cryostat (Molecular Histology, Gaithersburg, MD). Frozen sections were treated and stained with FITC-conjugated anti-Thy1.1, FITC-conjugated anti-VB13, PE-conjugated anti-perforin (H-315; Santa Cruz Biotechnology, Santa Cruz, CA), and active caspase-3 (C95-605; BD Biosciences) mAbs, then secondarily stained with Texas Red-conjugated, anti-goat, anti-rabbit (sc-2780; Santa Cruz Biotechnology). Images were obtained using a Leica TCS spectrophotometer confocal inverted microscope (NCI Core Fluorescence Imaging Facility, Bethesda, MD).

Statistics
Statistics were determined using factorial or repeated measure ANOVA (StatView statistical software; Abacus Concepts, Berkeley, CA). Differences were considered significant at p < 0.05.

Results
Pmel-1-transgenic CD8+ T cells traffic to B16, but only after activation
We explored whether T cells with tumor specificity were able to traffic to an Ag-expressing tumor site. We hypothesized that tumor activation of T cell precursors with specificity for tumor-associated Ags could trigger the extravasation and accumulation of T cells in the tumor. To assess whether tumor alone could activate tumor-specific CD8+ T cells, we used a TCR-transgenic mouse, called pme-l-1, which was developed in our laboratory. Pmel-1-transgenic mice express a TCR specific for the unmutated melanoma/melanocyte-associated Ag, gp100 (9).

We implanted the B16 mouse melanoma on transgenic mice and evaluated the activation of resident antitumor T cells in the spleen. As previously described, unmanipulated pme-l-1-transgenic splenocytes maintained a basal (Fig. 1A), but variable (data not shown), level of CD44 and CD62L expression, indicative of a relatively naive phenotype and similar to that in naive C57BL/6n mice. In the data shown in Fig. 1A, these distinctions were pronounced in the tumor-bearing transgenic mice. Pmel-1 T cell expression of CD44 (an activation marker) increased from 32% in unvaccinated mice to 81% in vaccinated transgenic mice. Likewise, the expression of CD62L (which moves in the opposite direction, i.e., lower) with Ag stimulation) on pme-l-1 T cells decreased from 60% in unvaccinated mice to 9% in vaccinated mice (9). Interestingly, the presence of the B16 tumor alone did not activate transgenic cells. Pmel-1 T cells could be activated, as defined by up-regulation of CD44 and down-regulation of CD62L, after IL-2 administration and immunization with recombinant fowlpox (rFPVhgp100) encoding an altered form of the target epitope, gp100(25–33), as previously described (9).

Despite the fact that >90% of the CD8+ T cells or roughly 20% of all splenocytes expressed a TCR specific for B16, no T cells were found in the tumor in unmanipulated pme-l-1 transgenic mice (Fig. 1B). However, these tumor-specific transgenic T cells could be found in the tumor tissue in large numbers after the administration of IL-2 and vaccination. Approximately 8.2% of all cells in the tumor were found to be CD8+/Vβ13+ T cells in a representative experiment (Fig. 1B). These data are consistent with the hypothesis that the Ag-specific vaccine, but not the B16 tumor, is responsible for the activation of pme-l-1 T cells in spleen and their accumulation in tumor.

As seen previously, tumor growth was unimpeded in unmanipulated pme-l-1-transgenic mice, similar to that in wild-type littermates (9) (Fig. 1C). We extended our earlier findings by showing that pme-l-1-transgenic mice that received rFPVhgp100 and IL-2 underwent tumor regression.

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4 Abbreviations used in this paper: MCA, methylcholanthrene; CM, complete medium; rFPV, recombinant fowlpox.
From these data it appeared that the presence of tumor alone did not activate naive pmel-1 T cells, nor did naive cells infiltrate into the tumor regardless of their abundance. Conversely, pmel-1 T cells appeared in the tumor in large numbers after activation, and this intensive T cell infiltration correlated with tumor destruction.

Role of target Ag expression in T cell trafficking

We studied the presence of T cells in tumor and specifically addressed what role the target Ag played in T cell migration. We used a tumor, MCA-205, that does not express mRNA for the target Ag, gp100 (Fig. 2A), and is not recognized by activated pmel-1 T cells unless pulsed with gp10025–33 peptide (Fig. 2B). To study the specificity of these cells in vivo, we implanted B16 and MCA-205 in the same mouse, on opposite flanks. We then treated these mice bearing two 7-day-old tumors using adoptive transfer of activated pmel-1 T cells, rFPVhgp100, and IL-2 (9). Adoptively transferred and in vivo-stimulated pmel-1 cells exhibited exquisite specificity for the in vivo destruction of Ag-expressing B16 tumor, but not Ag-negative MCA-205 (Fig. 2C).

We next sought to understand the bases for this tumor specificity. We hypothesized that cells must egress the circulation at the site of the tumor. Indeed, we observed (Fig. 1B) that tumor-specific T cells found their way into tumors after activation. To test the specificity of this extravasation, we applied the same dual tumor model, analyzing tissues at multiple times after treatment (Fig. 3A). Although B16 expresses the target Ag, gp100, and MCA-205 does not, we found equal percentages of pmel-1 T cells in both tissues, at multiple time points, and in repeated experiments. We observed similar T cell infiltration kinetics in spleen, liver, lung, peripheral blood, B16 draining inguinal lymph node, MCA-205 draining inguinal lymph node, and eye. In a repeated experiment examining the percentage of pmel-1 T cells at the peak of proliferation (day 5 after transfer), we observed 56.52% ± 3.7 and 51.27% ± 9.3% in the inguinal draining lymph nodes of B16 and MCA-205, respectively (n = 4). Few pmel-1 T cells were found in the kidney or brain (data not shown). There was a rapid increase in pmel-1 T cells in all tissues on day 5, followed by a dramatic decline on day 6. We and others have previously observed this explosive growth followed by a rapid contraction of activated CD8+ T cells in spleen (13–15). In this study we extend these findings to the periphery.

The absence of gp100 expression in the MCA-205 tumor had no impact on pmel-1 infiltration. To assess whether this surprising result was due merely to blood contamination derived from the vessels found in these highly vascularized tumors, we washed out the intravascular contents by a perfusion technique using the rupture of the right ventricle and infusion of the left ventricle with a large volume of heparinized saline and obtained similar results (not shown).
FIGURE 2. Pmel-1 T cells destroy tumor in an Ag-dependent fashion. A, B16, but not MCA-205, expresses gp100. mRNA was extracted from B16 and MCA-205 lysates. cDNA was synthesized and subjected to PCR using gp100 and β-actin intron-spanning primers for 25, 30, and 35 cycles. B, Activated pme1-1 T cells recognize B16, but not MCA-205, in vitro. T cells (10⁵) were cocultured in CM with 10⁵ target cells that were or were not pulsed with 1 μM human gp100²⁵⁻³³. Supernatants were collected after 24 h and assayed using a mouse IFN-γ ELISA kit. C, Pmel-1 T cells mediate the destruction of established B16, but not MCA-205. C57BL/6n mice were implanted with both B16 and MCA-205 tumors on opposing flanks and treated after 7 days with cultured pme1-1 T cells, with or without rFPVhgp100 and IL-2. Mice were randomized, and tumors were measured in a blinded fashion using digital calipers. The products of the perpendicular diameters are presented as the mean ± SEM. Experiments were performed independently at least three times with similar results.

FIGURE 3. Tumor-specific T cell trafficking and growth kinetics were similar in gp100⁺ and gp100⁻ tissues. A, Pmel-1 T cells traffic to Ag- and non-Ag-containing tissues. Seven-day established B16 and MCA-205 dual tumor-bearing C57BL/6n recipients were treated with cultured pme1-1 GFP⁺ T cells, with or without rFPVhgp100 and IL-2. On the days indicated, B16 and MCA tumors, eye, spleen (SPL), liver, lung, blood (PBL), B16 inguinal draining lymph node (B16 LN), and MCA-205 inguinal draining lymph node (MCA LN) were harvested and cytofluorometrically analyzed for pme1-1 (GFP⁺/CD8⁺). Values represent the percentage of GFP⁺/CD8⁺ events compared with total CD8⁺ cells. B, Equal numbers of pme1-1 T cells extravasated into both B16 and MCA-205. Tumors were harvested and imbedded in OCT (Miles, Elkhart, IN) from perfused mice 5 days after treatment with rFPVhgp100, IL-2, and pme1-1 T cells where indicated. Frozen sections were stained for Vβ13 (green) and examined using confocal microscopy. Results are representative of multiple fields. C, The total numbers of pme1-1 T cells were similar in both B16 and MCA-205. Five days after adoptive transfer of pme1-1 Thy1.1⁺ T cells and in vivo stimulation, tumors of comparable size (~20 mm²) were harvested from at least three mice and pooled. The product of the total percentage of Thy1.1⁺/CD8⁺ and the total cell count was used to calculate the total pme1-1. The results shown are presented as the mean ± SEM of three independently performed experiments.
Using confocal microscopy and Ab staining for Vβ13, we directly observed pmel-1 T cell infiltration into both tumors after intravascular flushing 5 days after adoptive transfer and in vivo stimulation (Fig. 3B). There were approximately equal numbers of pmel-1 T cells found in the B16 and MCA-205 tumors (Fig. 3B, lower panels). To more precisely enumerate the cells, we resected tumors of approximately equal size, then harvested and homogenized them into a single-cell suspension (Fig. 3C). Although the difference in the total number of pmel-1 found in tumor in treated vs untreated mice was significant ($p < 0.05$), there was no significant difference between the total pmel-1 in B16 or MCA-205 ($p = 0.52$) in treated mice.

It thus appears that in vivo-stimulated pmel-1 T cells infiltrate many organs regardless of whether they express the target Ag. Yet how do the cells achieve such wide distribution? We next determined whether the infiltration of pmel-1 T cell into all tissues was the result of proliferation in these tissues or of proliferation in one tissue and subsequent migration.

**Pmel-1 T cells proliferate in multiple tissues**

To determine where tumor-specific CD8$^+$ cells divide, we used the thymidine analog BrdU. This reagent is incorporated into the DNA of actively dividing cells and is detectable by FACS using a specific Ab-staining method. Four days after adoptive cell transfer, mice received a 2-h pulse of BrdU given i.p., then were bled and perfused, and organs were harvested and homogenized. Cells were stained for the expression of CD8, Vβ13, and BrdU.

After the administration of vaccine and IL-2, rapidly proliferating pmel-1 cells were found virtually everywhere, in lymph node, spleen, liver, and lung, but to a lesser extent in blood and kidney (Fig. 4A). Pmel-1 T cells transferred alone did not exhibit a profound proliferation and were not found in kidney (not shown). In

![FIGURE 4. Pmel-1 T cells proliferate in multiple tissues, regardless of Ag. A. After in vivo stimulation, pme1-1 T cells proliferate in many tissues. Four days after the indicated treatments, tumor bearing C57BL/6n mice were injected i.p. with BrdU; 2 h after injection, mice were bled and perfused, and organs were homogenized and extracellularly stained for Vβ13 and CD8 expression. To free anti-BrdU binding sites, samples were treated with DNase I, then intracellularly stained with anti-BrdU PE. In FACS plots, no treatment (NT) is gated on lymphocytes; pmel-1 and pmel-1 rFPV IL2 are gated on CD8$^+$ lymphocytes. B, Pmel-1 T cells proliferate in both gp100$^+$ and gp100$^-$ tissues after activation. In an experiment similar to that described above, B16 and MCA-205 tumors were harvested from dual tumor-bearing mice that received pmel-1 T cells, rFPVgp100, and IL-2. All Vβ13$^+$CD8$^+$ events were analyzed for BrdU staining. Experiments were independently performed twice with similar results.](http://www.jimmunol.org/Downloaded from)
a similar experiment, activated pmel-1 proliferated similarly in the Ag-expressing B16 and in the Ag-null MCA-205 (Fig. 4B). Our findings suggest that these highly activated CD8\(^+\) tumor/self-reactive T cells not only infiltrate nonspecifically, but also proliferate indiscriminately in multiple organs after stimulation. We explored why trafficking and proliferation were ubiquitous, but tumor destruction was specific.

**Differential functions of pmel-1 T cells on B16 and MCA-205**

To investigate the requirements for specific tumor destruction, we assessed intracellular IFN-\(\gamma\) production by pmel-1 T cells in gp100\(^+\) and gp100\(^-\) tumors in vivo. We found that pmel-1 T cells in the Ag-positive B16 tumor, but not those in the Ag-null MCA-205 tumor (Fig. 5A) or in spleen and lymph node (data not shown), up-regulate intracellular IFN-\(\gamma\). IFN-\(\gamma\) is important in viral clearance and
MHC up-regulation on target tissue (16), but may not be indicative of T cell-mediated tissue destruction. We chose to examine perforin release, which is more directly associated with T cell-mediated tissue destruction.

Perforin release by T cells in conjunction with granzymes induces an apoptotic cascade in target cells (17). To study whether there was differential release of lytic molecules in Ag-expressing tumor, we examined the release of perforin. B16 and MCA-205 tumors were harvest 5 days after adoptive cell transfer of pmel-1 T cells in conjunction with vaccine and IL-2 and were stained for pmel-1; perforin was examined using confocal microscopy (Fig. 5B). Consistent with the data presented in Fig. 3A, we observed approximately equal numbers of Vp138 pmel-1 cells in both tumors, but we only saw perforin staining in B16, not MCA-205, tumor (Fig. 5B) or spleen (data not shown). The downstream effect of perforin and granzymes includes the activation of caspases, in particular caspase 3 (18). With the transfer of in vivo-stimulated pmel-1, we observed staining of active caspase 3 in B16 (cover), but not MCA-205 (Fig. 5C) or spleen (data not shown). B16 from untreated mice did not stain for active caspase 3.

Discussion

In our present study we found that, contrary to what was previously thought, there is a lack of Ag-dependent specificity of tumor-reactive T cells at the level of infiltration and proliferation in the tumor. Tumor-reactive T cells infiltrate and proliferate in Ag-expressing and nonexpressing tumors in comparable numbers. However, tumor-reactive CD8+ T cells clearly mediate the regression of bulky, established tumor only if it expresses the target Ag.

Although the egress of activated CD8+ T cells into the tumor is not Ag specific (7), this infiltration may be required for tumor destruction (2, 3). Thus, workers have attempted to enhance T cell migration to the tumor. In an effort to circumvent the need for trafficking, direct intra-arterial infusion of T cells into the tumor can be successful (19), but is impractical in the case of widely metastatic disease. In nonaccessible tumor sites, induced expression of adhesion molecules, such as chemokine receptors, integrins, or selectins, may enhance T cell entry to the periphery (20–22). The induction of CXCR2 or CXCR4 into T cells may augment T cell targeting to melanoma or leukemias derived from narrow stromal cells that express their respective ligands, CXCL1 or stromal cell-derived factor-1 (23, 24). Other approaches include stimulation of the tumor environment to induce T cell infiltration (25). However, tumor-reactive T cell migration to the tumor alone does not reliably result in tumor regression (26–28). This may be due to the tumor’s inability to efficiently activate tumor-reactive T cells (29–32), although this may not be the case in all tumors (33). Thus, a potent T cell activation may be required to mount an effective immune response (14, 34).

Exogenous cytokine administration and, in some cases, Ag in the context of danger signals have been shown to stimulate adoptively transferred tumor-reactive T cells and enable them to induce tumor regression (1, 9, 28, 35). Transfer of these cells into a lymphopenic environment may accentuate these results (our unpublished observations) (10). The mechanisms involved in this tumor destruction have not been clearly delineated. Although it has been demonstrated that secondary stimulation of CD8+ T cells results in rapid proliferation, a peak, and subsequent contraction in lymphoid organs (15), little has been shown in peripheral organs. In the current study we found that in vivo stimulation of tumor-reactive T cells not only induces similar kinetics in spleen, blood, and lymph nodes, but also extends into multiple peripheral tissues. This rapid kinetic curve appears to occur simultaneously in all tissues, countering the idea of lymphoid proliferation and then peripheral migration. In addition, we found that several days after stimulation, tumor-reactive T cells were actively dividing in both Ag-positive and negative tumors. This phenomenon is in congruence with several recent studies demonstrating T cell programming and proliferation after a single antigenic exposure (36, 37). It is interesting to note that active T cell proliferation in the blood was not observed; perhaps some property of or in the peripheral tissues, but not the blood, is necessary for induction into S phase.

Although the migration and proliferation of these in vivo stimulated T cells are not tissue specific, tumor recognition and destruction are specific. IFN-γ is a reliable indicator of CD8+ T cell-specific target recognition (38, 39) that induces the up-regulation of MHC I on B16 (16). Furthermore, cytolytic molecules, such as perforin, can be observed in B16, but not MCA-205, treated with in vivo-stimulated pmel-1 T cells. The release of perforin in conjunction with granzymes by tumor-reactive T cells induces an apoptotic cascade in target tumor (40), mediating tumor cell death. This tumor killing is visualized by the staining of active caspase 3 in B16 infiltrated with pmel-1 T cells, but not in MCA-205, even in the presence of pmel-1 T cells.

The ability to raise large numbers of tumor-reactive T cells and induce ubiquitous T cell migration and the specific killing of tumor cells by T cells may have important clinical implications. The current vaccination strategies available can induce large quantities of tumor Ag-specific T cells, but these vaccinations do not reliably induce tumor regression (41). An alternative strategy, based on adoptive T cell transfer, involves the ex vivo expansion of large quantities of tumor-reactive T cells. This methodology obviates the requirement for continuous in vivo vaccination and enables the possibility of pretransplant lymphodepletion, which may enhance T cell-based immunotherapies. Furthermore, an increase in cell number simplifies the logistics involved in genetic modification of T cells with TCRs against tumor Ags (42) or with homing markers (23, 24).

Tumor-homing strategies are currently being explored in our laboratory and elsewhere, but the molecular bases of directing T cells specifically to tumor metastases remain largely unknown. As we have reported in the present manuscript, the in vivo stimulation of adoptively transferred cell circumvents the need for specific targeting, because cells migrate indiscriminately and ubiquitously. In the case of metastatic disease, tumor can infiltrate multiple organ systems. The ability to induce ubiquitous T cell infiltration, while maintaining specific-tumor killing, may be beneficial in the treatment of metastatic, noninflammatory tumor foci.

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