MHC Class II-Transfected Tumor Cells Directly Present Antigen to Tumor-Specific CD4+ T Lymphocytes

Todd D. Armstrong, Virginia K. Clements and Suzanne Ostrand-Rosenberg

*J Immunol* 1998; 160:661-666; http://www.jimmunol.org/content/160/2/661

---

**Why *The JI***?

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

---

**References**

This article cites 38 articles, 26 of which you can access for free at: http://www.jimmunol.org/content/160/2/661.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
MHC Class II-Transfected Tumor Cells Directly Present Antigen to Tumor-Specific CD4+ T Lymphocytes

Todd D. Armstrong, Virginia K. Clements, and Suzanne Ostrand-Rosenberg

We have developed and shown to be efficacious an immunotherapeutic strategy to enhance the generation of tumor-specific CD4+ T helper lymphocytes. The approach uses autologous tumor cells genetically modified to express syngeneic MHC class II genes as cell-based immunogens and is based on the hypothesis that tumor cells directly present tumor Ags to CD4+ T cells. Since the conventional pathway for CD4+ T cell activation is indirect via professional APC, induction of immunity following immunization with class II-transfected tumor cells was examined in bone marrow chimeric mice. Both tumor and host-derived cells are APC for tumor Ags, suggesting that the efficacy of tumor cell vaccines can be significantly improved by genetic modifications that enhance tumor cell Ag presentation. The Journal of Immunology, 1998, 160: 661–666.

Many of the recently developed strategies for enhancing immunity to autologous tumors involve immunization with genetically modified tumor cells. These approaches are based on the reasoning that tumor cells present potentially immunogenic tumor peptides, and that if modified appropriately, they could directly present tumor Ag and activate T lymphocytes, by-passing the requirement for transfer of tumor Ag (peptide) to host-derived professional APC.

Most of these studies have focussed on the direct activation of tumor-specific CD8+ T lymphocytes, either by providing appropriate costimulatory signals such as B7 (1–4) or by providing cytokines normally produced by CD4+ T helper lymphocytes, such as IL-2 or IL-4 (5, 6). In contrast, we and others have reasoned that optimal tumor-specific CD8+ T cells and long term immune memory will only be generated if sufficient CD4+ Th cells are also stimulated (7–10). As a result, we have targeted the activation of tumor-specific CD4+ T cells. We have hypothesized that class II-transfected tumor cells will present endogenously encoded peptides in the context of the transfected class II molecules and, therefore, directly present tumor peptides to CD4+ T helper lymphocytes. Reports from our laboratory as well as studies by others demonstrate that such genetically modified tumor cells are potent inducers of tumor-specific immunity in naive mice (7, 9, 11) as well as in tumor-bearing mice (12), demonstrating their potential as immunotherapeutic agents. Although these therapeutic results are consistent with the hypothesis that the genetically modified tumor cells directly present Ag to CD4+ T cells during the immunization process, there is no direct evidence demonstrating that the modified tumor cells directly present Ag to responding CD4+ T lymphocytes. Such a pathway would be unconventional, since CD4+ T cells are usually activated by the process of indirect Ag presentation or cross-priming, in which Ag is taken up and presented by host-derived professional APC (i.e., dendritic cells, macrophages, or B lymphocytes) rather than by the Ag-expressing cells themselves (13, 14). Since optimal exploitation of this immunotherapeutic approach will depend on a full understanding of the mechanism through which the genetically modified tumor cells activate T lymphocytes, the present studies were undertaken to identify the cell population(s) that serves as APC during the activation of tumor-specific CD4+ T cells following immunization with class II-transfected tumor cells.

Materials and Methods

Mice

C57BL/6, A/J, and (C57BL/6 × A/J)F1 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) or bred in the University of Maryland Baltimore County animal facility. Bone marrow donors and recipients were female mice, and chimeras were generated as previously described (15). Briefly, recipient (B6 × A/J)F1 mice were maintained on tetracycline water (0.2%) for 1 wk before and 5 wk after reconstitution and were given gentamicin sulfate s.c. (500 µg) for 7 consecutive days beginning 1 day before irradiation/reconstitution. Approximately 24 h before irradiation/reconstitution, recipients were taken off food. Recipients were irradiated with 875 rad total body irradiation using a 137Cs source (Kewaunee Scientific, Statesville, NC) and reconstituted i.v. with one femur-equivalent of donor bone marrow within 2 to 3 h of irradiation. Chimeras were maintained in a pathogen-free environment for 6 wk before use. All chimeras were tested by indirect immunofluorescence to ascertain bone marrow genotype and assure chimeric status as follows. Concurrent with the in vitro APC assays, spleens of chimeras were removed and stained for MHC class I (H-2Kb for A/J (mAb 16-3-1) (16); H-2Kb D b for B6; mAb B6-12.1) and class II molecules (mAb H-2Dd) and McAb anti-Thy 1.2. Chimeric mice were maintained in a pathogen-free environment for 6 wk before use. All chimeras were tested by indirect immunofluorescence to ascertain bone marrow genotype and assure chimeric status as follows. Concurrent with the in vitro APC assays, spleens of chimeras were removed and stained for MHC class I (H-2Kb for A/J (mAb 16-3-1); H-2KbDd for C57BL/6 (mAb 28-13-3)) (17) and class II molecules (mAb B6-12.1). Positively staining cells were gated relative to conjugate alone controls, and positive cells were compared with wild-type A/J and C57BL/6 splenocytes stained under identical conditions. The percentage of the donor phenotype was calculated by comparing the percentage of positive chimeric spleen cells vs that of wild-type cells. For example, if 98 and 2% of A/J→F1 chimeric splenocytes were stained with the 16-3-1 and 28-13-3 mAbs, respectively, then the A/J→F1 chimeras were considered 98% donor phenotype.
Cells, transfectants, and hybridomas

The SaI sarcoma and its transfectants were cultured as previously described (7). SaI sarcoma cells transfected with syngeneic MHC class II A<sup>k</sup> and A<sup>d</sup> genes (Sa/I/A<sup>k</sup>) have been previously described (7). SaI cells expressing I-A<sup>b</sup> class II molecules (Sa/I/A<sup>b</sup>) were generated by transfecting SaI cells with the A<sup>b</sup> and A<sup>3</sup>CDNAs contained in the pKCR3 plasmid (18) plus the pSV2.neo plasmid using the transfection procedure previously described (7). SaI, Sa/I/A<sup>k</sup>, or Sa/I/A<sup>b</sup> cells expressing an endoplasmic reticulum-retained hen egg lysozyme gene (HEL; Sa/I/HEL, Sa/I/A<sup>k</sup>/HEL, Sa/I/A<sup>b</sup>/HEL cells) were generated as previously described by transfecting with the BCMO-HEL plasmid containing the hygromycin<sup>k</sup> gene (19). Transfectants expressing MHC class II genes or HEL were maintained in medium supplemented with 400 &mu;g/ml G418 or 400 &mu;g/ml hygromycin, respectively. Double transfectants were maintained on both drugs. 3A9 is an I-A<sup>b</sup>-restricted HEL。<sup>a</sup>-specific T cell hybridoma (20) and was maintained as previously described (19); JK1290 is an I-A<sup>b</sup>-restricted HEL-specific hybridoma (21) and was maintained in Iscove’s modified Dulbecco’s medium supplemented with 10% Fetalclone I (HyClone, Logan, UT), 1% penicillin, 1% streptomycin, and 1% gentamicin.

Tumor challenges

For immunogenicity studies, mice were inoculated i.p. with live tumor cells, observed daily for survival, and killed when they became moribund. The inoculation dose was chosen based on previous titration studies (7). For immunization studies, chimeric mice were inoculated i.p. with 5 x 10<sup>6</sup> tumor cells and killed 6 or 7 days later. For tumor challenge studies, mice were inoculated i.p. with the indicated number of tumor cells and examined three times per week for tumor growth. Acsites tumors usually became palpable within 10 to 14 days of inoculation and grew progressively. Based on our previous experience with the SaI tumor, if mice do not develop palpable tumor within 2 mo of challenge, they will remain tumor free during their lifetime (7). Tumor incidence is the number of mice with progressively growing tumors divided by the total number of mice challenged. Tumor-bearing mice were killed according to University of Maryland Baltimore County institutional animal care and use committee guidelines when they became moribund. The mean survival time is the time between inoculation and sacrifice.

In vitro Ag presentation assays

Splenocytes from immunized mice were prepared from mechanically dissociated spleens, and B lymphocytes were removed by panning as previously described (22). Resulting T cells (5 x 10<sup>6</sup> cells/well) were cocultured in flat-bottom 96-well plates with fresh naive A/J or C57BL/6 splenocytes in a final volume of 250 &mu;L/well containing 1 mg/ml lysozyme. Responder to stimulator ratios were 10:1 and/or 50:1. Supernatants were harvested after 24 h and assayed for IL-2 content using ELISA kits as described by the manufacturer (Endogen, Boston, MA). Samples were run in triplicate, and the mean ± SD determined for each sample. In most cases, SDs were ≤5% of test values. Background values (IL-2 release in the absence of HEL) were subtracted from experimental values (IL-2 release in the presence of HEL) to obtain specific IL-2 release. Values were converted to picograms per milliliter using a standard curve incorporated into the IL-2 assay. In some experiments splenocytes were depleted of CD<sup>4+</sup> or CD<sup>8+</sup> T lymphocytes in vitro (22) or in vivo (12) before use in an APC assay. APC assays using splenocytes from chimeric plus T cell hybridomas 3A9 and JK1290 were performed as previously described (19).

Indirect immunofluorescence and flow cytometry

Tumor cells, transfectants, and splenocytes were monitored for cell surface Ag expression by indirect immunofluorescence as previously described (7) and analyzed on an Epics XL flow cytometer (Coulter, Hialeah, FL). The following mAbs were used: I-A<sup>k</sup> (10-3-6 or 10-2-16) (23), I-A<sup>d</sup> (34-5-3S) (17), R<sup>b</sup> (16-3-1) (16), D<sup>d</sup> (34-5-8) (24), and lysozyme (HyHEL 7 and 10) (25). Cells monitored for intracellular lysozyme were fixed with paraformaldehyde and stained with a mixture of the HyHEL 7 and 10 mAbs (25) as previously described (19). Isotype controls were performed for surface and cytoplasmically stained cells, and staining was essentially identical with that in fluorescent conjugate alone controls.

Results

In our previous studies the A<sup>d</sup>-derived (H-2<sup>k/d</sup>) SaI sarcoma, when transfected with syngeneic MHC class II A<sup>k</sup> and A<sup>d</sup> genes (Sa/I/A<sup>k/d</sup> tumor cells), induced potent CD<sup>4+</sup> T cell-dependent, tumor-specific immunity in syngeneic A/J and semisyngeneic (C57BL/6 x A/J)<sup>F<sub>1</sub></sup> mice. In vitro experiments using HEL-specific, I-A<sup>b</sup>-restricted T cell hybridomas showed that Sa/I/A<sup>b</sup> tumor cells transfected with a gene encoding an endoplasmic reticulum-retained HEL (Sa/I/A<sup>b</sup>/HEL tumor cells) present endogenously synthesized HEL peptide to CD4<sup>+</sup> T cells (19). The genetically modified tumor cells, therefore, are capable of presenting tumor-encoded Ag directly to T cells. To determine whether during the immunization process the tumor cells themselves are APC for endogenously encoded tumor Ags (direct Ag presentation) or if host-derived cells are the APC (indirect Ag presentation or cross-priming), we used the following genetic approach. (C57BL/6 x A/J)<sup>F<sub>1</sub></sup> mice are lethally irradiated and reconstituted with either C57BL/6 (H-2<sup>k</sup>/A<sup>d</sup>/D<sup>d</sup>) or A/J (H-2<sup>k</sup>/A<sup>d</sup>/D<sup>d</sup>) bone marrow so that the resulting chimeric mice (B6→F<sub>1</sub>, or A/J→F<sub>1</sub>, respectively) have host-derived APC of either the C57BL/6 or the A/J genotype, respectively. The chimeras are subsequently challenged with either Sa/I/A<sup>b</sup>/HEL or Sa/I/A<sup>k/d</sup>/HEL tumor cells, respectively, and the MHC restriction pattern of the response is determined. If the tumor cells are the exclusive APC for the tumor-encoded Ag (HEL), then the response will be restricted to the MHC class II genotype of the tumor cells. However, if host-derived cells are the exclusive APC for tumor-encoded HEL, then the T cell response will be restricted to the genotype of the reconstituting bone marrow in the chimeras. If both tumor cells and host cells are APC for tumor-encoded Ags, then the response will be restricted to both the tumor and bone marrow genotypes.

Sal sarcoma cells transfected with MHC class II and/or HEL genes express these gene products at the cell surface or intracellularly

Sa/I<sup>A</sup> and Sa/I<sup>A</sup>/HEL cells were available from previous experiments (7, 19). Sa/I<sup>A</sup> and Sa/I<sup>A</sup>/HEL cells were generated by gene transfection as described in Materials and Methods. The resulting transfectants were stained for cell surface expression of MHC class II molecules (live cells) or for intracellular expression of lysozyme (paraformaldehyde-fixed and saponin-permeabilized cells). As shown in Figure 1, cells transfected with the MHC class II I-A<sup>A</sup> and I-A<sup>b</sup> genes expressed comparable levels of these molecules, as measured by staining with the 10-2-16 and 34-5-3S mAbs, respectively (Fig. 1, i and j, and e and f for Sa/I<sup>A</sup> and Sa/I<sup>b</sup> cells, respectively). Similarly, cells transfected with the HEL construct expressed comparable levels of intracellular lysozyme as measured by staining with the mixture of HyHEL 7 and HyHEL 10 mAbs (Fig. 1, n, p, and r for Sa/I<sup>A</sup> and Sa/I<sup>b</sup> cells, respectively), while untransfected cells were negative for lysozyme (Fig. 1, m, o, and q). HEL transfectants were also stained for cell surface HEL expression and were negative (data not shown). Supernatants of the transfectants were assayed by ELISA for HEL secretion and showed low levels of soluble HEL (1-5 ng/ml/6.7 x 10<sup>6</sup> cells/24 h). The transfectants were also stained for MHC class I H-2<sup>k</sup>, H-2<sup>d</sup>, and H-2L<sup>d</sup> Ag expression, and these levels were approximately equivalent among all transfectants and parental SaI cells (data not shown).

Lysozyme peptides are presented by both I-A<sup>A</sup> and I-A<sup>b</sup> MHC class II molecules

Numerous in vitro studies have demonstrated that both I-A<sup>A</sup> and I-A<sup>b</sup> MHC class II molecules present HEL-derived peptides to CD4<sup>+</sup> T cells (21, 26-28). To ascertain that HEL peptides are presented by both class II alleles when the alleles are expressed by SaI sarcoma cells, semisyngeneic (C57BL/6 x A/J)<sup>F<sub>1</sub></sup> mice were challenged i.p. with parental SaI, Sa/I<sup>A</sup>, Sa/I<sup>A</sup>/HEL, Sa/I<sup>A</sup>/HEL, and Sa/I<sup>A</sup>/HEL tumor cells and followed for tumor incidence. As shown in Table I, wild-type SaI and Sa/I<sup>A</sup>/HEL tumor...
The Journal of Immunology

were significantly less lethal than SaI cells; however, they still presented peptides are presented by the I-A<sup>k</sup> and I-A<sup>b</sup> MHC class II molecules of the tumor cells and function as nominal Ag for T cell recognition.

Immunization of chimeric mice with tumor cell transfectants generates T cells restricted to both the tumor genotype and the host genotype

If the genetically modified tumor cells are the exclusive APC for tumor Ags, then A→F<sub>1</sub> bone marrow chimeras immunized with B tumor cells will have HEL-specific T cells restricted to the tumor (B) MHC genotype. In contrast, if host-derived cells are the APC, then the chimeras will have HEL-specific T cells restricted to the genotype of the host (A) regardless of the genotype of the tumor.

Table II. Efficiency of chimera formation and T cell depletion in A/J→F<sub>1</sub> and B6→F<sub>1</sub> bone marrow chimeric mice<sup>a</sup>

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Chimera</th>
<th>T Cell Depletion</th>
<th>% Cells</th>
<th>% Donor Genotype</th>
<th>Tumor Cells</th>
<th>Tumor Incidence</th>
<th>MST ± SE (days)</th>
<th>CD4&lt;sup&gt;+&lt;/sup&gt;</th>
<th>CD8&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A/J→F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>None</td>
<td>ND</td>
<td>ND</td>
<td>A/J</td>
<td>10/17</td>
<td>21 ± 5</td>
<td>CD4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>A/J→F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>None</td>
<td>ND</td>
<td>ND</td>
<td>A/J</td>
<td>10/10</td>
<td>21 ± 7</td>
<td>CD4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>A/J→F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Undepleted</td>
<td>19.0</td>
<td>9.2</td>
<td>A/J</td>
<td>10/20</td>
<td>28 ± 13</td>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD4&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>B6→F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>None</td>
<td>ND</td>
<td>ND</td>
<td>B6→F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0/5</td>
<td>≥ 90</td>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD4&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>B6→F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Undepleted</td>
<td>17.4</td>
<td>12.2</td>
<td>B6→F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0/5</td>
<td>≥ 90</td>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD4&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> To determine the efficiency of bone marrow reconstitution, splenocytes of bone marrow chimeric mice were tested by indirect immunofluorescence with donor and recipient genotype MHC class I-specific mAbs (16-3-1 for H-2<sup>K</sup><sub>k</sub>; 2B13-3 for H-2<sup>K</sup><sub>q</sub>). To determine efficiency of T cell depletion, splenocytes of CD4<sup>+</sup> or CD8<sup>+</sup> T cell depleted mice were tested by indirect immunofluorescence for CD4 and CD8 expression (GK1.5, 2.43 mAbs, respectively).

The chimeras were challenged i.p. with 10<sup>6</sup> tumor cells and mice were followed for tumor growth.

Table I. MHC class II/HEL-transfected tumor cells are rejected by semisynthetic (C57BL/6×A/J)<sub>F<sub>1</sub></sub> mice<sup>a</sup>

<table>
<thead>
<tr>
<th>Tumor Cells</th>
<th>Tumor Incidence</th>
<th>MST ± SE (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SaI</td>
<td>10/17</td>
<td>21 ± 5</td>
</tr>
<tr>
<td>SaI/HEL</td>
<td>10/10</td>
<td>21 ± 7</td>
</tr>
<tr>
<td>SaI/A&lt;sup&gt;k&lt;/sup&gt;</td>
<td>10/20</td>
<td>28 ± 13</td>
</tr>
<tr>
<td>SaI/A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4/10</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>SaI/A&lt;sup&gt;k&lt;/sup&gt;/HEL</td>
<td>0/5</td>
<td>≥ 90</td>
</tr>
<tr>
<td>SaI/A&lt;sup&gt;b&lt;/sup&gt;/HEL</td>
<td>0/5</td>
<td>≥ 90</td>
</tr>
</tbody>
</table>

<sup>a</sup> Semisynthetic (C57BL/6×A/J)<sub>F<sub>1</sub></sub> mice were challenged i.p. with 10<sup>6</sup> tumor cells and mice were followed for tumor growth.

![FIGURE 1. Indirect immunofluorescence staining of SaI (a, g, and m), SaI/HEL (b, h, and n), SaI/A<sup>k</sup> (c, i, and o), SaI/A<sup>b</sup>/HEL (d, j, and p), SaI/A<sup>k</sup>/HEL (f, l, and r) cells. Tumor cells were stained for I-A<sup>k</sup> with the 28-13-3 mAb (17) (g–l), or for lysozyme with the HyHEL 7 plus 10-2-16 mAb (23) (m–r). MHC class II staining was performed on live tumor cells; lysozyme staining was performed on fixed, permeabilized cells. The second-step, fluorescent conjugate FITC-goat anti-mouse IgG was used with all Abs. The solid line represents staining by fluorescent conjugate alone; the dotted line represents staining by specific Ab plus fluorescent conjugate.](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org)
To identify the responding splenic T cells, chimeric mice were depleted of CD4+ and CD8+ cells, respectively, demonstrating functional depletion of these populations. As shown in Table III, in both the depletion experiments, responding T cells were depleted for CD4+ or CD8+ T cells prior to assay.

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Tumor Cells</th>
<th>Chirera</th>
<th>T Cell Depletion</th>
<th>T Cell: APC Ratio</th>
<th>IL-2 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SaI/A+/HEL</td>
<td>A/J→F1</td>
<td>—</td>
<td>10:1</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50:1</td>
<td>607</td>
</tr>
<tr>
<td>2</td>
<td>SaI/A+/HEL</td>
<td>A/J→F1</td>
<td>—</td>
<td>10:1</td>
<td>430</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10:1</td>
<td>677</td>
</tr>
<tr>
<td>3</td>
<td>SaI/A+/HEL</td>
<td>A/J→F1</td>
<td>CD8</td>
<td>10:1</td>
<td>217</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7:3</td>
<td>137</td>
</tr>
<tr>
<td>4</td>
<td>SaI/A+/HEL</td>
<td>B6→F1</td>
<td>—</td>
<td>10:1</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7:3</td>
<td>33</td>
</tr>
<tr>
<td>5</td>
<td>SaI/A+/HEL</td>
<td>B6→F1</td>
<td>CD8</td>
<td>10:1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7:3</td>
<td>197</td>
</tr>
</tbody>
</table>

* A/J→F1, or B6→F1, bone marrow chimeric mice were challenged i.p. with SaI/A+/HEL or SaI/A+/HEL tumor cells, respectively, and splenic T cells were tested in vitro for IL-2 production in response to lysozyme plus I-Ab (C57BL/6) or I-Ak (A/J) APC, respectively. In some experiments, responding T cells were depleted for CD4+ or CD8+ T cells prior to assay.

Table III. Both tumor cells and host-derived APC present tumor-encoded Ag to CD4+ T cells in A/J→F1 and B6→F1 bone marrow chimeric mice

Discussion

During an in vivo immune response, CD4+ T lymphocytes are activated by an Ag-specific signal plus a second or costimulatory signal (29). The Ag-specific signal consists of antigenic peptide bound to a MHC class II molecule, which interacts with the corresponding TCR and CD4 complex on the responding T cell. The second signal consists of a costimulatory molecule, such as B7, which binds to its cognate receptor, CD28, on the responding T cell. Since only certain cells, such as dendritic cells, macrophages, activated B lymphocytes, or Langerhans cells, express MHC class II and either constitutively express or are inducible for costimulatory molecules (29), only these so-called professional APC are thought to activate CD4+ T lymphocytes. In our recently developed immunotherapeutic strategy, tumor cells are transfected with genes encoding syngeneic MHC class II molecules or MHC class II plus B7 molecules (4, 7). The class II transfectants are excellent immunogens for vaccinating tumor-free mice against subsequent challenge with wild-type tumor, and during the immunization process the transfectants are induced to express B7-1 and B7-2 molecules (30). Although the single transfectants (MHC class II) are not effective immunogens in tumor-bearing mice, double transfectants (MHC class II plus B7) are potent immunotherapeutic agents for the treatment of established solid tumors (12) and metastatic disease (31). We have hypothesized that such genetically modified tumor cells are APC for tumor-encoded Ags because they deliver both the Ag-specific signal and the costimulatory signal to CD4+ T cells. The studies reported here confirm this hypothesis by demonstrating that during the immunization process, class II-transfected tumor cells are APC for endogenously encoded tumor molecules. Our immunotherapeutic approach, therefore, provides an alternative pathway for activation of CD4+ T lymphocytes. Presumably, the ability of the class II transfected tumor cells to function as APC and directly activate CD4+ T cells is responsible for their potent immunotherapeutic effect.

Our data indicate that both tumor cells and host-derived cells are APC for tumor-encoded HEL. These experiments were designed to test Ag presentation of an intracellular, surrogate tumor Ag; however, ELISA assays of SaI/A+/HEL supernatants show low levels of secreted HEL. Since the SaI/A+/HEL transfectants are “leaky” for HEL, one cannot determine whether intracellular tumor Ag presentation is normally indirect via host-derived APC, or if cross-priming occurs because soluble HEL is available. If leakiness is the reason for the observed cross-priming, then the relative roles of direct vs indirect presentation for bona fide tumor Ags will be dependent on the cellular locale and behavior of tumor Ag.

Cross-priming or indirect Ag presentation is the usual route for activation of CD4+ T cells. In this process, tumor Ag (in this case, lysozyme) is released from tumor cells, traffics via the lymphatic and/or circulatory system to the closest regional lymph node(s), and is internalized by professional APC within the lymph node. Alternatively, Ag may be internalized in the periphery by professional APC and brought to the regional lymph node by the APC. In either case, internalized Ag is processed by the APC and presented as peptide in the context of MHC class II molecules on the surface of the APC (32). Since only a small percentage of CD4+ T cells has the appropriate receptor for a given Ag, localization of Ag to the regional lymph node allows exposure to the maximal epitope...
number of CD4^+ T cells. Trafficking of Ag to the regional lymph node during a conventional immune response, therefore, optimizes contact of Ag with the appropriate T cells.

If tumor cells are the APC, Ag presentation could occur either at the tumor site or in the regional lymph node. Presentation at the tumor site is unlikely, since unless a very large number of CD4^+ T cells traffic to the site, it is improbable that T cells with an appropriate TCR will be exposed to Ag. Trafficking of the transfectants to the regional nodes is more likely. Tumors are known to metastasize via the lymphatic circulation, and a recent study using genetically modified, nonmetastatic tumor cells demonstrated tumor cells in draining nodes (33). The class II-transfected tumor cells, therefore, may migrate to the draining lymph nodes; however, the precise logistics of the Ag presentation process remain unclear.

Although the activation of CD4^+ T cells by genetically modified tumor cells has not been previously studied, other investigators have examined the activation of CD8^+ T cells during immunization with transfected tumor cells. Three studies have examined Ag presentation by cytokine-modified tumor cells (granulocyte-macrophage CSF, IL-4, IL-7, or IL-3) and have found that cytokine expression facilitates cross-priming (34, 35) or that tumor-specific CD8^+ T cells are exclusively activated by host-derived APC (36). Two of these studies also investigated whether B7-transfected tumor cells directly or indirectly activated CD8^+ T cells. If tumor cells are the APC, Ag presentation could occur either at the tumor site or in the regional lymph node. Presentation at the tumor site or in the regional lymph node during a conventional immune response, therefore, optimizes cross-priming (34, 35) or that tumor-specific CD8^+ T cells are directly presented to CD8^+ T cells. If Ag presentation activity correlates with immunization potential, measurements of in vitro Ag presentation activity may be prognostic of therapeutic efficacy. Depending on the desired type of Th cell (Th1 vs Th2), immunizing cells could be tailored to facilitate activation of a particular subpopulation, perhaps via coexpression or simultaneous bolus administration of cytokines favoring differentiation of one or the other helper population (i.e., IL-12, IL-10, etc.).

Although concern has been voiced about using tumor cell-based immunogens in a clinical setting, a significant number of phase I or II clinical trials using tumor cell material have been completed or are in progress (http://cancer.gov.cancer.clinic.nih.gov). Since these trials have not identified any significant safety issues and in some cases have shown modest therapeutic responses (despite overwhelming tumor load), the clinical use of tumor cell-based immunogens is feasible. All the trials using cell-based strategies have been exclusively aimed at enhancing CD8^+ T cell responses. The data presented in this report combined with published therapeutic studies (12) strongly argue that an optimal cell-based vaccine should also target the activation of CD4^+ T lymphocytes.

Acknowledgments
We are very appreciative of the excellent care given to our mice by Ms. Sandy Mason. We also thank Drs. D. Mathis, H. Pelham, D. Pardoll, S. Adams, and N. Shastri for the I-A^b plasmids, erHEL construct, BCMG vector, and 3A9 and JK1290 hybridomas, respectively.

References


