Vaccination with Mouse Mammary Adenocarcinoma Cells Coexpressing B7-1 (CD80) and B7-2 (CD86) Discloses the Dominant Effect of B7-1 in the Induction of Antitumor Immunity

Alfonso Martín-Fontecha, Monica Moro, Maria Cristina Crosti, Fabrizio Veglia, Giulia Casorati and Paolo Dellabona

*J Immunol 2000; 164:698-704; doi: 10.4049/jimmunol.164.2.698
http://www.jimmunol.org/content/164/2/698

References
This article cites 57 articles, 35 of which you can access for free at:
http://www.jimmunol.org/content/164/2/698.full#ref-list-1

Why *The JI*? Submit online.
- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

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
Vaccination with Mouse Mammary Adenocarcinoma Cells Coexpressing B7-1 (CD80) and B7-2 (CD86) Discloses the Dominant Effect of B7-1 in the Induction of Antitumor Immunity

Alfonso Martín-Fontecha,2* Monica Moro,* Maria Cristina Crosti,* Fabrizio Veglia,† Giulia Casorati,3* and Paolo Dellabona3*

Nonreplicating TSA mammary adenocarcinoma cells expressing B7-2 (CD86) (TS/A-2) are more immunogenic than those expressing B7-1 (CD80) (TS/A-1), indicating that B7-1 and B7-2 display nonredundant costimulatory effects in inducing antitumor responses. Whereas transfection of B7-2 cDNA into TS/A-1 cells does not improve their immunogenicity, transfection of B7-1 cDNA into TS/A-2 cells (TS/A-2/1) decreases their immunogenicity in a manner that is directly related to the surface levels of B7-1. Ab blocking of B7-1 on TS/A-2/1 cells before their injection in vivo restores the higher immunogenicity characteristic of single B7-2 transfectants, indicating therefore that B7-1 actively modulates the B7-2-dependent costimulation. The expression of B7-1 also modifies quantitatively the balance of endogenous IFN-γ and IL-4 induced in vivo by TS/A-2 vaccines. In fact, we find that vaccination with TS/A-2/1 cells results in the production of more IFN-γ and less IL-4 than TS/A-2 vaccines, a pattern comparable to that induced by TS/A-1 cells. Thus, in the TS/A model of antitumor response, B7-1 modulates B7-2-dependent costimulatory effects in a dominant, noncompetitive way. The Journal of Immunology, 2000, 164: 698–704.

The interaction between B7-1 or B7-2 molecules with their T cell counter-receptors CD28/CTLA-4 plays a critical role in controlling T cell responses (1, 2). Although B7-1 and B7-2 bind with comparable low avidity to CD28 and high avidity to CTLA-4, they have distinct binding sites on either T cell counter-receptor (3–5). Furthermore, B7-1 and B7-2 display different kinetics of binding to either counter-receptor, B7-2 having a faster dissociation kinetics (3). The expression of B7-1 and B7-2 is also differently regulated on APCs: B7-2 is expressed constitutively on monocytes, dendritic cells, and resting B cells, and when both B7 molecules are up-regulated upon APC activation, B7-2 is up-regulated more rapidly (6–11). Altogether, these findings would support different costimulatory roles for each B7 molecule. A number of experimental evidences obtained both in the human and mouse systems have indeed indicated that the two B7 molecules exert nonredundant costimulatory effects. B7-2 but not B7-1 stimulated the production of IL-4 in vitro (12–14). B7-2 also plays a critical role in directing CD4+ Th2 differentiation in an animal model of helminth infection (15). Furthermore, blocking in vivo B7-1 with specific mAbs significantly accelerated the development of diabetes in nonobese diabetic mouse (16), whereas it prevented or cured established experimental allergic encephalomyelitis (17, 18). On the contrary, the treatment with anti-B7-2 mAb blocked the development of diabetes (16) while it exacerbated experimental allergic encephalomyelitis (17, 18). Thus, these data suggest that B7-1 and B7-2 ought to differentially activate the Th1/Th2 development pathways (19, 20). However, other experimental systems failed to reveal any significant difference between B7-1 and B7-2 in the induction of proliferation, cytokine production, and cytolytic activity (21, 22). Thus, additional factors, such as Ag dose, anatomical distribution, APCs, and genetic background may influence the outcome of B7-mediated costimulation.

Since B7-dependent costimulatory signals play a central role in T cell activation, it has been proposed that the lack of immunogenicity of many tumor types could be due to the lack of B7 expression (23, 24). Indeed, it was proved that transfection of B7-1 genes into different experimental mouse tumors greatly improved their immunogenicity (23, 24). However, when the efficacy of B7-1- and B7-2-dependent costimulation were compared on a more extended panel of mouse tumors, heterogeneous results were obtained. B7-1 and B7-2 equally induced protective and curative antitumor immunity when expressed into both immunogenic mastocytoma P815 (25, 26) and lymphoma RMA cells (27), but B7-1 was found to be superior to B7-2 when transfected into the 32Dc13 myeloid cell line (28) or into a subclone of P815 (29, 30). We have also found that B7-1 and B7-2 exert nonredundant costimulatory effects when expressed on TS/A mouse mammary adenocarcinoma cells. When used as nonreplicating cell vaccines, TS/A cells expressing B7-2 are more immunogenic than those expressing B7-1 (27). This differential effect between B7-1- and B7-2-dependent costimulation observed in the TS/A tumor model gave us the possibility to investigate whether the lower costimulatory efficacy of B7-1 was due to an active inhibitory effect by B7-1, or more simply to a weaker costimulatory activity of B7-1. For that, we

*Unità d’Immunochimica, DIBIT, Cancer Immunotherapy and Gene Therapy Program; and Unità di Biostatistica, Istituto Scientifico H. San Raffaele, Milan, Italy.

Received for publication December 21, 1998. Accepted for publication November 3, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by a grant from Associazione Italiana per la Ricerca sul Cancro. A.M.-F. was supported by a fellowship from the European Community.

2 Current address: Microbiology and Tumor Biology Center, Karolinska Institutet, S-171 77 Stockholm, Sweden.

3 Address correspondence and reprint requests to Dr. Paolo Dellabona or Dr. Giulia Casorati, Unità d’Immunochimica, DIBIT, Istituto Scientifico San Raffaele, Via Olgettina 58, Milan 20132, Italy. E-mail addresses: dellabona.paolo@hsr.it (P.D.) or casorati.giulia@hsr.it (G.C.)
coexpressed B7-1 and B7-2 on TS/A cells and compared nonreplicating double and single B7-1 or B7-2 transfectants for their capacity to 1) induce both protective and therapeutic immunity and 2) differentially induce IL-4 and IFN-γ in vivo. Our data show that B7-1 modulates B7-2-dependent antitumor response in an active, dominant, and noncompetitive way.

### Materials and Methods

#### Tumor cell lines

TS/A is a spontaneous, moderately differentiated mammary adenocarcinoma from BALB/c origin. The tumor cell line was maintained in RPMI 1640 with 5% FCS, 100 U/ml penicillin, 100 U/ml streptomycin, and 2.5 × 10⁻⁵ M 2-ME. Cells were not cultured for longer than 3–4 wk and were routinely screened for Mycoplasma contamination.

#### Generation of double-transfected TS/A cells

Vectors expressing mouse B7-1 or mouse B7-2 cDNAs were transfected by electroporation into TS/A expressing mouse B7-2 or mouse B7-1, respectively, as described (27). Double-transfected cells were selected in complete medium containing 2 mg/ml of G418, 50 µg/ml xantine, and 20 µg/ml of mycophenolic acid. Surviving cells expressing variable levels of the two B7 molecules were stained with anti-mouse B7-1 1G10 or anti-mouse B7-2 GL1 mAbs (PharMingen, San Diego, CA), selected by cell sorting using a FACStar flow cytometer (Becton Dickinson, Mountain View, CA), and cloned by limiting dilution in 96-well plates. Wells displaying single clones growing were reanalyzed by flow cytometry to verify the expression of the desired amount of each B7 costimulatory molecule.

Several TS/A-2/1 clones displaying high levels of B7-2 and low, medium, or high surface expression of B7-1 were found. Three clones expressing high levels of B7-1 and one representative clone expressing negative, low, or medium levels of B7-1 were selected for this study. Several TS/A-1/2 clones, coexpressing levels of both B7 molecules as high as those expressed by the single-transfected TS/A-1.26 and TS/A-2.22 clones, were also found. Four of them were selected for this study.

#### Mice

Female BALB/c mice (4–to 8-wk old), were purchased from Charles River Breeding Laboratories (Calco, Italy).

#### In vivo studies

All of the in vivo studies were approved by the Ethical Committee of the Istituto Scientifico San Raffaele and performed according to its guidelines. Nonreplicating cells were obtained by incubating 1 × 10⁷ cells/ml with 60 µg/ml of mitomycin C (Mit. C) (Sigma, St. Louis, MO) in RPMI 1640 for 30 min at 37°C. For vaccination experiments, mice were challenged in the left flank with a single inoculum of 1 × 10⁶ Mit. C-treated cells. Control vaccinations were done with nontransfected parental TS/A cells or TS/A cells transfected with empty vectors encoding for G418, guanine-xanthine-phosphoribosyl transferase (gpt), or both. After 2 wk, mice were challenged s.c. in the opposite flank with 10⁴ living nontransfected parental TS/A cells. Mit. C-treated cells maintained in vitro for 4 days did not show changes on the level of B7-1 and B7-2 expression (data not shown).

To block the effects of either B7-1 or B7-2 on double-transfected TS/A cells in vivo, mice were vaccinated s.c. with 10⁸ nonreplicating TS/A-2/1.4 cells, which were mixed in vitro for 10 min at 4°C with 2.5 × 10⁻⁵ M 2-ME. Cells were not cultured for longer than 3–4 wk and were routinely screened for Mycoplasma contamination.

### In vitro studies

To determine the persistence of the anti-B7-1 and B7-2 mAbs on their target molecules, 2 × 10⁶ nonreplicating TS/A-2/1.4 high cells were stained with 2 µg of either 1G10 or GL1 mAb for 20 min at 4°C. After washing and seeding in complete medium, aliquots of cells were drawn after 0, 1, 2, 4, 16, and 24 h of culture at 37°C, stained with a FITC-conjugated goat anti-rat antisera (Southern Biotechnology Associates, Birmingham, AL), and analyzed by flow cytometry. A regression curve was generated by interpolating the progressively decreased mean fluorescent intensities, which allowed calculation of a 50% dissociation time of about 12 h and 9 h for the anti-B7-1 and anti-B7-2 mAbs, respectively.

To determine the cytokine produced by T cells, primed in vivo by the different TS/A-B7 vaccines, three mice per group were vaccinated with 10⁶ nonreplicating parental TS/A, TS/A-1.26, TS/A-2.22, or TS/A-2/1 high-four cells, respectively, injected s.c. in the left flank. Three days later, mice were sacrificed, and the T cells extracted from pooled draining left inguinal and cervical lymph nodes were activated in vitro at 10⁶/ml using a combination of 50 ng/ml PMA (Sigma) and 1 µg/ml inomycin (Sigma) in complete medium. After 2 h at 37°C, brefeldin A was added at 10 µg/ml for another 2 h at 37°C, followed by fixation in 2% in 15 min at 4°C, and permeabilization in PBS containing 1% BSA and 0.5% saponin (Sigma). Fixed/permeabilized T cells were stained with FITC- or PE-conjugated isotype-matched mAbs (PharMingen). Control staining for cytokine production was performed using FITC- or PE-conjugated isotype-matched mAbs (PharMingen). Cells were analyzed on a FACSscan analyzer (Becton Dickinson, Mountain View, CA) by acquiring 3 × 10⁴ CD4+ or CD8+ T cells in each file.

### Statistical analysis

Unless specified in the text, all experiments in vivo were performed twice with groups of 5–15 mice, and the data were pooled. Frequencies were compared by the Fisher exact test or the χ² test for trend, when appropriate.

### Results

#### Expression of increasing levels of B7-1 into TS/A-2 cells reduces their immunogenicity in a dose-dependent manner

To determine the immunogenicity of TS/A adenocarcinoma cells expressing both B7-1 and B7-2 costimulatory molecules, we supertransfected B7-1 cDNA into the highly immunogenic TS/A-2.22 clone. Clones expressing increasing surface levels of B7-1, with constant levels of both B7-2 and MHC molecules, were selected (Fig. 1) and directly compared as nonreplicating cell vaccines for their protective efficacy against a lethal challenge with the nontransfected parental TS/A cells. As shown in Table I, TS/A-2/1 double transfectants that do not express surface B7-1 (clone.1) or express low (clone.2) or intermediate (clone.6) levels of B7-1 are as immunogenic as the single TS/A-2.22 clone. On the contrary, the protection obtained with clone TS/A-2/1 high.4, which expresses high levels of B7-1, decreases to 40%, a value comparable to that obtained with the single TS/A-1.26 clone. Two other TS/A-2/1 high clones, tested in independent experiments, gave similar results (data not shown). Since these data were suggesting a possible dominance of B7-1 in the induction of antitumor response, we supertransfected B7-2 cDNA into the TS/A-1.26 clone and tested double-transfected TS/A-1/2 clones coexpressing comparable high levels of both B7 molecules as nonreplicating cell vaccines. Interestingly, both the bulk culture and four independent TS/A-1/2 clones protected 40% of the mice from a challenge with living parental TS/A cells (data not shown), therefore displaying the lower immunogenicity typical of the TS/A-1 cells. Expression

---

*Abbreviation used in this paper: Mit. C, mitomycin C.*
of comparable levels of B7-1 and B7-2 on TS/A cells also decreased the efficacy of the original TS/A-2.22 clone to cure a 24-h-old tumor generated by the s.c. injection of $4 \times 10^4$ living TS/A cells (1 × minimum tumorigenic dose (MTD)). Whereas nonreplicating TS/A-2 cells cured 40% of the mice, nonreplicating TS/A-2/1 and TS/A-1 cells similarly cured 10% of the mice (data not shown).

Ab blocking of B7-1 in nonreplicating TS/A-2/1 cells abrogates its dominant effects

Taken together, the above data suggested that B7-1 plays an active role in modulating costimulation of antitumor immunity by B7-2. To confirm this hypothesis, we studied the effects of blocking either B7 molecule with specific mAbs on nonreplicating TS/A-2/1 vaccines at the time of injection into mice. Nonreplicating TS/A-2/1 cells were mixed in vitro with a saturating dose of either anti-B7-1 or anti-B7-2 mAb and injected s.c. Anti-B7-1 and anti-B7-2 mAbs displayed comparable stability of binding to transfected B7 molecules at 37°C (50% dissociation time at 37°C: 12 h and 9 h, respectively). Twelve hours later, mice received another i.p. injection of 100 μg of the appropriate Ab, and 2 wk later they were challenged contralaterally with 10^5 living parental TS/A cells and scored for tumor growth. The percentage of protected mice by TS/A-2/1-high.4 cells in the presence of anti-B7-1 vs anti-B7-2 mAb are significantly different (Fisher exact test, $p < 0.05$).

**TABLE I.** Dominant effect of B7-1 over B7-2 when coexpressed onto the TS/A adenocarcinoma line

<table>
<thead>
<tr>
<th>Tumor Vaccine</th>
<th>Tumor Take/Challenged Mice</th>
<th>Survival Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS/A</td>
<td>10/10 (0)</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>TS/A-2.22</td>
<td>4/20 (80)</td>
<td>21</td>
</tr>
<tr>
<td>TS/A-2/1 neg. 1</td>
<td>2/10 (80)</td>
<td>17</td>
</tr>
<tr>
<td>TS/A-2/1 low. 2</td>
<td>2/10 (80)</td>
<td>21</td>
</tr>
<tr>
<td>TS/A-2/1 med. 6</td>
<td>2/10 (80)</td>
<td>24</td>
</tr>
<tr>
<td>TS/A-2/1 high. 4</td>
<td>6/10 (40)</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>TS/A-1.26</td>
<td>18/30 (40)</td>
<td>23 ± 3</td>
</tr>
</tbody>
</table>

a BALB/c mice were vaccinated with a single s.c. inoculum of 10^6 nonreplicating TS/A-1, TS/A-2, or the indicated TS/A-2/1 clones. Fifteen days later, mice were challenged with a s.c. counterlateral inoculum of 10^5 living parental TS/A cells and scored for tumor growth.

b Number in parentheses indicate the percentage of protected mice.

c Time required for the tumor to reach 10 mm in mean diameter.

d Significantly different from the TS/A-2.22-immunized group (Fisher exact test, $p < 0.045$).

Ab blocking of B7-1 in nonreplicating TS/A-2/1 cells abrogates its dominant effects

We also determined whether B7-1 could modulate B7-2-dependent costimulatory effects when expressed in trans. Therefore, mice were vaccinated with a 1:2, 1:1, or 2:1 mixtures of nonreplicating TS/A-1 and TS/A-2 cells. As shown in Table II, the protective effects of TS/A-2 vaccines were never substantially modified by the presence of various doses of TS/A-1 cells, indicating that B7-1 exerted a dominant effect on B7-2 only when coexpressed on the same cell.

Vaccination with TS/A-2/1 or TS/A-1 cells similarly induces more IFN-γ and less IL-4 than TS/A-2 vaccines

Recent data have suggested that B7-1- and B7-2-dependent costimulations lead to different patterns of type 1 or type 2 cytokines produced during the immune response (19, 20). We therefore studied whether we could detect a difference in the balance of endogenous IFN-γ and IL-4 induced during the priming of the protective responses by nonreplicating TS/A-1, TS/A-2, or TS/A-2/1 vaccines.
High amounts of either anti-IFN-γ- or anti-IL-4–neutralizing mAb were injected into mice only within the first 7 days after vaccination, corresponding to the priming phase of the antitumor immunity. Fifteen days after vaccination, when no anti-cytokine mAb were detectable in their sera, mice were challenged with 10^5 living adenocarcinoma cells expressing B7-2 are more immunogenic than B7-1. The vaccination efficacy of TS/A-2.22 showed a significant negative trend (p = 0.025 by the χ^2 test for trend) upon its reduction below 50% in the cell-mixed vaccines, indicating absence of dominant effects by B7-1.

<table>
<thead>
<tr>
<th>Tumor Vaccinea</th>
<th>Tumor Take/Challenged Miceb</th>
<th>Survival Time (days)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS/A (%)</td>
<td>TS/A-1 (%)</td>
<td>TS/A-2 (%)</td>
</tr>
<tr>
<td>100</td>
<td>5/5 (0)</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>50</td>
<td>2/10 (80)</td>
<td>21</td>
</tr>
<tr>
<td>25</td>
<td>75 2/10 (80)</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>50</td>
<td>50 2/10 (80)d</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>75</td>
<td>25 4/10 (60)</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>100</td>
<td>100 6/10 (40)</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>50</td>
<td>50 6/10 (40)</td>
<td>22 ± 7</td>
</tr>
</tbody>
</table>

a Mice were vaccinated s.c. with 10^6 nonreplicating TS/A-1.6 and TS/A-2.22 cells mixed in a 1:0, 1:1, 1:2, or 2:1 ratio. After 15 days, mice were challenged contralaterally with 10^6 nontransfected parental TS/A cells and scored for tumor growth. Control groups were vaccinated with either 10^6 nonreplicating TS/A-1 or TS/A-2 mixed in a 1:1 ratio with nonreplicating parental TS/A cells, or with each type of cell vaccine alone.

b Number in parentheses indicate the percentage of protected mice.

c Time required for the tumor to reach 10 mm in mean diameter.

d The vaccination efficacy of TS/A-2.22 showed a significant negative trend (p = 0.025 by the χ^2 test for trend) upon its reduction below 50% in the cell-mixed vaccines, indicating absence of dominant effects by B7-1.

To determine the cellular basis for the unbalanced production of cytokines induced by TS/A-1, TS/A-2, and TS/A-2 vaccines, mice were vaccinated s.c. with a million of each type of nonreplicating B7 transfectant. Three days later, T cells purified from the draining lymph nodes were reactivated in vitro with a polyclonal stimulus, and the pattern of IFN-γ and IL-4 produced by either CD4^+ or CD8^+ T cells was determined by intracellular staining and flow cytometry analysis. As shown in Fig. 5, priming in vivo with TS/A-1.26 or TS/A-2/1 high.4 induced more CD4^+ T cells to produce IFN-γ and fewer cells to produce IL-4 than did TS/A-2.22 vaccines, thus substantially confirming the data obtained by titrating in vivo the relative amount of cytokines induced by nonreplicating TS/A vaccines. At variance with CD4^+ T cells, all three TS/A-B7 vaccines induced equivalent fractions of CD8^+ T cells to produce IFN-γ, but never IL-4 (data not shown).

Collectively, these data would therefore suggest that the expression of B7-1 modifies quantitatively the balance of endogenous IFN-γ and IL-4 induced in vivo by TS/A-2 vaccines.

Discussion

We have previously shown that nonreplicating TS/A mammary adenocarcinoma cells expressing B7-2 are more immunogenic...
FIGURE 4. Unbalanced induction of endogenous IFN-γ and IL-4 by TS/A-1 or TS/A-2/1 and TS/A-2 cells revealed by using subsaturating quantities of neutralizing mAbs in vivo of neutralizing mAbs. Mice (five per experimental group) received i.p. 400 μg of either 11B11 or AN18 mAb at days −2 and −1. At day 0, mice were vaccinated s.c. with 1 × 10^6 nonreplicating TS/A-1.26, TS/A-2.22, or TS/A-2/1 high.4 cells. The neutralizing regimen was continued by injecting 150 μg of mAb i.p. twice a week for 1 wk. As control, mice immunized with each B7-expressing TS/A clone received nonimmune rat IgG, following the same administration regimen. Fifteen days after the last Ab injection, mice were challenged s.c. controlaterally with 10^5 living TS/A and 10^6 nonreplicating TS/A-1.26, TS/A-2.22, or TS/A-2/1 high.4 cells. The neutralizing regimen was continued by injecting 150 μg of mAb i.p. twice a week for 1 wk. As control, mice immunized with each B7-expressing TS/A clone received nonimmune rat IgG, following the same administration regimen. Fifteen days after the last Ab injection, mice were challenged s.c. controlaterally with 10^5 living TS/A parental cells and scored for tumor growth. One of two independent experiments giving similar results is shown. The p value for anti-IL-4 effects in mice vaccinated with TS/A-1.26 and TS/A-2/1 high.4 is 0.003, whereas that for anti-IFN-γ effects in mice vaccinated with TS/A-2/1 high.4 is 0.0003 (Fisher exact test). Both p values have been calculated on two experiments.

Determined the pattern of endogenous IL-4 and IFN-γ balance, we show that B7-1 modulates in a dominant way the expansion and cytokine production by T cells, thus providing a molecular basis to explain our findings.

As a last criteria to measure the relative role exerted by each B7 molecule during the induction of antitumor immunity, we have determined the pattern of endogenous IL-4 and IFN-γ induced by each B7-expressing tumor vaccine. Also by this criteria, B7-1 is found to exerts a dominant role over B7-2 and makes the pattern of cytokines induced by double-transfected TS/A-2/1 vaccines similar to that induced by single TS/A-1 vaccines. In particular, in the TS/A tumor system, B7-2 induces more IL-4 and less IFN-γ than B7-1. Although these data indicate quantitative rather than qualitative differences in the pattern of cytokine induced by either B7 molecule, they appear to be substantially consistent with a number of other demonstrations, showing that B7-1 and B7-2 co-stimulate the induction of different patterns of cytokines in several experimental systems (12, 15, 17).

Interestingly, B7-1- and B7-2-expressing vaccine appears to induce unbalanced IFN-γ and IL-4 production in CD4^+ but not CD8^- T cells. Since TS/A is MHC class II negative and poorly responsive to IFN-γ up-regulation of MHC genes in vitro (M. Moro, unpublished observation), CD4^+ T cells are likely to be induced by indirect presentation of tumor-derived Ags by endogenous APCs, although they may still remain sensitive to B7-dependent costimulation provided by tumor cells in trans (33, 34).

Taken together, these findings may suggest a model of noncompetitive inhibition between two ligands for their receptor (35) in which B7-1 could displace B7-2 from binding to their T cell ligand, but not the other way round. Several molecular data generated in vitro indicate that B7-1 and B7-2 indeed recognize CD28 or CTLA-4 in a nonidentical manner and would support this hypothesis (4, 5, 36–38). Since CD28 activates while CTLA-4 inhibits T cell activation (20, 39–42), and the engagement of CD28 and CTLA-4 regulates positively and negatively, respectively, the induction of a Th2 response (43, 44), the differential ligation of CD28 or CTLA-4 by B7-1 or B7-2, would affect both clonal expansion and cytokine production by T cells, thus providing a molecular basis to explain our findings.
TheJournalofImmunology

Three independent reproducible experiments is shown.

-anti-CD8 mAb plus FITC-anti-IL-4 and PE-anti-IFN-
PMA

were purified from the draining left inguinal and cervical lymph nodes of

each mouse, pooled within each experimental group, and activated with

Unbalanced induction of endogenous IFN-γ and IL-4 by

FIGURE 5. Unbalanced induction of endogenous IFN-γ and IL-4 by

TS/A or TS/A-2/1 and TS/A-2 cells in CD4+ T cells. Three mice per
group were vaccinated s.c. in the left flank with 10^6 nonreplicating TS/A, TS/A-1.26, TS/A-2.22, or TS/A-23/1.4 high cells. After 3 days, T cells
were purified from the draining left inguinal and cervical lymph nodes of
each mouse, pooled within each experimental group, and activated with

PM + ionomycin as described in Materials and Methods. Fixed/perme-
abilized T cells were triple stained with either Cy-Chrome-anti-CD4 or

anti-CD8 mAb plus FITC-anti-IL-4 and PE-anti-IFN-γ mAbs. One of

three independent reproducible experiments is shown.

Other studies, performed on different antigenic systems, have also
suggested that B7-1-dependent costimulatory signal exerts a
somehow down-regulatory effect on the course of the immune re-

gponse. Sethna et al. (45) showed that transgenic mice constitutively
expressing B7-1 on B cells have a markedly reduced hu-
moral response. Kearny et al. (46) showed that expansion of
adaptively transferred CD4+ transgenic T cells upon Ag challenge
was consistently enhanced by the concomitant injection of anti-
B7-1-blocking mAb, whereas it was partially inhibited by anti-
B7-2 mAb. Furthermore, Ab blocking in vivo of B7-1 but not
B7-2 increases the germinal center formation in H. polygyrus-in-

culated mice (47).

Whether the superior immunogenicity of nonreplicating TS/A-2
cells may relate to the unbalance induction of IL-4 and IFN-γ
remains to be established. We can only speculate that a different
balance of these cytokines influences the development of T cell
subsets that are more efficient antitumor effectors than those in-
duced by TS/A-B7-1 vaccines. Because a burst of IL-4 is required
for both priming and expansion of CTLs (48, 49), as well as for
the development of a protective Th1 response against intracellular
parasites (50, 51), nonreplicating TS/A-2 cells may be more immu-
nogenic than TS/A-1 cells due to their superiority in eliciting an
initial burst of this cytokine. On the other hand, it is possible that
reduced induction of IFN-γ by TS/A-2 vaccines may also relate to

their higher immunogenicity. In fact, exposure to IFN-γ may lead
to depletion of recently activated T cells, leading to a smaller
clonal expansion (52, 53).

Altogether, these findings confirm the nonredundancy of the
two B7 molecules in regulating the immune response directed
against some tumors and show a model in which B7-1 modulates
B7-2-dependent costimulatory effects in a dominant noncompeti-
tive way.

Acknowledgments

We thank Drs. M. Bellone, P. Potti, and M. G. Roncarolo for the critical
reading of this manuscript and suggestions.

References

CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. Cell 71:
1065.

2. Lenschow, D. J., T. L. Walunas, and I. A. Bluestone. 1996. CD28/B7 system of

1994. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but

distinct kinetics to CD28 and CTLA-4 receptors [Published erratum appears in

eits cognate receptor CD80 (B7.1) and B70 (B7.2): analysis by site di-

5. Morton, P. A., X. T. Fu, J. A. Stewart, K. S. Giacoletto, S. L. White,
of C/CTA-4 substitutions on the binding of human CD80 (B7.1) and CD86 (B7.

parative analysis of B7-1 and B7-2 costimulatory ligands: expression and func-

7. Caux, C., B. Vanbervliet, C. Massacrier, M. Azuma, K. Okumura, L. L. Laniert,
and J. Banchereau. 1994. B70/B7-2 is identical to CD86 and is the major func-
tional ligand for CD28 expressed on human dendritic cells. J. Exp. Med. 180:
554.

8. Larsen, C. P., S. C. Ritchie, R. Hendrix, P. S. Linsley, K. S. Hathcock,
R. J. Hodes, R. L. Lowry, and T. C. Pearson. 1994. Regulation of immunostimu-
atory function and costimulatory molecule (B7-1 and B7-2) expression on mu-

H. Yagita, K. Okumura, P. S. Linsley, S. Ikehara, et al. 1994. The tissue distri-
bution of the B7-2 costimulator in mice: abundant expression on dendritic cells.

expression in Langerhans cells: differential regulation by T helper type I and T

B7-1 (CD80) and B7-2 (CD86) costimulatory molecules on mucosal macrophage
104.

12. Freeman, G. J., V. A. Boussiotis, A. Anumanthan, G. M. Bernstein, Y. Y. Ke,
do not deliver identical costimulatory signals, since B7-2 but not B7-1 preferen-
tially costimulates the initial production of IL-4. Immunity 2:523.

(CD80) is essential for the development of IL-4-producing T cells. J. Immunol.
156:549.

B7-2 requirement for helminth-induced granuloma formation and CD4


1997. B7-2 requirement for helminth-induced granuloma formation and CD4


and J. A. Bluestone. 1995. Differential effects of anti-B7-1 and anti-B7-2 mono-
clonal antibody treatment on the development of diabetes in the nonobese dia-

molecules activate differentially the Th1/Th2 developmental pathways: applica-
tion to autoimmune disease therapy. Cell 80:707.

P. J. Perrin. 1995. Distinct roles for B7-1 (CD80) and B7-2 (CD86) in the

19. Thompson, C. B. 1995. Distinct roles for the costimulatory ligands B7-1 and


cell ing cells lacking expression of CD80 or CD86. J. Immunol. 156:2713.


26. La Motte, R. N., M. A. Rubin, E. Barr, J. M. Leiden, J. A. Bluestone, and M. B. Mokry. 1996. Therapeutic effectiveness of the immunity elicited by P815 tumor cells engineered to express the B7-2 costimulatory molecule. Cancer Im-
munother. 42:161.


29. Gajewski, T. F., T. Fallarino, C. Uyttenhove, and T. Boon. 1996. Tumor rejection immunity elicited by tumor cells engineered to express the B7-2 costimulatory molecule. Cancer Im-
munother. 42:161.


33. Dung, L., and E. M. Shevach. 1994. Activation of CD4+ T cells by delivery of the B7 costimulatory signal on bystander antigen-presenting cells (trans-costimula-


35. Ross, E. M. 1996. Pharmacodynamics: mechanisms of drug action and the relation-
ship between drug concentration and effect. In The Pharmacological Basis of 


40. Lanier, L. L., S. O’Fallon, C. Somoza, J. H. Phillips, P. S. Linsley, K. Okumura, D. Ito, and M. Azuma. 1995. CD80 (B7) and CD86 (B70) provide similar co-
stimulatory signals to T cell proliferation, cytokine production, and generation of 

41. Natesan, M., Z. Razi-Wolf, and H. Reiser. 1996. Costimulation of IL-4 produc-
tion by murine B7-1 and B7-2 molecules. J. Immunol. 156:2783.
