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Interleukin 7 is a T-cell growth factor

(lymphocyte/cytokine/proliferation/phorbol ester)

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ABSTRACT **Interleukin 7 (IL-7) is a 25-kDa cytokine which was purified and its corresponding cDNA was cloned based upon its ability to stimulate the proliferation of pre-B cells. It has been shown that IL-7 can also function as a costimulator with Con A for the proliferation of T lymphocytes by inducing the production of interleukin 2 (IL-2). We demonstrate here that IL-7 in combination with phorbol 12-myristate 13-acetate can directly drive the proliferation of purified T cells and that this response is not inhibited by cyclosporine A or by antibodies to IL-2 and IL-4. Stimulation of T cells with phorbol myristate acetate and IL-2, IL-4, or IL-7 prepared T cells to respond to any of the three lymphokines. Although T cells activated *in vitro* by anti-CD3 or allogeneic cells failed to proliferate when challenged with IL-7, T cells primed *in vivo* to the same stimuli demonstrated a significant proliferative response when restimulated *in vitro* with IL-7. IL-7 can, therefore, function both as a growth factor for T cells in an IL-2-independent manner and as a competence factor for the induction of lymphokine responsiveness. The ability to induce IL-7 responsiveness via stimulation of the T-cell receptor complex *in vivo*, but not *in vitro*, raises the possibility that IL-7 may play a role in T-cell growth and differentiation *in vivo*.**

Although many of the interleukins were initially characterized as having functional effects on a single cell type, multiple studies over the past 5 years have demonstrated that almost all of the interleukins exhibit pleiotropic effects on a wide variety of both lymphoid and nonlymphoid cells. Interleukin 2 (IL-2) was originally considered to be the sole T-cell growth factor (1). However, IL-4, which was originally characterized as a B-cell growth factor (2), has been found to induce T-cell proliferation in an IL-2-independent manner (3), and IL-6, which was initially recognized as a B-cell differentiation and hybridoma growth factor (4), subsequently was also found to function as a T-cell growth factor in either an IL-2-dependent (5) or an IL-2-independent (6) manner.

IL-7 is a 25-kDa glycoprotein that was purified (7) and the corresponding cDNA was cloned (8, 9) based upon its ability to stimulate the proliferation of pre-B cells. Morrissey *et al.* (10) have recently shown that IL-7 can function as a costimulator for the proliferation of T cells depleted of accessory cells (AC) by inducing the production of IL-2. In the present study, we demonstrate that IL-7 and phorbol 12-myristate 13-acetate (PMA) induce resting T cells to synthesize DNA by an IL-2-independent pathway and also induce responsiveness to IL-7, IL-2, and IL-4.

MATERIALS AND METHODS

Animals. C57BL/6J and BALB/c female mice, 8 to 12 weeks of age, were obtained, respectively, from The Jackson Laboratories and from the National Cancer Institute, Frederick Cancer Research Facility (Frederick, MD).

Culture Medium and Conditions. Cultures, unless otherwise indicated, were performed in R10, which consists of RPMI 1640 medium (Biofluids, Rockville, MD) supplemented with L-glutamine (300 μg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (50 μM), and 10% heat-inactivated fetal calf serum (FCS).

Reagents and Monoclonal Antibodies (mAbs). Con A and PMA were purchased from Pharmacia and Sigma, respectively. Cyclosporine A (CsA) (Sandimmune I.V.) was a gift of Sandoz Pharmaceuticals. Low-Tox-M activated rabbit complement (C) was purchased from Cedarlane Laboratories. Recombinant human (rh) IL-2 was kindly provided by Cetus, recombinant murine (rm) IL-4 was the gift of W. Paul (National Institutes of Health), and rmIL-7 [from COS-7 cells transfected with expression plasmid pDC201 containing the IL-7 cDNA insert 1046B (8)] was generously supplied by Immunex. Supernatant from splenocytes cultured with Con A and then supplemented with methyl α-D-mannopyranoside (20 mg/ml, Sigma) was used as a source of murine IL-2. mAbs HO-13-4 (anti-Thy 1.2) (11), M5/114.15.2 (anti-Ia) (12), MAR 18.5 (anti-rat κ) (13), GK1.5 (anti-L3T4) (14), 53.6.7 (anti-Lyt-2) (15), S4B6.1 (anti-murine IL-2) (16), and 11B11 (anti-murine IL-4) (17) were used as dilute ascites fluid. mAb S4B6.1 ascites fluid was used at a final concentration of 1:40, at which it was able to specifically neutralize 50–100 units (U)/ml of murine IL-2 in a CTLL assay (3). mAb 11B11 ascites fluid was used at a final concentration of 1:400, at which it was able to specifically neutralize 2500–5000 U/ml of rmIL-4 in a bioassay. One unit of lymphokine is defined as that quantity which results in half-maximal [^{3}H]thymidine uptake by an indicator cell line in a bioassay. At the concentrations used, neither mAb S4B6.1 nor mAb 11B11 cross-reacted, respectively, with IL-4 or IL-2. Monoclonal antibody 145-2C11 (anti-CD3 ε) (18) was purified from culture supernatant by affinity chromatography on a staphylococcal protein A-Sepharose 4B column.

Preparation of Resting T Cells and AC. Highly purified T cells were prepared by passage of mesenteric lymph node cells twice over nylon wool columns followed by serial treatments with M5/114 + C and MAR 18.5 + C. T-cell subsets were prepared by the addition of either GK1.5 or 53.6.7 to the incubation mixture with M5/114. The purity of these populations was confirmed by fluorescent flow cytometric analysis. Non-T AC were prepared as previously described (19) and irradiated with 3000 rads (1 rad = 0.01 Gy).

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Abbreviations: AC, accessory cells; C, activated rabbit complement; CsA, cyclosporine A; IL, interleukin; mAb, monoclonal antibody; PMA, phorbol 12-myristate 13-acetate; rh, recombinant human; rm, recombinant murine; U, unit(s).

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***In Vitro* Activation of T Cells.** Lymphokine-activated T cells: Purified T cells (1×10^6 per well) were cultured for 36 hr in 24-well plates (2 ml per well) in control R10 medium or medium containing PMA (10 ng/ml), rhIL-2 (40 U/ml), rmIL-4 (800 U/ml), rmIL-7 (1000 U/ml) alone, or combinations of PMA and each lymphokine. Con A blasts: Purified T cells (2×10^6 per well) and AC (2×10^6 per well) were cultured for 72 hr in 24-well plates (2 ml per well) in R10 containing Con A (5 μg/ml). Blast cells were washed three times in methyl α-D-mannopyranoside. Immobilized anti-CD3: Several wells of 24-well culture plates were incubated with 250 μl of purified mAb 145-2C11 (2 μg/ml) in RPMI 1640 medium for 2 hr at 24°C. The wells were washed three times with 2 ml of R10 (20). Purified T cells (1×10^6 per well) were then added in a final volume of 2 ml and cultured for 72 hr. Mixed lymphocyte response: Purified BALB/c T cells (1.25×10^6 cells per ml) were cultured in R10 with C57BL/6J AC (2.5×10^6 cells per ml) for 96 hr in upright 75-cm^2 culture flasks in a final volume of 10 ml.

***In Vivo* Activation of T Cells.** Anti-CD3: Purified mAb 145-2C11 (25 μg) was injected into each hind footpad of BALB/c mice. The mice were sacrificed 24 hr later, and the draining popliteal lymph nodes were harvested and either treated with M5/114 + C followed by MAR 18.5 + C to remove non-T cells or treated with MAR 18.5 + C alone. Mixed lymphocyte response: Four days after being immunized in each rear footpad with 1.5×10^7 normal C57BL/6J spleen cells, BALB/c mice were sacrificed, their popliteal lymph nodes were harvested, and purified subsets were prepared as above.

Proliferation Assay. Resting T cells activated *in vitro* or *in vivo* (10^5 per well) were cultured in flat-bottomed 96-well microtiter plates in a final volume of 200 μl for 42–96 hr. [^{3}H]Thymidine (ICN) was added at 1 μCi (37 kBq) per well 4–18 hr before the end of the culture. The cells were then harvested on fiberglass filters and analyzed by liquid scintillation counting. All assays were performed in triplicate, and the data are expressed as the mean cpm of [^{3}H]thymidine incorporated by triplicate cultures. The SEM was always <10% of the mean.

RESULTS

IL-7 Is a Costimulant for Resting T Lymphocytes. It has been previously demonstrated that vigorous proliferative responses of resting murine T lymphocytes can be triggered by stimulation with either IL-2 or IL-4 in the presence of PMA (2). To evaluate whether IL-7 would also function as a costimulator of T-cell proliferation, highly purified T lymphocytes were cultured in the presence of IL-7, PMA, or the combination of IL-7 and PMA. Neither IL-7 nor PMA was mitogenic for resting T cells, but a marked proliferative response was observed when T cells were cultured in the presence of both agents (Fig. 1). In general, in multiple experiments of this type, the proliferative response to IL-7 and PMA was similar in magnitude to that seen with the combination of IL-4 and PMA but was consistently less than the response to IL-2 and PMA. Importantly, supernatants from mock-transfected COS-7 cells not containing the IL-7 cDNA failed, both in the presence and in the absence of PMA, to induce T-cell DNA synthesis (data not shown). It is unlikely that the proliferative response to IL-7 and PMA was mediated by a small subpopulation of the resting T cells or by contaminating B cells because 30% of the responding population exhibited a 2- to 4-fold increase in cell volume 36 hr after stimulation (data not shown). Furthermore, proliferative responses of a magnitude similar to that seen in the unfractionated population could be induced in highly purified CD4$^+$ and CD8$^+$ T-cell subsets (Table 1).

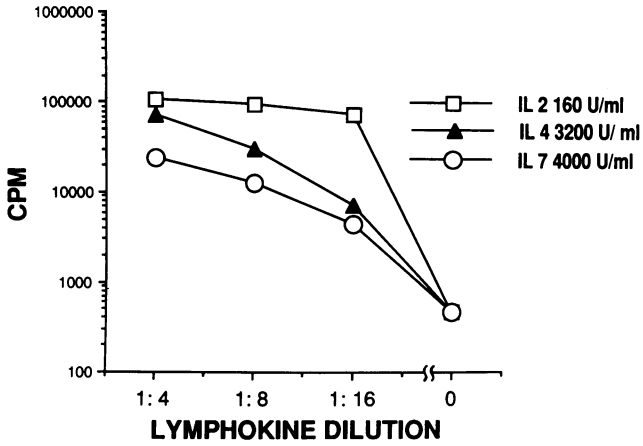

FIG. 1. IL-7 is a T-cell growth factor. Purified T cells were cultured in the presence of PMA and the indicated lymphokine for 72 hr. [^{3}H]Thymidine was added for the last 18 hr of the culture.

The Proliferative Response to IL-7/PMA Is IL-2 Independent. Although IL-7 appeared to function as a costimulant of resting T-cell proliferation, the possibility remained open that the effects of IL-7 were indirect and mediated by induction of the production of either IL-2 or IL-4, which would themselves trigger T-cell proliferation in the presence of PMA. However, supernatants of T cells stimulated with IL-7 and PMA did not contain quantities of IL-2 or IL-4 detectable in bioassays (data not shown). To definitively rule out the possibility of IL-2 or IL-4 production in the response to IL-7, highly purified T cells were stimulated with IL-7 and PMA in the presence of a neutralizing antibody to murine IL-2 (S4B6), a neutralizing antibody to murine IL-4 (11B11), or CsA, an immunosuppressive drug which has been shown to inhibit the transcription of IL-2 (21) and IL-4 (22) mRNAs. Neither S4B6 nor 11B11 had a significant effect on the proliferative response induced by IL-7 and PMA (Table 2). The addition of CsA produced a 30–50% decrease in the response to IL-7 and PMA but completely abrogated the proliferative response of Con A-stimulated T cells. It is unlikely that the concentrations of anti-lymphokine mAbs used were suboptimal, as the concentration of S4B6 used was capable of inhibiting the proliferative response of resting T cells to a supernatant which contained mouse IL-2 and PMA, while the concentration of 11B11 was similarly capable of inhibiting the proliferative response of the same cell population to stimulation by rmIL-4 and PMA.

Induction of Responsiveness to IL-7 by Stimulation with IL-2, IL-4, or IL-7 and PMA. To determine whether stimulation of T cells with the combination of IL-2, IL-4, or IL-7 and PMA induced a functional receptor for each of the lymphokines, highly purified T lymphocytes were cultured with PMA alone, lymphokine alone, or the combination of PMA and lymphokine for 36 hr, washed, and then stimulated in a secondary culture for 42 hr with medium or each of the

Table 1. IL-7 plus PMA induces a proliferative response of both CD4$^+$ and CD8$^+$ resting T lymphocytes

Stimulus	[^{3}H]Thymidine incorporation, cpm	
	CD4$^+$ T cells	CD8$^+$ T cells
IL-7 (1000 U/ml) + PMA	19,286	59,168
IL-2 (40 U/ml) + PMA	103,342	228,066
IL-4 (800 U/ml) + PMA	40,387	85,662

Highly purified CD4$^+$ and CD8$^+$ T lymphocytes were incubated with PMA (10 ng/ml) and the indicated lymphokine as in Fig. 1. Incubation of each T-cell subset with medium, PMA, or lymphokine alone yielded less than 1000 cpm.

Table 2. T-lymphocyte proliferative response to IL-7 plus PMA is independent of both IL-2 and IL-4

Stimulus	Addition	[^{3}H]Thymidine incorporation, cpm Exp. 1	Exp. 2
IL-7 (1000 U/ml) + PMA	—	40,789	23,342
	Anti-IL-2	32,623	24,920
	Anti-IL-4	47,771	22,535
	CsA	23,422	10,756
Con A + AC	—	34,887	81,224
	CsA	858	378
Murine IL-2 + PMA	—	143,450	30,065
	Anti-IL-2	37,342	346
IL-4 (800 U/ml) + PMA	—	52,686	87,995
	Anti-IL-4	681	332

Highly purified T lymphocytes were incubated either with PMA (10 ng/ml) and the indicated lymphokine or with Con A (5 μg/ml) and AC (10^5 per well) as in Fig. 1. In Exp. 1, the murine IL-2 containing supernatant was used at a 1:4 dilution; in Exp. 2, it was used at a 1:32 dilution. CsA was 0.25 μg/ml. Incubation of T cells with medium, PMA, Con A, or lymphokine alone yielded less than 1000 cpm.

lymphokines (Fig. 2). Preculture with each of the lymphokines and PMA, but not with PMA or any of the lymphokines alone, resulted in the induction of responsiveness in secondary culture to all three of the lymphokines tested. The magnitude of the response in secondary cultures was greatest for T cells precultured with IL-2 and PMA, while the responses of cells precultured with IL-7 and PMA or IL-4 and PMA were smaller. Thus, these results demonstrate that stimulation with any of one of these three lymphokines and PMA not only is capable of inducing a functional receptor for the lymphokine used during the preculture but also is capable of inducing functional receptors for the other two lymphokines.

Induction of IL-7 Responsiveness by Activation Via the CD3/T-Cell Receptor Complex. It was also of interest to examine whether physiologic pathways of T-cell activation *in vivo* or *in vitro* induced IL-7 responsiveness. T cells stimulated *in vitro* with Con A plus AC, anti-CD3, or allogeneic

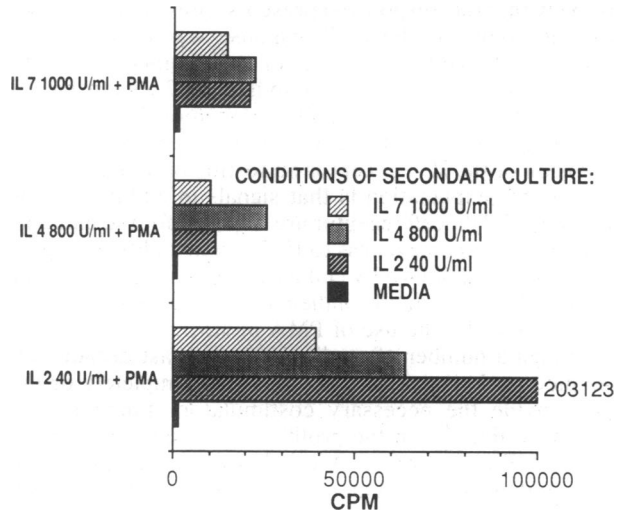

FIG. 2. IL-2, IL-4, or IL-7 in combination with PMA upregulate the IL-7 receptor. Purified T cells were cultured in the presence of IL-2, IL-4, or IL-7 in combination with PMA for 36 hr (see labels on the left), washed, and then recultured for an additional 42 hr in the presence of medium, IL-2, IL-4, or IL-7 as indicated in the key. [^{3}H]Thymidine was added for the last 18 hr of the secondary culture. The secondary responses of T cells precultured in medium, PMA, or lymphokine alone were uniformly less than 1500 cpm. Note that the response to IL-2 at the bottom goes off scale; the actual cpm is shown at the right of the bar.

cells failed to proliferate when restimulated with IL-7, although all three of the activated populations demonstrated a vigorous response to IL-2 and a lesser, but still significant, response to IL-4 (Fig. 3).

Very different results were observed with draining popliteal lymph node cells activated *in vivo* by injection of anti-CD3 or allogeneic cells in the footpads. All three lymphokines were capable of inducing a significant proliferative response of the unfractionated lymph node population, purified T cells (2C11-stimulated), or purified CD4 and CD8 subsets (allogeneic cell-stimulated) activated *in vivo* (Table 3). Furthermore, the effect of IL-7 on the *in vivo* activated cells appeared to be direct, as the addition of CsA, antilymphokine antibodies, or a combination of CsA and antilymphokine mAbs produced only a modest reduction in the magnitude of the proliferative responses to any of the lymphokines (Table 3).

IL-7 Functions as a Costimulator for Con A by an IL-2-Dependent Pathway. Morrissey *et al.* (10) have recently demonstrated that IL-7 has costimulatory activity for the Con A response of AC-depleted T-cell populations and that this costimulatory function is mediated by inducing the production of IL-2. Because our results on the stimulation of T-cell proliferation by IL-7 and PMA indicated that the proliferative response was independent of IL-2, we repeated the studies of Morrissey *et al.* and evaluated the costimulatory properties of IL-7 for the Con A response of highly purified T cells. In contrast to the results described by Morrissey *et al.*, we found in three of four experiments that IL-7 had only marginal ability to reconstitute the response of highly purified T cells to Con A (Table 4, Exp. 1); however, in some studies (Table 4, Exp. 2) a modest reconstitution of the Con A response was produced by the addition of IL-7. The response to the combination of IL-7 and Con A appeared to be mediated by an IL-2-dependent pathway, as the response was substantially inhibited by the addition of CsA or the addition of the anti-IL-2 mAb but not the addition of anti-IL-4. One possible explanation for the differences between our results and those of Morrissey *et al.* (10) is that an effect of IL-7 on the Con A response may require the presence of a small number of contaminating AC in the culture. Thus, the T-cell population in Table 4, Exp. 2 did demonstrate a small proliferative response to Con A in the absence of added AC, and flow cytometric analysis with anti-CD3 demonstrated a

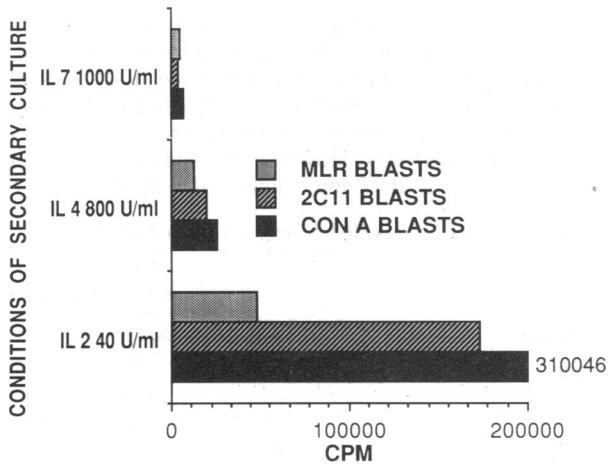

FIG. 3. Activation of T cells *in vitro* via the CD3/T-cell receptor complex fails to induce responsiveness to IL-7. T cells were cultured with Con A and AC (Con A blasts), mAb 2C11 bound to plastic (2C11 blasts), or allogeneic cells (MLR blasts) for 72 hr, washed, and then recultured for an additional 42 hr with IL-2, IL-4, or IL-7 as indicated on the left. [^{3}H]Thymidine was added for the last 18 hr of the secondary culture. Blast cells recultured with medium alone incorporated less than 1000 cpm.

Table 3. IL-7 induces proliferation of T lymphocytes activated *in vivo* through the CD3/T-cell receptor complex

Stimulus	Cell type	Addition	[^{3}H]Thymidine incorporation, cpm			
			Medium	IL-7	IL-2	IL-4
mAb 2C11	Lymph node	—	1969	20,106	125,650	39,127
		CsA	1536	15,538	96,220	28,239
		CsA + S4B6	1084	11,213		
		CsA + 11B11	1336	12,413		
	T cells	—	2802	23,268	164,436	46,989
		CsA	2570	17,912	131,360	49,141
		CsA + S4B6	1838	13,109		
		CsA + 11B11	1896	16,719		
Allogeneic cells	Lymph node	—	2365	21,925	131,591	ND
		CsA	214	13,632	114,248	ND
	CD4$^+$	—	690	25,476	158,277	ND
		CsA	235	20,350	174,562	ND
	CD8$^+$	—	780	31,865	123,030	ND
		CsA	646	31,971	157,509	ND

T cells activated *in vivo* by injection of mAb 2C11 (1×10^5 per well) or allogeneic cells (2×10^5 per well) were cultured with medium alone, rmIL-7 (1000 U/ml), rhIL-2 (10 U/ml), or rmIL-4 (800 U/ml) in the presence or absence of CsA (0.25 μg/ml) or CsA + mAbs for 42 hr. ND, not determined.

5–10% contamination by non-T cells (data not shown). In contrast, the T-cell population used in Table 4, Exp. 1, demonstrated less than 1% contamination by non-T cells and failed to respond to Con A alone.

DISCUSSION

IL-7 was originally identified as a stromal cell-derived soluble factor that was capable of supporting the growth of pre-B cells *in vitro* in the absence of any stromal cells. The availability of a specific bioassay for IL-7 activity facilitated its purification and subsequent cloning. Although the biologic activity of IL-7 was originally considered to be specific for pre-B cells, Morrissey *et al.* (10) have recently shown that IL-7 can provide a costimulatory signal for the *in vitro* proliferative response of purified murine T cells to Con A by inducing IL-2 production.

In the present report, we have extended these findings and demonstrated that IL-7 in combination with PMA can directly drive T-cell activation. The proliferative response to the combination of IL-7 and PMA was not inhibited by high concentrations of neutralizing antibodies to IL-2 or IL-4 and was largely resistant to the immunosuppressive effects of CsA, a drug which inhibits the transcription of a number of lymphokine genes, including those encoding IL-2, IL-4, and interferon-γ (22). These results strongly suggest that T-cell activation in this system is not mediated by an autocrine IL-2-dependent pathway. However, it is difficult to exclude the possibility that IL-7 may induce the production of a CsA-resistant lymphokine, and the partial susceptibility of the response to CsA may indicate that CsA induces the production of a CsA-sensitive T-cell growth factor other than IL-2 or IL-4.

Table 4. Response of purified T cells to Con A plus IL-7

Stimulus	AC	Addition	[^{3}H]Thymidine incorporation, cpm	
			Exp. 1	Exp. 2
Medium	−	—	327	917
Con A	−	—	804	2,298
	+	—	81,224	34,887
		CsA	378	858
IL-7	−	—	293	1,633
IL-7 + Con A	−	—	2,926	18,088
		CsA	ND	722
		Anti-IL-2	ND	7,384
		Anti-IL-4	ND	24,439

Highly purified T cells were cultured with IL-7 (1000 U/ml) and Con A (5 μg/ml) as in Fig. 1. When added, AC were 10^5 per well and CsA was 0.25 μg/ml. ND, not determined.

The induction of responsiveness to IL-7 exhibited a number of characteristics that are both similar and distinct from the induction of responsiveness to either IL-2 or IL-4. Stimulation of T cells with PMA and IL-2, IL-4, or IL-7 prepared T cells to respond to any of the three lymphokines. The secondary response to IL-2 was greater than the secondary response to either IL-7 or IL-4. Surprisingly, T cells activated *in vitro* via stimulation of the CD3/T-cell receptor complex failed to proliferate in secondary cultures to in response to IL-7, while responding as expected to stimulation with IL-2 or IL-4. In contrast, T cells obtained from the draining popliteal lymph nodes of animals injected in the footpad with anti-CD3 or allogeneic cells demonstrated a significant proliferative response when restimulated by IL-7. It is likely that this response represents a direct effect of IL-7 on proliferation, as the IL-7 response was only modestly reduced by the addition of CsA or anti-lymphokine mAbs. The significance of our failure to induce IL-7 responsiveness *in vitro* with Con A or anti-CD3 activation is not readily apparent and no data have as yet been presented on the expression of the IL-7 receptor on resting or activated cells. One possible explanation is that signals in addition to triggering the CD3/T-cell receptor are required for the induction of functional responsiveness to IL-7. Such additional signals may be provided *in vivo* by cell-associated or soluble factors produced by cells in the milieu of the draining activated lymph node or by the use of PMA *in vitro*.

Although a number of studies over the past decade have attempted to demonstrate that IL-1 can completely or partially provide the necessary costimulatory functions produced by viable AC in the proliferative response of resting peripheral T cells to Con A or phytohemagglutinin, more recent studies of both human and murine lymphocytes with recombinant IL-1 have been unable to confirm these older studies. A number of studies have claimed that some (23) or all (24) of the reconstitutive effects of AC on mitogen responses may be mediated by IL-6 via the induction of the IL-2 pathway. Morrissey *et al.* (10) have shown that IL-7 can act in a manner very similar to that described for IL-6 and reconstitute the response of AC-depleted murine T cells to Con A. However, in those studies it was impossible to estimate the extent of the reconstitution produced by the cytokine because parallel experiments with AC were not

included. We have confirmed the observations of Morrissey *et al.* (10) in that in certain experiments IL-7 was able to partially reconstitute the response of AC-depleted T cells to Con A and the reconstitution was mediated by the induction of an IL-2 pathway. However, in contrast to the uniform ability of the same T-cell population to respond to the combination of PMA and IL-7, significant reconstitution of the Con A response by IL-7 was only rarely seen in our experiments. One possible explanation for the difference between our findings and those of Morrissey *et al.* (10) is the extent of AC depletion of the responder T-cell population. It is thus possible that the production of another cytokine (IL-1 or IL-6?) by contaminating AC is needed for the costimulatory effects of IL-7 on the Con A response.

Although IL-7 was originally described as a cytokine with the pre-B cell as its specific target cell, as has become apparent with continued study of almost all cytokines, most are capable of acting on a wide variety of both lymphoid and nonlymphoid cells. Our studies clearly demonstrate that IL-7 can function both as a progression factor for T-cell growth and, in concert with PMA, as a competence factor for inducing responsiveness to IL-2 or IL-4. The physiologic role of IL-7 in T-cell activation is at present unknown, and most *in vitro* studies are consistent with the hypothesis that IL-2 and IL-4 are the major T-cell growth factors. However, the demonstration of the constitutive production of IL-7 mRNA by cells from lymphoid and nonlymphoid organs (8) coupled with our observation that responsiveness to IL-7 is induced *in vivo,* but not *in vitro,* by stimulation of T cells via the CD3/T-cell receptor complex raises the possibility that IL-7 may play a role equivalent to that of IL-2 or IL-4 in T-cell growth and development in the intact animal.

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1. Howard, M., Farrar, J., Hilfiker, M., Johnson, B., Takatsu,K., Hamaoka, T. & Paul, W. E. (1982) *J. Exp. Med.* **155,** 914–923.
2. Hu-Li, J., Shevach, E. M., Mizuguchi, J., Ohara, J., Mossmann, T. & Paul, W. E. (1987) *J. Exp. Med.* **165,** 157–172.
3. Brown, M., Hu-Li, J. & Paul, W. E. (1988) *J. Immunol.* **141,** 504–511.
4. Kishimoto, T. (1985) *Annu. Rev. Immunol.* **3,** 133–157.
5. Garman, R. D., Jacobs, K. A., Clark, S. C. & Raulet, D. H. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7629–7633.
6. Ceuppens, J. L., Baroja, M. L., Lorre, K., Damme, J. V. & Billiau, A. (1988) *J. Immunol.* **141,** 3868–3874.
7. Namen, A. E., Schmierer, A. E., March, C. J., Overell, R. W., Park, L. S., Urdal, D. L. & Mochizuki, D. Y. (1988) *J. Exp. Med.* **167,** 988–1002.
8. Namen, A. E., Lupton, S., Hjerrild, K., Wignal, J., Mochizuki, D. Y., Schmierer, A., Mosley, B., March, C. J., Urdal, D., Gillis, S., Cosman, D. & Goodwin, R. G. (1988) *Nature (London)* **333,** 571–573.
9. Goodwin, R. G., Lupton, S., Schmierer, A., Hjerrild, K. J., Jerzy, R., Clevenger, W., Gillis, S., Cosman, D. & Namen, A. E. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 302–306.
10. Morrissey, P. J., Goodwin, R. G., Nordan, R. P., Anderson, D., Grabstein, K. H., Cosman, D., Sims, J., Lupton, S., Acres, B., Reed, S. G., Mochizuki, D., Eisenman, J., Conlon, P. J. & Namen, A. E. (1989) *J. Exp. Med.* **169,** 707–716.
11. Marshak-Rothstein, A., Fink, P., Gridley, T., Raulet, D. H., Bevan, M. J. & Gefter, M. L. (1979) *J. Immunol.* **122,** 2491–2497.
12. Bhattacharya, A., Dorf, M. E. & Springer, T. A. (1981) *J. Immunol.* **127,** 2488–2495.
13. Lanier, L. L., Gutman, G. A., Lewis, D. E., Griswold, S. T. & Warner, N. L. (1982) *Hybridoma* **1,** 125–131.
14. Dialynas, D. P., Wilde, D. B., Marrack, P., Pierres, A., Wall, K. A., Havran, W., Otten, G., Loken, R., Pierres, M., Kappler, J. & Fitch, F. W. (1983) *Immunol. Rev.* **74,** 29–56.
15. Ledbetter, J. A. & Herzenberg, L. (1979) *Immunol. Rev.* **47,** 63–90.
16. Mossmann, T. R., Bond, M. W., Coffman, R. L., Ohara, J. & Paul, W. E. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5654–5658.
17. Ohara, J. & Paul, W. E. (1985) *Nature (London)* **315,** 333–336.
18. Leo, O., Foo, M., Sachs, D. H., Samelson, L. E. & Bluestone, J. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 1374–1378.
19. Malek, T. R., Ortega, G., Chan, C., Kroczek, R. A. & Shevach, E. M. (1986) *J. Exp. Med.* **164,** 709–722.
20. Yokoyama, W. M., Koning, F., Kehn, P. J., Pereira, G. M. B., Stingl, G., Coligan, J. E. & Shevach, E. M. (1988) *J. Immunol.* **141,** 369–376.
21. Krönke, M., Leonard, W. J., Depper, J. M., Arya, S. K., Wong-Staal, F., Gallo, R. C., Waldmann, T. A. & Greene, W. C. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5214–5218.
22. Gianelli-Piperno, A., Keane, M. & Steinman, R. M. (1988) *Transplantation* **46,** 53S–60S.
23. Garman, R. & Raulet, D. H. (1987) *J. Immunol.* **138,** 1121–1129.
24. Uyttenhove, C., Coulie, P. G. & Van Snick, J. (1988) *J. Exp. Med.* **167,** 1417–1427.