Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 3845–3849, May 1992
Immunology

Cells that present both specific ligand and costimulatory activity are the most efficient inducers of clonal expansion of normal CD4 T cells

YANG LIU* AND CHARLES A. JANEWAY, JR.

Section of Immunobiology, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510

Communicated by Edward Boyse, December 31, 1991 (received for review November 1, 1991)

ABSTRACT **Clonal expansion of naive CD4 T cells is a necessary step in most adaptive immune responses. Two distinct signals are required for clonal expansion to occur, ligation of T-cell receptors by an antigenic peptide bound to self major histocompatibility complex-encoded class II molecules (signal 1) and a costimulatory signal derived from an antigen-presenting cell (signal 2). To study whether these two signals need to be delivered by a single cell in order to induce clonal expansion of normal CD4 T cells, we have used anti-CD3 bound to Fc receptors as a ligand for the T-cell receptor to deliver signal 1 to all CD4 T cells, and we have inactivated signal 2 with a newly generated monoclonal antibody or by using Fc receptor-positive cells that lack the costimulator. Costimulation was delivered by cells whose Fc receptors were blocked with anti-Fc receptor monoclonal antibody. Our results indicate that delivery of ligand and costimulator on one cell is at least 30-fold more efficient than separate delivery. No significant clonal expansion was observed when signals 1 and 2 were delivered by different cells. We have also carried out experiments using fibroblast transfectants that can deliver either or both of these two signals. These studies show that separate delivery of these two signals is at least 80-fold less efficient than their combined delivery by one cell. These findings may explain why tissues can express autoantigens and contain active antigen-presenting cells without inducing autoimmunity.**

An essential event in the induction of virtually all adaptive immune responses is the clonal expansion of T lymphocytes and their differentiation to effector function (1). CD4 T cells are induced to clonally expand by antigen-presenting cells (APCs) that present peptide fragments of foreign antigens bound to class II molecules of the major histocompatibility complex, to CD4 T-cell receptors (TCR) (2). To induce clonal expansion of CD4 T cells, the APCs must also deliver a costimulatory signal (3, 4). Many studies indicate that stimulation of mature T cells via their TCR in the absence of a costimulatory signal leads to clonal inactivation in the form of clonal anergy (3, 5) or cell death (6). It is believed that this clonal inactivation mechanism plays a critical role in maintaining tolerance to most tissues (7). If clonal inactivation is to mediate self-tolerance *in vivo*, normal mature CD4 T cells must clonally expand only when both specific ligand and costimulatory activity are expressed by one cell. If this were not the case, professional APCs resident in tissues could provide costimulation to T cells recognizing self-antigens on the surface of tissue cells. Alternatively, separate encounters with cells expressing one or the other of these activities could induce T-cell activation. Either mechanism would be a threat to self-tolerance.

Studies on cloned T-cell lines have been interpreted to suggest that ligand and costimulator can be delivered by separate cells (5, 8–10), although the efficiency of this process has not been analyzed in detail. However, the critical question of whether one cell must present both ligand and costimulator to normal T cells in order to induce their initial clonal expansion has not been answered. We have examined this question by mixing cells that separately deliver these two signals to normal CD4 T cells. We find that clonal expansion of normal CD4 T cells is induced when both signals are expressed on the surface of the same APC, but does not occur when these are delivered on separate cells. This finding has important implications both for self-tolerance and for immunological surveillance of tissues.

MATERIALS AND METHODS

Mice, Cell Lines, and Antibodies. Male CBA/CaJ and BALB/cByJ mice were purchased from The Jackson Laboratories (Bar Harbor, ME) and used when 6–10 weeks old. The B-lymphoma cell line A20 (11) was cultured with Click's EHAA medium containing 5% fetal bovine serum. D10.G4.1, a cloned T_{h2} helper T-cell line, was generated in this laboratory and has been fully described (12). CTLL-2 cells were purchased from American Type Culture Collection and cultured with Click's EHAA medium containing 5% fetal bovine serum and 1% T-cell growth factor (12). Monoclonal antibodies (mAbs) against Fc receptor (FcR) (mAb 2.4G2; ref. 13) and heat-stable antigen (mAb 20C9; ref. 14) were purified from hybridoma supernatants through a protein G column. Anti-CD3 mAb YCD3-1 (15) was used as hybridoma supernatant.

Preparation of CD4 T Cells and Accessory Cells. Spleen CD4 T cells and B cells were prepared from CBA/CaJ and BALB/c mice as described (4). CD4 T-cell preparations contained >95% CD4 T cells and undetectable levels of $CD8^+$, Mac-1^+, or Ia^+ cells, and such cells do not respond to soluble anti-CD3 mAb when cultured at densities of up to 5×10^5 cells per well. Spleen B cells were activated with bacterial lipopolysaccharide (LPS, 10 μg/ml; *Escherichia coli*; Sigma) for 48 hr, and viable cells were isolated by centrifugation on lymphocyte separation medium (Organon Teknika–Cappel) and were either irradiated (2000 rads; 1 rad = 0.01 Gy) or fixed with 1% paraformaldehyde prior to being used as accessory cells (4). As specified in the text, mAbs 2.4G2 and 20C9 (100 μg/ml) were coated onto activated B cells (3×10^7 per ml) at 4°C for 60 min and washed extensively. These cells were then fixed with 1% paraformaldehyde and used as accessory cells.

Proliferation and Cytokine Assays. Proliferation of CD4 T cells was determined in 96-well tissue culture plates (4). In brief, CD4 T cells (10^5 per well) were mixed with various numbers of fixed accessory cells and anti-CD3 supernatant (1:40) and cultured in Click's EHAA medium containing 5%

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

Abbreviations: APC, antigen-presenting cell; FcR, Fc receptor; IL-4, interleukin 4; LPS, lipopolysaccharide; mAb, monoclonal antibody.

*Present address: Division of Immunology, Department of Pathology, New York University Medical Center, New York, NY 10016.

fetal bovine serum for 42 hr. These cultures were incubated with [^{3}H]thymidine (1 μCi per well; 1 μCi = 37 kBq) for an additional 6 hr, after which cells were harvested and [^{3}H]thymidine incorporation was determined. For the alloreactive response, CD4 T cells (5×10^5 per well) from BALB/cByJ mice were incubated for 96 hr with given numbers of either LPS-activated B cells (LPS-B) or LPS-activated B cells coated with anti-FcR (B-2.4G2) or 20C9 (B-20C9) prior to fixation, and the cultures were incubated with [^{3}H]thymidine for an additional 16 hr. Cytokine production by D10.G4.1 was determined on CTLL-2 cells (4).

Transfection of Fibroblasts with FcRIIB2 and B7 Genes. FcRIIB2 expression was determined using mAb 2.4G2 and B7 expression was determined using CTLA4Ig (kindly provided by Peter S. Linsley; ref. 16), which specifically binds to human B7. COS cell transfection was carried out by using DEAE-dextran as described (17). Three milligrams of either human B7 (18) or murine FcRIIB2 (19) cDNA or a mixture of the two was transfected per dish (60 × 15 mm) of COS cells. Forty-eight hours after transfection, COS cells were detached from the Petri dish with phosphate-buffered saline containing 0.5 mM EDTA and used either for staining or for functional assay. COS cells were treated with mitomycin C (100 μg/ml) for 1 hr at 37°C prior to use as accessory cells. CHO cell transfection using Lipofectin (BRL/Life Technologies) was carried out as described (20). Ten milligrams of human B7-pCDM8 cDNA was mixed with 1 mg of EBOpLPP vector carrying a hygromycin-resistance gene and transfected by lipofectin. Transfectants were selected with hygromycin (0.4 mg/ml). Positive cells were selected by fluorescence-activated cell sorting.

RESULTS

The major obstacle to studying clonal expansion of normal CD4 T cells is the low frequency of antigen-specific naive CD4 T cells. To avoid this problem, we have used anti-CD3 to ligate the receptor on all CD4 T cells, thus allowing us to characterize the signals required to induce clonal expansion of normal CD4 T cells. As APCs, we have used B cells activated with LPS for 48 hr and then fixed with paraformaldehyde. These fixed accessory cells will stimulate proliferation of purified CD4 T cells from normal syngeneic mice in the presence of anti-CD3 (Fig. 1*a* and ref. 4). This process requires two distinct activities in the APC: expression of a receptor for the Fc piece of the anti-CD3 (Fig. 1*a*) that can be blocked by anti-FcR antibody added prior to fixation, and a costimulatory activity that is fixation-sensitive on normal B cells but becomes fixation-resistant upon activation with LPS (4). The anti-FcR mAb does not interfere with costimulatory activity on these fixed LPS-activated B cells, as seen by its lack of effect on the response of normal CD4 T cells to allogeneic fixed LPS-activated B cells (Fig. 1*b*). However, the FcR is effectively blocked by anti-FcR treatment, as shown by the complete loss of the ability of antibody-treated cells to crosslink anti-TCR antibody on the D10 cloned T-cell line as measured by IL-4 production (Fig. 1*c*), a function that is independent of costimulation (12). Thus, these studies show that fixed LPS-activated B cells can provide ligand in the form of crosslinked anti-CD3 and a costimulatory activity required to induce clonal expansion of normal CD4 T cells. Earlier studies have shown that the predominant CD4 T cells responding in this assay are naive CD4 T cells expressing the high molecular weight isoforms of CD45 characteristic of such cells in mice, rats, and humans (4, 21, 22). Thus, this assay provides a good model for the induction of clonal expansion in naive CD4 T cells.

In a previous study, we showed that fixed cells of the FcR-bearing B-lymphoma line A20/2J could crosslink anti-CD3 but lacked a costimulatory activity required to induce normal CD4 T-cell proliferation (4). This allowed us to use two fixed APCs to separately deliver TCR crosslinking and costimulatory activity. Neither fixed anti-FcR treated, LPS-activated B cells that have costimulatory activity nor fixed A20 cells that have FcR but lack costimulatory activity can induce CD4 T-cell proliferation, and a mixture of these two cell types also lacks the ability to induce CD4 T-cell growth (Fig. 1*d*). When the response of CD4 T cells to this cell mixture is compared with the sum of the responses of CD4 T cells to each APC measured separately, little synergy between APCs is observed; the mixture of cells is at least

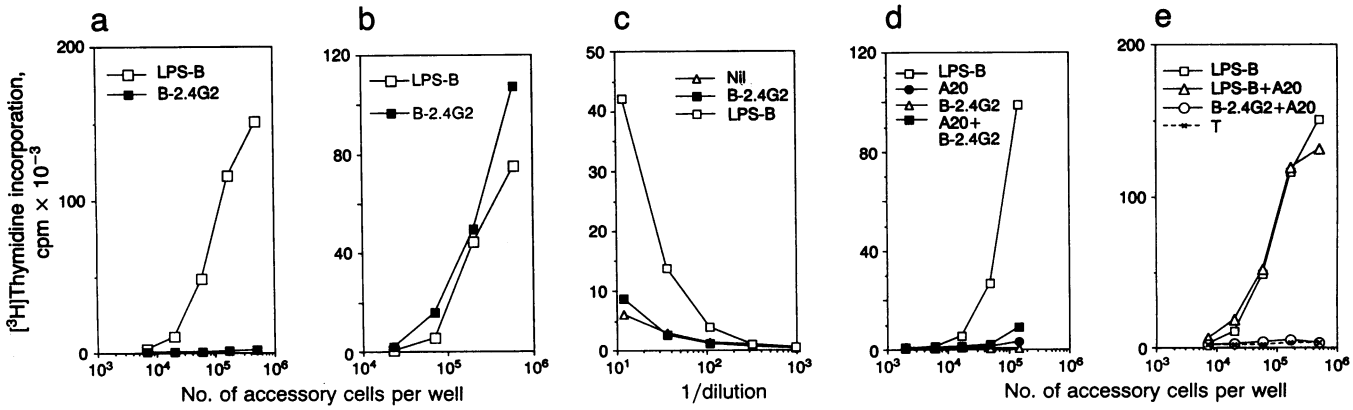

FIG. 1. Relative efficiency of separate versus linked delivery of a TCR ligand and costimulatory activity in inducing clonal expansion of normal CD4 T cells, with fixed A20 cells and anti-FcR mAb-treated, LPS-activated B cells as accessory cells. (*a*) Proliferation of CBA/CaJ CD4 T cells in response to anti-CD3 mAb YCD3-1 in the presence of either syngeneic fixed LPS-activated B cells (LPS-B, □) or LPS-activated B cells that have been treated with anti-FcR mAb 2.4G2 prior to fixation (B-2.4G2, ■). (*b*) Proliferation of allogeneic BALB/c ByJ (H-2^d) CD4 T cells in response to fixed LPS-B (□) and to B-2.4G2 (■). Data shown are means of duplicates with proliferation of CD4 T cells alone subtracted. (*c*) Efficacy of crosslinking anti-CD3 by LPS-B and B-2.4G2 as measured by their ability to enhance interleukin (IL-4) production by D10 cells. D10 cells (3×10^4 per well in 48-well plates) were stimulated with a 1:1000 dilution of YCD3-1 supernatant and no accessory cells (Nil, △) or 10^5 fixed B-2.4G2 (■) or LPS-B (□) cells per well. The supernatants were harvested 24 hr after stimulation, and IL-4 activity of dilutions of the supernatants was measured on CTLL-2 cells. The identity of IL-4 was confirmed with anti-IL-4 mAb 11B11 (data not shown). (*d*) Interaction of A20 and B-2.4G2 in anti-CD3-induced proliferation of CBA/CaJ CD4 T cells. Various numbers of fixed accessory cells (LPS-B, □; A20, ●; B-2.4G2, △; A20 plus B-2.4G2, ■) were incubated with CD4 T cells (10^5 per well) and 1:40 a dilution of anti-CD3 supernatant. For the group with two types of accessory cells, the two cell types were mixed at a 1:1 ratio and the numbers indicated are that of each cell type. (*e*) As in *d*, but the number of A20 cells used was constant (10^5 per well), while the *x* axis indicates the number of LPS-B (△) or B-2.4G2 (○) and the broken line represents the sum of cpm in groups containing the same numbers of each cell type added separately (×). □, LPS-B without A20.

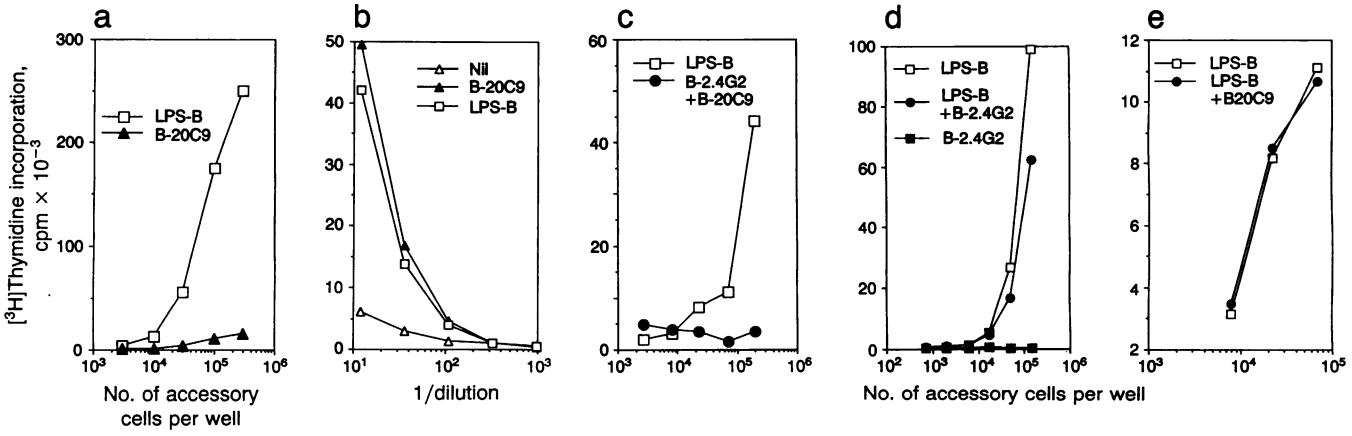

FIG. 2. Relative efficiency of separate versus linked delivery of a TCR ligand and costimulatory activity in the induction of clonal expansion of normal CD4 T cells, with mAb-treated, LPS-activated B cells as accessory cells. (*a*) Incubation of 20C9 (100 μg/ml) with LPS-B prior to washing and fixation inhibits anti-CD3-induced proliferation of CD4 T cells. (*b*) IL-4 production by D10 cells induced by anti-CD3 mAb in the presence of B-20C9 (▲) or LPS-B (□) or no accessory cells (Nil, △). (*c*) Lack of cooperation of B-2.4G2 and B-20C9 in CD4 T-cell proliferation. CD4 T cells (10^5 per well) were incubated with anti-CD3 mAb (1:40 dilution of hybridoma supernatant) and various numbers of accessory cells (LPS-B, □; B-2.4G2 plus B-20C9, ●). The cell numbers shown were those of each individual cell type. (*d*) Effect of fixed B-2.4G2 on accessory cell activity of fixed LPS-B; for details see *c*. (*e*) Effect of fixed B-20C9 on accessory cell activity of fixed LPS-B.

30-fold less efficient than fixed LPS-activated B cells and may lack activity altogether (Fig. 1*e*).

The results of these studies suggest that TCR crosslinking and delivery of a costimulatory signal must occur on the same APC surface in order to induce clonal expansion of CD4 T cells. However, the failure to observe clonal expansion of normal CD4 T cells when fixed A20 cells are used alone leaves some doubt as to the potency of the signal delivered via the FcR of these cells, although previous studies have revealed equivalent functional activities of FcR on fixed A20 and fixed LPS-activated B cells (4). To address this problem, we used the newly generated anti-heat-stable antigen mAb 20C9, which can inhibit the proliferation of CD4 T cells by blocking the delivery of a costimulatory activity (14). Treatment of LPS-activated B cells with 20C9 inhibits their ability to induce the proliferation of CD4 T cells (Fig. 2*a*). This treatment does not affect the crosslinking of the anti-CD3 mAb on T cells as it does not reduce cytokine production by D10 cells induced by anti-CD3 mAb and LPS-activated B cells as a crosslinker (Fig. 2*b*). Thus, by treating LPS-activated B cells with specific mAbs that inhibit either the FcR or costimulation, we could again ask whether TCR crosslinking and costimulatory signal had to be delivered by one cell, or whether a mixture of the two cells each blocked by one of these antibodies could synergize in this assay. LPS-activated B cells treated with either antibody prior to fixation did not induce significant CD4 T-cell proliferation in the presence of anti-CD3 (Figs. 1*a* and 2*a*). More important, a mixture of LPS-activated B cells treated independently with anti-FcR or with 20C9 prior to fixation did not stimulate CD4 T-cell proliferation in this assay (Fig. 2*c*). The failure of these cells to stimulate is not due to leakage of antibodies from one APC to the other, as shown by the failure of the singly treated APCs to inhibit CD4 T-cell stimulation when mixed with untreated APCs (Fig. 2 *d* and *e*).

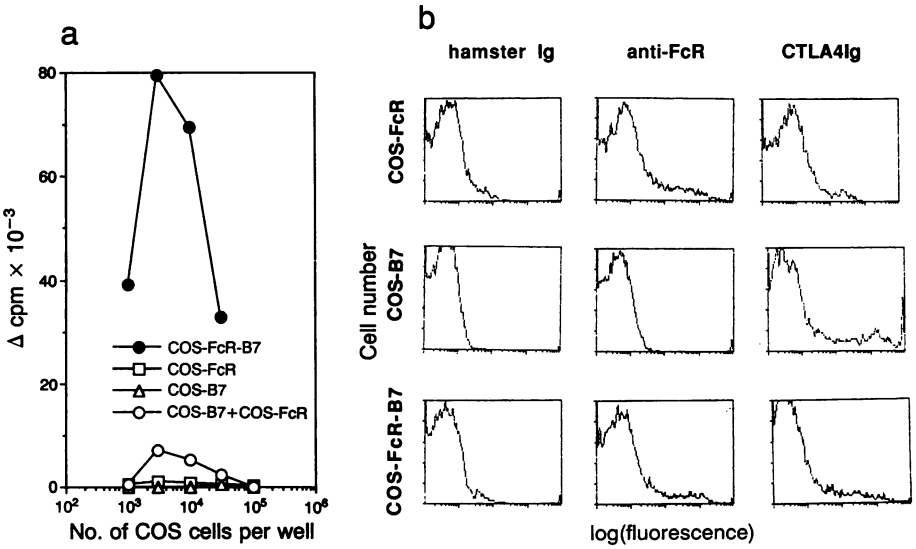

FIG. 3. (*a*) Relative efficiency of separate versus linked delivery of a TCR ligand and costimulatory activity in inducing clonal expansion of normal CD4 T cells using as accessory cells COS cells transfected with B7 (△), FcRIIB2 (□), or both genes (●) or using a mixture of COS-B7 and COS-FcRII transfectants (○). Various numbers of mitomycin C-treated COS cell transfectants were mixed with CD4 T cells (10^5 per well) from CBA/CaJ mice, and proliferation was determined at 48 hr as described in the legend to Fig. 1. When a mixture of two transfectants was used in one well, the numbers represent those of each cell type. (*b*) Expression of B7 and FcRIIB2 on the COS cell transfectants as determined by a FACScan instrument (Beckman). FcRIIB2 expression was determined with mAb 2.4G2, and B7 expression was determined with CTLA4Ig.

Recently, Linsley *et al.* (20) have demonstrated that fibroblasts transfected with cDNA encoding the human B-cell activation antigen B7 have costimulatory activity for human T cells. As shown in Fig. 3*a*, COS cells cotransfected with human B7 and FcRIIB2 cDNA provide excellent stimulation for murine CD4 T cells in contrast to COS cells transfected only with FcR cDNA. This allowed us a third system using unfixed cells in which to test whether costimulator and TCR ligand need to be on one cell to induce optimal clonal expansion of normal CD4 T cells. Using COS cells transfected with either B7, FcR, or both, we found that a mixture of COS cells transfected with either B7 or FcR failed to induce proliferation of T cells (Fig. 3*a*). Flow cytofluorimetry indicates that the expression of FcR and B7 is comparable on the double transfectant and on single transfectants (Fig. 3*b*).

Taken together, these studies using mixtures of APC that can deliver either TCR crosslinking or costimulatory activity, but not both, clearly indicate that induction of clonal expansion of normal CD4 T cells requires one cell to deliver both signals. These data appear to conflict with earlier results obtained by using cloned or activated T cells (5, 8–10), where separate expression of the TCR ligand and costimulatory signals were sufficient to induce proliferation (although the efficiency of this process has not been reported, it appears to be low; ref. 3). This difference in results could be due to differences in the activation state of the responder T cells used. The requirement of the naive CD4 T cells for the coexpression of the costimulator and TCR ligand on one cell could reflect a requirement for receiving these two signals at the same time or a higher activation threshold of naive CD4 T cells. Either of these requirements could be altered after the naive CD4 T cells become activated. The cloned T cells used in earlier studies were activated T cells, whereas the normal CD4 T cells that respond in our model system are mainly CD4 T cells with a CD45 phenotype consistent with that of naive T cells (4). To determine whether activated CD4 T cells respond to costimulatory signals differently from normal T cells, we preactivated normal CD4 T cells with anti-CD3 mAb. As shown in Fig. 4*a*, activated T cells proliferated when added to CHO cells transfected with B7, in agreement with experiments of Linsley *et al.* (20) using phytohemagglutinin-stimulated blast cells, whereas normal CD4 T cells do not respond in such cultures. Parental CHO cells did not stimulate activated CD4 T-cell proliferation.

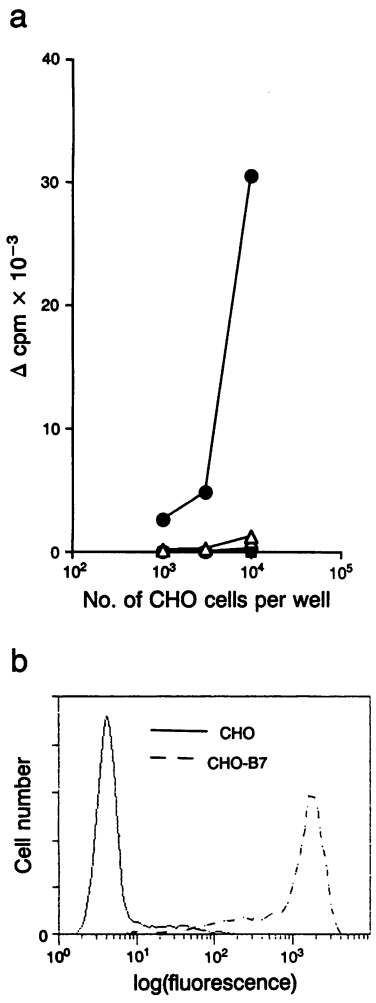

FIG. 4. Activated, but not normal, CD4 T cells can respond to a costimulatory signal in the absence of a TCR ligand. CD4 T cells from C57BL/6J mice were activated with YCD3-1 supernatant (1:40 dilution) and mitomycin C-treated spleen cells. Viable cells were isolated 7 days after activation and used for the proliferation assay. (*a*) Proliferation of CD4 T cells in response to CHO cells transfected with human B7. Activated (●, ■) or normal (○, △) CD4 T cells (5×10^4 per well) were incubated with CHO cells (■, △) or CHO-B7 transfectants (●, ○). Proliferation was determined at 48 hr. (*b*) Expression of B7 as determined by FACScan with CTLA4Ig.

DISCUSSION

The requirement documented here for the coexpression of the TCR ligand and a costimulator on one APC in order to induce clonal expansion of normal CD4 T cells has important implications for the generation and maintenance of self-tolerance. It is known from studies of allogeneic tissue grafting that APCs able to stimulate clonal expansion of T cells reside in virtually all tissues (23). By contrast, most tissue cells appear to lack this activity, as shown by their poor immunogenicity after depletion of APCs and by various transgenic mouse experiments (23–27). If these "passenger" APCs could provide costimulatory signals to CD4 (or presumably CD8) T cells whose receptors were bound to a tissue antigen, then induction of autoreactivity should be virtually unavoidable. Our data indicate that if this "bystander costimulation" occurs at all, then it is sufficiently inefficient to allow tolerance to tissues to be normally maintained. The ability of activated T cells to respond to bystander costimulation is compatible with self-tolerance, since autoreactive CD4 T cells will be deleted or will not become activated in the first place.

Data from many laboratories point to two mechanisms for maintaining self-tolerance in CD4 T cells. CD4 T cells that respond to self-antigens presented by syngeneic APCs are clonally deleted during intrathymic development (28, 29); here, cells expressing costimulators are at least as effective as cells that do not (30). CD4 T cells specific for antigens expressed only on selected cell sets in the periphery appear to be rendered tolerant by binding ligand on the surface of cells that do not express the costimulator required for clonal expansion (23, 24). Such cells do not die, but they also cannot respond to antigen upon restimulation with competent APCs, remaining in a state of anergy for a significant period of time. Moreover, when activated effector CD4 T cells are stimulated by ligand in the absence of costimulatory signals, they rapidly die after releasing effector cytokines; indeed, the mechanism of cell death is dependent on release of the cytokine interferon γ (6). The residual viable cells are mainly anergic. Like clonal anergy, this mechanism of clonal elimination and inactivation can be prevented by costimulation. Thus, the mechanisms of tolerance that have been defined in the periphery can be overcome by providing costimulation. The major finding of this paper is that presentation of the specific TCR ligand and the costimulator by one APC is the most effective way to induce clonal expansion of normal CD4 T cells. This requirement presumably plays an important role

in preventing T-cell activation by autoantigens, while still allowing tissue APCs to efficiently present the antigens of local infectious agents in a form that will rapidly induce a protective immune response. These data support a premise of the two-signal hypothesis of Bretscher and Cohn (31).

We thank Ira Mellman for the gift of hybridoma 2.4G2 and for FcRIIB2 cDNA, Peter Linsley for CTLA4Ig, Lee Nadler for human B7 cDNA, Jane Dunn for making mAbs, Susan Morin for secretarial assistance, and Drs. A. Rudensky and P. Linsley for helpful discussion. This work was supported by National Institutes of Health Grant AI26810. Y.L. is the recipient of an Irvington Institute fellowship.

1. Janeway, C. A., Jr. (1989) *Cold Spring Harbor Symp. Quant. Biol.* **54,** 1–13.
2. Watts, T. H. & McConnell, H. M. (1987) *Annu. Rev. Immunol.* **5,** 461–475.
3. Jenkins, M. K., Pardoll, D. M., Mizuguchi, J., Quill, H. & Schwartz, R. H. (1987) *Immunol. Rev.* **95,** 113–115.
4. Liu, Y. & Janeway, C. A., Jr. (1991) *Int. Immunol.* **3,** 323–332.
5. Otten, G. R. & Germain, R. N. (1991) *Science* **251,** 1228–1231.
6. Liu, Y. & Janeway, C. A., Jr. (1990) *J. Exp. Med.* **172,** 1735–1739.
7. Schwartz, R. H. (1989) *Cell* **57,** 1073–1082.
8. Jenkins, M. K., Ashwell, J. D. & Schwartz, R. H. (1988) *J. Immunol.* **140,** 3324–3330.
9. Gilbert, K. M., Hoang, K. D. & Weigle, W. O. (1990) *J. Immunol.* **144,** 2063–2071.
10. Roska, A. K. & Lipsky, P. E. (1985) *J. Immunol.* **135,** 2053–2061.
11. Kim, K. J., Kanellopoulis-Langvin, C., Merwin, R. M., Sachs, D. H. & Asofsky, R. (1979) *J. Immunol.* **122,** 549–554.
12. Kaye, J., Gillis, S., Mizel, S. B., Shevach, E. M., Malek, T. R., Dinarello, C. A., Lachman, L. B. & Janeway, C. A., Jr. (1984) *J. Immunol.* **133,** 1339–1364.
13. Mellman, I. S. & Unkeless, J. C. (1980) *J. Exp. Med.* **152,** 1048–1060.
14. Liu, Y., Jones, B., Aruffo, A., Sullivan, K. M., Linsley, P. M. & Janeway, C. A., Jr. (1992) *J. Exp. Med.* **175,** 437–445.
15. Portoles, P., Rojo, J., Golby, A., Bonneville, M., Gromkowski, S. H., Greenbaum, L., Janeway, C. A., Jr., Murphy, D. B. & Bottomly, K. (1989) *J. Immunol.* **142,** 4169–4175.
16. Linsley, P. S., Brady, W., Urnes, M., Grosmaire, L. S., Damle, N. K. & Ledbetter, J. A. (1991) *J. Exp. Med.* **173,** 721–730.
17. Seed, B. & Aruffo, A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3385–3389.
18. Freeman, G. L., Freeman, A. S., Segil, J. M., Lee, G., Whitman, J. E. & Nadler, L. M. (1989) *J. Immunol.* **143,** 2174–2181.
19. Lewis, V. A., Koch, T., Plutner, H. & Mellman, I. (1986) *Nature (London)* **324,** 372–375.
20. Linsley, P. S., Brady, W., Grosmaire, L., Aruffo, A., Damble, N. K. & Ledbetter, J. A. (1991) *J. Exp. Med.* **173,** 721–730.
21. Sanders, M. E., Makagoba, M. W. & Shaw, S. (1988) *Immunol. Today* **9,** 195–199.
22. Dianzani, U., Luqman, M., Rojo, J., Yagi, J., Baron, J. L., Woods, A., Janeway, C. A., Jr., & Bottomly, K. (1990) *Eur. J. Immunol.* **20,** 2249–2257.
23. Lafferty, K. J., Prowse, J. & Simeonovich, S. J. (1983) *Annu. Rev. Immunol.* **1,** 143–174.
24. Lo, D., Burkly, L. C., Widera, C. T., Cowing, C., Flavell, R. L., Palmiter, R. D. & Brinster, R. L. (1988) *Cell* **53,** 159–168.
25. Burkly, L., Lo, D., Kanagawa, O., Brinster, R. L. & Flavell, R. A. (1990) *Nature (London)* **342,** 562–564.
26. Allison, J., Campbell, I. L., Morahan, G., Mandel, T. E., Harrison, L. C. & Miller, J. F. A. P. (1988) *Nature (London)* **333,** 529–533.
27. Morahan, G., Allison, J. & Miller, J. F. A. P. (1989) *Nature (London)* **333,** 539–543.
28. Kappler, J. W., Roehm, N. & Marrack, P. (1987) *Cell* **49,** 273–280.
29. von Boehmer, H., Teh, H. S. & Kisielow, P. (1989) *Immunol. Today* **10,** 61–66.
30. Matzinger, P. & Guerder, S. (1989) *Nature (London)* **338,** 74–76.
31. Bretscher, P. & Cohn, M. (1970) *Science* **169,** 1042–1049.