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Importance of B7-1-Expressing Host Antigen-Presenting Cells for the Eradication of B7-2 Transfected P815 Tumor Cells

Ross N. La Motte,²* Arlene H. Sharpe,† Jeffrey A. Bluestone,³† and Margalit B. Mokyr³,4*

We have previously shown that B7-2 (CD86)-transfected P815 tumor cells elicit tumor-eradicating immunity that leads to the regression of the B7-2⁺ P815 tumor after transient growth in normal DBA/2 mice. Here, we show that both the B7-2 and B7-1 (CD80) molecules contribute to the eradication of B7-2⁺ P815 tumors as treatment of the mice with both anti-B7-2 and anti-B7-1 mAb was required to prevent B7-2⁺ P815 tumor regression. The cells that expressed the B7-1 molecule following inoculation of B7-2⁺ P815 tumor cells into normal mice were not the tumor cells but rather host APCs including MAC-1⁺ cells present in the draining lymph nodes. Moreover, B7-1-expressing host APCs were found to be important for the rejection of B7-2⁺ P815 tumors as anti-B7-2 mAb alone, which was ineffective in preventing B7-2⁺ P815 tumor rejection by normal wild-type mice, was effective in preventing B7-2⁺ P815 tumor rejection by mice in which the B7-1 gene was disrupted. Finally, consistent with the importance of B7-1-expressing host APCs for the generation of tumor-eradicating immunity against B7-2⁺ P815 tumor cells, CD4⁺ T cells (not only CD8⁺ T cells) were found to participate in tumor-eradicating immunity against B7-2⁺ P815 tumor cells. Thus, in addition to eliciting tumor-eradicating immunity directly, B7-2⁺ P815 tumor cells elicit tumor-eradicating immunity indirectly through B7-1-expressing host APCs that present tumor-associated Ags to CD4⁺ T cells. The Journal of Immunology, 1998, 161: 6552–6558.

Two distinct signals are required for T cell activation. The first signal is Ag-specific and originates from the interaction of TCR with Ag presented on APCs in the context of MHC. The second signal is provided either by soluble factors such as IL-2 or by the interaction of costimulatory receptors and their ligands (1, 2). One of the most important costimulatory receptors on T cells appears to be the CD28 molecule, as blockade of CD28 signaling for T cell activation can cause clonal anergy or deletion (3, 4).

The ligands for the CD28 receptor belong to the B7 family, which consists of at least two members, the B7-1 (CD80) (5) and the B7-2 (CD86) (6, 7). The B7-1 molecule is normally expressed at low levels on professional APCs such as dendritic cells, macrophages, and thymic epithelial cells, and is up-regulated on these APCs as well as on B cells following activation by soluble factors (e.g., endotoxins and cytokines) or ligation of surface molecules (e.g., MHC class II or CD40) (8–11). In contrast, the B7-2 molecule is constitutively expressed at substantial levels on dendritic cells and macrophages and is rapidly up-regulated on B cells following activation (e.g., by cross-linking of surface Ig receptors or CD40 or addition of a variety of cytokines) (8, 10–12).

The discovery that the B7/CD28 interaction is important for T cell activation has stimulated interest in transfecting B7-negative tumor cells with the B7-1 or the B7-2 gene with the hope that the transfected tumor cells would provide a more effective stimulus for the generation of tumor-eradicating immunity. Indeed, several studies have demonstrated that B7-1 transfectants of selected tumors can trigger the development of sufficient tumor-eradicating immunity to lead to their rejection and provide immunoprotection against a challenge with unmodified B7-negative parental tumor cells (13–20). B7-2 transfectants of tumor cells were also found to trigger the development of tumor-eradicating immunity upon inoculation into normal mice, although some controversy exists whether B7-2 transfectants are equal to or inferior to B7-1 transfectants in doing so (16–20).

The effectiveness of tumor cells transfected with the B7 gene(s) in eliciting tumor-eradicating immunity was attributed to the ability of the tumor cells to function as APCs, thereby stimulating directly CD8⁺ CTLs specific for the tumor (20–22). However, a recent study by Huang et al. (23) has challenged the above dogma by demonstrating that although B7-1⁺ colon carcinoma cells expressing influenza nucleoprotein can directly prime naïve CTLs in vivo, most of the CTL priming in mice inoculated with these B7-1⁺ tumor cells is done by host APCs.

The current studies were undertaken to determine the importance of B7-expressing host APCs for the in vivo generation of tumor-eradicating immunity following inoculation of B7-expressing P815 tumor cells into normal mice. For this purpose, we used our B7-2 transfectant of P815 tumor cells that was previously shown to be comparable to a B7-1 transfectant of P815 tumor cells in terms of its ability to elicit tumor-eradicating immunity in normal DBA/2 mice and provide immunoprotection against a challenge with B7-negative parental P815 tumor cells (17). Here, we show that in addition to the known ability of B7-expressing tumor cells to stimulate the generation of tumor-eradicating immunity

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directly, such tumor cells can also stimulate the generation of potent tumor-eradicating immunity indirectly by triggering the presentation of tumor-associated Ags by B7-1-expressing host APCs, which in turn stimulate CD4+ cells.

Materials and Methods

**Tumor cell lines**

All studies employed the B7-2 transfectant of P815 tumor cells (H-2b) that was previously described (17) and which was maintained in vitro in low-glucose DMEM (Life Technologies, Grand Island, NY) supplemented with 10% FBS (Sigma, St. Louis, MO), 1% penicillin and streptomycin and G418 sulfate at a concentration of 0.8 mg/ml (Life Technologies).

**Mice**

Our studies employed primarily female DBA/2 mice (H-2d), 8–12 wk old, that were obtained from the Charles Rivers Breeding Laboratories (Wilmington, MA). In some experiments, we have used mice in which the B7-1 gene was disrupted (B7-1 KO mice) (7, 24) and which were bred back to BALB/c mice (that are H-2d like DBA/2 mice) for 10 generations. In these experiments, we also used wild-type BALB/c mice (Charles Rivers Breeding Laboratories) as controls. Routinely, mice were inoculated s.c. with 3 × 10⁶ B7-2+ P815 tumor cells.

**Flow cytometric analysis**

Assessment of B7-1 and B7-2 expression on B7-2+ P815 grown in vivo for 7 days was conducted with FITC-conjugated anti-B7-2 mAb and biotinylated anti-B7-1 mAb followed by phycoerythrin (PE)-conjugated streptavidin (all of which were purchased from PharMingen, San Diego, CA). The tumor cells were identified based on light scatter properties and intensity of B7-2 staining (i.e., the product of the transfected B7 gene). In experiments assessing up-regulation of B7-1 and B7-2 expression on host APCs, lymph node cells derived from three to five mice per group were stained simultaneously for B7 expression (with biotinylated anti-B7-1 or anti-B7-2 mAb followed by PE-conjugated streptavidin) and for the expression of B220 or MAC-1 molecules (by the use of the appropriate FITC-conjugated mAb purchased from PharMingen). Finally, flow cytometric analysis of 20,000 viable cells was conducted on a Coulter EPICS Elite ESP (Coulter Electronics, Hialeah, FL). Each experiment was repeated at least three times and the results of a representative experiment are provided in the form of a histogram.

**mAb treatments**

In experiments assessing the importance of B7-1 and/or B7-2 expression for the rejection of B7-2+ P815 tumor cells, mice were given an i.p. injection of either a) anti-B7-1 (16-10A1 [25]), b) anti-B7-2 (GL-1 [7]), c) anti-B7-1 plus anti-B7-2, or d) normal rat IgG (NlgG; Sigma) at a dose of 100 μg/mouse starting 1 h before tumor inoculation and every other day thereafter for the duration of the experiment. In experiments assessing the importance of CD4+ and/or CD8+ T cells for the rejection of B7-2+ P815 tumor cells, mice were given an i.p. injection of either a) anti-CD4 (GK1.5), b) anti-CD8 (11b), c) anti-CD4 plus anti-CD8, or d) NlgG at a dose of 1 mg/mouse starting 2 days before tumor inoculation, and every 5 days thereafter for the duration of the experiment. This protocol of anti-CD4 and of anti-CD8 mAb treatments was found (by indirect immunofluorescence staining followed by flow-cytometric analysis of cells from the draining lymph nodes of the mice) to lead to >95% depletion of CD4+ cells or >90% depletion of CD8+ cells, respectively, with no decrease, but actually some increase, in the percentage of the other T cell subset. In all experiments, tumor growth was monitored three times a week with the aid of calipers, and the average diameter of two perpendicular measurements was determined. In adherence to animal care policy, mice were sacrificed when their tumor diameter approached 20 mm or mice became moribund. Each experiment was repeated at least twice, and the results of a representative experiment are provided as mean tumor diameter (± SE) for all mice in a given group.

**Statistical analysis**

To determine the significance of differences in the fraction of mice surviving following different treatments, the generalized Savage (Mantel-Cox) test was used. For all other statistical analyses, the Student’s t test was employed. A p value of 0.05 or lower was considered significant in both tests.

**Results**

**Importance of B7-1 expression for the rejection of B7-2+ P815 tumor cells**

Experiments were conducted to determine whether the ability of normal mice to reject P815 tumor cells transfected with the B7-2 gene depends only on the B7-2 molecule or also on the other member of the B7 family. Accordingly, we determined whether anti-B7-2 and/or anti-B7-1 mAb treatment can prevent (or slow down) the rejection of B7-2+ P815 tumor cells. As seen in Fig. 1, mice inoculated with B7-2+ P815 tumor cells developed tumors that grew transiently and then completely regressed. Administration of either anti-B7-2 or anti-B7-1 mAb alone did not prevent or slow down tumor regression. However, administration of anti-B7-2 together with anti-B7-1 mAb prevented B7-2+ P815 tumor regression in all mice. Thus, not only the B7-2 molecule, but also the B7-1 molecule, contributes to the eradication of B7-2+ P815 tumor cells by normal mice.

**Lack of up-regulation of B7-1 expression on B7-2+ P815 tumor cells grown in vivo**

In an attempt to identify the B7-1-expressing cell type that contributes to the eradication of B7-2+ P815 tumors by normal mice, we determined initially if B7-2+ P815 tumor cells are induced in vivo to express the B7-1 molecule. Such a possibility was considered in light of reports that in some situations B7-negative tumor cells can be induced in vivo to express the B7-1 and the B7-2...
molecules (26). Due to difficulties in obtaining viable single-cell suspensions from the s.c. tumor nodules of mice inoculated with P815 tumor cells, B7-2+ P815 tumor cells grown i.p. were examined for B7-1 and B7-2 expression. For this purpose, we used two-color immunofluorescence staining followed by flow cytometric analysis and gated on the tumor cells based on light scatter properties and bright staining for B7-2 expression (i.e., the product of the B7 gene that was transfected into the tumor cells) (Fig. 2). These determinations revealed that essentially all cells identified as tumor cells were negative for B7-1 expression, indicating that B7-2+ P815 tumor cells are not induced in vivo to express B7-1.

**Up-regulation of B7-1 and B7-2 expression on APCs from the draining lymph nodes of mice inoculated with B7-2+ P815 tumor cells**

Studies were next conducted to determine whether s.c. inoculation of B7-2+ P815 tumor cells into normal mice leads to up-regulation of B7-1 and/or B7-2 expression on APCs isolated from the draining lymph nodes of the tumor-bearing mice. Flow cytometric studies showed that B7-2 expression was maximal on cells from the draining lymph nodes of the tumor bearers 4–5 days after tumor inoculation, while B7-1 expression did not peak until 1–2 days later (data not shown). Therefore, subsequent studies examining B7-2 expression on individual cell subsets [B220+ (Fig. 3) and MAC-1+ (Fig. 4)] from the draining lymph nodes of mice inoculated with the B7-2+ P815 tumor cells were conducted on day 4–5 after tumor inoculation, while studies examining B7-1 expression were conducted on day 6–7. As illustrated in Figs. 3 and 4 (which provide the results of one representative experiment of a total of three experiments), inoculation of B7-2+ P815 tumor cells into normal mice led to a substantial up-regulation of B7-2 but not B7-1 expression on B220+ cells from the draining lymph nodes. In addition, MAC-1+ cells from naive mice stained quite brightly for B7-2 and to a lesser extent for B7-1 expression, and inoculation of B7-2+ P815 tumor cells led to a substantial up-regulation of B7-1 but not B7-2 expression on the MAC-1+ cells. Statistical analysis (paired Student’s t test) of the data of all three experiments revealed that the mean of the percentage of B220+ cells that were positive for B7-2 expression increased as a consequence of inoculation of B7-2+ P815 tumor cells from 17.0 ± 8.6 to 54.8 ± 7.7 (p = 0.01), while the percentage of B220+ cells that were positive for B7-1 expression did not increase significantly (i.e., 1.0 ± 0.7 vs 2.1 ± 1.0; p = 0.3). The statistical analysis also revealed that the mean of the percentage of MAC-1+ cells that were positive for B7-1 expression increased as a consequence of inoculation of B7-2+ P815 tumor cells from 22.6 ± 9.1 to 48.7 ± 5.5 (p = 0.03), while the percentage of MAC-1+ cells that were positive for B7-2 expression did not increase significantly (i.e., 72.3 ± 4.5 vs 75.0 ± 1.8; p = 0.5). Thus, inoculation of B7-2+ P815 tumor cells into...
normal mice leads to a substantial up-regulation of B7-1 expression on MAC-1⁺ cells and a substantial up-regulation of B7-2 expression on B220⁺ cells from the draining lymph nodes.

**Importance of B7-1 expression on host APCs for the generation of tumor-eradicating immunity against B7-2⁺ P815 tumor cells**

The above results demonstrated that the B7-1 molecule a) contributes to the eradication of B7-2⁺ P815 tumor cells by normal mice (Fig. 1), and b) is selectively up-regulated on host APCs and not tumor cells following inoculation of B7-2⁺ P815 tumor cells (Figs. 2 and 4). Therefore, we conducted studies to determine directly the importance of B7-1-expressing host APCs for the eradication of B7-2⁺ P815 tumor cells. Specifically, we determined if anti-B7-2 mAb treatment alone, which is unable to prevent the regression of B7-2⁺ P815 tumors in wild-type mice, can prevent the regression of B7-2⁺ P815 tumors in mice in which the B7-1 gene was disrupted and therefore cannot express B7-1 molecules on host APCs (i.e., in B7-1 KO mice). As seen in Fig. 5, B7-1 KO mice, like wild-type mice, which were inoculated with B7-2⁺ P815 tumor cells, developed tumors that grew transiently and then completely regressed. However, while prevention of tumor regression in wild-type mice inoculated with B7-2⁺ P815 tumor cells was achieved only when the mice received anti-B7-2 mAb together with anti-B7-1 mAb, anti-B7-2 mAb alone was sufficient to prevent tumor regression in the B7-1 KO mice. Thus, B7-1-expressing host APCs contribute to the regression of B7-2⁺ P815 tumors in wild-type mice.

**Importance of CD4⁺ T cells for the generation of tumor-eradicating immunity against B7-2⁺ P815 tumor cells**

Because B7-1-expressing host APCs were found to contribute to the eradication of B7-2⁺ P815 tumor cells by normal mice, we considered the possibility that B7-expressing host APCs are indirectly presenting tumor-associated Ags to CD4⁺ T cells which in turn are involved in the acquisition of tumor-eradicating immunity against B7-2⁺ P815 tumor cells that do not express MHC class II molecules. Accordingly, we determined whether anti-CD4 and/or anti-CD8 mAb treatment can prevent the rejection of B7-2⁺ P815 tumors by wild-type mice. As seen in Fig. 6, anti-CD4 mAb alone or anti-CD8 mAb alone, although anti-CD8 mAb alone was ineffective in preventing the regression of B7-2⁺ P815 tumors, although anti-CD8 mAb alone, but not anti-CD4 mAb alone, slowed down the rate of tumor regression substantially. However, anti-CD4 mAb together with anti-CD8 mAb prevented B7-2⁺ P815 tumor regression in all mice. Thus, not only CD8⁺ T cells, but also CD4⁺ T cells, participate in the development of tumor-eradicating immunity against B7-2⁺ P815 tumor cells.

**Discussion**

Over the last 5 years, multiple studies have been initiated to examine the potential of ectopic expression of the CD28 ligands, B7-1 and B7-2, in the generation of tumor-eradicating immunity (14–22). These studies formed the basis for the conviction that ectopic expression of B7 on tumor cells leads to direct CD8⁺ T cell activation (14, 20–22). Here, we show that P815 tumor cells transfected with the B7-2 gene elicit tumor-eradicating immunity...
We show here that treatment of mice with anti-B7-2 mAb was not sufficient to prevent the regression of B7-2\(^+\) P815 tumors. The fact that the B7-2 transfectant of P815 tumor cells did not behave like B7-negative parental P815 tumor cells (17) in mice treated with anti-B7-2 mAb is not unique to our B7-2 transfectant. In fact, the same protocol of anti-B7-2 mAb treatment was also ineffective in preventing the regression of a different B7-2 transfectant of P815 tumor cells that was generated independently of our transfectant (R. N. La Motte, P. E. Fields, R. Finch, J. A. Bluestone, and M. B. Mokyr, unpublished observations). At present, we do not know why the B7-2 transfectant of P815 tumor cells does not behave like B7-negative parental P815 tumor cells in mice treated with anti-B7-2 mAb. Interestingly, increasing the dose and the frequency of anti-B7-2 mAb treatment from 100 μg/mouse every other day to 250 μg/mouse every day did not alter the results (R.N.L., J.A.B., and M.B.M., unpublished observations). It is possible, that even with this aggressive protocol of anti-B7-2 mAb treatment we did not mask continuously all the B7-2 molecules on the B7-2\(^+\) tumor cells, and the transiently unmasked residual B7-2 molecules expressed on the tumor cells were sufficient to trigger an enhanced killing of the P815 tumor cells (i.e., beyond the level of killing of parental tumor cells), thereby making tumor-associated Ags more readily available to host APCs (23, 27). Another possibility is that the anti-B7-2 mAb that bound to the B7-2\(^+\) P815 tumor cells led to the killing of some tumor cells (e.g., through Ab-dependent cell-mediated cytotoxicity), which in turn increased Ag release and availability to host APCs. Regardless of the exact reason(s) why B7-2\(^+\) P815 tumor cells do not behave like B7-negative parental P815 tumor cells in mice inoculated with anti-B7-2 mAb, the current studies illustrate the importance of both B7-2 and B7-1 molecules for the eradication of B7-2\(^+\) P815 tumors by demonstrating that treatment with both anti-B7-2 and anti-B7-1 mAb is required to prevent B7-2\(^+\) P815 tumor regression.

In an attempt to identify the B7-1-expressing cell type that contributes to the eradication of B7-2\(^+\) P815 tumors, we focused our attention initially on the possibility that B7-2\(^+\) tumor cells are induced in vivo to express the B7-1 molecule. Such a possibility was considered in light of the report by Basker et al. (26) that under some conditions B7-negative tumor cells can be induced to express both B7-1 and B7-2 molecules, and B7 expression on the tumor cells was implicated in the acquisition of tumor-eradicating immunity. Such a mechanism is apparently not operative in our system, as B7-2\(^+\) P815 tumor cells were not induced in vivo to express the B7-1 molecule. On the other hand, B7-1 expression on host APCs is important for the eradication of B7-2\(^+\) P815 tumors as evident from our observations that while both anti-B7-2 and anti-B7-1 mAb were required to prevent B7-2\(^+\) P815 tumor regression in wild-type mice, anti-B7-2 mAb alone was sufficient to prevent the regression of B7-2\(^+\) P815 tumors in B7-1 KO mice. It is interesting to note that although the focus of the aforementioned study by Basker et al. was on the induction of B7-1 and B7-2 expression on B7-negative tumor cells grown in vivo, host APCs were also found to up-regulate B7 expression. Thus, based on our current findings, it is conceivable that B7-expressing host APCs also contributed to tumor eradication in their system.

Yang et al. (28) have recently shown that a B7-1 transfectant of P815 tumor cells led to up-regulation of B7-2 expression on CD3\(^+\) cells in the draining lymph nodes of normal mice. Here, we extend their observations by demonstrating that a B7-2 transfectant of P815 tumor cells also leads to up-regulation of B7-2 expression on CD3\(^+\) cells including B20\(^+\) cells from the draining lymph nodes of normal mice. Moreover, we show that the B7-2 transfectant of P815 tumor cells also leads to up-regulation of B7-1 expression on MAC-1\(^+\) cells in the draining lymph nodes of the mice and that B7-1-expressing host cells elicit potent tumor-eradicating immunity against B7-2\(^+\) P815 tumor cells. These observations, coupled with reports that draining lymph nodes are important for the generation of antitumor immunity (28, 29), suggest that the B7-1-expressing MAC-1\(^+\) cells are important for the generation of tumor-eradicating immunity against B7-2\(^+\) P815 tumor cells. At present, it is not known through which mechanism(s) B7-1 expression on host APCs is up-regulated, but it is likely to involve inflammatory cytokines (30) and/or increased cell surface interactions such as CD40/CD40L (11, 31). In addition, it remains to be determined if the host APCs that display up-regulation of B7 expression following inoculation of B7-2\(^+\) P815 tumor cells are APCs that were present in the lymph nodes before tumor inoculation or represent APCs that migrated into the lymph nodes subsequent to the B7-2\(^+\) P815 tumor inoculation.

Although B7 expression on host APCs is important for the acquisition of tumor-eradicating immunity against P815 tumor cells (as evident from our experiments with B7-1 “knockout” mice), a recent study by Yang et al. (16) illustrated that B7 expression on host APCs alone is not sufficient for the eradication of P815 tumors.
and B7 expression on tumor cells is also required. Specifically, this study showed that although inoculation of B7-negative parental tumor cells led to up-regulation of B7 expression on host APCs in the draining lymph nodes, B7-negative parental P815 tumor cells, in contrast to B7-1+ or B7-2+ P815 tumor cells, established progressively growing tumors (16). Moreover, Yang et al. concluded that B7 expression on host APCs is important for the generation of an anti-tumor immune response against B7-negative parental P815 tumor cells as blockade of B7/CD28 interaction through the use of CTLA4Ig facilitated the growth of B7-negative P815 tumors. It should be pointed out that the study by Yang et al. (16) did not identify the B7-expressing CD3+ cells in the draining lymph nodes of mice inoculated with B7-negative parental P815 tumor cells, and therefore it is not known at present if they belong to the same population of APCs as the cells that display up-regulation of B7-1 expression following inoculation of B7-2+ P815 tumor cells.

At least three models, which are not mutually exclusive, can be advanced to explain the role of B7 expression on tumor cells for the full realization of tumor-eradicating immunity. In the first model, B7-expressing tumor cells function as APCs to stimulate directly CD8+ T cells with tumor-eradicating activity. In the second model, B7 expression on tumor cells leads to increased lysis of the tumor cells, which facilitates subsequent Ag uptake and processing by host APCs (23, 32, 33). In the third model, the induction of T cell-dependent tumor-eradicating immunity occurs in the draining lymph nodes following stimulation with B7+ APCs, and is followed by the maturation/amplification of the T cell-dependent tumor-eradicating immunity within the tumor node wherein B7 expression on tumor cells plays an important role (28). Regardless of the exact mechanism(s) through which B7-2 expression on tumor cells contributes to the generation of tumor-eradicating immunity, in vivo up-regulation of B7-1 expression on the B7-2+ P815 tumor cells is not essential for this purpose.

Our observation that CD4+ T cells, and not only CD8+ T cells, participate in the development of potent tumor-eradicating immunity against B7-2+ P815 tumor cells is consistent with the importance of B7-expressing host APCs for this process, as P815 tumor cells do not express MHC class II molecules. However, the mechanism(s) through which the CD4+ T cells exert their antitumor effects against B7-2+ P815 tumor cells remains to be elucidated. Although it is tempting to conclude from our observations with anti-CD4 and/or anti-CD8 mAb treatments that CD4+ T cells lead to the development of potent tumor-eradicating immunity in the absence of CD8+ T cells, such a conclusion is premature based on the current observations. In other words, the fact that anti-CD8 mAb alone did not prevent the regression of B7-2+ P815 tumors and both anti-CD4 and anti-CD8 mAb were required for this purpose does not necessarily mean that CD4+ T cells exerted their tumor-eradicating activity in a CD8+ T cell-independent manner. The possibility exists that in the presence of CD4+ T cells the CD8- T cells developed a much more potent tumor-eradicating immunity than was required to cause the regression of the B7-2+ P815 tumors (i.e., >10-fold greater than needed), and, even after the depletion of 90% of the CD8+ T cells, as a consequence of anti-CD8 treatment, the residual CD8- T cell-mediated tumor-eradicating immunity was still sufficient. However, when the CD4+ T cells were depleted, the amplifying effect of the CD4+ T cells for the generation of CD8+ T cell-mediated tumor-eradicating immunity was removed, and the resultant CD8+ T cell-mediated tumor-eradicating immunity (which was still sufficient to cause tumor regression) was no longer >10-fold greater than that required for the eradication of the B7-2+

P815 tumors. Consequently, a 90% depletion of CD8+ T cells in mice that were also depleted of the amplifying effect of CD4+ T cells reduced the anti-CD8+ T cell-mediated tumor-eradicating immunity below the level required for the eradication of the B7-2+ P815 tumors.

Taken together, the results presented herein illustrate B7-2-expressing tumor cells can stimulate the generation of potent tumor-eradicating immunity indirectly through presentation of tumor-associated Ag by B7-1-expressing host APCs, which in turn activate CD4+ T cells. Consequently, our observations suggest that it may be possible to enhance the tumor-eradicating immunity elicited by B7-transfected tumor cells by combining this strategy with methods to enhance the differentiation and activation of host APCs (e.g., through the administration of granulocyte-macrophage-CSF or IFN-γ), thereby enhancing the effectiveness of B7-transfected tumor cells as tumor cell vaccines.

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