Reversal of CD8+ T Cell Ignorance and Induction of Anti-Tumor Immunity by Peptide-Pulsed APC

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*J Immunol* 2000; 165:6731-6737; doi: 10.4049/jimmunol.165.12.6731

http://www.jimmunol.org/content/165/12/6731
Reversal of CD8+ T Cell Ignorance and Induction of Anti-Tumor Immunity by Peptide-Pulsed APC

Nava Dalyot-Herman,* Oliver F. Bathe, † and Thomas R. Malek2*

In the present report, we have studied the potential of naive and activated effector CD8+ T cells to function as anti-tumor T cells to a solid tumor using OVA-specific T cells from TCR-transgenic OT-I mice. Adoptive transfer of naive OT-I T cells into tumor-bearing syngeneic mice did not inhibit tumor cell growth. The adoptively transferred OT-I T cells did not proliferate in lymphoid tissue of tumor-bearing mice and were not anergized by the tumor. In contrast, adoptive transfer of preactivated OT-I CTL inhibited tumor growth in a dose-dependent manner, indicating that E.G7 was susceptible to immune effector cells. Importantly, naive OT-I T cells proliferated and elicited an anti-tumor response if they were adoptively transferred into normal or CD4-deficient mice that were then vaccinated with GM-CSF-induced bone marrow-derived OVA-pulsed APC. Collectively, these data indicate that even though naive tumor-specific T cells are present at a relatively high fraction they remain ignorant of the tumor and demonstrate that a CD8-mediated anti-tumor response can be induced by Ag-pulsed APC without CD4 T cell help. The Journal of Immunology, 2000, 165: 6731–6737.

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t is now well-established that some tumors express Ags that result in induction of anti-tumor immune responses (1, 2). Although in some cases such a response results in eradication of the tumor, more often an anti-tumor immune response is ineffective, and the tumor ultimately grows. There are a number of specific mechanisms by which a tumor evades an ongoing immune response. These vary widely and include: development of tumor-Ag loss variants; down-regulation of surface MHC molecules or other molecules required for Ag presentation by the tumor cells (3, 4); induction of anergy of tumor-specific T cells due to lack of costimulatory molecules on the tumor cells (5–7); and suppression of the immune response by tumor secretion of inhibitory cytokines (8, 9). Tumor Ags by nature are usually dysregulated self-proteins or variants of self-Ag. Therefore, anti-tumor immunity may also fail due to a weak response to the Ags expressed by the replicating tumor or because potentially tumor-reactive T cells are tolerant to the tumor Ags.

Although the fate and function of adoptively transferred tumor-specific effector T cells have been extensively studied (10–12), comparatively little is known concerning the initial activation of tumor-specific T cells in vivo, primarily due to their low frequency in the peripheral lymphocyte pool. With the introduction of TCR-transgenic mice, this problem has been overcome. Analysis of an Ag-specific T cell response to nominal Ags is facilitated by adoptive transfer of a relatively low, but detectable, number of TCR-transgenic T cells to normal mice and then challenging such animals with the appropriate Ag (13). This approach allows direct phenotypic and functional characterization of the responding Ag-specific transgenic T cells during the course of the immune response and avoids the complications inherent in direct Ag stimulation of the TCR-transgenic mouse, in which all the T cells are Ag responsive.

More recently, this method has been adapted to study the induction of anti-tumor immunity in vivo. In this setting, a pre-determined number of naive TCR-transgenic T cells are adoptively transferred to mice bearing a tumor that was transfected with an Ag recognized by the transgenic T cells (14–18). The present study employed this TCR-transgenic strategy to compare the capacity of naive and activated effector-transgenic CD8+ T cells to generate an anti-tumor immune response to a solid tumor. We used OT-I TCR-transgenic T cells that are specific for OVA (19) as the source of anti-tumor-specific T cells and the OVA-transfected EL4 cell line, E.G7, as the tumor cells expressing a tumor-specific Ag, i.e., OVA (20). We demonstrated that the naive OT-I T cells are functionally blind or ignorant of the OVA tumor Ag. This failure of OT-I T cells to respond to this tumor was overcome by proper Ag presentation, as supplied by peptide-pulsed professional APC, leading to an effective anti-tumor immune response. These findings provide a relevant strategy to overcome tumor Ag ignorance for cancer immunotherapy.

Materials and Methods

Animals

The OT-I TCR-transgenic mice (19) were maintained by breeding heterozygous OT-I TCR-transgenic mice to wild-type C57BL/6. The progeny mice were screened by PCR for the expression of the OVA-TCR gene. Six to 10-wk-old female C57BL/6 or CD4-deficient mice on the C57BL/6 background were purchased from The Jackson Laboratory (Bar Harbor, ME).

Cell lines

EL-4, a thymoma derived from the C57BL/6 mouse (H-2b), was obtained from American Type Culture Collection (ATCC, Manassas, VA). OVA-transfected EL-4, designated as E.G7 (20), was a gift from Dr. M. Bevan (University of Washington, Seattle, WA). These cell lines were maintained in RPMI 1640 containing 5% FCS, glutamine (30 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), and 2-ME (5 x 10-5 M) (complete medium).
Abs and other reagents

OVA peptide (SIINFEKL) (21) was synthesized by Research Genetcs (Huntsville, AL). Directly conjugated mAbs, including CyChrome-conjugated anti-CD8α, PE-conjugated anti-mouse Vα2 TCR, and FITC-conjugated anti-mouse Vβ5.1, 5.2 TCR, were purchased from Pharmingen (San Diego, CA). CFSE was purchased from Molecular Probes (Eugene, OR). Cells were labeled with CFSE as previously described (22). Briefly, cells (2 × 10^6/ml) were incubated with 5 μM CFSE (from a 5 mM stock in DMSO) in serum-free medium for 10 min at 37°C and washed twice with cold complete medium and twice with HBSS.

Tumor challenge and adoptive transfer of transgenic T cells

Normal C57BL/6 or CD4^-/- mice were injected with 1 × 10^6 E.G7 cells in 0.2 ml HBSS s.c. into the midline of the abdomen. The tumor cells were freshly thawed and grown in culture for 5–10 days before each injection. Spleen cell suspensions from heterozygous OT-I mice (≥6 wk of age) were prepared as previously described (15). Splenocytes containing the indicated number of transgenic OT-I T cells were injected i.v. in 0.5 ml HBSS 5–7 days after tumor challenge.

FACS analysis

Spleens and draining lymph nodes (LN) ^1 (inguinal, brachial) were collected and subjected to FACS analysis as previously described (15). Between 50,000 and 100,000 events per sample were collected on a FACScan flow cytometer (Becton Dickinson, San Jose, CA) and analyzed using CellQuest software (Becton Dickinson). OT-I-transgenic T cells were enumerated either by three-color staining for CD8, Vα2-TCR, and Vβ5.1, 5.2-TCR or by the fraction of CFSE-labeled (FITC) OT-I spleen cells that also stained for CD8. The latter approach is valid because essentially all CD8^+ cells in OT-I mice express the transgenic TCR.

Purification of CD8^+ OT-I cells

OT-I T cells were purified by a combination of negative and positive selection. First, B cells were depleted on anti-Ig-coated plates, followed by further depletion by incubation of the nonadherent cells with anti-CD24 ( stained for CD8, Vα2-TCR, and Vβ5.1, 5.2-TCR or by the fraction of CFSE-labeled (FITC) OT-I spleen cells that also stained for CD8. The latter approach is valid because essentially all CD8^+ cells in OT-I mice express the transgenic TCR.

Culture for APCs

A single-cell suspension of bone marrow cells from normal C57BL/6 mice was cultured at 0.5 × 10^6/ml in complete medium containing 2 ng/ml murine GM-CSF (PeproTech, Rocky Hill, NJ). Four to 5 days later, adherent cells were collected after incubation with PBS containing 5 mM EDTA at 37°C for 15 min. The cells were washed with HBSS, incubated with 1 μM OVA peptide for 1 h at 37°C, and washed three times with HBSS. Mice were injected i.v. with the OVA-pulsed APC in 0.5 ml of HBSS.

Generation and assay of CTL

Splenocytes (1 × 10^6) from OT-I-transgenic mice were cultured in complete medium containing 1 nM OVA peptide, 20 U/ml IL-2, and 40 U/ml IL-4. After 3 days, the cells were collected, washed, and re-cultured at 1 × 10^6 cells/ml in complete medium containing 20 U/ml IL-2 and 40 U/ml IL-4 for 2 days. The CTL activity of the cells was measured by a standard ^51Cr release assay as previously described (23) using ^51Cr-labeled EL4 or E.G7 cells as the targets.

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^1 Abbreviation used in this paper: LN, lymph node.

Results

Activation of OT-I in vitro

In this study, the OVA-specific MHC class I-restricted (H2^b) OT-I TCR-transgenic CD8^+ T cells were used as tumor-specific T cells by adoptive transfer to mice bearing the E.G7 tumor (OVA-transfected EL4) as a solid tumor. The presence of the OT-I cells in vivo was assessed by the coexpression of Vβ5 and Vα2 of the transgenic TCR on CD8^+ T cells. In our initial studies, we determined the potential to functionally measure low numbers of OT-I T cells in peripheral lymphoid tissue by evaluating the proliferative response to OVA peptide or E.G7 in vitro. Dose-response studies demonstrated that OT-I T cells developed strong proliferative responses to 10^-7–10^-9 M OVA peptide, with detectable responses in cultures containing as little as 10^-15 M OVA peptide (Fig. 1B). Furthermore, by mixing OT-I spleen cells with normal C57BL/6 spleen cells in such a manner that the fraction of transgenic OT-I cells was predetermined, readily detectable proliferative responses were routinely generated by a relatively high dose of OVA peptide (10^-9 M) when the cultures contained as few as 1 × 10^3 OT-I T cells, which is only 0.5% of the total number of spleen cells in culture (Fig. 1A). Thus, OT-I cells are exquisitely sensitive to OVA peptide, and OT-I T cells were functionally detected in lymphoid tissues when present at a frequency of 1 in 200.

The E.G7 cells used in this study secreted 560 pg of OVA/animal/1 × 10^6 cells after a 24-h culture as determined by ELISA (data not shown). The addition of as much as 1.0 mg/ml of native soluble OVA protein induced only minimal proliferation of OT-I T cells in vitro (data not shown). Despite this relatively low level of

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FIGURE 1. Response of OT-I cells in vitro. A, Response by limiting number of OT-I cells. B, Dose response to OVA peptide. C, Response to cell-associated-OVA. D, Response by purified OT-I cells. C57BL/6 spleen cells (2 × 10^6/well) containing the indicated number of OT-I T cells (A) or 2 × 10^6/well OT-I T cells (B and C) were stimulated for 3 days with the indicated concentration of OVA peptide, anti-CD3 (5% supernatant), or the indicated number of irradiated EL4, E.G7, or OVA-pulsed APC. D, A total of 2.5 × 10^4 purified OT-I cells were stimulated for 3 days with the indicated concentration of OVA peptide or the indicated number of irradiated E.G7 in the presence or absence of 1 × 10^3 accessory cells. Data are representative of two to five experiments.
secretion of OVA, the E.G7 cells were immunogenic, as the irradiated E.G7, but not the parental EL4 thymoma, induced proliferation by OT-I cells in a fashion comparable to peptide-pulsed APC (Fig. 1C). Taken together, these data suggest that cell-associated OVA is much more immunogenic than soluble OVA protein. Furthermore, E.G7 directly stimulated substantial proliferation by highly purified OT-I T cells in the absence of accessory cells (Fig. 1D). By contrast, the response of these purified T cells to soluble OVA peptide was dependent upon accessory cells. These results indicate that the E.G7 can directly present OVA to OT-I.

OT-I T cells lack anti-tumor activity in vivo

Given the potent immunogenicity of E.G7 for OT-I T cells in vitro, we examined the ability of OT-I T cells to mount an anti-tumor immune response in vivo. Five days after C57BL/6 mice received 10⁶ E.G7 s.c., OT-I T cells were adoptively transferred to these tumor-bearing mice, and tumor size was measured over time. Each mouse received 2.5 × 10⁶ OT-I T cells, yielding mice in which ~5% of their CD8⁺ T cells were OT-I. As reported by Jenkins and coworkers (13), this number of transgenic T cells seeds the peripheral immune compartment so that the transgenic T cells are present at a frequency that is still detectable by FACS analysis, but these cells are not at such a high level as to cause imbalance in the peripheral immune compartment. When compared with control mice that did not receive OT-I cells, it is quite apparent that rate of tumor growth was comparable between both groups of mice (Fig. 2). Transfer of a larger number (4 × 10⁶) of OT-I T cells still failed to affect tumor cell growth (data not shown). Thus, OT-I cells did not generate as obvious an anti-tumor response to E.G7 growing as did a solid tumor.

To examine whether the adoptively transferred OT-I cells proliferated to E.G7 in vivo, we determined the proportion of OT-I T cells in the spleen and draining LN of tumor-bearing animals by three-color FACS analysis by staining for CD8, Vα2, and Vβ5. In a normal mouse, ~0.2% and ~0.5% of spleen and LN cells, respectively, express these three surface molecules. After adoptive transfer of OT-I T cells, the fraction of cells bearing these markers initially increased to ~0.6% for the spleen and ~1.3% for the draining LN (Fig. 3A). Importantly, these numbers decreased over time for both control and tumor-bearing animals to a level similar to that seen in normal mice. The total spleen and LN recovery, and the proportion of CD8⁺ T cells from mice that were adoptively transferred with OT-I cells, in the presence or absence of E.G7, was always comparable (data not shown), further indicating that there was no obvious lymphoid cell expansion by the tumor. These findings suggest that OT-I T cells did not proliferate in response to the E.G7 tumor.

To further evaluate whether OT-I cells responded to E.G7 in vivo, CFSE-treated OT-I T cells were adoptively transferred to E.G7-bearing mice. CFSE is an intracellular fluorescent label for which the fluorescence intensity decreases proportionally upon cell division (22). When CD8 cells in the draining LN and spleens of control and tumor-bearing mice were subjected to FACS analysis, the large majority of cells maintained the original fluorescence intensity (Fig. 3B), indicating that OT-I cells did not proliferate in response to E.G7 in vivo.
immunity. Naive OT-I T cells were adoptively transferred to mice 5 days after s.c. injection of E.G7, and 24 h later the mice received OVA-pulsed bone marrow-derived APC. As shown earlier, the tumor grew quickly in mice that received tumor in the presence of naive OT-1 (Fig. 5A). By contrast, coadministration of OT-1 and OVA-pulsed APC resulted in a substantial delay in the progression of the tumor. In 40% of the mice, no tumor was detected 45 days after injection of E.G7, and several mice that were observed longer remained tumor-free on day 60. Importantly, there was no inhibition of growth when tumor-bearing mice received OVA-pulsed APC in the absence of OT-I T cells. This result indicates that the inhibition of tumor growth

**Table I. E.G7 tumor cells do not anergize OT-I cells**

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Days after Adoptive Transfer</th>
<th>Cells from:</th>
<th>% OT-I&lt;sup&gt;a&lt;/sup&gt;</th>
<th>cpm × 10&lt;sup&gt;5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spleen</td>
<td>LN</td>
<td>Spleen</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>Tumor-bearing mice</td>
<td>0.53 ± 0.03</td>
<td>1.39 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal mice</td>
<td>0.67 ± 0.03</td>
<td>1.23 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Tumor-bearing mice</td>
<td>0.56 ± 0.08</td>
<td>1.50 ± 0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal mice</td>
<td>0.55 ± 0.12</td>
<td>1.48 ± 0.21</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>Tumor-bearing mice</td>
<td>0.58 ± 0.02</td>
<td>0.94 ± 0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal mice</td>
<td>0.60 ± 0.07</td>
<td>1.10 ± 0.15</td>
</tr>
</tbody>
</table>

<sup>a</sup> Normal C57BL/6 mice were injected with E.G7 (1 × 10<sup>6</sup>). Five day later mice received 4 × 10<sup>5</sup> OT-I cells (experiment 1) and 2.5 × 10<sup>6</sup> OT-I cells (experiments 2 and 3). Spleen and LN were harvested at the indicated days, after adoptive transfer, and were subjected to analyses. Data shown contain three to four mice per group in each experiment.

<sup>b</sup> Determined by FACS analysis of three color positive cells for CD8, Vα2, and Vβ5.

<sup>c</sup> Determined by proliferation of spleen and LN after culture for 3 days with 1 nM OVA peptide.
is dependent upon the presence of the adoptively transferred transgenic T cells. Thus, after appropriate activation either in vitro or in vivo, E.G7 was susceptible to tumor (OVA)-specific OT-I effector cells.

To determine whether this anti-tumor response required CD4⁺ T cells, we transferred OT-I T cells to tumor-bearing CD4-deficient mice (Fig. 5B). Similar to E.G7-bearing C57BL/6 normal mice, the growth of E.G7 was delayed in the CD4⁻/⁻ recipient mice, if they received OVA-pulsed APC, and two of six mice remained tumor-free after 50 days.

The E.G7 tumor eventually grew in all mice that received in vitro-induced OT-I effector cells and in some mice that were stimulated with OVA-pulsed APC in vivo. The E.G7 cells were excised from one such mouse in each of the treatment groups and grown in culture for at least 7 days. These cells were then used as targets for OT-I CTL generated by in vitro culture. The E.G7 cells obtained from the mice treated with ex vivo-induced OT-I effector cells were nearly as good targets for OVA-specific CTL as the parental E.G7 (Fig. 6). Thus, the tumor outgrowth from this mouse appears to be the result of a failure of the adoptively transferred CTL to completely kill the tumor. However, E.G7 from the in vivo APC-treated mice were not lysed by the OVA-specific CTL, suggesting that tumor outgrowth in this case was caused by the selection of a tumor variant that escaped the effector OT-I CTL. In addition, ELISA analysis confirmed that these cells failed to secrete a detectable OVA level (data not shown). Collectively, these data raise the possibility that in vivo-induced effector T cells induced a more potent anti-tumor immune response than adoptive T cell immunotherapy.

Discussion

In the present study, we showed that a tumor-immune response could not be elicited in tumor-bearing mice after the adoptive transfer of a relatively high number of naive transgenic tumor-specific CD8⁺ T cells. Importantly, as assessed at several different time points after adoptive transfer, the tumor-specific transgenic T cells were not activated to proliferate in both the spleen and the draining LN in the presence of the growing solid tumor. The failure to activate the T cells was not because the tumor anergized them, because OT-I T cells derived from tumor-bearing mice readily proliferated upon Ag challenge in vitro. Therefore, our data indicate that failed anti-tumor responses to E.G7 were due to immunological ignorance, i.e., a failure of the host immune system to recognize OVA, in this case in the context of a tumor cell, during the induction phase of the immune response.

It was surprising that the OT-I T cells remained ignorant of E.G7 when adoptively transferred in vivo considering the robust proliferative response of the T cells to irradiated E.G7 tumor cells in vitro. The activation of OT-I T cells in vivo has been shown to be dependent on Ag presentation by short-lived bone marrow-derived APC within the draining LN (24). Furthermore, when OVA is cell associated, e.g., in transgenic β-islet cells, activation of OT-I T cells is dependent upon cross-priming, i.e., the β-islet-associated OVA is ultimately processed by an exogenous class I pathway and presented to OT-I T cells by professional APC (24). Cross-priming is facilitated by either destruction of the Ag-containing cells and/or a high level of expression of the cell-associated Ag (25). In the case of β-islet cells, when cross-priming was not facilitated, the OVA-containing β-islets were ignored by OT-I T cells (26).

The failure of OT-I T cells to recognize E.G7 is not analogous to that described for OVA-containing β-islets, because E.G7 were able to directly present OVA to OT-I. It is likely that two factors promoted ignorance of E.G7. First, ignorance in our model appears to be at least in part the result of lack of contact between the OT-I tumor-specific T cells and OVA associated with the E.G7 tumor cells. Naive OT-I T cells appear not to readily migrate to the site of the tumor, preventing their direct activation by E.G7. Consistent with this hypothesis, so far we have not detected OT-I cells within the tumor site as determined by using CFSE-labeled OT-I cells and FACS analysis of the excised tumors (data not shown). Similarly, E.G7 does not obviously traffic to the spleen or the LN following s.c. or i.v. injections (data not shown). These observations suggest that OT-I T cells do not encounter E.G7 either at the site of the tumor or within the draining LN. Second, there is no indication that the E.G7 was able to cross-prime OT-I T cells by host APC. E.G7 produced relatively low levels of OVA, and minimal destruction of the rapidly growing E.G7 tumor is expected, especially early after injection of the tumor, conditions that would not favor cross-priming by the APC of the recipient mice. The observation that professional APC consistently activated OT-I T cells in the spleen and draining LN of E.G7-bearing mice demonstrates that ignorance to E.G7 is not due to a failure of the naive OT-I T cells to ultimately migrate to secondary lymphoid tissue, or to generalized immune suppression by E.G7, although we cannot exclude other means by which OT-I are ignorant of E.G7.
The value of using adoptive transfer of TCR-transgenic T cells to mice bearing tumors transfected with a model Ag is that this approach provides insight into the strength and duration of an anti-tumor T cell response. So far, two major outcomes have been observed in these types of studies. As we have seen for E.G7 as a solid tumor, tumor-specific TCR-transgenic T cells have been reported to be ignorant of Ld-transfected AG104A fibrosarcoma (27) and a glycoprotein of lymphocytic choriomeningitis virus after transfection into either Lewis lung carcinoma or the MC57G fibrosarcoma (17, 18). In the latter case, T cell ignorance required that the tumor be transplanted as a solid tumor fragment rather than s.c. injection of a single-cell suspension. These observations and our results suggest that T cell ignorance represents one important reason for failed anti-tumor immunity. Our data indicate that immunological ignorance may pertain to tumor-specific T cells bearing a high affinity for TCR, as OT-I T cells are extremely sensitive to OVA-peptide and proliferate to as little as 1 pM of peptide in vitro, a dose that is 1000-fold lower than that required to activate the lymphocytic choriomeningitis virus-specific TCR-transgenic T cells (17).

In other studies of the adoptive transfer of TCR-transgenic T cells to Ag-transfected tumors, an initial transient anti-tumor response was observed (14, 15, 28). In several cases, failed anti-tumor immunity was shown to be due to anergy of the tumor-specific T cells. This has been observed for both MHC class I- and II-restricted TCR-transgenic T cells. Interestingly, in the case of the MHC class I T cell response, OT-I T cells and E.G7 tumor cells also served as the model system (15). In that study, the E.G7 cells were injected into the peritoneal cavity 1 day after adoptive transfer of the OT-I T cells, which led to initial activation and proliferation of the OT-I T cells in vivo. Effective anti-tumor immunity failed in part due to CTLA4-mediated down-regulation of endogenous CD4 helper activity (29). This finding markedly contrasts with our result in which we found that OT-I T cells were ignorant of E.G7 as a solid tumor. We have compared the E.G7 subline maintained in our laboratory with that used by Srikant et al. (15) and found that our subline failed to activate OT-I after injection i.p. whereas the E.G7 subline used by Srikant and coworkers activated OT-I when present as a solid tumor. This indicates that the different pattern of results is unlikely to be due to differences in experimental protocols and/or responses to a systemic vs solid tumor. Furthermore, both sublines of E.G7 produce similar levels of OVA. Therefore, it is most likely that these two cell lines express some intrinsic undefined difference, as the cells were independently passaged for a considerable period of time.

Although in our study naive OT-I T cells failed to inhibit the growth of E.G7, anti-tumor immune responses were elicited by OT-I effector cells, confirming that the OT-I T cells have sufficient affinity to specifically attack the developing E.G7 solid tumor. Effector OT-I T cells were generated either ex vivo in culture or by in vivo stimulation of the naive adoptively transferred OT-I T cells with peptide-pulsed bone marrow-derived APC. This illustrates that T cell ignorance can be overcome simply by proper Ag presentation of a tumor Ag. Furthermore, by using CD4-deficient mice, we demonstrated that this anti-tumor activity was independent of CD4 T cell help. Although many other studies clearly indicate a need for CD4+ T cells for anti-tumor responses (30–33), our data, similar to that reported by Wick et al. (27), demonstrate that direct activation of CD8 T cells can be sufficient for potent anti-tumor immunity.

Several other studies have demonstrated that bone marrow-derived APC effectively inhibit tumor growth (17, 34), perhaps by activation of a population of ignorant tumor-specific T cells. It is interesting to note that so far we have only “cured” E.G7 with OT-I cells when the mice were immunized with OVA-pulsed APC. These findings raise the prospect that effective anti-tumor immunity may be facilitated by approaches that both increase the frequency of tumor-specific T cells and induce activation of such T cells by vaccination with tumor-Ag-containing APC.

Acknowledgments

We thank Dr. E. Codias for critically reading this manuscript, P. Scibelli and T. Nguyen for technical assistance, and the Sylvester Comprehensive Cancer Center for support of the FACS facilities.

References

biased toward high dose antigens and those released during cellular destruction. J. Exp. Med. 188:409.


