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IL-6 AND IL-1 SYNERGIZE TO STIMULATE IL-2 PRODUCTION AND PROLIFERATION OF PERIPHERAL T CELLS[1]

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Purified T cells can be induced to proliferate and to produce the autocrine growth factor IL-2 with mAb to the TCR and costimulatory cytokines. In a previous report we demonstrated that human IL-6 stimulates IL-2 production and proliferation of purified T cells, in conjunction with the insolubilized anti-TCR Vβ8 mAb, F23.1. Here we show that when $CD4^+$ T cells are rigorously purified to >99% $CD4^+CD8^-$, they respond only weakly to F23.1 and IL-6. Instead, there is an additional requirement for IL-1, which dramatically synergizes with IL-6 to induce prolonged (>7 days) proliferative responses and IL-2 production. Similar results were observed when the highly mitogenic anti-CD3 mAb 145-2C11 was substituted for F23.1. The proliferation induced by F23.1, IL-1, and IL-6 was substantially (>80%) inhibited by a mAb to mouse IL-2, and was not inhibited by an anti-IL-4-mAb. In accordance with this finding, medium conditioned by the activated $CD4^+$ cells contained large amounts of IL-2, which increased over a 7-day culture period. These results demonstrate that IL-6 and IL-1 stimulate T cell proliferation by inducing production of the autocrine growth factor IL-2. In addition, the two lymphokines must be present simultaneously for activation to occur. The possible roles of IL-6 and IL-1 in IL-2 gene regulation and in Ag-induced T cell activation are discussed.

Ag-induced proliferation of T cells depends on the autocrine growth factors IL-2 (1, 2) and IL-4 (3, 4). Considerable evidence suggests that in addition to presenting processed, MHC-associated Ag for TCR recognition (5), APC provide Ag-nonspecific costimulatory signals (6–9) necessary for the secretion of the growth factors by the T cells and increased expression of functional cell-surface receptors for them (10–13). The nature of these costimulatory signals and their regulation is of considerable interest because they play a pivotal role in controlling an early step in immune responses.

We (8, 9) and others (14–16) have previously shown that stimulation of the Ag receptor complex of purified lymph node T cells is not sufficient to induce transcription of the IL-2 gene and secretion of IL-2. We identified and partially purified a soluble molecule produced by mouse spleen cells (TAF)[3] that in conjunction with TCR cross-linking stimulates IL-2 production and T cell proliferation (8). By screening expression cDNA libraries of a human T cell line we subsequently found that human IL-6, a molecule of diverse biologic activities, also stimulates IL-2 production and T cell proliferation, and is of similar size and chromatographic properties to TAF (9). Recently we demonstrated that TAF is, in fact, mouse IL-6, by using a neutralizing anti-mouse IL-6 antiserum kindly provided by Drs. R. Nordan, D. Hilbert, and S. Rudikoff (National Institutes of Health, Bethesda, MD) (R. Garman and D. Raulet, unpublished). Thus, we proposed that IL-6 may play a role as a costimulatory signal in T cell activation. Notably, we could not demonstrate any such activity with IL-1, which has been proposed to function as a costimulatory signal in some studies (6, 7, 17) but not in others (11, 18–20).

We have continued to investigate the role of IL-6 in T cell activation, focusing on its effects on Th cells that have been rigorously purified to >99% $CD4^+CD8^-$. To our surprise we found that upon culture with an antibody to cross-link the TCR, the cells failed to proliferate vigorously in response to IL-6, nor did they respond to IL-1. Here we show that the two lymphokines dramatically synergize to stimulate IL-2 production and Th cell proliferation. The possible roles of IL-6 and IL-1 in T cell activation and in the regulation of IL-2 gene expression are discussed.

MATERIALS AND METHODS

Mice. BALB/c AnN mice were bred and maintained in the Center for Cancer Research Animal Facility, Massachusetts Institute of Technology, Cambridge, MA.

Cytokines. Human rIL-1α was a generous gift of Hoffmann-La-Roche (Nutley, NJ). Human rIL-2 was generously provided by Biogen Research (Cambridge, MA). Murine rIL-4 was kindly provided by Dr. Steven Gillis (Immunex Corporation, Seattle, WA). Conditioned medium from monkey COS-1 cells transfected with an IL-6 expression plasmid (pCSF-309) was kindly provided by Drs. K. Jacobs and S. Clark (Genetics Institute, Cambridge, MA) and was used as a source of human rIL-6. The conditioned medium contained ~2.1 μg/ml of IL-6, as determined by comparison to purified human rIL-6 in a proliferation assay with the use of the IL-6-dependent hybridoma, B9. Purified COS cell-derived human rIL-6 was a generous gift of Catherine Turner (Genetics Institute).

mAb. Anti-mouse IL-2 (S4B6.1) (21) and anti-mouse IL-4 (11B11) (22) were provided by Dr. D. Parker and were purified from ascites fluids by the use of protein G-Sepharose and protein A-Sepharose (Pharmacia Corp., Piscataway, NJ) respectively. A preparation of purified rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) was used as a negative control in the mAb-blocking

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[3] Abbreviations used in this paper: TAF, T cell-activating factor.

experiments. The anti-Vβ8 mAb F23.1 (15) and anti-CD3 mAb 145-2C11 (23) (hereafter called 2C11) were provided by Dr. M. Bevan and Dr. J. Bluestone, respectively. GK1.5 (anti-CD4) (24) and AD4(15) (anti-CD8) (25) were provided by Dr. Frank Fitch and Dr. Paul Gottlieb, respectively. F23.1 and 2C11 were coupled to cyanogen bromide-activated Sepharose beads (Sigma, St. Louis, MO) at 2 mg of protein/ml of bead suspension (26).

T cell proliferation experiments. Culture medium used in all experiments was RPMI 1640 supplemented with 5% FCS, 50 μM 2-ME, 0.2 M HEPES, and antibiotics. Peripheral lymph node cells from 6- to 10-wk-old mice were passed over nylon wool columns. The nonadherent cells at 1×10^7/ml were treated with anti-Ia mAb (BP107 and 14.4.4) and dilutions of AD4(15) (anti-CD8) ascites for 30 min on ice, followed by a mixture of rabbit (1/50 final dilution) and guinea pig (1/10 final dilution; GIBCO, Grand Island, NY) C for 45 min at 37°C. Viable cells were isolated by using Ficoll/Isopaque, and were then incubated in PBS containing 5% FCS on plates coated with 2.5 μg/ml GK1.5 (anti-CD4) antibody. After 1.5 h at 4°C, nonadherent cells were removed by extensive washing, and then the adherent cells were isolated by vigorous pipetting. These adherent cells were >99% $CD4^+CD8^-$ as assessed by flow cytometry, using GK1.5 and 53.67 (anti-CD8) antibodies, followed by a fluorescein-ated goat anti-rat IgG secondary mAb (not shown). The $CD4^+$ cells were cultured in half area, flat bottomed microculture wells (Costar 3696, Cambridge, MA) at 1×10^5 or 2×10^4/well in the presence of anti-TCR mAb (either conjugated to Sepharose beads or to the well bottoms directly) and various lymphokines (final volume, 0.1 ml). Positive control cultures included irradiated (3000 rad) syngeneic spleen cells (1×10^5/well) and 2 μg/ml Con A. Proliferation of the cells was assessed at various time points by adding [^{3}H]TdR (0.5 μCi/well, 6.7 Ci/mmol; New England Nuclear, Boston, MA) to the cultures 4 h before processing them for scintillation counting by using a Skatron cell harvester (Skatron, Sterling, VA). 2C11 was directly coated to the bottoms of half area, 96-well plates by loading 25 μl of purified antibody (0.5 μg/ml) per well. The plates were incubated at 37°C for 2 h, then washed twice with RPMI 1640 supplemented with 5% FCS. The plates were further incubated with RPMI-FCS for 30 min at 37°C, washed once again, and then used in T cell activation experiments. These conditions gave maximal proliferative responses in preliminary titration experiments.

IL-2 assay. Medium conditioned by cultured $CD4^+$ cells was isolated and the IL-2 content was determined as described by Gillis et al. (27) by using a subline of the CTLL-2-15H indicator cell line that responds to IL-2 but not to IL-4 (9). The indicator cells (2000/well) were cultured for 20 h with the indicated samples and pulsed for 8 h with [^{3}H]TdR (0.5 μCi/well). Unit values were assigned based upon comparisons with an IL-2 standard included in each experiment. One unit is ~820 pg of IL-2.

RESULTS

IL-6 and IL-1 synergize in the activation of $CD4^+$ cells. Purified resting T cells depleted of accessory cells will produce IL-2 and proliferate in response to exogenously added IL-6 and agarose beads conjugated with the anti-Vβ8 mAb, F23.1, which cross-links the TCR of approximately 20% of BALB/c T cells (9). To determine whether these results could be reproduced with more highly purified cells, $CD4^+CD8^-$ T cells were purified by using a more rigorous protocol that includes a "panning" step to positively select for $CD4^+$ cells (see *Materials and Methods*). This method yields a preparation of >99% $CD4^+$ T cells as assessed by flow cytometry, and results in a more thorough functional depletion of accessory cells. When these cells were cultured with F23.1-conjugated beads and IL-6 only a small proliferative response was observed on day 2.5 of culture and diminished sharply thereafter (Fig. 1). Parallel cultures containing IL-1α yielded no detectable response. IL-6 combined with IL-1, however, produced a vigorous proliferative response that was evident as late as day 7.5 (see also Fig. 6). The level of the response to the combination of F23.1 beads, IL-6, and IL-1 was comparable to that observed in control cultures containing Con A and irradiated spleen cells as a source of accessory cells (see legend to Fig. 1). Essentially similar

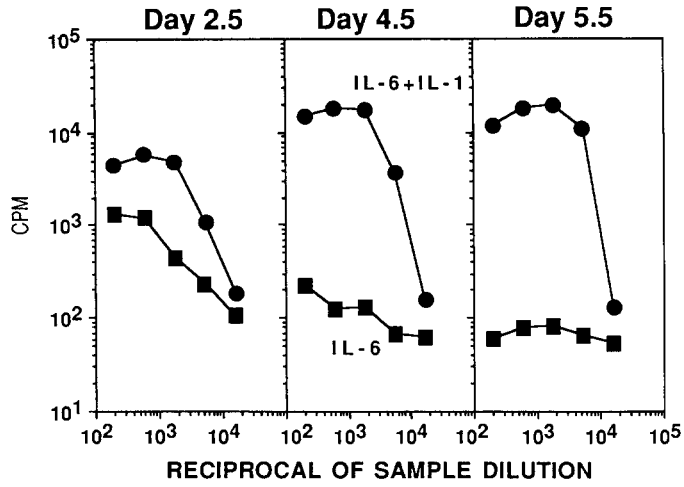

Figure 1. Synergy between IL-1 and IL-6 in the induction of $CD4^+$ cell proliferation. Highly purified $CD4^+$ cells (10^5/well) were cultured with F23.1 conjugated beads and a titration of a COS cell supernatant containing human rIL-6, with or without 10 U/ml of human rIL-1α (see *Materials and Methods*). The response to cells cultured in medium alone, with F23.1 beads alone, or with F23.1 beads + IL-1 was <100 cpm on all 3 days. $CD4^+$ cells cultured with 10^5 irradiated (3000 r from ^{137}Cs source) syngeneic spleen cells and 2 μg/ml Con A gave 11,032 cpm on day 2.5, 21,732 cpm on day 4.5, and 31,874 cpm on day 5.5. The data are the mean of duplicate cultures. SE were generally less than 20% of the mean.

results were obtained in 18 of 20 independent experiments, although the ratio of the responses to IL-6 plus IL-1 and the positive control with Con A and irradiated spleen cells varied from one-third to unity. In two experiments the T cells did not respond to IL-6 plus IL-1, but did respond to Con A plus irradiated spleen cells.

The requirement for both IL-6 and IL-1 for high rate DNA synthesis evident in these experiments contrasts with our earlier finding that IL-6 alone costimulates IL-2 production and T cell proliferation (8, 9). In addition, the duration of the proliferative responses observed in the present experiments, and the levels of IL-2 produced in these cultures (see below), far exceed that observed in our previous experiments. In those studies, however, we used less rigorously purified T cells, and it is likely that IL-1 produced by small numbers of contaminating macrophages in those cultures synergized with the exogenously added IL-6.

The possibility that the panning step with anti-CD4 antibodies alters the responsiveness of the cells was assessed in separate experiments by eliminating this step (data not shown). In general, we obtained similar results to those described above when the panning step was omitted, although the responses of these cells to IL-6 without IL-1 was usually somewhat higher than those of "panned" cells. It is therefore likely that the CD4-panning step is a more reproducible means of depleting residual accessory cells.

The results above, obtained by determining thymidine incorporation, were corroborated in a separate experiment by performing direct cell counts of cultures over a 7.5-day period. As shown in Figure 2, only cultures containing both IL-1 and IL-6 yielded increased viable cell numbers (about fourfold) over the culture period; these cells were mostly large blasts. This amounts to a 20-fold specific increase in cell numbers when it is considered that only Vβ8 cells (~20% of T cells) should respond in these cultures. In contrast, viable cell counts in cultures with F23.1 beads and IL-1 alone, like the control cultures

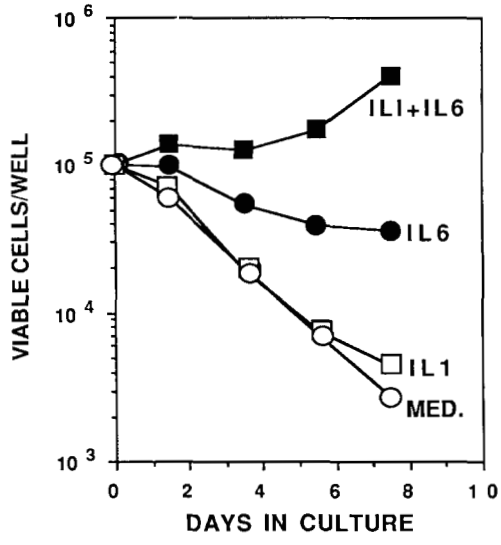

Figure 2. Direct cell counts. CD4+ cells at 10^5/well were cultured with F23.1 beads and the indicated lymphokines. At selected times the contents of each well were mixed thoroughly and samples were removed for counting on a hemacytometer. Viable cells were identified by the exclusion of trypan blue.

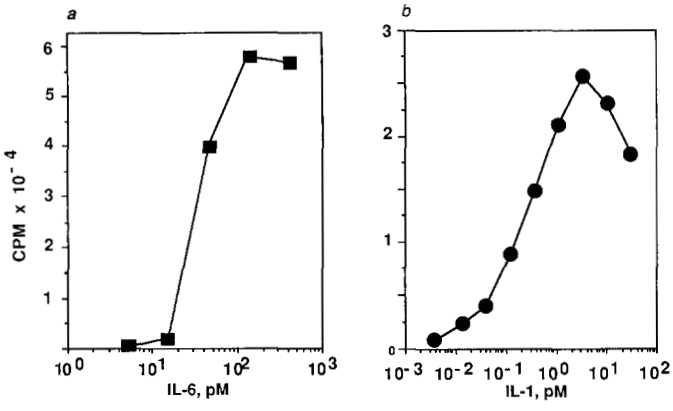

Figure 3. Determination of concentrations of IL-6 and IL-1 required for half maximal responses. Purified CD4+ cells were cultured at 10^5/well with F23.1 beads and *a*) a titration of purified human rIL-6 with 10 U/ml (30 pM) of human rIL-1α, or *b*) a titration of IL-1α with a saturating amount of IL-6 (400 pM). Incorporation of [^{3}H]TdR was measured on day 3.5. *a*, The response to F23.1 beads and IL-1 was 154 ± 32 cpm, and the response to F23.1 beads and saturating IL-6 (400 pM) without IL-1 was 884 ± 150 cpm; *b*, the response to F23.1 beads and IL-6 was 1760 ± 405 cpm. Cells cultured in medium alone or with F23.1 beads alone gave <100 cpm in both experiments. The data are means of triplicate cultures. SE were generally less than 20% of the mean.

with no stimulants, declined steadily until by day 5.5 very few living cells remained. Interestingly, although cell numbers declined in cultures stimulated by F23.1 beads and a saturating amount of IL-6, significant numbers of living cells were detected as late as day 7.5. The decline in cell number in these cultures may be attributed to the death of Vβ8$^-$ T cells, because they are not recognized by the F23.1 mAb; the combination of IL-6 and direct stimulation of the TCR may maintain the viability of Vβ8$^+$ T cells in these cultures (see below).

In the presence of 10 U/ml of IL-1α, the concentration of purified (COS cell-derived) human rIL-6 required for a half maximal response is approximately 40 pM (~1 ng/ml; Fig. 3*a*). This concentration corresponds roughly to that required for other IL-6 mediated activities, and fits with the published K_d of 10^{-11} M reported for the high affinity human IL-6R (28). In the presence of saturating amounts of IL-6, 0.2 pM of *Escherichia coli*-derived rIL-1α is required for a half maximal response, which is within the effective dose range reported for other IL-1 bioactivities (Fig. 3*b*) (29, 30).

Extension of the system to mAb 145-2C11. To demonstrate that the synergistic activation of T cells by IL-1 and IL-6 is not a special property of cells stimulated with F23.1-beads, the highly mitogenic mAb 145-2C11, which recognizes the ϵ-chain of the CD3 complex expressed by all mature T cells (23) was compared to F23.1. When CD4$^+$CD8$^-$ cells were cultured at a density of 2×10^4/well with 2C11-conjugated agarose beads, the synergy between IL-6 and IL-1 was readily apparent: the cells failed to respond to IL-6 or IL-1 alone, but responded vigorously to a combination of the two lymphokines (Table I; Expt. 1). However, when the cells were cultured with 2C11-beads at a higher density of 1×10^5 cells/well significant responses to either IL-6 or IL-1 alone were observed, and the response to IL-6 and IL-1 together was only threefold higher than the response to IL-6 alone.

This density dependence was reproduced in a separate experiment when purified 2C11 was coated directly to the surfaces of the culture wells at a predetermined optimal concentration (Table I: Expt. 2). When cultured at 2×10^4 CD4$^+$ cells/well, a requirement for both IL-6 and IL-1 to stimulate vigorous proliferation was clearly evident. However, when cultured at the higher density of 1×10^5 cells/well, a vigorous response to IL-6 alone and a modest response without lymphokines was evident. It is possible that at the higher cell densities the concentrations of IL-6 and IL-1 produced by small numbers of contaminating macrophages are sufficient for a low level of activation of the CD4$^+$ cells by the highly mitogenic 2C11 antibodies. Alternatively, at high cell densities the cells stimulated with 2C11 may be activated to a small extent by a mechanism unrelated to IL-6 and IL-1.

Interestingly, cells cultured at 5×10^3/well with plate-bound 2C11 antibodies failed to respond to IL-1 plus IL-6 (data not shown). This observation suggests that a critical cell-cell interaction necessary for the response may become limiting at lower cell densities.

The proliferation induced by IL-6 and IL-1 is mediated by IL-2. In order to identify the autocrine growth

TABLE I

Synergy of IL-6 and IL-1 in stimulating T cells activated with anti-CD3 antibodies[a]

Lymphokines Added	Response (cpm) on Day 3.5		
Expt. 1	F23.1 beads 1×10^5 cells/well	2C11 beads 1×10^5 cells/well	2C11 beads 2×10^4 cells/well
None	78 ± 8%	1,167 ± 11%	65 ± 2%
IL-1, 10 U/ml	79 ± 2%	23,784 ± 24%	86 ± 7%
IL-6, 1/200[b]	1,659 ± 4%	30,760 ± 2%	97 ± 11%
IL-6 + IL-1	57,867 ± 2%	97,162 ± 5%	8,010 ± 11%
Expt. 2	F23.1 beads 1×10^5 cells/well	2C11 plates 1×10^5 cells/well	2C11 plates 2×10^4 cells/well
None	77 ± 12%	5,899 ± 18%	58 ± 5%
IL-1, 10 U/ml	140 ± 6%	8,574 ± 5%	79 ± 11%
IL-6, 1/200	6,429 ± 23%	71,431 ± 8%	1,053 ± 50%
IL-6 + IL-1	67,373 ± 2%	77,440 ± 2%	11,208 ± 2%

[a] Purified CD4$^+$ cells were cultured at the indicated densities with F23.1-beads, 2C11-beads (Expt. 1), or on 2C11-coated plates (Expt. 2), and with the indicated lymphokines. Proliferation of the cells was measured on day 3.5 by pulsing the cells with 0.5 μCi/well of [^{3}H]TdR and harvesting them for scintillation counting. The data are the arithmetic means and SE of triplicate cultures.

[b] Diluted COS-cell supernatant.

factor that mediates proliferation in this system, culture supernatants from activated CD4+ T cells were assayed for IL-2 content by using an IL-2 indicator subline that responds to IL-2 and not IL-4. When F23.1-beads were used to stimulate the CD4+ cells, IL-2 (up to 25 U/ml) could only be measured when both IL-6 and IL-1 had been added to the cultures; neither IL-6 nor IL-1 alone stimulated detectable IL-2 production (Fig. 4). With 2C11-coated plates the results were even more dramatic: IL-6 and IL-1 together stimulated the production of >135 U/ml of IL-2 6.5 days after the initiation of culture; in contrast, IL-1 alone and IL-6 alone produced only <0.2 and 3.5 U/ml, respectively. That the active lymphokine is indeed IL-2 was confirmed in experiments in which an anti-IL-2 mAb, but not an anti-IL-4 mAb, completely inhibited the response of the indicator cells to the relevant supernatant (data not shown). These results demonstrate that IL-1 and IL-6 together stimulate the production of the autocrine growth factor IL-2.

It is striking that in this system IL-2 levels are still increasing at day 7 of culture (Fig. 4), indicating that secretion of the lymphokine continues for at least a week. In contrast, IL-2 levels in lectin-stimulated spleen cells (27, 31) or T cell lines (17, 31) peak by 24 to 48 h of culture. Moreover, the levels of IL-2 eventually produced in cultures stimulated with IL-6 plus IL-1 far exceeds that produced in mitogen-induced cultures with splenic accessory cells (usually only a few U/ml, not shown).

To determine whether the proliferation of purified T cells induced by IL-6 and IL-1 together is mediated by the IL-2 they produce, CD4+ T cells were stimulated by saturating amounts of IL-6, IL-1, and F23.1 beads in the presence of mAb that neutralize either murine IL-2 or murine IL-4 (Fig. 5). Neither the rat IgG control nor the anti-IL-4 mAb significantly inhibited the response to IL-6 plus IL-1, even though in a control experiment the anti-IL-4 mAb blocked proliferation in response to 3200 U/ml of murine IL-4 (unit definition of Genzyme Corp., Boston, MA). The anti-IL-2 mAb, however, achieved a 70% inhibition of proliferation at a concentration of 2 μg/ml (Fig. 5a); in a separate experiment the anti-IL-2 mAb added at 20 μg/ml achieved a maximum of 83% inhibition (Fig. 5b). As a control for nonspecific effects of the antibody, we found that it did not inhibit proliferation mediated by *human* IL-2 (not shown). Thus, in contrast to some reports (32, 33), we find that proliferation of T cells supported by IL-6 (9) and by IL-6 and IL-1 (this report) is mediated largely by IL-2. Little or none of the response can be attributed to IL-4. Because 100% inhibition of proliferation was not attained with the anti-IL-2 mAb, we cannot rule out the possibility that a small fraction of the proliferative response proceeds by an IL-2-independent pathway.

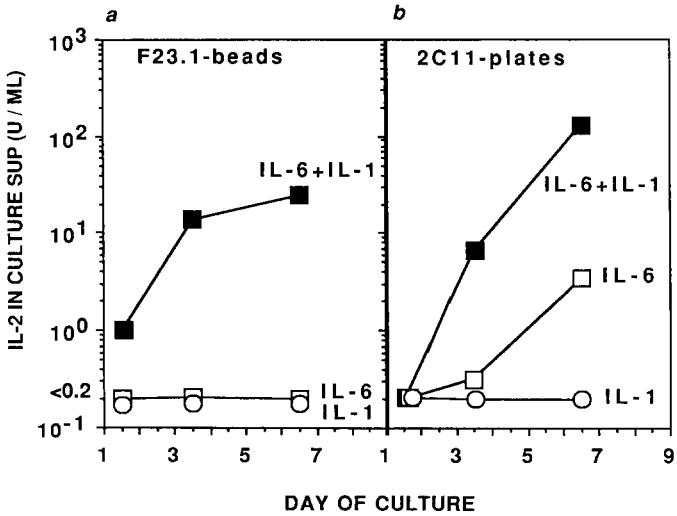

Figure 4. IL-2 content in media conditioned by stimulated CD4+ cells. *a*, CD4+ cells at 1 × 10⁵/well were stimulated with F23.1 beads and saturating amounts of the indicated lymphokines; *b*, CD4+ cells at 2 × 10⁴/well were stimualted on 2C11-coated plates with saturating amounts of the indicated lymphokines. In both experiments, cells cultured with insolubilized anti-TCR antibodies alone produced <0.2 U/ml of IL-2 at all time points.

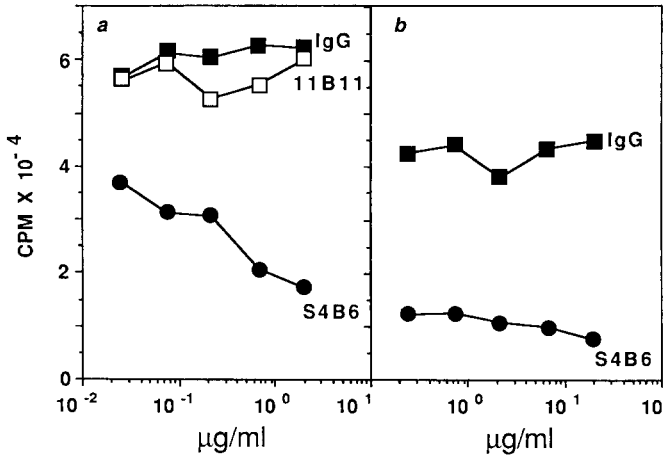

Figure 5. Proliferation of CD4+ cells stimulated by IL-6 + IL-1 is inhibited by a mAb to IL-2. CD4+ cells were cultured at 10⁵/well with F23.1 beads, IL-1 (10 U/ml), saturating IL-6 (1/1000 dilution of COS cell supernatant in *a*, 1/1500 in *b*), and a titration of the indicated mAb: either rat IgG, anti-mIL-4 mAb 11B11, or anti-mIL-2 mAb S4B6. Incorporation of [³H]TdR was measured on day 4.5 in *a* and day 3.5 in *b*. The proliferative responses to medium alone, F23.1 beads alone, and F23.1 beads and IL-1 were <200 cpm in both experiments. The responses to F23.1 beads and IL-6 were 3012 ± 813 cpm in *a* and 1281 ± 77 cpm in *b*. SE were generally less than 15% of the mean of triplicate cultures.

IL-6 and IL-1 may act simultaneously to induce the proliferative response. A delayed addition experiment was designed to address whether the two synergizing lymphokines must be present simultaneously or can act sequentially. CD4+ cells were purified as before and cultured with F23.1 beads. Replicate sets of cultures received excess IL-6, IL-1, IL-6 + IL-1, or no lymphokines. After 2 or 3.5 days of culture, some sets of cultures were supplemented with IL-6, IL-1, or both lymphokines. The kinetics of the proliferative responses were compared with a standard kinetic response from cultures that received both lymphokines at day 0, with no further additions. The results in Figure 6 show that IL-1 added on day 2 to cultures already containing IL-6 elicited a delayed but vigorous response, and when added on day 3.5 it induced modest proliferation that was still increasing on day 9. In contrast, cells cultured with F23.1 beads and IL-1 did not respond when IL-6 was added on day 2. In addition, cells cultured in medium alone for 2 days failed to respond to a combination of the two lymphokines. The failure to achieve a response upon late addition of IL-6 may be caused in part by the rapid decline in viable cell numbers in cultures lacking IL-6 (Fig. 2). However, since there are still significant numbers of viable cells at day 2 in such cultures, the failure to respond to IL-6 added at that time implies that the T cells rapidly become anergic to IL-6 upon in vitro culture. Taken together, these results

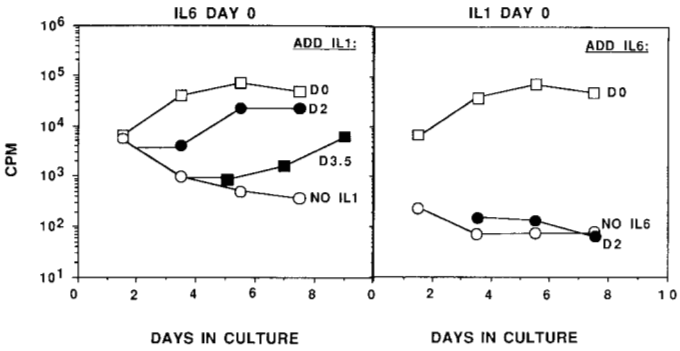

Figure 6. Delayed addition experiment. *a*, CD4+ cells were cultured with F23.1 beads and IL-6 (75 pM), and 10 U/ml of IL-1 was added at the indicated times. *b*, CD4+ cells were cultured with F23.1 beads and 10 U/ml of IL-1. At the indicated times IL-6 (75 pM) was added. [^{3}H]TdR incorporation was assessed at various times as indicated. Cells cultured with F23.1 beads did not proliferate when IL-6 and IL-1 were added together on day 2 or on day 3.5.

suggest that IL-1 and IL-6 must be present simultaneously to induce T cell proliferation.

DISCUSSION

In this communication we have demonstrated that IL-6 and IL-1 synergize in vitro to stimulate IL-2 production and proliferation of highly purified CD4+ cells. It is important to consider how these lymphokines may function in vivo as costimulatory signals in T cell activation. We emphasize that our results in no way demonstrate that IL-6 or IL-1 are obligatory signals for T cell activation mediated by APC. Nonetheless, the fact that macrophages produce both IL-6 (34) and IL-1(35) and also function effectively as APC for resting T cells (36, 37) raises the possibility that the two lymphokines are necessary cofactors for macrophage-induced T cell activation. Alternatively, IL-6 and IL-1 produced by macrophages during inflammatory responses may amplify T cell activation mediated by undefined costimulatory signals from other APC. Indeed, recent reports that dendritic cells do not produce IL-1 (35), despite their potent activity as accessory cells (38), suggest that IL-1 may not play an essential role in dendritic cell-induced T cell activation. IL-1 has been demonstrated to *augment* dendritic cell function, however (19). Whether dendritic cells produce IL-6 is currently under investigation. To our knowledge, the possibility that different types of APC use different pathways to activate T cells is not ruled out by the current literature.

Recent studies of the activation of cloned IL-2 producing T cell lines have suggested that none of the known lymphokines can substitute for viable APC (39) (R. Schwartz, personal communication). Thus it is possible that our assay systems miss an undefined costimulatory signal, which may be provided only by direct cell contact (39). Indeed, a requirement for a limiting cell-cell interaction may explain our observation that responses to IL-6 plus IL-1 fall off rapidly at low cell densities. Whether IL-6 and IL-1 are necessary (rather than sufficient) for activation of cloned IL-2-producing T cells as well as peripheral T cells is currently under investigation. In any case, the activation requirements of cloned T cells may not completely overlap with those of resting peripheral T cells.

Several possible mechanisms can explain how IL-6 and IL-1 affect IL-2 gene expression. Our previous results (8) as well as recent unpublished results indicate that IL-6 and IL-1 act at least at the level of regulating IL-2 transcript levels. The signals transduced by their respective receptors might induce distinct transcription factors that are necessary for transcription. Indeed, such a model involving the induction of different IL-2 enhancer-binding proteins by independent stimulatory signals has been proposed (40). Alternatively, one lymphokine may induce transcription and the other may prevent down-regulation of IL-2 expression, perhaps by stabilizing the mature IL-2 mRNA. This model accomodates the observation that early after the initiation of culture IL-6 alone stimulates a significant degree of proliferation, and that the synergy between IL-6 and IL-1 becomes more dramatic at later timepoints (see Fig. 1 and Fig. 6). Experiments to distinguish between these models are in progress.

Finally, the synergy of IL-6 and IL-1 has been demonstrated in other bioassays recently (33, 41–43), and may implicate these two lymphokines in a general pathway of activation involving a variety of cell types.

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