

Activation of CD4⁺ T lymphocytes from interleukin 2-deficient mice by costimulatory B7 molecules

ZIBA RAZI-WOLF*†, GEORG A. HOLLÄNDER‡§¶, AND HANS REISER*†||

Departments of *Pathology and ‡Pediatrics, Harvard Medical School, Boston, MA 02115; and Divisions of †Lymphocyte Biology and §Pediatric Oncology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115

Communicated by Baruj Benacerraf, Dana-Farber Cancer Institute, Boston, MA, December 20, 1995 (received for review November 30, 1995)

ABSTRACT Interleukin 2 (IL-2)-deficient (IL-2^{-/-}) mice develop hemolytic anemia and chronic inflammatory bowel disease. Importantly, the induction of disease in IL-2-deficient mice is critically dependent on CD4⁺ T cells. We have studied the requirements of T cells from IL-2-deficient mice for costimulation with B7 antigens. Stable B7-1 or B7-2 chinese hamster ovary (CHO) cell transfectants could synergize with anti-CD3 monoclonal antibody (mAb) to induce the proliferation of CD4⁺ T cells from IL-2^{-/-} mutant mice. Further mechanistic studies established that B7-induced activation resulted in surface expression of the α chain of the IL-2 receptor. B7-induced proliferation occurred independently of IL-4 and was largely independent of the common γ chain of the IL-2, IL-4, IL-7, IL-9, and IL-15 receptors. Finally, anti-B7-2 but not anti-B7-1 mAb was able to inhibit the activation of IL-2^{-/-} T cells induced by anti-CD3 mAb in the presence of syngeneic antigen-presenting cells. The results of our experiments indicate that IL-2^{-/-} CD4⁺ T cells remain responsive to B7 stimulation and raise the possibility that B7 antagonists have a role in the prevention/treatment of inflammatory bowel disease.

Activation of primary T lymphocytes depends on costimulatory molecules that are typically provided by the antigen-presenting cell. The most potent costimulatory molecules known to date are the B7 proteins. The B7 subfamily of the immunoglobulin superfamily includes 45- to 60-kDa cell-surface glycoproteins that bind to two receptors on the T-cell surface, CD28 and CTLA-4 (1, 2). Two members of the B7 family, B7-1 (CD80) (3–5) and B7-2 (CD86) (6–8), have been cloned, and a third member, B7-3, has been postulated (9). In the case of CD4⁺ T lymphocytes, transfected cell lines that express B7-1 or B7-2 as well as B7-Ig fusion proteins can synergize with anti-CD3-, lectin-, or antigen-stimulated T-cell activation (6–8, 10–15). Engagement of the CD28 receptor by B7 molecules results in the production of cytokines such as interleukin 2 (IL-2) (10) and interleukin 4 (IL-4) (13), in the cell-surface expression of cytokine receptors (15), and in the expression of the *bcl-x_L* survival gene (16). Thus, costimulation with B7 molecules can promote growth of primary T cells by a multitude of mechanisms, although it is still unclear whether B7-1 and B7-2 induce identical or different gene effector programs (17).

Whereas the participation of the B7 costimulatory molecules in the activation of T cells by alloantigens and foreign antigens has been studied extensively (18–23), their potential role in the induction and maintenance of autoimmune and inflammatory responses is less clear. We are studying the dysregulated immune response in mice with a targeted disruption in the IL-2 gene. IL-2^{-/-} mice are normal at birth but eventually develop a wasting disorder that is characterized by lymphoproliferation, hemolytic anemia, and inflammatory

bowel disease (24). Importantly, while the induction of hemolytic anemia in IL-2^{-/-} mice appears to require both T and B cells, the development of inflammatory bowel disease is B-cell-independent but critically depends on T cells (25). In fact, available evidence suggests that CD4⁺ T cells are sufficient for disease induction (26).

Despite their potential involvement in inflammatory bowel disease induction, the activation requirements of IL-2^{-/-} CD4⁺ T cells have remained largely unexplored (27). Given the ability of the costimulatory B7 molecules to regulate the function of IL-2^{+/+} T cells, we have analyzed their potential role in the activation of IL-2^{-/-} CD4⁺ T cells. In the present report we document the capacity of stable B7-1 and B7-2 transfectants to costimulate the activation of purified CD4⁺ T cells from IL-2^{-/-} mice. The proliferative responses of IL-2^{-/-} CD4⁺ T cells are mostly independent of cytokines that use the common γ chain (γ_c) of cytokine receptors. We also show that the anti-CD3 monoclonal antibody (mAb)-induced proliferation of unfractionated IL-2^{-/-} lymph node cells is inhibited by anti-B7-2 but not by anti-B7-1 mAb.

MATERIALS AND METHODS

Animals. IL-2-deficient mice were provided by I. Horak (Universität Würzburg). IL-2^{-/-} mice or IL-2^{+/+} littermate controls on a C57BL6 \times 129 background were used in all experiments. All mice were genotyped by Southern blotting before use in functional studies.

Cell Lines and Antibodies. The CHO-B7-1 cell lines and vector-transfected CHO control transfectants have been described (16). CHO-B7-2 transfectants will be described elsewhere. These cells express B7-2 at a level comparable to that of B7-1 on CHO-B7-1 cells when analyzed by immunofluorescence and flow cytometry (28).

The following mAbs were used in this study: mAb 145-2C11 = anti-CD3 (29); mAb M5/114 = anti-I-A^{b,d,q}, anti-I-E^{d,k} (30); mAb ADH4 = anti-CD8.2 (31); 16-10A1 = anti-B7-1 (32); GL1 = anti-B7-2 (33); mAb 11B11 = anti-IL-4 (34); 7D4 = anti-IL-2R α (35); TUGm2 = anti- γ_c (36); mAb TM- β 1 = anti-IL-2R β (PharMingen); anti-IL-7 mAb (Genzyme).

Purification of Murine Spleen Cells. Splenocytes were depleted of erythrocytes by treatment with Tris/NH₄Cl. Splenic T cells were purified by nylon wool enrichment (37). CD4⁺ T cells were isolated as described (16), except that CD8⁺ T cells and major histocompatibility complex class II⁺ cells were depleted by 2-fold treatment with specific mAbs followed by adsorption on magnetic beads. The resulting population contains >98% CD4⁺ T cells (ref. 15; data not shown).

Abbreviations: RT-PCR, reverse transcriptase PCR; IL-2, interleukin 2; IL-4, interleukin 4; IL-2R, interleukin 2 receptor; mAb, monoclonal antibody; mmC, mitomycin C; TCR, T-cell receptor; γ_c , common γ chain; Sup Dil, supernatant dilution.

¶Present address: Pediatric Immunology, Department of Research, Kantonsspital and Kinderspital, Hebelstrasse 20, CH-4031 Basel, Switzerland.

||To whom reprint requests should be addressed at the † address.

Cell Cultures. Microcultures were set up in triplicate as described (16, 38), except that CHO transfectants were treated with mitomycin C (mmC; Sigma) before addition to cultures. The precise culture constituents and incubation periods are described in the respective experimental protocols. All cultures were pulsed with 1 μ Ci (37 kBq) of [3 H]thymidine per well for the last 12–14 hr of the 48- to 72-hr incubation period to assay for T-cell proliferation. To assay for lymphokine secretion, 100 μ l of culture supernatant was harvested 24 or 48 hr after initiation of the culture. IL-2 and IL-4 contents were determined by using the HT-2 and CT.4S indicator cell lines, respectively (13). The SEM for cytokine and proliferative responses were usually <15%.

Semiquantitative Reverse Transcriptase-PCR (RT-PCR). Total RNA was purified by a single-step, guanidinium thiocyanate-phenol-chloroform extraction method, using TRIzol (GIBCO/BRL), following the manufacturer's specifications. Briefly, cells were lysed directly in culture wells by adding TRIzol, followed by extraction with chloroform. After centrifugation, RNA contained within the aqueous phase was precipitated in isopropanol. Precipitates were washed once in 75% ethanol and then resuspended in water treated with diethylpyrocarbonate (Sigma). Semiquantitative RT-PCR was done as described in detail elsewhere (28). The number of amplification cycles was determined experimentally to yield a detectable amount while remaining within the linear range of the assay. The IL-2 primers used were as follows: IL-2 sense 5'-TGATGGACCTACAGGAGCTCCTGAG-3'; IL-2 antisense 5'-GAGTCAAATCCAGAACATGCCGAG-3'. The IL-4 primers used were as follows: IL-4 sense 5'-CGAAGAACACCACAG-AGAGTGAGCT-3'; IL-4 antisense 5'-GACTCATTTCATGGTGCAGCTTATCG-3'. The β -actin primers were: β -actin sense 5'-TGGAATCCTGTGGCATCCATGAAC-3'; β -actin anti-sense 5'-TAAAACGCAGCTCAGTACAGTCCG-3'. Negative controls included amplification of a sham reverse transcription reaction (incubated without enzyme) and amplification of a reaction mixture with no added cDNA (data not shown). Positive controls included PCR reactions using a cytokine control plasmid (39). An aliquot of each PCR reaction mixture was electrophoresed on a 2% agarose gel, stained with ethidium bromide, and photographed. The sizes of the amplified PCR products were 167 bp for IL-2 primers, 180 bp for IL-4 primers, and 348 bp for β -actin primers, as expected (39).

RESULTS

Activation of CD4⁺ T Lymphocytes from IL-2-Deficient Mice by B7-1 or B7-2 Transfectants. Previous studies by

Schorle *et al.* (40) had demonstrated that T cells from IL-2-deficient mice can still proliferate in response to T-cell receptor (TCR) stimuli, although at significantly reduced level. Our studies on IL-2^{+/+} mice (15) prompted us to ask whether B7-1 and/or B7-2 molecules were involved in the activation of T cells from IL-2^{-/-} mice. We initially asked whether B7-1 and B7-2 transfectants could costimulate the proliferation of purified IL-2^{-/-} CD4⁺ T cells. For this purpose, we purified CD4⁺ (>98% pure) T cells from either IL-2^{+/+} or IL-2^{-/-} mice. The resulting populations were assayed for their capacity to proliferate in response to soluble anti-CD3 mAb. A representative experiment is shown in Fig. 1. CHO-B7-1 transfectants could costimulate anti-CD3 mAb-induced proliferation of T cells from IL-2^{-/-} mice. The effect of CHO-B7-1 transfectants was specific, as it was blocked by anti-B7-1, but not by anti-B7-2 mAb (Fig. 1B, and data not shown). Moreover, control CHO transfectants did not support T-cell proliferation (Fig. 1B). Under maximal stimulation conditions [2×10^4 transfectants per well; 1/500 supernatant dilution (Sup Dil) of anti-CD3 mAb 145-2C11], B7-1 transfectants costimulated the proliferation of CD4⁺ T cells from IL-2^{-/-} mice at levels that were 31.2–61.2% of the proliferative response of IL-2^{+/+} CD4⁺ T cells. Addition of exogenous IL-2 was able to reconstitute the proliferative response of IL-2^{-/-} CD4⁺ T cells to wild-type levels (data not shown). Finally, as shown in Fig. 2, CHO-B7-2 transfectants could also costimulate the proliferation of CD4⁺ T cells from IL-2^{-/-} mice. As in the case of IL-2^{+/+} CD4⁺ T cells (28), CHO-B7-1 had slightly stronger costimulatory activity than CHO-B7-2 cells (Fig. 2).

B7-induced Costimulation of CD4⁺ T Cells from IL-2^{-/-} Mice Leads to Cell-Surface Expression of IL-2R α . We had previously shown that incubation of IL-2^{+/+} T cells with B7 transfectants not only leads to IL-2 production but also enhances the cell-surface expression of the IL-2 receptor (IL-2R) (15). Given our observation that B7 transfectants could costimulate the proliferation of IL-2^{+/+} CD4⁺ T cells, it was of interest to determine whether the transfectants could enhance TCR-induced cell-surface expression of IL-2R α . As for IL-2^{+/+} T cells, incubation of purified IL-2^{-/-} CD4⁺ T cells with ConA in the presence of CHO-B7-1 cells led to enhanced cell-surface expression of IL-2R α . In contrast, incubation of purified IL-2^{-/-} CD4⁺ T cells with ConA in the presence of vector-transfected CHO control cells had only a marginal effect on the IL-2R α surface expression (Fig. 3A, A5 and A6). Thus, the effect of B7-1 is specific. Incubation of purified T cells with CHO-B7-1 cells in the absence of a TCR stimulus did not enhance cell-surface expression of IL-2R α (data not shown).

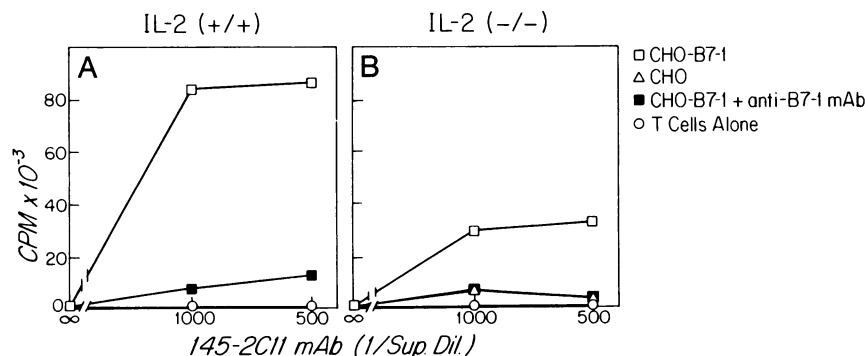


FIG. 1. Costimulation of T cells from IL-2-deficient mice by B7-1 molecules. Microcultures were set up with 10^5 purified CD4⁺ T cells from either IL-2^{+/+} mice (A) or IL-2^{-/-} mice (B). Where indicated, 2×10^4 mmC-treated CHO-B7-1 (□, ■) or CHO (△) cells were added to the cultures. Anti-B7-1 mAb 16-10A1 (2 μ g/ml) was added as indicated (■). As an additional control, T cells were incubated in the absence of CHO transfectants (○). Indicated on the x axis in A and B are the concentrations of anti-CD3 mAb 145-2C11 added to cultures. To assay T-cell proliferation, cultures in A and B were pulsed after 60 hr with 1 μ Ci of [3 H]thymidine per well for the last 14 hr of incubation. This figure represents one of six experiments. Sup Dil, supernatant dilution.

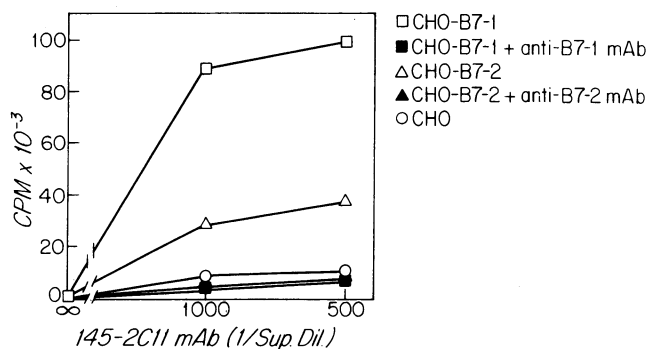


FIG. 2. Both B7-1 and B7-2 transfectants can costimulate activation of T cells from IL-2-deficient mice. Microcultures were set up with 10^5 purified CD4⁺ T cells from IL-2^{-/-} mice. Twenty thousand mmC-treated CHO-B7-1 (□, ■), CHO-B7-2 (△, ▲), or CHO (○) cells were added to the cultures. Anti-B7-1 mAb 16-10A1 (1 μg/ml, ■) and anti-B7-2 mAb GL1 (1 μg/ml, ▲) were added as indicated. Displayed on the x axis are the concentration of anti-CD3 mAb 145-2C11 added to cultures. All cultures were pulsed after 60 hr with 1 μCi of [³H]thymidine per well for the last 14 hr of incubation to assay T-cell proliferation. This experiment was done on a different day than that of Fig. 1 and is representative of five experiments.

B7-Induced Proliferation of CD4⁺ T Cells from IL-2^{-/-} Mice Is Independent of IL-4. Initial experiments confirmed

that IL-2^{-/-} CD4⁺ T cells did not produced detectable levels of IL-2 in response to costimulation with B7-1 or B7-2 transfectants, as determined by the HT-2 bioassay or by semiquantitative RT-PCR (Fig. 3B and data not shown). In subsequent experiments, we studied whether B7-costimulated proliferation of IL-2^{-/-} CD4⁺ T cells was due to IL-4 production (13, 17). However, at no point did IL-2^{-/-} CD4⁺ T cells secrete detectable levels of IL-4 in response to stimulation with anti-CD3 mAb and either CHO-B7-1 or CHO-B7-2 cells, as determined in the CT.4S assay (data not shown). Similarly, we were unable to detect IL-4 mRNA by semiquantitative RT-PCR (data not shown). However, the above experiments did not rule out the possibility that a very small amount of IL-4 was sufficient to promote the proliferation of CD4⁺ T cells from IL-2^{-/-} mice. To investigate this point further, we tested whether the B7-1- or B7-2-induced proliferation of T cells from IL-2^{-/-} mice could be blocked by anti-IL-4 mAb 11B11. As shown in Fig. 4A, this explanation could be excluded. In parallel cultures, 11B11 mAb blocked the proliferation of CT.4S cells to exogenous IL-4 very efficiently (Fig. 4B). Similar results were obtained when B7-2 transfectants were used instead of B7-1 transfectants (data not shown). Taken together, our findings demonstrate that the B7-induced proliferation of T cells from IL-2^{-/-} mice occurs independently of both IL-2 and IL-4.

Effect of Anti-γ_c mAb on B7-Induced Proliferation of IL-2^{-/-} CD4⁺ T Cells. Additional mAb blocking experiments

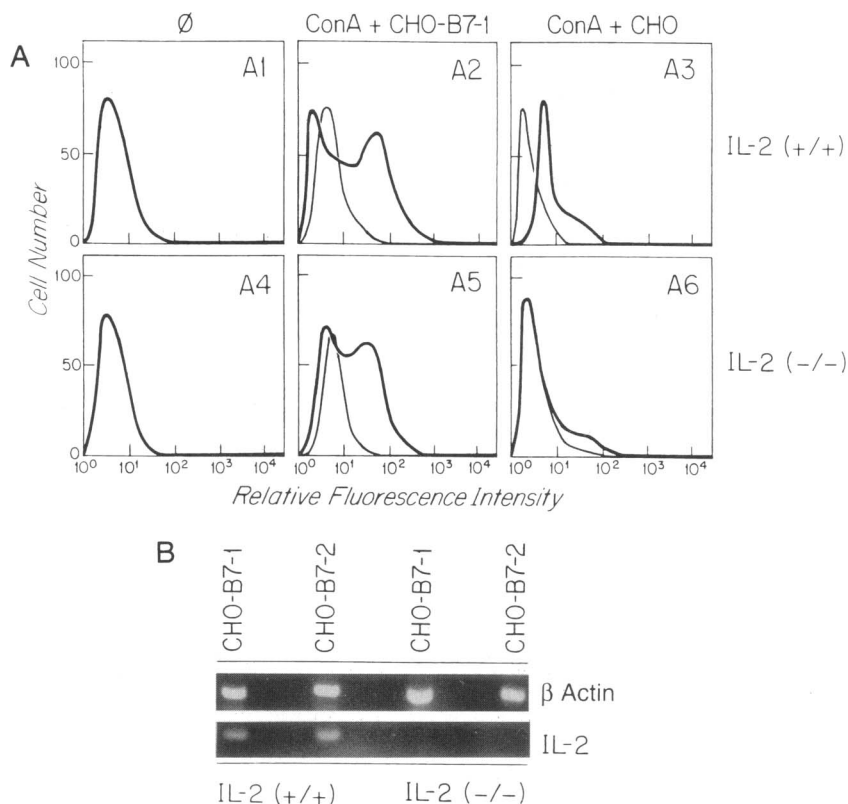


FIG. 3. (A) Costimulation with B7-1 transfectants induces cell-surface expression of the IL-2R α chain. Purified CD4⁺ T cells (10^7) from either IL-2^{+/+} mice (A1–A3) or IL-2^{-/-} mice (A4–A6) were incubated in 4 ml of culture medium in 6-well tissue-culture plates. T cells were either incubated with medium alone (∅; A1 and A4) or with the following additions: ConA at 1 μg/ml and 10^6 mmC-treated CHO-B7-1 cells (A2 and A5); ConA at 1 μg/ml and 10^6 mmC-treated CHO cells (A3 and A6). Cells were analyzed after 20 hr with the rat anti-mouse anti-IL-2 receptor mAb 7D4 followed by a second-step fluorescein isothiocyanate–goat anti-rat immunoglobulin antibody (Kirkegaard and Perry Laboratories) (thick line). Staining with second-step reagent only was done as a negative control (thin line). In A1 and A4, the thin and thick lines are superimposed. Samples were analyzed on a FACScan (Becton Dickinson). Five thousand cells were analyzed per sample. (B) Analysis of IL-2 mRNA levels in IL-2^{+/+} or IL-2^{-/-} CD4⁺ T cells by RT-PCR. Purified CD4⁺ T cells (5×10^5 cells) from IL-2^{+/+} or IL-2^{-/-} mice were incubated with 5×10^4 mmC-treated CHO-B7-1 or CHO-B7-2 cells and anti-CD3 mAb as indicated (1:1000 dilution of 145-2C11 hybridoma culture supernatant). Cells were harvested 9 hr after initiation of the cultures and RNA was isolated. Expression of IL-2 and β-actin was analyzed by semiquantitative RT-PCR as described. The induction of IL-2 mRNA in IL-2^{+/+} CD4⁺ T cells strictly depended on the addition of B7 transfectants and anti-CD3 mAb to the culture (data not shown). An experiment representative of three experiments is shown.

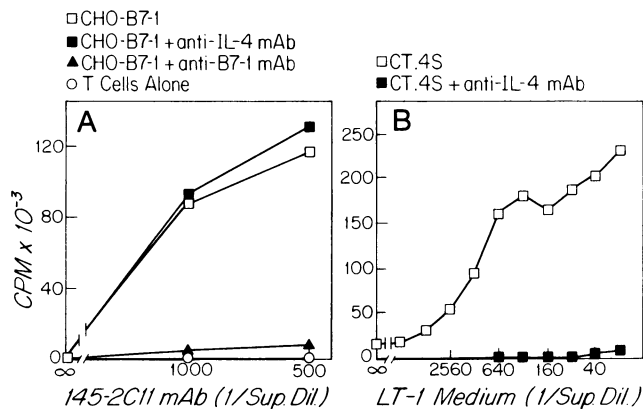


FIG. 4. B7-costimulated proliferation of T cells from IL-2^{-/-} mice is not blocked by anti-IL-4 mAb. (A) Microcultures were set up with 10⁵ purified CD4⁺ T cells and either 2 × 10⁴ mmC-treated CHO-B7-1 (□, ■, ▲) or medium (○). Where indicated, anti-B7-1 mAb (1 μg/ml 16-10A1, ▲) or anti-IL-4 mAb (1:1000 ascites diluted 11B11, ■) were added to cultures. The concentrations of anti-CD3 mAb added to the cultures are indicated on the x axis in A. Cultures in A were pulsed after 48 hr with 1 μCi of [³H]thymidine per well for the last 14 hr of incubation to assay T-cell proliferation. (B) Microcultures were set up with 10⁴ CT.4S cells, and the concentration of IL-4 (dilutions of LT-1 supernatant) is indicated on the x axis. Medium (□) or anti-IL-4 mAb (1:1000 dilution of 11B11 ascites, ■) were added to cultures. CT.4S cultures were pulsed after 48 hr with 1 μCi of [³H]thymidine per well for the last 14 hr of incubation. This experiment is representative of three experiments.

revealed that the B7-induced proliferation of IL-2^{-/-} CD4⁺ T cells was also not inhibited by mAbs to IL-7 (data not shown). In subsequent experiments we therefore asked whether B7-mediated activation of CD4⁺ T cells from IL-2^{-/-} mice involved the γ_c chain, a component of the receptors for at least IL-2, IL-4, IL-7, IL-9, and IL-15. For this purpose we determined whether IL-2^{-/-} T-cell proliferation could be blocked by the anti-γ_c mAb TUGm2 (36). In four independent experiments, we found only marginal suppression of the B7-1-induced proliferation of IL-2^{-/-} CD4⁺ T cells by the TUGm2 mAb. Even combinations of anti-IL-2Rβ and anti-γ_c mAbs, known to be optimal for the inhibition of wild-type T cells (36), resulted only in ≈20% inhibition of the B7-1-induced proliferation of T cells from IL-2^{-/-} mice (Fig. 5). In contrast, the same combination of reagents blocked the proliferation of IL-2^{+/+} T cells by ≈80% (Fig. 5). Similar results were obtained when CHO-B7-2 cells were used instead of CHO-B7-1 cells to costimulate the proliferation of IL-2^{-/-} CD4⁺ T cells (data not shown). We tested a range of concentrations of anti-IL-2Rβ and anti-γ_c mAb and found that concentrations other than those used in this experiment did not give qualitatively different results, using T cells from either IL-2^{-/-} or IL-2^{+/+} mice (data not shown). Taken together, the results of this and similar experiments suggest that most of the proliferation induced by B7 molecules occurs independently of cytokines that signal through γ_c.

Effects of Anti-B7-1 and B7-2 mAbs on the Anti-CD3-Induced Proliferation of IL-2^{-/-} Unfractionated Lymph Node Cells. Available evidence suggests that in the case of IL-2^{+/+} CD4⁺ T cells, B7-2 is a more important costimulatory ligand than B7-1 in polyclonal stimulation assays (41). To determine whether the activation requirements of IL-2^{-/-} T cells were similar to IL-2^{+/+} T cells, we carried out mAb inhibition studies in the presence of syngeneic antigen-presenting cells (APCs). For this purpose, IL-2^{-/-} or IL-2^{+/+} unfractionated lymph node cells were stimulated with anti-CD3 mAb. As expected (40), IL-2^{-/-} lymphocytes proliferated significantly less well than IL-2^{+/+} lymphocytes. In three independent experiments proliferative responses of IL-2^{-/-} lymphocytes were <20% of those from

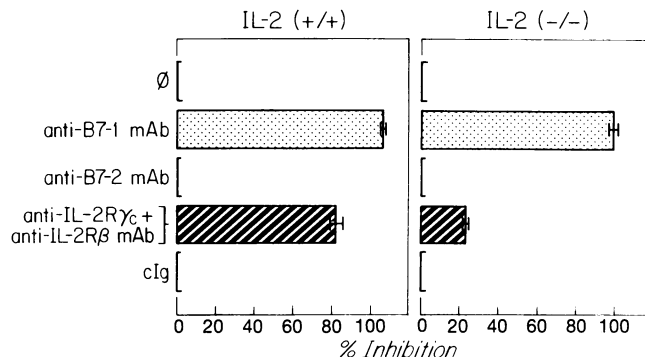


FIG. 5. Costimulation of T cells from IL-2-deficient mice by B7-1 molecules in the presence of anti-IL-2Rβ and anti-IL-2Rγ mAbs. Microcultures were set up with 2 × 10⁵ purified CD4⁺ T cells from either IL-2^{+/+} mice (Left) or IL-2^{-/-} mice (Right), 2 × 10⁴ mmC-treated CHO-B7-1 cells, and anti-CD3 mAb 145-2C11 [1:1000 supernatant dilution (Sup Dil)]. Anti-B7-1 mAb 16-10A1 (1 μg/ml), anti-B7-2 mAb GL1 (1:1500 ascites dilution), or a combination of anti-IL-2Rβ (4 μg/ml) and anti-γ_c mAb (1:100 ascites dilution, TUGm2 mAb) were added as indicated. Cultures in A and B were treated after 48 hr with 1 μCi of [³H]thymidine per well for the last 14 hr of incubation to assay T-cell proliferation. Indicated on the x axis is inhibition (%) of specific proliferation induced by anti-CD3 mAb and CHO cells. These control values (∅) were 67,675 cpm for IL-2^{+/+} T cells and 41,280 cpm for IL-2^{-/-} T cells. This experiment is representative of four experiments. cIg, control immunoglobulin.

normal littermates. A representative experiment is shown in Fig. 6. Importantly, as for IL-2^{+/+} lymphocytes (Fig. 6A; ref. 33), anti-B7-2 mAb was far superior to anti-B7-1 mAb at inhibiting the proliferation of IL-2^{-/-} lymphocytes (Fig. 6B). This result strongly suggests that the physiologic activation of T lymphocytes from IL-2^{-/-} mice is much more dependent on B7-2 than on B7-1.

DISCUSSION

IL-2-deficient mice develop a form of chronic IBD that closely resembles ulcerative colitis in humans. Although available evidence suggests that CD4⁺ T cells are intimately involved in disease induction, the activation requirements of IL-2^{-/-} CD4⁺ T cells have remained largely unexplored. Given the established role of the CD28/B7 costimulatory pathway in the expansion of IL-2^{+/+} CD4⁺ T cells, we have examined the potential role of B7-1 and B7-2 molecules in the activation of IL-2^{-/-} CD4⁺ T cells. The results of our experiments, using B7 transfectants as well as specific B7 antagonists, indicate that IL-2^{-/-} CD4⁺ T cells remain responsive to B7 stimulation and raise the possibility that B7 antagonists have a role in the prevention/treatment of inflammatory bowel disease.

The results of our studies using stable B7-1 and B7-2 transfectants show that B7-1 and B7-2 molecules are sufficient costimulatory ligands for the activation of CD4⁺ T cells from IL-2^{-/-} mice. The results of these experiments raise a number of interesting issues. (i) B7-1 transfectants were able to costimulate the proliferation of CD4⁺ T cells from younger as well as older (>3 mo) IL-2^{-/-} mice (data not shown). Thus even T cells from mice with significant proliferative disease were still responsive to stimulation with B7 transfectants. (ii) The observation that B7 transfectants can costimulate the cell-surface expression of the IL-2Rα chain in IL-2^{-/-} mice (Fig. 3A) extends our prior observations on normal CD4⁺ T lymphocytes (15) and formally demonstrates that the upregulation of IL-2Rα is not a mere consequence of the increased production of IL-2. (iii) As for IL-2^{+/+} T cells (28), CHO-B7-1 cells had a slightly higher costimulatory activity than CHO-B7-2 cells. This finding is consistent with previous binding

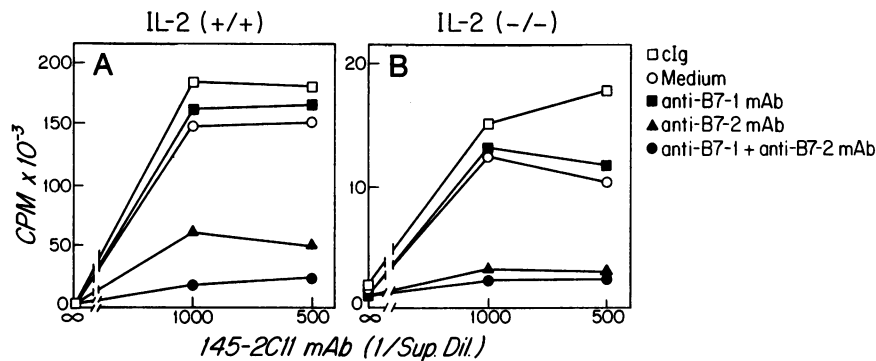


Fig. 6. Anti-B7-2 mAb GL1 blocks the anti-CD3 mAb-induced proliferation of T cells from IL-2^{-/-} mice. Microcultures were set up with 4×10^5 unfractionated lymph node cells from either IL-2^{+/+} mice (A) or IL-2^{-/-} mice (B). T-cell proliferation was induced by the addition of anti-CD3 mAb 145-2C11 as indicated on the x axis. Medium (○), irrelevant control antibody (1 μ g/ml; □), anti-B7-1 mAb 16-10A1 (1 μ g/ml, ■), anti-B7-2 mAb GL1 (1:1500 ascites dilution, ▲), or a combination of anti-B7-1 and anti-B7-2 mAb (●) were added to the cultures. Cultures were pulsed after 56 hr with 1 μ Ci of [³H]thymidine per well for the last 14 hr of incubation to assay T-cell proliferation. To resolve the differences between the experimental groups of IL-2^{-/-} lymphocytes, B is displayed at a scale different from A. cIg, control immunoglobulin.

measurements by Linsley *et al.* (42) in the human system, who have shown that B7-1 binds CD28 with 2- to 3-fold higher avidity than B7-2 (42), although we cannot rule out the possibility that CHO-B7-1 cells expressed costimulatory ligands at slightly higher levels than CHO-B7-2 cells. It is noteworthy that, where studied, we have not found differences in the patterns of cytokines induced by CHO-B7-1 and CHO-B7-2 transfectants (28). (iv) Our mechanistic studies indicate that the B7-induced proliferation of IL-2^{-/-} CD4⁺ T lymphocytes is independent of IL-4. This result was true in the case of both B7-1 and B7-2 transfectants. In fact, even IL-2^{+/+} CD4⁺ T lymphocytes from the backgrounds used in our study (C57BL6 \times 129) are low producers of IL-4 upon costimulation with B7 transfectants (H. R., unpublished work). A mAb to IL-7 also did not block the proliferation of IL-2^{-/-} CD4⁺ T cells (data not shown). The results of additional antibody blocking experiments raise the possibility that B7 antigens induce two pathways in IL-2^{-/-} CD4⁺ T cells, one that is γ_c -dependent and one that is γ_c -independent. This interpretation is consistent with recent experiments by Cao *et al.* (43), who have demonstrated that T cells from mice lacking the γ_c chain can respond to activation by B7 molecules, although to what extent the T-cell populations in IL-2- and γ_c -deficient mice are comparable remains to be determined.

We have also analyzed the activation of T cells from IL-2^{-/-} mice in the presence of syngeneic APCs. Consistent with the results of Schorle *et al.* (40), lymph node cells from IL-2-deficient mice responded significantly less well to anti-CD3 mAb than those from normal littermates. In fact, the differences between IL-2^{+/+} and IL-2^{-/-} lymphocytes in these experiments were more dramatic than in experiments in which transfectants were used to stimulate purified T cells. The most likely explanation for this difference is that our transfectants express B7 molecules higher than those present on normal APCs. In fact, when purified CD4⁺ T cells were stimulated with anti-CD3 mAb and CHO transfectants under more limiting conditions, differences in proliferative responses between IL-2^{+/+} and IL-2^{-/-} T cells became more dramatic (data not shown).

Finally, as in the case of IL-2^{+/+} mice, anti-CD3 mAb-induced activation of T lymphocytes by syngeneic splenic APCs appears to depend on B7-2 but not on B7-1 molecules, suggesting that physiologic activation of CD4⁺ T cells from IL-2^{-/-} mice involves a signal delivered by the B7-2 costimulatory ligand. This finding also raises the interesting possibility that B7 molecules participate in the initiation or maintenance of dysregulated immunity in IL-2-deficient mice. Finck *et al.* (44) have reported that treatment of lupus-prone NZB/NZW F₁ mice with B7

antagonists blocks autoantibody production and prolongs life expectancy.

We thank Dr. Baruj Benacerraf for encouragement and discussions. We are grateful to Dr. Richard Hodes (National Institutes of Health) and Dr. Kazuo Sugamura (Tohoku University School of Medicine) for providing valuable antibody reagents and to Dr. Ivan Horak (Universität Würzburg) and Dr. Steven Simpson (Division of Immunology, Beth Israel Hospital, Boston) for providing IL-2-deficient mice. This study was supported by Grant AI-33679 from the National Institutes of Health and by a Junior Faculty Research Award from the American Cancer Society (H.R.).

- Linsley, P. S., Clark, E. A. & Ledbetter, J. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5031–5035.
- Linsley, P. S., Brady, W., Urnes, M., Grosmaire, L. S., Damle, N. K. & Ledbetter, J. A. (1991) *J. Exp. Med.* **174**, 561–564.
- Freedman, A. S., Freeman, G., Horowitz, J. C., Daley, J. & Nadler, L. M. (1987) *J. Immunol.* **139**, 3260–3267.
- Freeman, G. J., Gray, G. S., Gimmi, C. D., Lombard, D. B., Zhou, L. J., White, M., Fingerhuth, J. D., Gribben, J. G. & Nadler, L. M. (1991) *J. Exp. Med.* **174**, 625–631.
- Croft, M., Bradley, L. M. & Swain, S. L. (1994) *J. Immunol.* **152**, 2675–2685.
- Azuma, M., Ito, D., Yagita, H., Okumura, K., Phillips, J. H., Lanier, L. L. & Somoza, C. (1993) *Nature (London)* **366**, 76–79.
- Freeman, G. J., Borriello, F., Hodes, R. J., Reiser, H., Gribben, J. G., Ng, J. W., Kim, J., Goldberg, J. M., Hathcock, K., Laszlo, G., Lombard, L. A., Wang, S., Gray, G. S., Nadler, L. M. & Sharpe, A. H. (1993) *J. Exp. Med.* **178**, 2185–2192.
- Freeman, G. J., Gribben, J. G., Boussiotis, V. A., Ng, J. W., Restivo, V. A., Jr., Lombard, D. A., Gray, G. S. & Nadler, L. M. (1993) *Science* **262**, 909–911.
- Boussiotis, V. A., Freeman, G. J., Gribben, J. G., Daley, J., Gray, G. & Nadler, L. M. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 11059–11063.
- Linsley, P. S., Brady, W., Grosmaire, L., Aruffo, A., Damle, N. K. & Ledbetter, J. A. (1991) *J. Exp. Med.* **173**, 721–730.
- Azuma, M., Cayabyab, M., Buck, D., Phillips, J. H. & Lanier, L. L. (1992) *J. Exp. Med.* **175**, 353–360.
- Damle, N. K., Klussman, K., Linsley, P. S. & Aruffo, A. (1992) *J. Immunol.* **148**, 1985–1992.
- Galvin, F., Freeman, G. J., Razi-Wolf, Z., Benacerraf, B., Nadler, L. & Reiser, H. (1992) *J. Immunol.* **149**, 3802–3808.
- Norton, S. D., Zuckerman, L., Urdahl, K. B., Shefner, R., Miller, J. & Jenkins, M. K. (1992) *J. Immunol.* **149**, 1556–1561.
- Reiser, H., Freeman, G., Razi-Wolf, Z., Gimmi, C., Benacerraf, B. & Nadler, L. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 271–275.
- Boise, L. H., Minn, A. J., Noel, P. J., June, C. H., Accavitti, M. A., Lindsten, T. & Thompson, C. B. (1995) *Immunity* **3**, 87–98.
- Freeman, G. J., Boussiotis, V. A., Anumanthan, A., Bernstein, G. M., Ke, X.-Y., Rennert, P. D., Gray, G. S., Gribben, J. G. & Nadler, L. M. (1995) *Immunity* **2**, 523–532.

18. Lenschow, D. J., Zeng, Y., Thistlethwaite, J. R., Montag, A., Brady, W., Gibson, M. G., Linsley, P. S. & Bluestone, J. A. (1992) *Science* **257**, 789–792.
19. Linsley, P. S., Wallace, P. M., Johnson, J., Gibson, M. G., Greene, J. L., Ledbetter, J. A., Singh, C. & Tepper, M. A. (1992) *Science* **257**, 792–795.
20. Turka, L. A., Linsley, P. S., Lin, H., Brady, W., Leiden, J. M., Wei, R., Gibson, M. L., Zhen, X., Myrdal, S., Gordon, D., Bailey, T., Bolling, S. F. & Thompson, C. B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 11102–11105.
21. Lin, H., Bolling, S. F., Linsley, P. S., Wei, R.-Q., Gordon, D., Thompson, C. B. & Turka, L. A. (1993) *J. Exp. Med.* **178**, 1801–1806.
22. Blazar, B. R., Taylor, P. A., Linsley, P. S. & Vallera, D. A. (1994) *Blood* **83**, 3815–3825.
23. Wallace, P. M., Johnson, J. S., Macmaster, J. F., Kennedy, K. A., Gladstone, P. & Linsley, P. S. (1994) *Transplantation* **58**, 602–610.
24. Sadlack, B., Merz, H., Schorle, H., Schimpl, A., Feller, A. C. & Horak, I. (1993) *Cell* **75**, 253–261.
25. Ma, A., Datta, M., Margosian, E., Chen, J. & Horak, I. (1995) *J. Exp. Med.* **182**, 1567–1572.
26. Simpson, S. J., Mizoguchi, E., Allen, D., Bhan, A. K. & Terhorst, C. (1995) *Eur. J. Immunol.* **25**, 2618–2625.
27. Powrie, F. (1995) *Immunity* **3**, 171–174.
28. Natesan, M., Razi-Wolf, Z. & Reiser, H. (1996) *J. Immunol.* **156**, in press.
29. Leo, O., Foo, M., Sachs, D. H., Samelson, L. E. & Bluestone, J. A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1374–1378.
30. Bhattacharya, A., Dorf, M. E. & Springer, T. A. (1981) *J. Immunol.* **127**, 2488–2495.
31. Gottlieb, P. D., Marshak-Rothstein, A., Auditore-Hargreaves, K., Berkoben, D. B., August, D. A., Rosche, R. M. & Benedetto, J. D. (1980) *Immunogenetics* **10**, 545–555.
32. Razi-Wolf, Z., Freeman, G. J., Galvin, F., Benacerraf, B., Nadler, L. & Reiser, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4210–4214.
33. Hathcock, K. S., Laszlo, G., Dickler, H. B., Bradshaw, J., Linsley, P. & Hodes, R. J. (1993) *Science* **262**, 905–907.
34. Ohara, J. & Paul, W. E. (1985) *Nature (London)* **315**, 333–336.
35. Malek, T. R., Robb, R. J. & Shevach, E. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5694–5698.
36. Kondo, M., Takeshita, T., Ishii, N., Nakamura, M., Watanabe, S., K.-i, A. & Sugamura, K. (1993) *Science* **262**, 1874–1877.
37. Julius, M. F., Simpson, E. & Herzenberg, L. A. (1973) *Eur. J. Immunol.* **3**, 645–649.
38. Reiser, H. (1990) *J. Immunol.* **145**, 2077–2086.
39. Platzter, C., Richter, G., Überla, K., Müller, W., Blöcker, H., Diamantstein, T. & Blankenstein, T. (1992) *Eur. J. Immunol.* **22**, 1179–1184.
40. Schorle, H., Holtschke, T., Hünig, T., Schimpl, A. & Horak, I. (1991) *Nature (London)* **352**, 621–624.
41. Guinan, E. C., Gribben, J. G., Boussiotis, V. A., Freeman, G. J. & Nadler, L. M. (1994) *Blood* **84**, 3261–3282.
42. Linsley, P. S., Greene, J., Brady, W., Bajorath, J., Ledbetter, J. A. & Peach, R. (1994) *Immunity* **1**, 793–801.
43. Cao, X., Shores, E., Hu-Li, J., Anver, M., Kelsall, B. L., Russel, S. M., Drago, J., Noguchi, M., Grinberg, A., Bloom, E. T., Paul, W. E., Katz, S. I., Love, P. E. & Leonard, J. (1995) *Immunity* **2**, 223–238.
44. Finck, B. K., Linsley, P. S. & Wofsy, D. (1994) *Science* **265**, 1225–1227.