B7-1/CD80-transduced Tumor Cells Elicit Better Systemic Immunity than Wild-Type Tumor Cells Admixed with Corynebacterium parvum

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ABSTRACT

Tumor cells genetically modified by transduction of B7 (B7-1/CD80), a natural ligand for the T-cell costimulatory molecules CD28 and CTLA-4, can elicit potent tumor immunity, and they can be effective for treatment of established cancers in animal models. In this study, three tumor lines, the EL4 lymphoma, the P815 mastocytoma, and the MCA102 sarcoma were transduced with recombinant retrovirus containing the murine B7 gene, and their potency to induce systemic immunity protective against challenge with wild-type tumor was compared to that of the same tumor cells admixed with the commonly used adjuvant Corynebacterium parvum. No systemic immunity was induced by B7-origin, and the P815 mastocytoma are of DBA/2 (H-2d) origin. They were all

MATERIALS AND METHODS

INTRODUCTION

Interactions between costimulatory molecules on T-lymphocytes and their ligands on antigen-presenting cells play an important role in the induction of efficient immune responses against many antigens (1, 2), including tumor antigens (3). The effect of B7 (B7-1/CD80), a natural ligand for the T-cell costimulatory molecules CD28 and CTLA-4, on activation of T-cell immunity against tumors, has been underscored in recent studies. B7-transfected tumor cells have been used as an immunogen to induce systemic tumor immunity, including an enhanced induction of CD8+ CTLs (4–8) and CD4+ helper T-cells (8, 9) against tumors. Studies using a panel of tumor lines transduced with B7 revealed that tumor immunogenicity is a key factor determining the effect of costimulation by B7 (6).

To determine the effectiveness of B7-transduced tumor cells on the induction of tumor immunity, we have compared the effect of B7-transduced tumor cells with that of tumor cells admixed with C. parvum, a commonly used immunoadjuvant (10, 11), on the induction of tumor immunity. As shown in this paper, B7-transduced cells from immunogenic tumors could, as demonstrated previously (6), induce systemic immunity to wild-type tumors, whereas B7-negative tumor cells mixed with C. parvum could not.

RESULTS

The EL4 lymphoma and the P815 mastocytoma are categorized as weakly immunogenic since immunization of mice by either a single injection of irradiated cells at 1 or 10 times the minimal tumorigenic dose, or the surgical removal of growing tumor nodules, does not provide any significant protection against subsequent challenge, whereas protection against the outgrowth of EL4 and P815 in syngeneic mice can be achieved if multiple boosts are given after the initial immunization (6). The MCA102 sarcoma is nonimmunogenic since the same procedure of multiple immunizations does not provide protective immunity (6, 12). These three tumor lines were injected into immunocompetent, syngeneic mice after either transduction by the murine B7 gene or admixture with C. parvum. After B7 transduction, the EL4 and P815 lines grew transiently and regressed completely in all mice, while the growth of the MCA102 sarcoma was not affected (Fig. 1); these data are in concordance with our previously published findings (6). In contrast, admixture of C. parvum led to regression of all 3 tumor lines tested (Fig. 1; Table 1).
Mice that had undergone tumor regression at the site of injection were challenged with the respective wild-type tumor cells at a distant site in order to detect systemic immunity. None of the mice that had been immunized with C. parvum-admixed, nontransduced tumor cells rejected wild-type tumors derived from any of the 3 lines tested. Similar results were observed when a higher dose of C. parvum (500 μg) was used (data not shown). In contrast, B7-transduced cells from the weakly immunogenic EtA and P815 tumors elicited a strong protective immunity against challenge with the respective wild-type tumors. Immunization with B7-transduced MCA1O2 could, on the other hand, not elicit any protective immunity. We conclude that B7 transduction, but not admixture of C. parvum, can make a tumor capable of inducing systemic immunity if it is immunogenic.

Several methods for immunization of mice with B7-transduced tumor cells were then compared. In the EL4 and P815 tumor models, we have shown previously that injection of live tumor cells followed by either spontaneous regression or surgical removal of tumor nodules before they had time to regress, had elicited strong systemic immunity (6), and the present findings confirm this (Table 2). Single immunization with γ-irradiated EL4 or P815 tumor cells, as used in the present experiments, did not induce protection against challenge of the respective wild-type tumor cells. As also shown in Table 2, γ-irradiated B7-transduced tumor cells were no better than B7-negative wild-type cells in inducing protective tumor immunity. To our surprise, admixture of C. parvum with B7 EL4 cells induced a less potent protective immunity than did B7 EL4 cells alone (Table 2).

Finally, the CTL activity was examined in mice which had been immunized with irradiated EL4 cells, B7-transduced EL4 cells, or EL4 cells admixed with C. parvum. Spleen cells from these immunized mice were harvested and stimulated in culture with irradiated EL4 cells for 5 days. Immunization with B7-transduced EL4 generated more CTL activity than did immunization with either irradiated or C. parvum-admixed EL4 cells. CTLs lysing EL4 did not lyse a syngeneic thymoma TIMI.4 (Fig. 2). The cytolytic activity of CTL induced by immunization with B7+ EL4 cells was abrogated by in vitro treatment with the anti-CD8 mAb HO2.2, but not by the anti-CD4 mAb GK1.5 (Fig. 3).

Table 1 shows the regression of tumors in situ in mice either transduced by B7 or admixed with the adjacent C. parvum.

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>WT* tumor cells alone</th>
<th>B7-transduced tumor cells</th>
<th>WT cells mixed with C. parvum</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL4</td>
<td>9/9</td>
<td>0/15</td>
<td>0/10</td>
</tr>
<tr>
<td>P815</td>
<td>9/10</td>
<td>1/10</td>
<td>0/10</td>
</tr>
<tr>
<td>MCA102</td>
<td>18/18</td>
<td>20/20</td>
<td>1/10</td>
</tr>
</tbody>
</table>

a WT, wild type (B7 negative).
b Significantly different from the wild-type tumor cell-alone group (P < 0.01) by χ² test.

Table 2 shows the comparison of different methods to immunize mice against challenge with wild-type tumor cells.

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>B7 transduction</th>
<th>Surgical removal of growing tumors</th>
<th>Spontaneous regression</th>
<th>γ-irradiated tumor cells</th>
<th>Admixture of C. parvum</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL4</td>
<td>–</td>
<td>9/9</td>
<td>0/20</td>
<td>15/15</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0/10</td>
<td></td>
<td>8/10</td>
<td>8/10</td>
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<tr>
<td>P815</td>
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<td>9/10</td>
<td>1/15</td>
<td>9/10</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1/10</td>
<td></td>
<td>9/10</td>
<td>ND</td>
</tr>
<tr>
<td>MCA102</td>
<td>–</td>
<td>5/5</td>
<td>15/15</td>
<td>4/4</td>
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</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

a Significantly different from the group in which growing tumors were surgically removed (P < 0.01 by χ² test).
b ND, not done.
IMMUNIZATION AGAINST TUMOR WITH B7-TRANSDUCED CELLS

Fig. 2. Comparison of CTL activity generated from mice immunized with irradiated, B7-transduced, or C. parvum-admixed tumor cells. Wild-type (irradiated), C. parvum-admixed wild-type (nonirradiated), or B7-transduced EL4 cells (nonirradiated) at 1 × 10⁶ were injected s.c. into syngeneic mice. Four to 10 weeks later, spleen cells were prepared and cocultivated with irradiated (10,000 rad) EL4 cells for 5 days. CTL activity against ⁵¹Cr-labeled EL4 or TIMI.4 target cells was measured as described in "Materials and Methods." The SD for all points was <5%. There were significant differences between the B7-transduced EL4 group and the other groups at every effector:target (E:T) ratio (P < 0.01) by χ² test.

DISCUSSION

We have shown that tumor cells admixed with C. parvum, a classic adjuvant, which has been used clinically with modest success, can elicit a strong host response at the site of tumor injection, which can lead to local regression of tumors, regardless of their immunogenicity. However, regression caused by C. parvum failed to induce systemic immunity against a challenge with wild-type tumor cells. In contrast, the weakly immunogenic tumor lines EL4 and P815 transduced with the murine B7 gene elicited effective systemic immunity, a finding which is in agreement with our previous observations (6).

One reason why B7 transduction is superior to admixture of C. parvum for the generation of systemic tumor immunity may be that B7-transduced tumor cells produce a stronger CTL response as shown in the EL4 tumor model (Fig. 2). Furthermore, injection of B7-expressing tumor cells causes inflammatory infiltrates which predominantly consist of T-cells (4, 7), especially CD8+ CTL (7). In vitro depletion of CD4+ T-cells by mAb GK1.5 did not affect the CTL activity in the EL4 system, while treatment with anti-CD8 mAb HO2.2 abolished it (Fig. 3), suggesting that CD8+ CTL are crucial for tumor rejection. However, in vivo depletion studies with the same mAbs that are needed to exclude that recruitment of CD4+ T-cells also play a role in B7-mediated costimulation of tumor immunity. The effect of C. parvum on inhibition of local tumor growth may work through different cellular mechanisms; injection of C. parvum induces inflammatory infiltrates which predominantly consist of neutrophils and macrophages (11). Our finding that admixture of C. parvum with B7+ EL4 decreased, rather than increased, systemic antitumor immunity (Table 2) supports the view that the immune response induced by C. parvum and B7 costimulation are different.

Immunization with irradiated B7+ cells at 1 × 10⁶/mouse did not induce any significant protection (Table 2), and an increasing number of irradiated B7+ cells (up to 5 × 10⁶/mouse) were also ineffective (data not shown). Since γ-irradiated cells do not proliferate, the tumor-antigen dose provided by such cells will be much less than that provided by tumors, which grew for a while after injection before they either spontaneously regressed or were surgically removed, and it is possible that a threshold dose of tumor antigen higher than that provided by up to 5 × 10⁶ tumor cells is required to induce protective immunity. There is evidence that neither γ-irradiation (7) nor paraformaldehyde fixation (15) of cells destroys the costimulatory activity of B7 in vitro. Further experiments are needed to clarify the effect of tumor cell irradiation on B7-mediated costimulation of tumor immunity in vivo, since cells which are made incapable of dividing are most likely going to be needed in any clinical trial using B7-transfected tumors as immunogen.

Hock et al. (16) reported recently that tumor cells transduced with genes encoding certain lymphokines, including interleukin 2, interleukin 4, interleukin 7, tumor necrosis factor, or γ-interferon, were not more effective in inducing systemic immunity to wild-type tumors than were tumor cells admixed with C. parvum (16). Since we did not study the immunization capacity of tumor cells transduced with the B7 gene as compared to genes encoding those lymphokines, we cannot conclude that B7-transduced cells are superior to cells transduced with a lymphokine gene, particularly since the potency of "engineered tumor vaccines" varies in different tumor systems. Nevertheless, our data contrast the profound local and lacking systemic effects seen with C. parvum admixed with three tumor lines, two of which were immunogenic and one that was not.

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REFERENCES


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