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CD28-induced Costimulation of T Helper Type 2 Cells Mediated by Induction of Responsiveness to Interleukin 4

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Summary

Type 1 and type 2 cloned T helper (Th) cells are believed to require different antigen-presenting cell (APC)-derived costimuli for proliferation. In the case of Th1-cloned T cells, CD28 signaling costimulates production of autocrine interleukin 2 (IL-2). Th2 cells produce their autocrine growth factor, IL-4, without costimulation, but require APC-derived costimuli, or IL-1, to respond to IL-4. Here we demonstrate that engagement of CD28 on Th2 cells with anti-CD28 antibody or with APC-associated B7 costimulates Th2 responsiveness to IL-4 but does not affect IL-4 or IL-2 production by Th2 cells. Costimulation of Th2 cells via CD28 appears to involve the induction of IL-1 production by Th2 cells, which acts in an autocrine fashion to induce IL-4 responsiveness. These results suggest that CD28-induced costimulation plays an important role in responses mediated by both types of Th cells.
scribed previously. The T32 Th1 clone was kindly provided by Drs. T. Briner and M. Gefter (Massachusetts Institute of Technology, Cambridge, MA), and has been described previously (16). The HT2 indicator cell line has also been previously described (21). RPMI media was obtained from Bio Whittaker, Inc. (Walkersville, MD) and fetal bovine serum (FBS) from Sigma Chemical Co. (St. Louis, MO). The anti-CD28 mAb (37.51) was generously provided by Dr. J. P. Allison (University of California, Berkeley, CA) and has been described previously (22). CTLA4-Ig is a fusion molecule containing the cytoplasmic domain of mouse CTLA4 and the hinge, Cy2, Cy3, and Cy4 domains of human IgG1. CTLA4-Ig-expressing transfectants were generously provided by P. Lane (Basel Institute for Immunology, Basel, Switzerland). CTLA4-Ig was isolated from the culture supernatant of these cells using a protein A-Sepharose column. ITA receptor agonist (tLA) (23) and murine IL-2 were purchased from R&D Systems, Inc. (Minneapolis, MN) and Cetus Corp. (Emeryville, CA), respectively. Murine IL-4 and human IL-1α were generously provided by the DNAX Research Institute for Immunology, Basel, Switzerland). CTLA4-Ig was isolated from the culture supernatant of these cells using a protein A-Sepharose column. ITA receptor agonist (tLA) (23) and murine IL-2 were purchased from R&D Systems, Inc. (Minneapolis, MN) and Cetus Corp. (Emeryville, CA), respectively. Murine IL-4 and human IL-1α were generously provided by the DNAX Research Institute (Palo Alto, CA) and Hoffmann-La Roche (Nutley, NJ), respectively.

**Cell Culture Conditions.** The Th2 cell lines used in this study were maintained as described previously (19, 20). Briefly, 10^6 D10.G4 or CDC-35 cloned Th2 cells were restimulated weekly in 24-well plates with 200 μg/ml conalbumin (ConAlb) or 100 μg/ml rabbit gamma globulin (RGG), respectively, in complete RPMI media supplemented with 10% FBS, 2 × 10^5 T cell-depleted (CD4^−, CD8^−, Thy-1^−) C3H/He] or BALB/cByJ (Jackson Laboratories, Bar Harbor, ME) irradiated (3,000 rad) splenocytes, 5% Con A-stimulated rat spleen cell culture supernatant, and 25 mM α-methyl mannoside. At the end of a 7-d stimulation cycle, the T cells were isolated by centrifugation on a Ficoll-Isopaque gradient. Where indicated, Th2 cells were further purified by passing the cells over a nylon wool column to remove adherent cells, and IE^+^ cells were depleted by incubating the cells with an anti-IE mAb (14.4.4) (24) and complement. Viable Th2 cells were then isolated by centrifugation on a Ficoll-Isopaque gradient.

**Proliferation Assays.** For proliferation assays involving splenocytes, 10^5 Th2 cells were cultured with the indicated number of T cell-depleted irradiated (3,000 rad) syngeneic spleen cells, antigen, and the indicated additions for 48 h at 37°C in a total volume of 100 μl in 96A/2 flat-bottom microtiter plates (Costar, Cambridge, MA). T cell proliferation was determined by pulsing cultures with 0.5 μCi of [3H]thymidine for an additional 18 h, harvesting with a sample harvester (Skatron, Sterling, VA) and counting incorporated [3H]thymidine.

Antibody-coated plates were prepared with 96 A/2 flat-bottom microtiter plates (Costar) as described previously (25). In brief, wells were incubated with 50 μl PBS containing 4 μg/ml of the anti-α/β TCR mAb H57-597 (26) at 22°C for 2 h, followed by a postcoat with 5% FBS in PBS at 37°C for 30 min and multiple washes with PBS containing 5% FBS. 10^4 Th2 cells were cultured in mAb-coated wells with the indicated additions in a total volume of 100 μl for 48 h at 37°C, pulsed with 0.5 μCi of [3H]thymidine for 18 h, and harvested for [3H]thymidine counting.

**Lymphokine Assays.** HT2 cells (21) were obtained from A. Abbas (Harvard Medical School, Boston, MA) and maintained in complete media supplemented with 5% FBS, 10% Con A-stimulated rat spleen cell culture supernatant, and 25 mM α-methyl mannoside. To determine IL-2 and IL-4 levels, dilutions of T cell culture supernatants, harvested 24 h after T cell stimulation, were cultured with 2,000 HT2 cells in 96-well A/2 plates for 24 h at 37°C. The cultures were pulsed with 0.5 μCi of [3H]thymidine the last 18 h, and the [3H]thymidine incorporation was determined. IL-2 versus IL-4 content was determined by including 10 μg/ml of either purified anti-IL-4, 11B11 (27), or purified anti-IL-2, 5B4B6 (28) mAb in the cultures.

**Flow Cytometry.** 10^6 cells/ml were incubated in PBS containing 5% FBS alone or with anti-CD28 mAb (1/10 dilution of ascites fluid) for 30 min on ice, washed extensively, and incubated with a 1/12 dilution of FITC-conjugated anti-hamster IgG mAb (CALTAG Laboratories, South San Francisco, CA) for an additional 20 min on ice. After washing the cells extensively, they were analyzed on a FACS® IV analyzer (Becton Dickinson & Co., Mountain View, CA).

**IL-1 PCR Analysis.** RNA was prepared from stimulated D10.G4 or resident peritoneal cells with a guanidinium-isothiocyanate extraction in the presence of carrier bacterial rRNA and ultracentrifugation through a cesium chloride cushion (29). The D10.G4 cells were purified before activation by passage over nylon wool and depleted of IE^+^ cells to minimize contamination with APC (see above). Variable amounts of total cellular RNA corresponding to calculated input cell equivalents were subjected to RT-PCR to measure the levels of IL-1 transcripts. PCR was carried out using conditions suggested by the manufacturer of the IL-1 primers (Stratagene Inc., La Jolla, CA). To amplify IL-1α transcripts, reverse transcription was primed with an 820-843-bp antisense primer, and the cDNA was amplified with the same primer and a 301-324-bp sense primer. To amplify IL-1β transcripts, a 756-776-bp antisense primer and a 350-350-bp sense primer were used. The positive control β-tubulin transcripts were amplified with a 24-54-bp sense transcript and 210-239-bp antisense transcript. PCR was carried out for 50 cycles (30). The PCR products were analyzed by electrophoresis on a 4% agarose gel.

**Results**

**Anti-CD28 mAb Can Costimulate Th2 Cell Proliferation.** To determine if Th2 cells express surface CD28, the AKR-derived Th2 clone D10.G4 (19) and BALB/c-derived Th1 clone T32 were stained with mAb 37.51 (anti-CD28) (22) and complement. Viable Th2 cells were further purified by passing the cells over a nylon wool column to remove adherent cells, and IE^+^ cells were depleted by incubating the cells with an anti-IE mAb (14.4.4) (24) and complement. Viable Th2 cells were then isolated by centrifugation on a Ficoll-Isopaque gradient.

Engagement of the TCR by antigen–MHC class II complexes can be mimicked by aggregation of the TCR with the use of immobilized anti-TCR antibodies. To determine if anti-CD28 mAb (mAb 37.51) costimulated Th2 proliferation, D10.G4 and CDC-35 cells were cultured in wells coated with anti-pan TCR-β mAb, H57-597 (26) and variable concentrations of soluble anti-CD28 mAb. Anti-CD28 mAb costimulated vigorous proliferation of both T cell clones (Fig. 1, a and c). Both clones could also be costimulated by IL-1α (Fig. 1, see legend). As expected, little proliferation was observed when Th2 cells were cultured with anti-TCR mAb alone, or with anti-TCR mAb and an irrelevant control hamster mAb (anti-Vγ3 TCR) (Fig. 1, b and c). In no case did anti-CD28 mAb alone stimulate D10.G4 cell proliferation (data not shown). These experiments indicate that CD28 sig-
naling can substitute for exogenously added IL-1 in costimulating Th2 cell proliferation.

Costimulation of Th2 Cell Proliferation by Anti-CD28 mAb

Results from Stimulation of Responsiveness to IL-4. The response of D10.G4 cells stimulated with immobilized anti-TCR antibody and anti-CD28 mAb was IL-4 dependent. D10.G4 cells were cultured with plate-bound anti-TCR-β mAb and the indicated concentrations of soluble purified anti-CD28 mAb (11). Parallel sets of cultures also contained 10 μg/ml anti-IL-2 mAb, S4B6 (28) (Fig. 2 a). The proliferative responses of cultures stimulated with plate-bound anti-TCR-β mAb and 1 U/ml IL-4 were 39,729 cpm in the case of D10.G4 cells (b) and 55,426 cpm in the case of CDC-35 cells (c).

CD28-B7-mediated Costimulation Does Not Increase Growth Factor Production by Th2 Cells. CD28 costimulation with anti-CD28 mAb did not increase production of the growth factors IL-4 or IL-2 by Th2 cells. This was shown by testing the capacity of activated D10.G4 or CDC-35 cell culture supernatants to induce proliferation of HT-2 indicator cells (21), which respond to both IL-4 and IL-2 (4). The titration curves revealed that the IL-4 content of culture supernatants of CD28-costimulated cells was similar to that of cells stimulated by anti-TCR mAb alone (Fig. 3, a and b). The active growth factor in the culture supernatants was identified as IL-4 by inhibition of the proliferation of the HT-2 indicator cells with anti-IL-4 mAb but not with anti-IL-2 mAb (Fig. 3, a and b). Titrations of rIL-4 are shown for comparison. Therefore,
creased growth factor (IL-2) production by Th1 cells, it failed to increase the level of IL-4 production by these cells, although CD28-mAb-mediated costimulation induced increased growth factor (IL-2) production by Th1 cells, it failed to induce production of IL-2 by Th2 cells and did not increase the level of IL-4 production by these cells.

Some evidence suggests that Th2 cells costimulate proliferative responses of Th1 cells (31, 32), raising the possibility that Th2 cells express costimulatory molecules such as B7. These observations raise the possibility that Th2 cells can receive costimulatory signals from other Th2 cells in culture. However, the level of B7 expressed by Th2 cells is apparently insufficient to costimulate IL-4 responsiveness or proliferation of Th2 cells (Figs. 1 and 2). Nevertheless, the possibility remained that the interaction of CD28 on Th2 cells with B7 on other Th2 cells is necessary for IL-4 production by Th2 cells in our experiments. To test this possibility, we used the B7 antagonist CTLA4-Ig, a genetically engineered soluble fusion protein derived from CTLA4, a second high-affinity receptor for B7. A saturating dose of CTLA4-Ig, sufficient to prevent B7-mediated costimulation of resting T cells and Th1 cells ([33]; Fig. 6 and data not shown), was included in Th2 cultures stimulated with anti-TCR mAbs and the IL-4 content of the culture supernatants was subsequently assayed with the use of HT-2 indicator cells. A comparison of the titration curves revealed that the inclusion of CTLA4-Ig in

![Figure 3](image-url) Figure 3. CD28-B7 costimulation of Th2 cell proliferation does not affect IL-4 production. (a and b) Costimulation with anti-CD28 mAb does not increase IL-4 or IL-2 release by D10.G4 or CDC-35 cells. Cell culture supernatants of D10.G4 cells (a) or CDC-35 cells (b) were titrated for growth-promoting activity on the IL-2/IL-4-responsive HT2 indicator cells. Supernatants from cells activated with plate-bound anti-TCR-β mAb (4 μg/ml) or with anti-TCR-β mAb and 75 μg/ml soluble CD28 mAb (●) were tested. In both cases the active growth factor was IL-4, as shown by the inhibition of HT-2 proliferation with anti-IL-4 mAb (open symbols), but not anti-IL-2 mAb (hatched symbols). Titrations of IL-4 (initial sample concentrations were 190 U/ml (Fig. 3a) and 60 U/ml (Fig. 3b) are shown for comparison (Δ). The assays shown in a, b, and c were performed on separate days. (c and d) CTLA4-Ig does not inhibit IL-4 release by TH2 cells. Similar levels of HT-2 proliferation were induced by cell culture supernatants of D10.G4 cells (●) or CDC-35 cells (△) that were activated with plate-bound anti-TCR-β mAb (4 μg/ml) alone (●) or with anti-TCR-β mAb and 18 μg/ml CTLA4-Ig (△). Supernatants from D10.G4 or CDC-35 cells cultured with 10^5 T cell-depleted syngeneic splenocytes without antigen served as a control to show that IL-4 release required TCR engagement (Δ). The [3H]thymidine incorporation was determined 48 h later. The data in b and d are from the same experiment.

![Figure 4](image-url) Figure 4. Anti-CD28 mAb costimulation of Th2 cell proliferation is IL-1 dependent. (a and c) CD28 costimulation of anti-TCR stimulated Th2 cells is IL-1 dependent. D10.G4 (a) or CDC-35 (c) cells were stimulated with plate-bound anti-TCR-β mAb with or without the addition of 75 μg/ml purified anti-CD28 mAb. Parallel sets of cultures stimulated with plate-bound anti-TCR mAb and anti-CD28 mAb were also incubated with 250 ng/ml IL-1 receptor antagonist (IL1 RA) or a mixture of 10 μg/ml anti-IL-1-α mAb and 10 μg/ml anti-IL-1-β mAb (ANTI-IL1; a). The inhibition by IL-1 antagonists was not due to nonspecific toxicity because D10.G4 cell proliferation was restored with the addition of 5 U/ml IL-2 (IL1 RA/IL-2, 42,099 cpm; ANTI-IL1/IL-2, 24,885 cpm). Both D10.G4 and CDC-35 cells were depleted of IL^+ cells, and D10.G4 cells were further depleted of adherent cells as described in Materials and Methods. (b and d) Responsiveness of Th2 cells to IL4 is IL-1 dependent. D10.G4 (b) or CDC-35 (d) cells were stimulated with 5 U/ml IL-2 alone or with the further addition of 75 μg/ml purified anti-CD28 mAb, and the proliferative responses were measured. Parallel cultures contained in addition 250 ng/ml IL-1 receptor antagonist (IL1 RA) or a cocktail of 10 μg/ml anti-IL-1-α mAb and 10 μg/ml anti-IL-1-β mAb (ANTI-IL1; b). The inhibition by IL1 antagonists was not due to nonspecific toxicity because D10.G4 cell proliferation was restored with the addition of 5 U/ml IL-2 (IL1 RA/IL-2, 42,738 cpm; ANTI-IL1/IL-2, 26,180 cpm).
cultures of D10.G4 or CDC-35 cells had no effect on the levels of IL-4 release induced by anti-TCR mAb (Fig. 3, c and d). HT-2 proliferation induced by each of these culture supernatants was abrogated with the addition of an anti-IL-4 mAb, but not with an anti-IL-2 mAb (data not shown). Together these data suggest that IL-4 production by Th2 cells is independent of the B7-CD28 signaling pathway.

**CD28 Costimulation of Th2 Cells Is IL-1 Dependent.** Because responsiveness of Th2 cells to IL-4 was stimulated by either anti-CD28 mAb or by IL-1, it was possible that the effects of CD28 signaling were mediated indirectly by IL-1. IL-1 inhibitors were used to test this possibility. Vigorous D10.G4 cell proliferation was observed in cultures stimulated with anti-TCR mAb and anti-CD28 mAb, but the response was inhibited to background levels by purified IL-1 RA or by a mixture of anti-IL-1α and anti-IL-1β mAbs (Fig. 4 a). Similarly, the costimulation of CDC-35 cell proliferation with anti-CD28 mAb was inhibited by the addition of IL-1 RA (Fig. 4 c). The IL-1 inhibitors also prevented the induction by anti-CD28 mAb of enhanced responsiveness of D10.G4 and CDC-35 cells to exogenously added IL-4, in the absence of TCR engagement (Fig. 4, b and d). These results demonstrate that costimulation through CD28 of Th2 cell IL-4 responsiveness and proliferation involves an IL-1–dependent mechanism.

In the preceding experiments, no APC or other cells were added to the Th2 cell cultures, and possible contaminating APC were depleted by passage of the Th2 cells over nylon wool columns and/or cytotoxic elimination of MHC class II+ cells with anti-IE mAb plus complement (see legend to Fig. 4). These considerations suggest that the source of the IL-1 is the Th2 cells themselves.

To determine if IL-1 transcripts were induced in Th2 cells after culture with anti-CD28 mAb, RT-PCR analysis was performed. IL-1α transcripts were detected as a 265-bp PCR product in RNA from D10.G4 cells costimulated with anti-CD28 mAb (Th2/TCR/CD28), but not in RNA from cultures stimulated with anti-TCR-β mAb alone (Th2/TCR) (Fig. 5 a). This was confirmed by Southern blot analysis with an internal IL-1-α–radiolabeled probe (Fig. 5 b). Based on comparisons of the titration of the RNA samples in this and another experiment, IL-1α transcript levels were approximately 5–10-fold lower in costimulated Th2 cells than in LPS-activated peritoneal cells, a source of macrophages. Equivalent amounts of intact RNA were used, as shown by RT-PCR amplification of β-tubulin transcripts. It is unlikely that the IL-1α transcripts in the Th2 cultures arise from contaminating macrophages, because the LPS-activated macrophages, a potent source of IL-1, produce only 5–10 times more IL-1α transcripts than the Th2 cells. Furthermore, the Th2 cells had been depleted
of APC before stimulation (see Materials and Methods). Also arguing against the possibility that contaminating macrophages were the source of the IL-1 transcripts was the observation that no IL-1β transcripts (expected product, 447 bp) were detected in the costimulated Th2 cell RNA. IL-1β transcripts were easily detected in RNA from control-activated macrophages (Mφ/LPS). These results indicate that CD28 costimulation induces IL-1α (but not IL-1β) transcripts in Th2 cells, and suggests that induction of IL-1α production is the mechanism of CD28-mediated costimulation of Th2 cells.

Costimulation of Th2 Cells by Splenic APC Is Mediated by the CD28-B7 Pathway. The previous experiments demonstrate that engagement of CD28 with an mAb is costimulatory to Th2 cells, but do not indicate whether APC can costimulate Th2 cells via a B7–CD28–mediated interaction. To address this question, we investigated the effects of the B7 antagonist, CTLA4-Ig, on proliferation of Th2 cells induced by antigen and splenic APC (Fig. 6). CTLA4-Ig inhibited antigen-dependent proliferation of both D10.G4 and CDC-35 Th2 cells in a dose-dependent fashion (Fig. 6, a and b). The proliferative responses of the CTLA4-Ig–containing cultures were restored by addition of IL-2, demonstrating that the CTLA4-Ig was not toxic for the cells. The effects of CTLA4-Ig on these responses suggest that B7 signaling plays an important role in costimulation of Th2 cell proliferation by splenic APC.

The effects of CTLA4-Ig and IL-1 RA were compared for their capacity to inhibit proliferative responses of D10.G4 cells induced by antigen and splenic APC (Fig. 6 c). As in the previous experiment, CTLA4-Ig strongly blocked D10.G4 proliferation induced by antigen and APC. IL-1 RA also blocked D10.G4 proliferation, consistent with earlier reports (7) and with our finding that costimulation mediated by anti-CD28 mAb is an IL-1–dependent process. The combination of the two inhibitors was only slightly more inhibitory than either alone. The inhibition of D10.G4 proliferation by CTLA4-Ig and IL-1 RA was not due to toxicity of the reagents, because the addition of IL-2 restored the proliferative response. Taken together, these results indicate that B7–CD28–mediated costimulation plays an important role in the responses of Th2 cells to antigen presented by splenic APC. The dependence of the responses on IL-1 production, presumably of Th2 cells, suggests that IL-1 production occurs as an intermediate step.

Costimulation of Th2 Cell Proliferation by IL-1 Is Independent of the B7–CD28 Pathway. The preceding data do not address the possibility that IL-1– and CD28–mediated signals are both essential to synergistically activate Th2 cells. Although Th2 cells are costimulated by exogenous IL-1 in the absence of APC, Th2 cells may themselves express costimulatory molecules, as noted earlier. To determine whether costimulation of Th2 cells mediated by exogenous IL-1 requires B7–mediated interactions, we used the B7 antagonist CTLA4-Ig. The addition of increasing concentrations of CTLA4-Ig, up to 10 μg/ml, failed to inhibit the proliferative response costimulated by exogenous IL-1α (Fig. 7). This concentration of CTLA4-Ig is sufficient to strongly inhibit various B7–mediated costimulation events, including costimulation of Th1 cells ([34]; data not shown) and costimulation of Th2 cells by splenic APC (Fig. 6). Furthermore, titrations of IL-1α together with CD28 mAb revealed no synergy in induction of proliferation of Th2 cells stimulated with anti-TCR mAb (data not shown). Together these results suggest that costimulation in vitro by exogenous IL-1 is independent of B7–CD28 interactions.

Figure 6. B7-CD28 signaling is involved in stimulation of Th2 cells by splenic APC and antigen. CDC-35 (a) and D10.G4 (b) cells were stimulated with 2 × 10^6 T-depleted irradiated (3,000 rad) syngeneic splenocytes and antigen (RGG or Con Alb, respectively) and increasing concentrations of CTLA4-Ig (▲). As a control for toxicity of the CTLA4-Ig, the inhibition caused by the highest dose of CTLA4-Ig was reversed with the addition of 5 U/ml rIL-2 (O). The Th2 cells were also stimulated with 4 μg/ml plate-bound anti-TCR mAb and the indicated concentration of CTLA4-Ig (▲). (c) D10.G4 cells were stimulated with the indicated number of T-cell-depleted irradiated syngeneic splenocytes and 100 μg/ml antigen (Con Alb) (▲). Parallel sets of cultures contained 10 μg/ml CTLA4-Ig (▲), 250 ng/ml IL-1 RA (△), or both (▲), and the proliferative responses were determined by [3H]thymidine incorporation 48 h after stimulation. As a control for toxicity of the reagents, proliferation of antigen-stimulated D10.G4 cells inhibited with a mixture of CTLA4-Ig and IL-1 RA was restored by the further addition of 10 U/ml of rIL-2 (O).
Discussion

Based on the finding that IL-1 costimulates Th2 cell proliferation, it has often been assumed that Th2 cell activation requires costimulation by APC-derived IL-1. We have now shown that CD28–B7 interactions can costimulate proliferation of two independently derived Th2 clones. The costimulation of Th2 cell responses mediated by CD28 provides an explanation for the ability of cells that produce little or no IL-1, such as dendritic cells and activated B cells (18, 34), to costimulate Th2 cell proliferation. Both of these APC types express B7 (35, 36). Further studies will be necessary to determine whether a similar mechanism holds in the case of IL-4–dependent Th2 cells in vivo.

B7-mediated signaling is apparently the predominant source of Th2 costimulation in splenocyte populations, based on the demonstration that the B7 antagonist CTLA4-Ig strongly blocked Th2 cell proliferation. Although cells capable of IL-1 production (e.g., macrophages) are present among splenic APC, and could in principle directly costimulate Th2 cells by releasing IL-1, our results suggest that they fail to secrete sufficient IL-1 to trigger Th2 cells. Perhaps these cells lack a sufficient activating signal to induce maximal IL-1 release, at least in the in vitro culture system used. Instead, our results suggest that signaling via direct T cell–APC contact may be required for effective Th2 costimulation. Consequent autocrine IL-1 production, and IL-4 responsiveness. If this holds true for IL-4–dependent Th2 cells in vivo, these costimulatory requirements may help to tightly regulate antigen-specific responses of Th2 cells under steady state conditions. On the other hand, in cases of serious infections, where circulating IL-1 can achieve sustained high levels, the requirement for costimulation mediated by cell contact may be relaxed in the interests of promoting a vigorous and rapid response of Th2 cells. In this case, antigen presented even by B7-negative APC, such as resting B cells, could lead to a Th2 cell response.

Costimulation of Th1 cell proliferation has been previously shown to involve increased IL-2 production (5, 37). In contrast, the costimulation of Th2 cell proliferation by anti-CD28 mAb is not mediated through increased IL-2 or IL-4 secretion. Rather, CD28 costimulation induces IL-1 production, apparently by the Th2 cells themselves, which then acts in an autocrine fashion to induce increased responsiveness of the cells to IL-4. It is unlikely that APC contamination was responsible for the IL-1 production in our experiments, because APCs were depleted from the Th2 population and because IL-1 was absent in the D10.G4 RNA preparations.

We do not yet know whether costimulation of IL-1 production by Th2 cells involves induction of IL-1 gene transcription and/or stabilization of IL-1 transcripts. Both mechanisms are involved in costimulation of IL-2 gene expression in Th1 cells by CD28 mAb (14, 39). Regulated stabilization of cytokine transcripts has been associated with the presence of specific sequence repeats (AUUUA) in the 3′ untranslated segment of the mRNA. These sequences have been found in all of the transcripts previously known to be stabilized by CD28 mAb costimulation (40, 41). Interestingly, such sequences are also found in the 3′ untranslated region of the IL-1α transcript (40), suggesting that message stabilization may be involved in costimulation of IL-1 production via CD28 signaling.

Other groups have reported that Th2 cells synthesize IL-1 (42, 43). However, Zubiaga et al. (43) reported that exogenous costimuli are not required for proliferation of Th2 cells cultured at high cell densities. Under those conditions, Th2 cells reportedly produce IL-1α and proliferate after stimulation with a variety of mitogens. This effect may be related to the findings that Th2 cells, in sufficiently high numbers, can costimulate proliferative responses of Th1 cells (31, 32), suggesting that Th2 cells can express costimulatory ligands. Presumably, the dependence of our responses on exogenous costimulation is due to the use of lower Th2 cell concentrations. In vivo, it is likely that the concentrations of activated costimulatory Th2 cells are too low in most circumstances to circumvent the necessity for APC-derived costimuli.

Th1 cells can also proliferate in response to exogenously provided IL-4. Interestingly, responsiveness of Th1 cells to IL-4 was shown to depend on a costimulatory signal that is sensitive to chemical fixation (44). Th1 cell responsiveness to IL-4 was not mediated by IL-2. Although the identity of the costimulatory molecule mediating IL-4 responsiveness by Th1 cells has not yet been reported, it appears likely to be B7. Interestingly, in contrast to Th2 cells, there is no evidence to indicate that Th1 cells produce IL-1 or that they can respond to it (7, 8). In fact, Th1 cells reportedly lack cell surface receptors for IL-1 (7, 45). These considerations raise the possibility that IL-4 responsiveness of Th1 cells is mediated by a distinct mechanism from that of Th2 cells.

With the addition of our results, it appears that CD28–B7–mediated costimulation plays a role in the activation of most if not all types of T cells, suggesting that this signaling mechanism arose early in the evolution of T cells. A practical vein, these findings enhance optimism that therapeutic approaches based on blocking the B7 costimulation pathway of Th cells will be effective. A therapeutic approach with particular promise is based on the premise that blocking costimulatory signals during immune responses to transplanted tissue, autoantigens, or allergens will render the individual
tissue, autoantigens, or allergens will render the individual unresponsive to subsequent challenges. However, anergy induction has not been demonstrated in the case of Th2 cells, raising some doubts as to whether the approach would be effective in the case of Th2-dependent responses. With the knowledge that Th2 cells are responsive to CD28–B7-mediated costimulatory signals, the possibility that blockade of B7 will lead to anergy of Th2 cells can be directly examined.

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