Role of IL-6, IL-1, and CD28 Signaling in Responses of Mouse CD4$^+$ T Cells to Immobilized Anti-TCR Monoclonal Antibody[1]

Matthew A. Holsti,* James McArthur,* James P. Allison,*† and David H. Raulet[2]*†

*Division of Immunology, Department of Molecular and Cell Biology, and †The Cancer Research Laboratory, University of California, Berkeley, CA 94720

Purified CD4$^+$ T cells require TCR engagement and Ag-nonspecific co-stimulatory signals to produce IL-2 and proliferate. A number of recent studies have demonstrated that the interaction of the B7 molecule expressed on APC with the T cell-associated CD28 molecule provides a potent co-stimulatory signal to both freshly isolated CD4$^+$ T cells and cloned Th1 cells. Earlier reports have described the role of cytokines, in particular IL-6 and IL-1, as co-stimulatory molecules for T cell activation. We previously reported that IL-6 and IL-1 synergize to co-stimulate proliferation of purified mouse CD4$^+$ T cells in conjunction with anti-TCR mAb. In this report we explore the interaction of IL-6, IL-1, and CD28 signaling in the activation of mouse CD4$^+$ T cells, and demonstrate that the co-stimulatory requirements of the cells vary depending on the mode of TCR stimulation. CD28 signaling is not sufficient to co-stimulate responses of high buoyant density CD4$^+$ T cells to anti-TCR-conjugated agarose beads; there is an additional requirement that can be supplied by exogenous IL-6 but not by IL-1. In contrast, in responses to anti-TCR mAb that is passively bound to the bottom of culture wells, CD28 stimulation is sufficient to co-stimulate proliferation, resulting in a very high level of IL-2 production; there is no additional requirement for exogenous IL-6 or IL-1. Possible explanations for the differential requirement for IL-6 in the two systems are discussed. Our results are consistent with the notion that CD28 signaling plays a central role in co-stimulating T cell responses. However, the results also suggest that, depending on the nature of the TCR stimulus, T cell activation may also require additional co-stimulatory signals provided by cytokines. *Journal of Immunology,* 1994, 152: 1618.

Activation of resting T cells involves two types of signals that are provided by APC. First, an antigenic peptide bound to an MHC molecule engages the TCR (1). Second, the APC provides Ag nonspecific co-stimulatory signals (2–5). Together, these two types of signals stimulate secretion of the autocrine growth factor IL-2 by the responding T cell and up-regulation of high affinity cell surface receptors for IL-2 (2, 3, 6, 7). Clonal expansion of the stimulated T cell ensues, along with the development of effector functions.

Two types of co-stimulatory molecules have been reported. Recent studies have focused on cell surface-associated co-stimulatory molecules, particularly the interaction between the B7/BB1 molecule expressed by APC and the T cell-associated CD28 molecule (8–12). These studies have provided compelling evidence that the B7-CD28 interaction provides a potent co-stimulatory signal to cloned Th1 cells responding to fixed Ag-pulsed APC (12) and to freshly isolated T cells responding to insolubilized (plate-bound) anti-TCR mAb (8, 12, 13). Moreover, blocking studies suggest that in several experimental systems responses to Ag presented by APC require the CD28-mediated signal. For example, monomeric F(ab) fragments of an anti-murine CD28 mAb strongly inhibit the response of cloned Th1 cells to Ag presented by splenic APC (12). Anti-CD28 F(ab) fragments also inhibit the provision of co-stimulatory signals by LPS-activated splenocytes to freshly isolated CD4$^+$ T cells responding to plate-bound anti-CD3 mAb (12). And a soluble ligand of B7, the CTLA4-Ig fusion protein, partially blocks the MLR (14). Taken together, these results suggest that the B7-CD28 interaction plays a central role in the co-stimulation of various T cell responses.

Received for publication May 17, 1993. Accepted for publication November 16, 1993.

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 in part by Grant RO-1 AI21069 to D.H.R. from the National Institutes of Health.

[2] Address correspondence and reprint requests to Dr. David H. Raulet, MCB Division of Immunology, Room 489 LSA, University of California, Berkeley, CA 94720.

Copyright © 1994 by The American Association of Immunologists

0022-1767/94/$02.00

Earlier studies focused on the possible role of cytokines, particularly IL-6 and IL-1, as co-stimulatory molecules (15–22). We reported that IL-6 strongly augmented the proliferative responses of purified, freshly isolated T cells to T cell mitogens or immobilized anti-TCR mAb (4). Later studies in which contaminating APCs were more rigorously depleted from the responding T cell population demonstrated that IL-6 was by itself not sufficient to co-stimulate the response of purified mouse $CD4^+$ T cells to immobilized anti-TCR mAb. Instead, an additional co-stimulatory signal was required that could be supplied by IL-1 (22). IL-6 and IL-1 synergistically induced production of IL-2 by the responding T cells, whereas neither cytokine alone stimulated significant IL-2 production. Based on these and other data, it was proposed that both IL-6 and IL-1 play a role in co-stimulation of freshly isolated $CD4^+$ T cells.

However, further studies on the role of IL-6 and IL-1 as co-stimulatory molecules suggested that their action requires additional co-stimulatory signals. First, responses of freshly isolated $CD4^+$ T cells to IL-6 plus IL-1 fell off very rapidly as the concentration of cells in the cultures was progressively decreased (22). This suggested that the response required a cell-cell interaction in addition to the cytokines. Second, IL-6 plus IL-1 failed to co-stimulate the response of cloned Th1 cells to Ag presented by carbodiimide-fixed APC (5; J. McArthur and D. Raulet, unpublished data). Instead, CD28 signaling, in the absence of exogenous IL-6 and IL-1, provides a sufficient co-stimulatory signal for cloned Th1 cells (12). Given these data suggesting the existence of multiple co-stimulatory molecules, it was of interest to study how IL-6, IL-1, and CD28 signaling interact in the activation of purified resting $CD4^+$ T cells.

In this report we provide evidence that the co-stimulatory requirements of freshly isolated T cells vary depending on the mode of TCR stimulation. CD28 stimulation is not sufficient to co-stimulate the response of purified, high buoyant density $CD4^+$ T cells to anti-TCR mAb that is covalently bound to agarose beads; there is an additional signaling requirement that can be supplied by exogenously added or APC-derived IL-6. In contrast, in the response of similarly purified $CD4^+$ T cells to anti-TCR mAb that is passively bound to microtiter wells, CD28 signaling, without the addition of IL-6 or IL-1, provides a strong co-stimulatory signal. In this system, co-stimulation of CD28 has an extremely potent effect on IL-2 production and the addition of the cytokines has little additional effect. All of these results are consistent with the possibility that CD28 signaling is a requisite co-stimulatory signal in both of the two systems studied and in other systems of T cell activation. However, the results also suggest that, depending on the mode of TCR stimulation, effective responses of resting T cells may in some circumstances require the co-stimulatory effects of cytokines in addition to CD28 signaling.

Materials and Methods

Mice

BALB/c and BALB/cByJ mice were purchased from Bantin and Kingman (Fremont, CA) and Jackson Laboratories (Bar Harbor, ME), respectively, and were maintained in the Life Sciences Addition Animal Facility at the University of California, Berkeley, CA. Animals used for experiments were 6 to 12 wk old.

Cytokines and Abs

Human rIL-1α was a generous gift of Hoffmann-LaRoche (Nutley, NJ). Human rIL-2 was generously provided by Biogen Research (Cambridge, MA) and by the Biological Response Modifiers Program of the National Cancer Institute (Frederick, MD). Purified COS cell-derived human rIL-6 was a generous gift of Catherine Turner (Genetics Institute). A crude bacterial lysate containing mouse rIL-6 was generously provided by Dr. D. Hilbert (Laboratory of Genetics, NCI, Bethesda, MD). The following Abs were used in this study. H57-597 (anti-TCR-αβ; 23) and GK1.5 (anti-CD4; 24) were purified from hybridoma culture supernatants and ascites fluid, respectively, using protein A Sepharose chromatography. S4B6.1 (anti-mouse IL-2; 25) was purified from ascites fluids by protein G Sepharose chromatography. 6B4 (anti-mouse IL-6; 26) was purified from ascites fluid using a goat anti-rat IgG affinity column as described (27). BP107 (anti-I-$A^{d/b}$; 28), 14.4.4 (anti-I-E; 29), and AD4-15 (anti-CD8; 30) were used as diluted ascites fluid. J1j (anti-Thy-1.2; 31) was used as diluted culture supernatant. The anti-murine CD4 mAb RM4-5 was purchased from PharMingen Corp., San Diego, CA. A preparation of purified rat IgG used as a negative control was purchased from Jackson Immunoresearch Laboratories (West Grove, PA).

The anti-mouse CD28 mAb 37N51.1.3.7.1 (12) was purified using protein G Sepharose chromatography or was used as diluted ascites fluid. Monomeric F(ab) fragments of the anti-mouse CD28 mAb were prepared by digestion of protein G-purified Ab with bead-immobilized papain (Pierce Biochemicals, Rockford, IL) and passage through protein A Sepharose to remove undigested Ab.

Anti-TCR-conjugated agarose beads

The anti-TCR-αβ mAb H57-597 and control hamster anti-Vγ3 mAb were coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia LKB Biotechnology, Piscataway, NJ) following the protocol provided by the manufacturer. In all cases essentially all the added protein was coupled to the beads, as determined by measuring the residual soluble protein concentration after conjugation. For most experiments the beads were conjugated with 1 mg Ab protein/ml of bead suspension, but titrated dosages were tested in some experiments as indicated in the figure legends. For T cell activation experiments, a volume of bead suspension containing 1 to 2 μl of packed beads was added to each half-area (96/2) microtiter well. This dose of beads served to cover the bottom surface area of the wells.

Anti-TCR-coated microtiter wells

Various dosages of H57-597 were bound to the bottoms of microculture wells as described previously (22). The anti-TCR-coated wells were always prepared on the same day as the initiation of the T cell cultures.

$CD4^+$ T cell proliferation experiments

$CD4^+$ peripheral lymph node cells were purified essentially as described (22) by passage over nylon wool columns and treatment of the nylon wool-nonadherent cells with anti-MHC class II and anti-CD8 mAbs plus activated complement, followed by positive "panning" on anti-CD4-coated petri dishes. In some experiments the anti-CD4 mAb RM4-5 was used instead of GK1.5 to prepare the anti-CD4-coated panning plates.

In some experiments the $CD4^+$ T cell population was fractionated according to cell density using Percoll discontinuous gradient centrifugation. The purified $CD4^+$ T cells in 2 ml of medium were carefully overlaid onto a discontinuous gradient consisting of 2-ml layers of p = 1.07, 1.08, 1.085, 1.09, and 1.10 g/ml of Percoll (Pharmacia LKB Biotechnology). The gradient was centrifuged at 1500 rpm and 4°C for 30 min. At equilibrium the majority of cells forms a major band located at

Table I. *High density CD4⁺ cells fail to respond to IL-6 + IL-1*[a]

	Response (cpm) on Day 4.5		
	Low density CD4⁺ ($p \leq 1.08$)	High density CD4⁺ ($p \geq 1.085$)	Unfractionated CD4⁺
αTCR beads[b]	34 ± 10[c]	50 ± 6[d]	41 ± 7[c]
αTCR beads + IL-1[e]	61 ± 24	32 ± 2	48 ± 10
αTCR beads + IL-6[f]	64 ± 11	53 ± 13	68 ± 15
αTCR beads + IL-1 + IL-6	11,539 ± 657	482 ± 64	6,545 ± 238
T cell-depleted spleen cells + Con A	69,329 ± 2,574	82,037 ± 670	53,323 ± 8,712

[a] CD4⁺ T cells were purified as described in *Materials and Methods*. Some cells were set aside and used as the unfractionated CD4⁺ population (right column). The remaining cells were separated into high buoyant density ($p \geq 1.085$; middle column) and low density ($p \leq 1.08$; left column) populations using a discontinuous Percoll density gradient (see *Materials and Methods*). Cells were cultured with anti-TCR-conjugated agarose beads and saturating dosages of the indicated cytokines.

[b] 2-μl packed beads/half area well.

[c] Mean ± SE of triplicate cultures.

[d] Mean ± range of duplicate cultures.

[e] Human rIL-1α, 10 U/ml.

[f] Human rIL-6, 3 ng/ml.

or slightly below the 1.08/1.085 boundary. This major band of cells and cells in the lower density layers were carefully removed and pooled and comprised the low density ($p \leq 1.08$) CD4⁺ population. This population typically comprised at least 90% of the recovered population. Cells in the higher density layers were removed and pooled and comprised the high density ($p = \geq 1.085$) population. The high density population typically comprised 10% or less of the recovered cell population. Both the high and low density CD4⁺ populations were washed extensively in medium before addition to the cultures.

Purified CD4⁺ cells were cultured in the presence of anti-TCR mAb (either covalently bound to agarose beads or passively bound to the culture wells) and various co-stimulants in a final volume of 0.1 ml in the wells of flat-bottomed half-area 96-well plates. Positive control cultures contained 1×10^5 irradiated syngeneic spleen cells and 2 μg/ml Con A or 2.5×10^3 3000 rad-irradiated, thioglycollate-elicited PEC[3]. Proliferation of the cells was assessed at various time points by adding [^{3}H]TdR (0.25 μCi/well, 6.7 Ci/mmol; NEN Research Products, DuPont Co., Boston, MA) to the cultures 4 h before they were processed for scintillation counting with a Skatron cell harvester (Skatron, Sterling, VA).

APC populations

Cell populations used to provide co-stimulatory signals were either PEC, spleen cells, or T cell-depleted spleen cells. Thioglycollate-induced PECs were prepared as described (32). T cell-depleted spleen cells were prepared by treating spleen cells with a cocktail of anti-CD4 (GK1.5), anti-CD8 (AD4-15), and anti-Thy-1.2 (J1j) mAbs and rabbit and guinea pig complement for 1 h at 37°C. Viable cells were isolated using Ficoll/Isopaque. APC populations were γ-irradiated (3000 rad from ^{137}Cs source) or mitomycin C-treated (see below) before culture with T cells.

Mitomycin C treatment

Cells were incubated at 5×10^7/ml in PBS containing 25 μg/ml mitomycin C (Sigma Chemical Co., St. Louis, MO) for 30 min at 37°C. The cells were washed several times with medium before being added to the cultures. Viable mitomycin C-treated spleen cells and T cell-depleted spleen cells did not incorporate [^{3}H]TdR after a 48-h incubation with 2 μg/ml Con A, thus demonstrating the effectiveness of the treatment. In addition, low density CD4⁺ T cells treated with mitomycin C did not proliferate in response to anti-TCR-conjugated beads and IL-6 plus IL-1 (data not shown).

IL-2 assay

Medium conditioned by cultured CD4⁺ cells was isolated and the IL-2 content was determined as previously described by titrating the samples for induction of growth of the CTLL-2 indicator cells (22). One unit, as defined by the Biological Response Modifiers Program (National Cancer Institute), corresponds to approximately 1 ng of pure human rIL-2.

[3] Abbreviations used in this paper: PEC, peritoneal exudate cells; ECM, extracellular matrix.

Results

IL-6 and IL-1, alone or in combination, are not sufficient to co-stimulate the proliferative response of high buoyant density CD4⁺ T cells to anti-TCR-conjugated agarose beads

To investigate the possible role of residual APC in the proliferative response of purified CD4⁺ T cells to anti-TCR-conjugated beads IL-6 and IL-1, we used discontinuous Percoll density gradients to purify high buoyant density CD4⁺ cells and deplete low buoyant density macrophages, dendritic cells, and activated T cells. The high ($p \geq 1.085$) and low ($p \leq 1.08$) buoyant density populations corresponded to approximately 10 and 90% of the cells recovered from the Percoll gradients, respectively.

As we observed in our earlier experiments, the density-unfractionated population responded to IL-6 plus IL-1, and both cytokines were required for the proliferative response (Table I). Similar results were obtained with the low buoyant density fraction. In marked contrast, the high buoyant density CD4⁺ population gave an approximately 24-fold lower response to IL-6 plus IL-1 than that of the low density CD4⁺ population. There was no deficiency in the potential responsiveness of the high density CD4⁺ cells because they responded as well as the low density cells did to irradiated spleen cells plus Con A.

IL-6, IL-1, and APC synergize to co-stimulate high buoyant density CD4⁺ T cell responses to anti-TCR beads

The response of the high density CD4⁺ population to anti-TCR beads and IL-6 plus IL-1 was restored when low density CD4⁺ cells, pretreated with mitomycin C to prevent their proliferation, were added back to the cultures (Table II). The low density CD4⁺ cells were by themselves

Table II. *Mitomycin C-treated spleen cells and low density CD4⁺ T cells synergize with IL-6 + IL-1 to co-stimulate high density CD4⁺ T cell responses to anti-TCR beads*[a]

Stimulus	Response (cpm) on Day 4.5	
	Low density CD4⁺ ($p \leq 1.08$)	High density CD4⁺ ($p \geq 1.085$)
αTCR beads[b]	44 ± 5[c]	62 ± 12[d]
αTCR beads + IL-1 + IL-6[e]	35,122 ± 1,622	2,619 ± 869
αTCR beads + Spleen cells[f]		
250,000	ND	144 ± 54
100,000	ND	113 ± 8
αTCR beads + IL-1 + IL-6 + spleen cells[f]		
250,000	ND	29,930 ± 3,866
100,000	ND	28,875 ± 6,115
αTCR beads + Low density CD4⁺ cells[f]		
250,000	ND	24 ± 8
100,000	ND	60 ± 15
αTCR beads + IL-1 + IL-6 + low density CD4⁺ cells[f]		
250,000	ND	31,136 ± 186
100,000	ND	15,828 ± 1,126
Spleen cells + Con A[g]	14,027 ± 2,206	43,940 ± 3,244

ND, not done.

[a] CD4⁺ T cells were purified and separated into low density ($p \leq 1.08$; left column) and high density ($p \geq 1.085$; right column) populations using a Percoll discontinuous gradient. The cells were cultured with anti-TCR beads and the indicated co-stimulants.

[b] 2-μl packed beads/half area well.

[c] Mean ± SE of triplicate cultures.

[d] Mean ± range of duplicate cultures.

[e] Human rIL-1α at 10 U/ml; mouse rIL-6 at 1/25,000 dilution of bacterial lysate containing IL-6.

[f] Mitomycin C pretreated.

[g] 3000 rad-irradiated.

not co-stimulatory. Thus, the response of high density CD4⁺ T cells to anti-TCR beads requires a low density cell type present in the purified (density-unfractionated) CD4⁺ fraction, in addition to IL-6 and IL-1.

In the same experiment we assessed the co-stimulatory activity of splenic APC. Splenocytes, like the low density CD4⁺ population, were not co-stimulatory for the high density CD4⁺ T cell population in the absence of IL-6 and IL-1 (Table II). This result was surprising, because splenic APCs are usually capable of providing complete APC function (12, 33, 34). However, mitomycin C-treated splenocytes, like the low density CD4⁺ population, restored the response to anti-TCR beads and IL-6 plus IL-1 (Table II).

Splenocytes were capable of providing this synergistic co-stimulatory activity even when T cells were depleted from the spleen cell population, indicating that IL-2 production by splenocytes is not responsible for the effect (Fig. 1). These results suggest that a splenic APC (i.e., not a T cell) can synergize with IL-6 plus IL-1 to co-stimulate responses of T cells in this system.

We also investigated whether both IL-6 and IL-1 were required for co-stimulation in the presence of splenic APC. With low dosages of T cell-depleted splenic APC, both IL-6 and IL-1 were required for a strong T cell response (Fig. 1). As the dose of APC was increased, however, the requirement for IL-1 decreased, and at the highest dose of splenic APC the response without IL-1 was nearly equivalent to that with IL-1.

Taken together, these results indicate that neither IL-6 plus IL-1 nor splenic APC provide a sufficient co-stimulatory signal for T cell activation by anti-TCR-conjugated beads. At low dosages of splenic APC, these three co-stimulants synergistically activate the CD4⁺ T cells; at

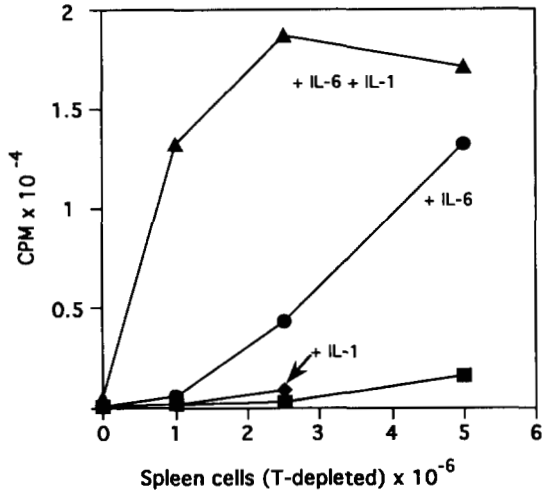


FIGURE 1. Splenic APC synergize with IL-6 plus IL-1 to co-stimulate high density CD4⁺ T cell responses to anti-TCR beads. High buoyant density CD4⁺ T cells were purified as described in *Materials and Methods*. The cells were cultured with anti-TCR beads and increasing dosages of mitomycin C-pretreated, T cell-depleted spleen cells. Where indicated, saturating dosages of human rIL-1α (10 U/ml) and mouse rIL-6 (1/25,000 dilution of a bacterial lysate containing IL-6) were included in the cultures. Proliferation of the cells was measured on day 4.5.

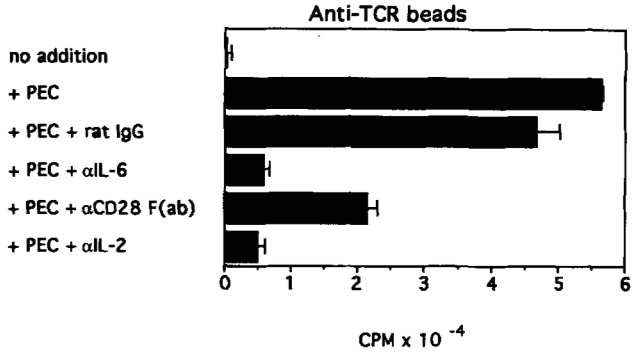


FIGURE 2. Co-stimulation of high density CD4+ T cells by PEC is mediated in part by IL-6 and CD28. High density CD4+ T cells were cultured with anti-TCR beads with or without the addition of 25,000 3,000 rad-irradiated, thioglycollate-elicited PECs. Where indicated, the following Abs were added at 10 μg/ml: either control rat IgG, anti-mouse IL-6 mAb 6B4, anti-CD28 F(ab) fragments, or anti-mouse IL-2 mAb S4B6. Proliferation of the cells was measured on day 4.5. The data are the mean of duplicate cultures. Error bars indicate the range. None of the Abs used inhibited the response of low density CD4+ T cells to 2 μg/ml Con A plus 25 U/ml of human rIL-2 (data not shown).

high dosages of splenic APC the requirement for exogenous IL-1 is diminished considerably. It can be inferred that splenic APC, at all of the dosages tested, fail to supply sufficient IL-6 for T cell activation in this system.

Co-stimulatory activity of activated peritoneal macrophages depends on both IL-6 and CD28-mediated signaling

To directly examine the co-stimulatory signals provided by potential APC in the response to anti-TCR beads, we used activated macrophages from thioglycollate-induced PECs as a source of APC. We studied PEC because macrophages have been reported to produce substantial quantities of IL-6 (35–37). Unlike splenic APC, syngeneic PECs were by themselves capable of co-stimulating strong responses of high buoyant density CD4+ T cells to anti-TCR beads (Fig. 2). The response to PEC was dependent on IL-2 production, because an antimurine IL-2 mAb inhibited the response of T cells to PEC by 90% (Fig. 2).

To determine whether co-stimulation mediated by PEC is mediated in part by IL-6, the effects of a neutralizing anti-mouse IL-6 mAb were examined. The anti-IL-6 mAb inhibited the response of high buoyant density CD4+ T cells to anti-TCR beads and irradiated PEC by approximately 90% (Fig. 2). In control cultures stimulated with Con A and human IL-2 the anti-IL-6 mAb had no effect, which establishes that the reagent was not toxic (data not shown). Thus, the co-stimulatory effect of PEC is mediated in part by IL-6.

To investigate the potential role of CD28 signaling mediated by PEC, we tested whether F(ab) fragments of the anti-CD28 mAb could inhibit the response. Although intact anti-CD28 mAb can substitute for APC in several systems of T cell activation by providing co-stimulatory signals to T cells (8, 9, 12), F(ab) fragments of the anti-CD28 mAb fail to activate in these systems and indeed inhibit the provision of co-stimulatory signals to T cells from APC (12). As shown in Figure 2, anti-CD28 F(ab) fragments inhibited the response by 62% ($p < 0.005$; Student's t-test). The incompleteness of the inhibition may be due to the low affinity of the F(ab) fragments for B7. The inhibition observed was not due to toxicity of the F(ab) fragments because they did not inhibit the response of T cells to Con A plus human IL-2 (data not shown). These results suggest that in addition to providing IL-6, PEC provide a co-stimulatory signal mediated through CD28.

Synergy between IL-6 and the anti-CD28 mAb in T cell activation

To further examine whether the co-stimulation of CD4+ T cells in this system is mediated by both IL-6 and a CD28-mediated signal, we tested whether CD28 signaling and IL-6 synergize to co-stimulate proliferation. A strong synergistic co-stimulation was observed when graded dosages of purified anti-CD28 mAb were combined with saturating dosages of IL-6 in cultures of CD4+ T cells stimulated with anti-TCR beads (Fig. 3*A*). The synergy between IL-6 and the anti-CD28 mAb was observed with CD4+ T cells that were not density fractionated and with high and low buoyant density CD4+ T cell populations (Figs. 3 and 4).

In marked contrast to results observed in several other systems of T cell activation, the anti-CD28 mAb without exogenous cytokines was insufficient to co-stimulate proliferation in this system (Figs. 3*A* and *B* and 4). The failure of anti-CD28 mAb by itself to co-stimulate the T cells was observed over a large range of dosages of stimulating anti-TCR mAb conjugated to the beads (Fig. 3*B*), as was the synergistic co-stimulation by a mixture of anti-CD28 mAb and IL-6. No proliferative responses to the co-stimulating agents were observed when an irrelevant hamster mAb instead of anti-TCR mAb was conjugated to the beads (Fig. 3*C*).

The effect of IL-1 in cultures stimulated with anti-CD28 and/or IL-6 was also examined (Fig. 4). The further addition of IL-1 to cultures containing IL-6 and anti-CD28 mAb did not increase the magnitude of the response. Thus, exogenous IL-1 is not necessary for synergistic activation of T cells mediated by anti-CD28 and IL-6 in this system. Furthermore, IL-1 did not significantly synergize with anti-CD28 in the absence of IL-6. As in the earlier experiments (see Table I), in the absence of the anti-CD28 mAb, IL-6 and IL-1 synergistically activated the low buoyant density CD4+ T cell population (which contains co-stimulatory cells; see above) but had little effect on the high buoyant density population.

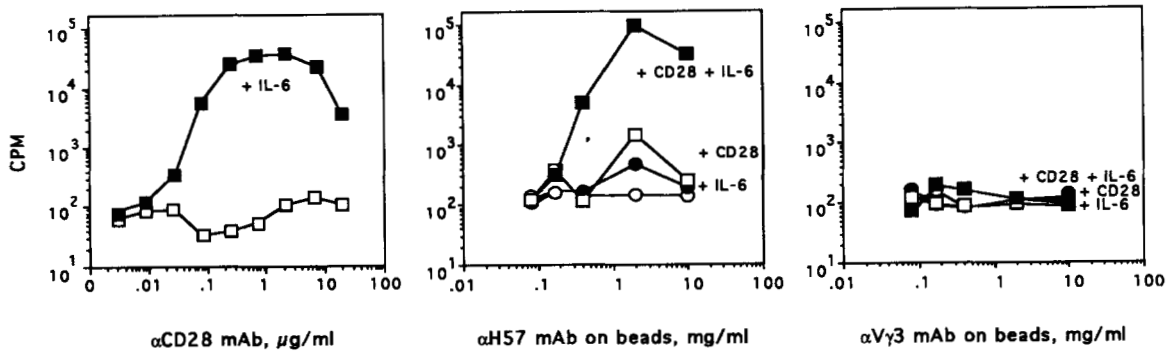


FIGURE 3. IL-6 and anti-mouse CD28 synergize to co-stimulate CD4+ T cell responses to anti-TCR beads. *A:* CD4+ T cells were purified and cultured with anti-TCR beads and increasing dosages of purified anti-CD28 in the presence or absence of mouse rIL-6 (1/25,000 dilution of bacterial lysate containing IL-6). Cell proliferation was measured on day 4.5. The data are the mean of triplicate cultures. The SEs were generally less than 20% of the mean. *B:* Purified CD4+ T cells were cultured with or without a constant dose of anti-CD28 mAb (a 1/50 dilution of ascites fluid) and/or mouse rIL-6 (same dose as in *A*), with the addition of beads that had been conjugated with graded dosages of anti-TCR mAb. Proliferation was measured as above. *C:* Identical to panel *B*, except beads conjugated with graded dosages of irrelevant anti-Vγ3 mAb were used.

The synergistic effect of cytokines and the anti-CD28 mAb in this system was also observed at the level of IL-2 production. Medium conditioned by cultures of high density CD4+ cells stimulated with both the anti-CD28 mAb and cytokines (IL-6 plus IL-1) contained more than 40-fold more IL-2 than those stimulated with either the anti-CD28 mAb or cytokines alone (Table III).

Taken together, the results suggest that in the case of TCR stimulation with anti-TCR-conjugated beads, co-stimulation requires both IL-6 and a CD28-mediated signal that synergize to stimulate increased IL-2 production. PEC provide both of these signals to T cells. The CD28-mediated signal can be provided by splenic APC but they do not provide sufficient IL-6 for the response. Exogenous IL-1 is not required for the response; however, exogenous IL-1 augments the response when limiting dosages of splenic APC are present.

Co-stimulatory requirements of CD4+ T cells stimulated with anti-TCR mAb bound to microtiter wells

An experimental system commonly used to study T cell activation in vitro involves the use of anti-TCR mAb passively bound directly to the bottoms of plastic microtiter wells (plate-bound anti-TCR mAb). We therefore extended our investigation to study the role of IL-6, IL-1, and CD28 signaling in the response of CD4+ T cells to plate-bound anti-TCR mAb.

The proliferative response of purified but density-unfractionated mouse CD4+ T cells to the anti-TCR-αβ mAb passively bound to culture wells is shown in Figure 5. In the absence of any exogenous co-stimulants, high dosages of plate-bound anti-TCR mAb stimulated a significant proliferative response from the CD4+ T cells. Addition of either anti-CD28 mAb or IL-6 to the cultures augmented the response to plate-bound anti-TCR mAb. The effects of both reagents were dramatic at low dosages of anti-TCR mAb. At high dosages of anti-TCR mAb, where there was a significant response without added co-stimulants, the addition of anti-CD28 or IL-6 increased the response approximately two- to fourfold.

To examine the possible role of low buoyant density co-stimulatory cells in the proliferative response in this system, CD4+ T cells were fractionated into high and low buoyant density fractions and tested for their responsiveness to co-stimulants at high dosages of plate-bound anti-TCR mAb (Fig. 6). The low density population behaved much like density-unfractionated CD4+ cells, in that the anti-CD28 mAb and cytokines (in this case a mixture of IL-6 and IL-1) modestly augmented the proliferative responses to anti-TCR mAb; the effect was approximately two- to threefold over the response to anti-TCR mAb alone. A mixture of the anti-CD28 mAb and IL-6 plus IL-1 did not result in any further increase in the response.

The responses of the high density population to the anti-CD28 mAb and to IL-6 plus IL-1 were slightly lower than those of the low density cells; a mixture of anti-CD28 and the cytokines had an approximately additive effect (Fig. 6). These data corroborate earlier studies that demonstrated that IL-6 plus IL-1 and the anti-CD28 mAb each co-stimulate proliferative responses of CD4+ T cells to plate-bound anti-TCR mAb (12, 22). However, the results contrast markedly with those obtained with bead-bound anti-TCR mAb, where the anti-CD28 mAb and IL-6 are by themselves insufficient to co-stimulate the response but synergize to yield a strong response.

Anti-CD28 mAb has a greater effect on IL-2 production than does IL-6 plus IL-1

A somewhat different picture emerged when we examined IL-2 secretion of the cultures activated with plate-bound

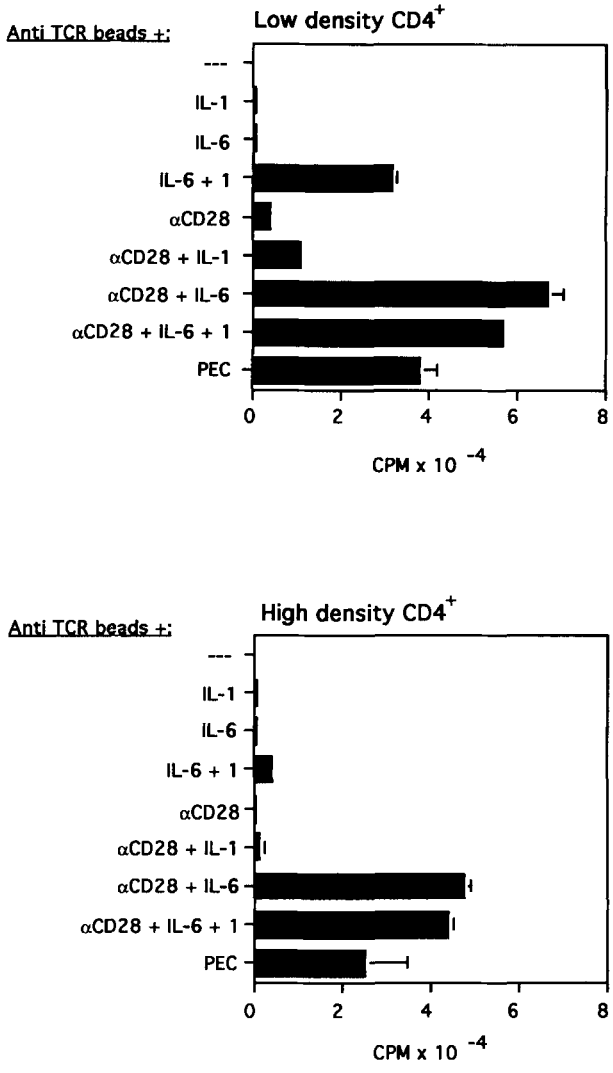


FIGURE 4. High and low buoyant density CD4+ T cell responses to anti-TCR beads and combinations of IL-1, IL-6, and anti-CD28. CD4+ T cells were purified and separated into high ($p \geq 1.085$) and low ($p \leq 1.08$) density populations using a discontinuous Percoll density gradient. The cells were cultured with anti-TCR beads and the indicated additions: human rIL-1α (10 U/ml), mouse rIL-6 (1/400 dilution of bacterial lysate containing IL-6), anti-CD28 ascites (1/400 dilution), or 25,000 thioglycollate-elicited, 3000 rad-irradiated PEC. Proliferation of the cells was measured on day 4.5. The data for low density CD4+ T cells are the mean of triplicate cultures; error bars indicate the SE. The data for the high density CD4+ T cells are the mean of duplicate cultures and the error bars indicate the range.

Table III. *IL-2 production of high density CD4+ T cells stimulated with anti-TCR beads*[a]

Stimulus	IL-2 Production (U/ml)[b]
Anti-TCR beads	<0.5
Anti-TCR beads + IL-1 + IL-6[c]	2
Anti-TCR beads + anti-CD28[d]	<0.5
Anti-TCR beads + IL-1 + IL-6 + anti-CD28	83

[a] High density CD4+ T cells were cultured with anti-TCR beads and the indicated co-stimulants. On day 4.5 samples (25 μl/culture well) of medium conditioned by the cells were withdrawn for the determination of IL-2 content.
[b] In the absence of IL-2, CTLL-2 cells did not proliferate in the presence of IL-1, IL-6, anti-CD28, or to any combination of the cytokines and anti-CD28. In addition, the anti-CD28 mAb did not alter the responsiveness of CTLL-2 cells to exogenous IL-2 (data not shown).
[c] Human rIL-1α at 10 U/ml; mouse rIL-6 at 1/25,000 dilution of a crude bacterial lysate containing rIL-6.
[d] 1/2,000 dilution of ascites fluid containing anti-CD28.

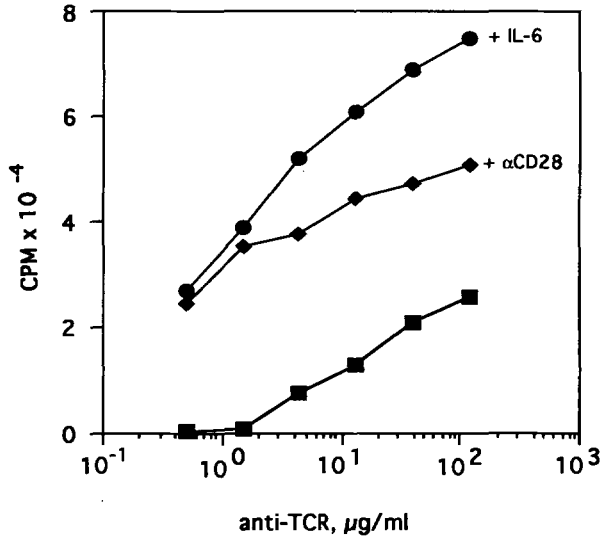


FIGURE 5. IL-6 and anti-CD28 by themselves co-stimulate CD4+ T cell responses to plate-bound anti-TCR mAb. Purified (density-unfractionated) CD4+ T cells were cultured in wells precoated with graded dosages of anti-TCR mAb. Anti-CD28 ascites (diluted 1/2000) or mouse rIL-6 (1/25,000 dilution of bacterial lysate containing IL-6) were added as indicated. Proliferation of the cells was measured on day 4.5. Cultures did not respond to anti-CD28 or IL-6 in the absence of anti-TCR mAb. The data are the mean of triplicate cultures. The SEs were generally less than 20% of the mean.

anti-TCR mAb (Fig. 7). Low density CD4+ T cells stimulated with anti-TCR mAb alone secreted significant quantities of IL-2, and the addition of IL-6 and IL-1 resulted in a substantial (approximately eightfold) increase. In contrast, IL-6 plus IL-1 had little effect on IL-2 production by the high density CD4+ cells, increasing it by less than twofold. This data suggests that the ability of the two cytokines to stimulate IL-2 production may depend on the presence of low density co-stimulatory cells whose numbers are depleted in the high density population.

More importantly, the amount of IL-2 secreted by both high and low density CD4+ populations stimulated with plate-bound anti-TCR mAb and anti-CD28 was much higher than that stimulated by IL-6 plus IL-1 (Fig. 7). Anti-CD28 co-stimulated secretion of 1000 to 3000 U/ml of IL-2 into the culture supernatants, an increase of 30- to

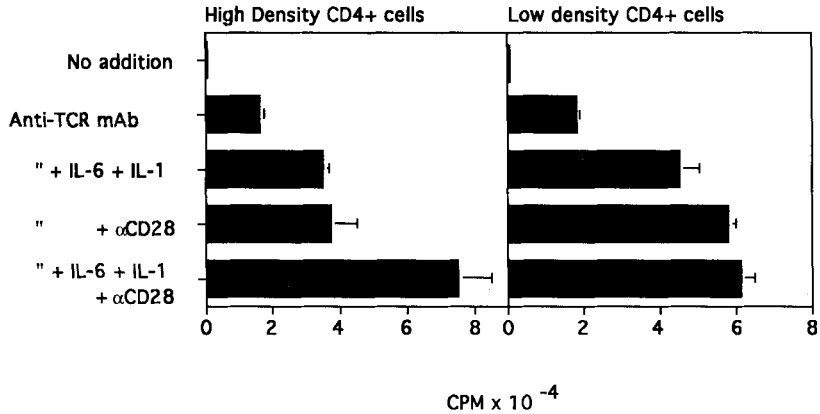
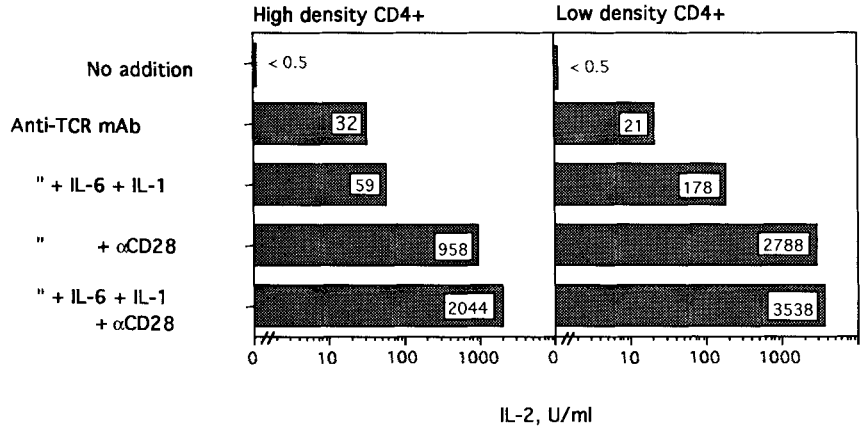


FIGURE 6. Responses of high and low density CD4+ T cells to plate-bound anti-TCR mAb with IL-6 plus IL-1 and/or anti-CD28. Purified CD4+ T cells were separated into high and low density fractions using discontinuous Percoll gradient centrifugation. The cells were cultured in wells precoated with 40 μg/ml of anti-TCR mAb. Saturating dosages of human rIL-1α (10 U/ml) and mouse rIL-6 (1/25,000 dilution of crude bacterial lysate containing IL-6) and anti-CD28 ascites (1/50 dilution) were added where indicated. The cultures were incubated for 2.5 days and then 25 μl of each culture supernatant was collected and tested for IL-2 content, as shown in Figure 7. The cultures were then pulsed with [^{3}H]TdR and harvested 4 h later for scintillation counting. The data for high density CD4+ cells are the mean of duplicate cultures; the error bars indicate the range. The data for low density CD4+ cells are the mean of triplicate cultures; the error bars denote the SE. IL-1 + IL-6 and anti-CD28, either by themselves or combined, did not stimulate proliferation in the absence of plate-bound anti-TCR mAb (data not shown).

FIGURE 7. IL-2 production by high and low density CD4+ T cells to plate-bound anti-TCR mAb with IL-6 plus IL-1 and/or anti-CD28. Conditioned medium was collected from the cultures of high and low density CD4+ T cells stimulated as indicated in Figure 6. IL-2 content of the samples was measured using the IL-2-dependent CTLL-2 indicator cell line, as described in *Materials and Methods*. Unit values were assigned based on the titration of a Bioresponse Modifiers Program human rIL-2 standard.



0-fold over the background levels secreted in the absence of anti-CD28. The mixture of anti-CD28 and IL-6 plus IL-1 resulted in a further increase but this effect was only modest, being less than twofold.

Thus, both proliferation and IL-2 production data indicate that IL-6 plus IL-1 and the anti-CD28 mAb do not have significant synergistic effects in the plate-bound anti-TCR system. Even in the presence of limiting dosages of anti-TCR mAb, there was no evidence for a significant synergistic co-stimulation by these reagents (data not shown). It should also be noted that the maximum amounts of secreted IL-2 detected in the plate-bound anti-TCR system greatly exceeded the levels measured in the bead-bound anti-TCR system (compare Fig. 7 and Table III).

Discussion

Role of CD28-mediated co-stimulation in CD4+ T cell responses to anti-TCR-conjugated beads and IL-6 plus IL-1

In several earlier studies a combination of the two cytokines IL-6 and IL-1 seemed to provide a sufficient co-stimulatory signal to freshly isolated T cells (20–22). However, the results of the present study indicate that high buoyant density CD4+ T cells respond poorly if at all to the mixture of anti-TCR-conjugated beads, IL-6, and IL-1. Our earlier results can be explained by the presence of contaminating low buoyant density cells in the CD4+ population that provide co-stimulatory signals necessary for

responses to IL-6 plus IL-1 (see Table II). The residual co-stimulatory activity may be due to contaminating dendritic cells. However, given the high purity of the $CD4^+$ T cell population, it is also possible that the relevant contaminating low density cells correspond to activated T cells that have been implicated as co-stimulatory cells in other studies (5, 38–40). A related possibility is that low buoyant density $CD4^+$ T cells do not require CD28 stimulation for activation and in response to IL-6 plus IL-1 produce IL-2, which the high density $CD4^+$ T cells then use to initiate proliferation.

Regardless of the nature of the co-stimulatory low density cell in the $CD4^+$ population, the observation that T cell-depleted splenic APCs are able to restore the response indicates that APC-derived signals synergize with the cytokines to co-stimulate high density $CD4^+$ T cells in this system. In this case, the high buoyant density T cells must produce their own IL-2. The experiments using PEC as APC and those using anti-CD28 mAb suggest that the essential APC co-stimulatory signal corresponds to a CD28 ligand, which acts synergistically with IL-6 to activate the T cells.

Differential requirement for IL-6 depending on the mode of TCR stimulation

In contrast, when $CD4^+$ T cells are activated with anti-TCR mAb immobilized on the bottom of plate wells, co-stimulation through CD28 is apparently sufficient to induce secretion of extremely high levels of IL-2 and proliferation of the T cells. Exogenous provision of IL-6 plus IL-1 is not required for IL-2 secretion or for proliferation in this system. The cytokines by themselves do induce some proliferation of the cells but stimulate comparatively little IL-2 secretion from the high buoyant density $CD4^+$ T cell population. Thus, a central conclusion of this study is that the co-stimulatory requirements of $CD4^+$ T cells differ significantly depending on the mode of TCR stimulation.

It is unlikely that the differential requirement for IL-6 is due to quantitative differences in TCR stimulation in the two systems. The lack of a requirement for IL-6 was observed when the concentration of anti-TCR mAb bound to the plate wells was varied over several orders of magnitude (Fig. 5; data not shown). Conversely, the requirement for both IL-6 and anti-CD28 mAb for co-stimulation was observed over a broad range of concentrations of anti-TCR mAb conjugated to beads (Fig. 3*B*). Furthermore, the requirement for IL-6 in the bead system was not due to a nonspecific toxic effect of the beads, because the addition of beads (conjugated with irrelevant hamster IgG) did not inhibit the response of $CD4^+$ T cells to plate-bound anti-TCR mAb plus anti-CD28 mAb (data not shown). Finally, the different results cannot have resulted from alterations in the signaling requirements of the cells due to the method used to purify them, which involved panning on plates coated with anti-CD4 mAb. First, the cells used in each system were prepared identically. Second, when $CD4^+$ T cells were prepared by elimination of $CD8^+$ T cells and the panning step eliminated, the requirement for both IL-6 and anti-CD28 mAb for responses to bead-bound Ab remained (data not shown). Previous studies have demonstrated that the anti-CD28 mAb is sufficient to co-stimulate responses to plate-bound mAb (12).

We therefore favor an alternative explanation for the differential requirement for IL-6, i.e., that cells stimulated with plate-bound anti-TCR mAb receive some other co-stimulatory signal that substitutes for IL-6 and that is not provided to cells stimulated with anti-TCR-conjugated beads. For example, T cells stimulated by plate-bound anti-TCR mAb might receive a co-stimulatory signal from the ECM coating the bottom of the culture wells. The ECM is composed of serum-borne and cell-derived proteins such as fibronectin, laminin, and collagens that have been shown recently to augment $CD4^+$ T cell proliferation when they are co-immobilized with limiting dosages of plate-bound anti-TCR mAb (41–44). Perhaps cells stimulated by anti-TCR-conjugated beads—which presumably do not support the formation of an ECM on their surface—do not receive the putative ECM-derived signal because some of the cells are suspended away from the bottom of the well. And cells at the well bottoms would not be expected to receive the ECM-derived signal at the same site where TCR aggregation induced by anti-TCR beads is occurring. The signal induced by IL-6 may feed into the same signaling pathway as the putative ECM signal and partially substitute for it.

The physiologic significance of this model depends on whether there are circumstances in which IL-6 is required for T cell activation in vivo. According to the above model, IL-6 might be a critical co-stimulant in sites where ECM is limiting. The findings that IL-6 augments essentially all primary T cell responses in vitro suggests, at the least, that IL-6 produced at sites of inflammation or other tissue damage will play a role in augmenting weak immune responses. In many circumstances, however, it is likely that IL-6 is not an essential co-stimulant for T cells in vivo.

Role of IL-6 and IL-1 in T cell responses to anti-TCR-conjugated beads

An important question concerning the responses stimulated with anti-TCR beads is whether IL-6 and/or IL-1 act directly on T cells or rather act indirectly to augment the co-stimulatory activity of residual APC. In the case of IL-6, the data suggest that it acts directly on T cells. In the presence of anti-CD28 mAb, IL-6 is required for the activation of the high buoyant density $CD4^+$ T cell population, which is a very pure population of T cells (>99% $CD4^+CD8^-$ as assessed by flow cytometry; data not

shown). Moreover, because CD28 signaling is also required, it cannot be argued that the role of IL-6 is to augment the ability of APC to provide the CD28-mediated signal.

On the other hand, based on these results, we suspect that the action of IL-1 may be at least partly indirect. In contrast to IL-6, exogenous IL-1 is not required when high density $CD4^+$ T cells are co-stimulated with anti-CD28 mAb, which suggests that IL-1 might be involved in the B7-CD28 pathway, perhaps by augmenting limited co-stimulatory activity of residual APC. In support of this hypothesis, when splenic APC rather than anti-CD28 mAb are used as a co-stimulus for high buoyant density $CD4^+$ T cells, IL-1 is required only at limiting dosages of the APC (Fig. 1). Furthermore, previous reports demonstrate that IL-1 augments the co-stimulatory function of dendritic cells (45). It will be interesting to determine whether IL-1 induces B7 expression by dendritic cells or other APC. Despite these considerations, IL-1 may also be acting directly on the resting T cells, which have been reported to express IL-1 receptors (46). One possibility consistent with our data is that IL-1 augments the sensitivity of T cells to CD28-mediated signaling.

Role of IL-6 and IL-1 in T cell activation mediated by plate-bound anti-TCR

Exogenous IL-6 and IL-1 are not required for proliferation when $CD4^+$ T cells are stimulated with plate-bound anti-TCR mAb and the anti-CD28 mAb. However, the two cytokines in the absence of the anti-CD28 mAb augmented proliferation of the low density and density-unfractionated $CD4^+$ T cell populations and caused an eightfold increase in IL-2 production over background (see Figs. 6 and 7). These results corroborate earlier observations that IL-6 plus IL-1 co-stimulates the response of T cells to plate-bound anti-TCR mAb (22).

In contrast, when high buoyant density $CD4^+$ T cells were tested, the two cytokines caused only a twofold increase in IL-2 production over background levels and had little additional effect on IL-2 production co-stimulated by the anti-CD28-mAb. However, the effects of the cytokines on the high density $CD4^+$ population were more apparent at the level of proliferation (see Fig. 6). By themselves, IL-6 plus IL-1 caused as much proliferation as the anti-CD28 mAb and in the presence of anti-CD28 mAb they augmented the proliferative response. The increase in proliferation is unlikely to be related to the increased IL-2 production, because the amount of IL-2 secretion induced by the anti-CD28 mAb alone should have far exceeded the capacity of the cells to use it. Hence, it is likely that the effects of IL-6 plus IL-1 in this system are mediated in part by promoting the response to IL-2 (21). Such effects may include, but are not limited to, effects on the level of cell surface IL-2 receptors.

Acknowledgments

We thank Drs. R. Kubo, J. Sprent, D. Parker, D. Hilbert, P. Gottlieb, and F. Fitch for gifts of Ab reagents and cell lines essential for this study and R. West and E. Becale for technical assistance.

References

1. Jorgensen, J. L., P. A. Reay, E. W. Ehrich, and M. M. Davis. 1992. Molecular components of T-cell recognition. *Annu. Rev. Immunol. 10:835.*
2. Larsson, E.-L., and A. Coutinho. 1979. The role of mitogenic lectins in T-cell triggering. *Nature 280:239.*
3. Smith, K. A. 1980. T-cell growth factor. *Immunol. Rev. 51:337.*
4. Garman, R. D., K. A. Jacobs, S. C. Clark, and D. H. Raulet. 1987. B-cell-stimulatory factor 2 (β, interferon) functions as a second signal for interleukin 2 production by mature murine T cells. *Proc. Natl. Acad. Sci. USA 84:7629.*
5. Mueller, D., M. Jenkins, and R. Schwartz. 1989. Clonal expansion versus functional clonal inactivation: a costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. *Annu. Rev. Immunol. 7:445.*
6. Robb, R. J., A. Munck, and K. A. Smith. 1981. T cell growth factor receptors: quantitation, specificity, and biological relevance. *J. Exp. Med. 154:1455.*
7. Malek, T. R., J. A. Schmidt, and E. M. Shevach. 1985. The murine IL 2 receptor. III. Cellular requirements for the induction of IL-2 receptor expression on T cell subpopulations. *J. Immunol. 134:2405.*
8. Thompson, C. B., T. Lindsten, J. A. Ledbetter, S. L. Kunkel, H. A. Young, S. G. Emerson, J. M. Leiden, and C. H. June. 1989. CD28 activation pathway regulates the production of multiple T-cell-derived lymphokines/cytokines. *Proc. Natl. Acad. Sci. USA 86:1333.*
9. Jenkins, M. K., P. S. Taylor, S. D. Norton, and K. B. Urdahl. 1991. CD28 delivers a costimulatory signal involved in antigen-specific IL-2 production by human T cells. *J. Immunol. 147:2461.*
10. Linsley, P., W. Brady, L. Grosmaire, A. Aruffo, N. Damle, and J. Ledbetter. 1991. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J. Exp. Med. 173:721.*
11. Koulova, L., E. A. Clark, G. Shu, and B. Dupont. 1991. The CD28 ligand B7/BB1 provides costimulatory signal for alloactivation of $CD4^+$ T cells. *J. Exp. Med. 173:759.*
12. Harding, F., J. McArthur, D. Raulet, and J. Allison. 1992. CD28-mediated signalling costimulates T cell activation and prevents induction of anergy in T cell clones. *Nature 356:607.*
13. Gimmi, C. D., G. J. Freeman, J. G. Gribben, K. Sugita, A. S. Freedman, C. Morimoto, and L. M. Nadler. 1991. B-cell surface antigen B7 provides a costimulatory signal that induces T cells to proliferate and secrete interleukin 2. *Proc. Natl. Acad. Sci. USA 88:6575.*
14. Linsley, P. S., W. Brady, M. Urnes, L. S. Grosmaire, N. K. Damle, and J. A. Ledbetter. 1991. CTLA-4 is a second receptor for the B cell activation antigen B7. *J. Exp. Med. 174:561.*
15. Larsson, E.-L., N. N. Iscove, and A. Coutinho. 1980. Two distinct factors are required for induction of T-cell growth. *Nature 283:664.*
16. Smith, K. A., L. B. Lachman, J. J. Oppenheim, and M. F. Favata. 1980. The functional relationship of the interleukins. *J. Exp. Med. 151:1551.*
17. Raulet, D. H. 1984. Regulation of IL-2 production by a T cell factor. In *Regulation of the Immune System.* F. Sercarz, H. Cantor, and L. Chess, eds. Alan R. Liss, New York, p. 335.
18. Lichtman, A. H., J. Chin, J. A. Schmidt, and A. K. Abbas. 1988. Role of interleukin 1 in the activation of T lymphocytes. *Proc. Natl. Acad. Sci. USA 85:9699.*
19. Garman, R. D., and D. H. Raulet. 1987. Characterization of a novel murine T cell-activating factor. *J. Immunol. 138:1121.*
20. Houssiau, F. A., P. G. Coulie, D. Olive, and J. Van Snick. 1988. Synergistic activation of human T cells by interleukin 1 and interleukin 6. *Eur. J. Immunol. 18:653.*
21. Houssiau, F., P. Coulie, and J. Van Snick. 1989. Distinct roles of IL-1 and IL-6 in human T cell activation. *J. Immunol. 143:2520.*

22. Holsti, M. A., and D. H. Raulet. 1989. IL-6 and IL-1 synergize to stimulate IL-2 production and proliferation of peripheral T cells. *J. Immunol. 143:2514.*

23. Kubo, R. T., W. Born, J. W. Kappler, P. Marrack, and M. Pigeon. 1989. Characterization of a monoclonal antibody which detects all murine αβ T cell receptors. *J. Immunol. 142:2736.*

24. Dialynas, D. P., D. B. Wilde, P. Marrack, A. Pierres, K. A. Wall, W. Havran, G. Otten, M. R. Loken, M. Pierres, J. Kappler, and F. W. Fitch. 1983. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. *Immunol. Rev. 74: 29.*

25. Mosmann, T. R., M. W. Bond, R. L. Coffman, J. Ohara, and W. E. Paul. 1986. T cell and mast cell lines respond to B cell stimulatory factor-1. *Proc. Natl. Acad. Sci. USA 83:5654.*

26. Vink, A., P. G. Coulie, P. Wauters, R. P. Nordan, and J. Van Snick. 1988. B cell growth and differentiation activity of interleukin-HP1 and related murine plasmacytoma factors: synergy with interleukin 1. *Eur. J. Immunol. 18:607.*

27. Harlow, E., and D. Lane. 1988. *Antibodies: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

28. Symington, F., and J. Sprent. 1981. A monoclonal antibody detecting an Ia specificity mapping in the I-A or I-E subregion. *Immunogenetics 14:53.*

29. Ozato, K., N. Mayer, and D. Sachs. 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. *J. Immunol. 124:533.*

30. Raulet, D. H., P. Gottlieb, and M. J. Bevan. 1980. Fractionation of lymphocyte population with monoclonal antibodies specific for Lyt-2.2 and Lyt-3.1. *J. Immunol. 125:1136.*

31. Bruce, J., F. W. Symington, T. J. McKearn, and J. Sprent. 1981. A monoclonal antibody discriminating between subsets of T and B cells. *J. Immunol. 127:2496.*

32. Mishell, B. B., and S. M. Shiigi. 1980. *Selected Methods in Cellular Immunology.* W. H. Freeman and Company, San Fransisco, CA.

33. Sprent, J., and M. Schaefer. 1985. Properties of purified T cell subsets. I. In vitro responses to class I vs. class II H-2 alloantigens. *J. Exp. Med. 162:2068.*

34. Jenkins, M. K., C. Chen, G. Jung, D. L. Mueller, and R. H. Schwartz. 1990. Inhibition of antigen-specific proliferation of type 1 murine T cell clones after stimulation with immobilized anti-CD3 monoclonal antibody. *J. Immunol. 144:16.*

35. Horii, Y., A. Muraguchi, S. Suematsu, T. Matsuda, K. Yoshizaki, T. Hirano, and T. Kishimoto. 1988. Regulation of BSF-2/IL-6 production by human mononuclear cells: macrophage-dependent synthesis of BSF-2/IL-6 by T cells. *J. Immunol. 141:1529.*

36. Navarro, S., N. Debili, J.-F. Bernaudin, W. Vainchenker, and J. Doly. 1989. Regulation of the expression of IL-6 in human monocytes. *J. Immunol. 142:4339.*

37. Krutmann, J., R. Kirnbauer, A. Kock, T. Schwarz, E. Schopf, L. T. May, P. B. Sehgal, and T. A. Luger. 1990. Cross-linking Fc receptors on monocytes triggers IL-6 production: role in anti-CD3-induced T cell activation. *J. Immunol. 145:1337.*

38. Evavold, B., and J. Quintans. 1989. Accessory cell function of Th2 clones. *J. Immunol. 143:1784.*

39. Evavold, B., A. Yokoyama, R. Hirsch, J. Bluestone, and J. Quintans. 1989. T helper 2 (Th2) but not Th1 clones co-stimulate resting T cells in the presence of anti-CD3 monoclonal antibody. *Int. Immunol. 1:443.*

40. Azuma, M., H. Yssel, J. H. Phillips, H. Spits, and L. L. Lanier. 1993. Functional expression of B7/BB1 on activated T lymphocytes. *J. Exp. Med. 177:845.*

41. Matsuyama, T., A. Yamada, J. Kay, K. M. Yamada, S. K. Akiyama, S. F. Schlossman, and C. Morimoto. 1989. Activation of CD4 cells by fibronectin and anti-CD3 antibody: a synergistic effect mediated by the VLA-5 fibronectin receptor complex. *J. Exp. Med. 170:1133.*

42. Dang, N. H., Y. Torimoto, S. F. Schlossman, and C. Morimoto. 1990. Human CD4 helper T cell activation: functional involvement of two distinct collagen receptors, 1F7 and VLA integrin family. *J. Exp. Med. 172:649.*

43. Davis, L. S., N. Oppenheimer-Marks, J. L. Bednarczyk, B. W. McIntyre, and P. E. Lipsky. 1990. Fibronectin promotes proliferation of naive and memory T cells by signaling through both the VLA-4 and VLA-5 integrin molecules. *J. Immunol. 145:785.*

44. Shimizu, Y., G. A. Van Seventer, K. J. Horgan, and S. Shaw. 1990. Costimulation of proliferative responses of resting $CD4^+$ T cells by the interaction of VLA-4 and VLA-5 with fibronectin or VLA-6 with laminin. *J. Immunol. 145:59.*

45. Koide, S. L., K. Inaba, and R. M. Steinman. 1987. Interleukin 1 enhances T-dependent immune responses by amplifying the function of dendritic cells. *J. Exp. Med. 165:515.*

46. Lowenthal, J. W., and H. R. MacDonald. 1987. Expression of interleukin 1 receptors is restricted to the $L3T4^+$ subset of mature T lymphocytes. *J. Immunol. 138:1.*