Interleukin 7 Enhances Cytolytic T Lymphocyte Generation and Induces Lymphokine-activated Killer Cells from Human Peripheral Blood

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Summary

The effects of purified recombinant interleukin 7 (IL-7) on the generation of cytolytic T lymphocytes (CTL) in mixed lymphocyte culture (MLC) and on the induction of lymphokine-activated killer (LAK) cells in autologous cultures of human peripheral blood mononuclear cells were investigated. IL-7 was found to induce the generation of both CTL and LAK cells in bulk cultures. The appearance of peak CTL activity in MLC established with exogenous IL-7 was delayed in comparison with replicate cultures containing exogenous IL-2, but both cytokines stimulated quantitatively similar levels of antigen-specific lytic activity. An IL-2-neutralizing antiserum inhibited substantially, but not completely, the effect of IL-7 on CTL generation, implying the existence of both an indirect component of IL-7 activity via IL-2 utilization, as well as an IL-2-independent component. Cell surface phenotypic analysis of IL-2- or IL-7-generated CTL effector cells revealed that CD8+ cells were responsible for the vast majority of lytic activity. Limiting dilution analysis (LDA) revealed that essentially identical frequencies of CTL precursors (CTL-P) were capable of clonal expansion and/or differentiation in the presence of exogenous IL-2, IL-4, or IL-7, supporting the concept that all three of these cytokines are capable of exerting a major influence on T cell growth and differentiation. Approximately half of the CTL-P that responded in IL-7-supplemented LDA cultures did so in an IL-2-independent manner. IL-7 stimulated the development of LAK cells in autologous bulk cultures, but only weakly in comparison with IL-2. In contrast to its effects on CTL generation, the induction of LAK cells by IL-7 was virtually independent of IL-2. LAK cells induced by IL-7, like those induced by IL-2, were phenotypically heterogeneous and included CD8+, CD56+, and γ/δ+ cells. Limiting dilution analysis indicated that IL-2 stimulated fivefold more LAK-P than IL-7 and 220-fold more than IL-4. Collectively, these data suggest that IL-7 has potent regulatory effects on human cytolytic cell populations and, either alone or in combination with other cytokines, could be important for the in vitro expansion of cells for adoptive immunotherapy.

A ctivation of resting T lymphocytes into cytolytic cells normally requires both interaction with processed antigen through the TCR complex and the collaborative action of soluble cytokines (1). Among the cytokines with reputed roles in T cell proliferation and/or differentiation are IL-1, -2, -4, -5, and -6, TNF, IFN-γ, and granulocyte-macrophage CSF (2–8). The most recent addition to the list of T cell–active cytokines was IL-7. IL-7 was originally purified and its cDNA cloned based upon the ability to cause the growth of pre-B cells (9). More recently, it has been shown to be a potent costimulus for the proliferation of mitogen-activated murine and human T cells (10–13). Notably, IL-7 has been shown to expand numerically both CD4+ and CD8+ T cells. The cytokine IL-2, in addition to promoting

the growth and differentiation of T cells, including CTL, also has been shown to induce a killer cell population in the absence of overt antigen that is able to lyse fresh tumor cells and NK-resistant tumor cell lines (14). These cytolytic cells, termed lymphokine-activated killer (LAK)[1] cells, are also regulated in both a positive and negative manner by other cytokines, including IL-4 (15–18).

In this study, we assessed the activity of purified rIL-7 on the generation of CTL from human PBL in MLC, and on the induction of LAK cells in autologous cultures, both alone and in combination with IL-2 and IL-4. Exogenous IL-7 was

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[1] *Abbreviations used in this paper:* CTL-P, CTL precursor; LAK, lymphokine-activated killer; LDA, limiting dilution analysis.

found to be a potent enhancer of antigen-specific CTL gener-
ation with activity equivalent to IL-2. However, optimal lytic
activity generated by IL-7 was detected later than in cultures
supplemented with IL-2. The effect of IL-7 on the generation
of CTL could be partially inhibited by antibodies against IL-
2, though there remained a portion of IL-2-independent ac-
tivity. IL-7 also induced LAK cells in cultures containing no
overt antigenic stimulus, but did so far less efficiently than
IL-2. In contrast to the generation of CTL, the induction
of LAK activity by IL-7 was not inhibited by anti-IL-2. Thus,
the effects of IL-7 on the development of cytolytic cells in
vitro appear to have both IL-2-dependent and IL-2-independent
components, depending upon the culture system (MLC vs.
autologous) and corresponding cell types (CTL vs. LAK) in-
volved. These results implicate IL-7 as an important regula-
tory cytokine in human lymphocyte growth and differen-
tiation.

Materials and Methods

Cell Cultures. PBMC were isolated from heparinized blood by
centrifugation over Ficoll-Hypaque and washed three times with
culture medium. Culture medium consisted of RPMI 1640 (Gibco
Laboratories, Grand Island, NY) supplemented with 10% FCS (Hy-
clone Laboratories, Logan, UT), 50 U/ml penicillin, 50 μg/ml strep-
tomycin, 2 mM glutamine and 5 × 10^{-5} M 2-ME. 10^6 PBMC
were cultured with 10^6 irradiated stimulating cells (3,500 rad,
using a ^{137}Cs source) in 16-mm wells (3524; Costar, Cambridge,
MA) in 2 ml of culture medium. Cultures were incubated at 37°C
in 5% CO_2 for 7–14 d. The tumor cell lines Daudi and K562 were
obtained from the American Type Culture Collection (Rockville,
MD) and maintained in continuous passage.

Cytokines. Human rIL-2 and rIL-4 were purified from superna-
tants of yeast cells expressing the corresponding cDNA as described
previously (15, 19). Purified IL-2 had a specific activity of 3 × 10^5
U/μg in the CTLL assay (20). Purified IL-4 had a specific activity
of 10^4 U/μg in a B cell comitogenesis assay (15).

A human IL-7 cDNA was cloned as previously described (21)
and expressed in *Escherichia coli*. IL-7 was extracted from an *E. coli*
cell paste by soaking the cells in 6 M guanidine HCl, 0.15 M so-
dium sulfite, 0.05 M potassium tetrathionate, 0.1 M Tris, pH 8,
for 16 h at room temperature. The resulting lysate was clarified
by centrifugation (30,000 g, 30 min, 20°C) and applied to a column
of NTA-Sepharose (22). The column was washed with 6 M guani-
dine HCl, 0.1 M Tris, pH 8, and then eluted with 6 M guanidine
HCl, 1% acetic acid. The purified IL-7 was then refolded and dia-
lyzed to 50 mM sodium phosphate, pH 7.2. Protein concentration
was determined by amino acid analysis and biological activity was
determined using the murine pre-B cell assay (9). Specific activity
of IL-7 expressed in *E. coli* and mammalian cells was equivalent.

Cytotoxicity Assay. A 4-h ^{51}Cr release assay was used to assess
cytolytic activity of cultured cells, as previously described (15).
Briefly, tumor cell lines or PBMC cultured for 3 d with PHA (PHA
blasts) were radiolabeled by incubation with 100 μCi of ^{51}Cr for
1 h at 37°C. Cell cultures to be assessed for cytolytic activity were
washed twice in culture medium and serially diluted in 96-well
V-bottomed plates (3896; Costar). ^{51}Cr-labeled targets (2 × 10^3)
were added to make a final volume of 0.2 ml/well. After 4 h at
37°C, plates were centrifuged (150 g for 5 min), and 150 μl of
supernatant were harvested from each well. ^{51}Cr content of super-
natants was determined using an Auto gamma counter (5780;

Packard Instrument Co., Downers Grove, IL). 1 LU was defined
as the fraction of the initial culture giving rise to 25 or 50% lysis
of the target cell population (PHA blasts and Daudi, respectively),
and was determined from dose-response curves. Percent specific
^{51}Cr release was calculated according to the formula: 100× (ex-
perimental cpm − spontaneous cpm)/(maximum cpm − sponta-
neous cpm), where spontaneous cpm = cpm released in the ab-
sence of effector cells and maximum cpm = cpm released in the
presence of 1 N HCl.

Anti-IL-2 Serum. A rabbit antiserum raised against purified
human rIL-2 was used to neutralize IL-2 bioactivity. The antiserum
was used at a 1:500 dilution, a concentration that could totally
neutralize up to 10 ng/ml of IL-2 activity.

Limiting Dilution Analysis (LDA). Limiting numbers of re-
sponding cells (ranging from 10 to 10,000 cells/well) were cultured
in 24 replicates per cell concentration in 96-well round-bottomed
microtiter plates (25850; Corning Glass Works, Corning, NY) with
10^5 irradiated, autologous (LAK-P analysis) or allogeneic (CTL-P
analysis) PBMC. After a 13–14-d incubation, the culture superna-
tants were removed and the cellular contents of individual wells
were resuspended in 100 μl of fresh medium. The cells were then
transferred to the wells of V-bottomed plates, and 2 × 10^3 ^{51}Cr-
labeled targets in 100 μl of medium were added per well. The E/T
mixtures were incubated in a 4-h ^{51}Cr release assay, as described
above.

Statistical Analysis. CTL-P and LAK-P frequencies were deter-
mined from the Poisson distribution relationship between the
number of cells per microculture and the percent cytolytically nega-
tive microcultures using the minimum χ^2 method, as described
by Taswell (23). Cultures were considered positive if the specific
^{51}Cr release exceeded by 3 SD the mean background ^{51}Cr release
exhibited by control cultures comprised of feeder cells with cytokines
but no responding cells.

Separation of Effector Cell Subsets by Immunoadsorbent Panning. The
following murine mAbs against human cell surface antigens were
used for immunoadsorbent panning: OKT4 (CD4), OKT8 (CD8),
Leu-19 (CD56, expressed on NK cells and cytotoxic T cells that
mediate non–MHC-restricted cytotoxicity; Becton Dickinson &
Co., Mountain View, CA), and TCR-δ1 (recognizing the δ chain
of the TCR; T Cell Sciences, Cambridge, MA). Cells were sus-
pended in 100 μl of culture medium supplemented with 10 μg/ml
of mAb at 4°C. After 30 min, cells were washed three times with
PBS containing 1% FCS. Labeled cells were then incubated on plastic
petri dishes that had previously been coated with goat anti–mouse
IgG (Sigma Chemical Co., St. Louis, MO) at 5 μg/ml for 2 h.
Petri dishes were incubated at 4°C for 40 min. Nonadherent cells
were collected by washing gently three times with 3 ml of PBS
containing 1% FCS. Adherent cells were recovered using a cell
scraper and collected by washing. Isolated cell populations were
assessed for purity by flow cytometry.

Results

IL-7 Augments Antigen-Specific CTL Generation. Freshly
isolated PBMC from human donor VB were cultured in MLC
in the presence of irradiated stimulating cells from allogeneic
donor KB and 10 ng/ml of either human rIL-2 or human
rIL-7. Cytolytic activity against PHA blasts was assessed at
day 7 using a 4-h ^{51}Cr release assay. Culture of cells from
these two donors in MLC under the conditions used in this
study results in a detectable but weak CTL response that can
be augmented significantly by cytokines that promote CTL
development (15). Addition of either IL-2 or IL-7 at the be-

Table 1. *Effects of IL-2 and IL-7 on the Generation of Human Cytolytic Cell Populations*

Target	Culture	Culture supplement		
		Medium	IL-2	IL-7
KB	VB anti-KB	~1*	208	59
	VB anti-VB	<1	~2	<1
VB	VB anti-KB	<1	<1	<1
	VB anti-VB	<1	<1	<1
Daudi	VB anti-KB	<1	270	36
	VB anti-VB	<1	540	29

Cell cultures were established with 10^6 VB effector cells and 10^6 irradiated VB- or KB-stimulating cells. IL-2 or IL-7 was added at 10 ng/ml, and cultures were assessed for cytolytic activity at day 7 against ^{51}Cr-labeled KB or VB PHA blasts or Daudi target cells.
* Data represent LU per culture. LU were calculated from the dose-response curves (see Figs. 1 and 2) using 25% specific ^{51}Cr release for KB and VB PHA blasts and 50% specific ^{51}Cr release for Daudi targets. The lysability of VB targets was previously established with a control KB anti-VB culture supplemented with IL-2, which exhibited >150 LU of anti-VB activity.

ginning of culture significantly enhanced the generation of cytolytic activity against KB targets prepared from the stimulating cell donor, when compared with cultures containing no exogenous cytokine (Fig. 1). As shown in Table 1, anti-KB lytic activity recovered on day 7 of VB anti-KB MLC was enhanced ~200-fold by inclusion of exogenous IL-2 in culture, and 60-fold by IL-7 in comparison with cultures containing no exogenous cytokine. In a series of five identical experiments where VB responding cells were cultured with irradiated KB-stimulating cells and assayed for lytic activity against KB PHA blast targets at day 7-10, nonsupplemented cultures contained an average of ~1 LU/culture (range, <1-2), compared with 49 LU/culture for IL-7-supplemented cultures (range, 16-88) and 115 LU/culture for IL-2-supplemented cultures (range, 28-215). In addition, CTL
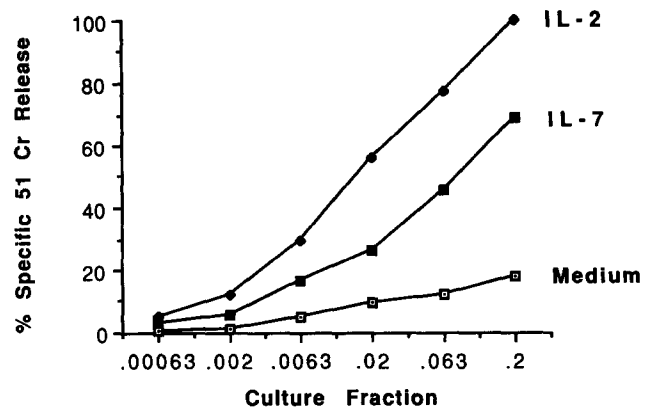

Figure 1. Enhancement of CTL generation in MLC by IL-7 and IL-2. VB anti-KB MLC were supplemented with 10 ng/ml of either IL-2 or IL-7 and assayed at day 7 for their ability to lyse ^{51}Cr-labeled PHA blasts from donor KB in a 4-h ^{51}Cr release assay. Cell recoveries were 92% for nonsupplemented cultures and 270 and 208%, respectively, for IL-2- and IL-7-supplemented cultures.

generated in either IL-2- or IL-7-supplemented MLC were specific for KB targets in that they were unable to cause significant lysis of control VB targets syngeneic with the responding cell donor. Table 2 shows the results of an experiment with three different blood donors. In all cases, the generation of lytic activity against PHA blast target cells from the stimulating cell donor was significantly enhanced (>100-fold) by the addition of either IL-2 or IL-7. As anticipated, cross-reactive lytic activity against third-party targets was also enhanced by either IL-2 or IL-7, however, minimal lytic activity was observed against target cells from the responding cell donor.

IL-7 Stimulates the Formation of LAK Cells. Autologous cultures of VB PBMC containing either IL-2 or IL-7 but no overt antigen were assessed for cytolytic activity against the LAK-sensitive, NK-resistant tumor cell line Daudi. Supplementation of cultures with either IL-2 or IL-7 resulted in the appearance of a LAK cell population that lysed Daudi targets (Fig. 2). However, IL-7 induced 10-20-fold less LAK activity per culture than did IL-2 (Table 1). In a series of five

Table 2. *IL-7 Enhances the Generation of Allospecific CTL*

Culture	Target LB			Target MH			Target PS		
	Medium	IL-2	IL-7	Medium	IL-2	IL-7	Medium	IL-2	IL-7
LB anti-MH	<1*	<1	<1	<1	2,380	1,250	<1	238	238
LB anti-PS	<1	<1	<1	<1	300	238	<1	323	526
MH anti-LB	~1	500	769	<1	<1	<1	<1	29	94
MH anti-PS	~1	116	116	<1	<1	<1	<1	109	139
PS anti-LB	<1	364	1,300	<1	98	455	<1	<1	<1
PS anti-MH	10	435	1,162	8	417	1,000	<1	<1	<1

Cell cultures were established with 10^6 responding cells and 10^6 stimulating cells. Cytokines (10 ng/ml) were added at the initiation of culture and cytolytic activity was tested against ^{51}Cr-labeled PHA blasts on day 9.
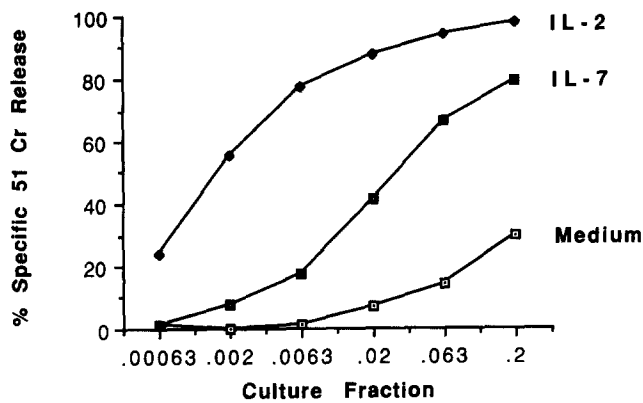* Results are expressed as LU per culture.

Figure 2. LAK activity is induced by IL-7. Autologous cultures were established using VB effector cells and irradiated VB filler cells. Cultures were supplemented with 10 ng/ml of either IL-2 or IL-7 and assayed at day 7 for cytolytic activity against ^{51}Cr-labeled Daudi target cells. Cell recoveries were 57% for nonsupplemented cultures and 167 and 156%, respectively, for IL-2- and IL-7-supplemented cultures.

identical experiments where VB-responding cells were cultured with autologous irradiated cells and assayed for lytic activity against Daudi targets at day 7-10, nonsupplemented cultures contained an average of ~1 LU/culture (range, <1-2), compared with 21 LU/culture for IL-7-supplemented cultures (range, 15-29) and 352 LU/culture for IL-2-supplemented cultures (range, 241-540). Similar results were obtained with PBMC from several other donors (data not shown).

Kinetics of CTL and LAK Cell Generation by IL-7. Table 3 shows data derived from a kinetics experiment in which the lytic activity of allogeneic and autologous cultures was assessed at days 7, 10, and 14. In IL-2-supplemented cultures, CTL activity was maximal at day 7 (215 LU) and gradually decreased by day 10 (143 LU) and day 14 (62 LU). The effect of IL-7 on the CTL response was delayed in comparison with IL-2 such that maximal activity (182 LU) was detected on day 10. However, the levels of peak lytic activity arising in cultures supplemented with either IL-2 or IL-7 were similar. In contrast to CTL activity, optimal LAK cell activity induced by either IL-2 or IL-7 was detected at day 10 (900 and

Table 3. *Kinetics of CTL and LAK Cell Generation in Cultures Supplemented with Either IL-2 or IL-7*

Culture supplement	CTL			LAK		
	Day 7	Day 10	Day 14	Day 7	Day 10	Day 14
Medium	~2*	22	21	<1	~5	~4
IL-2	215	143	62	480	900	286
IL-7	88	182	91	28	80	56

Cultures comprised 10^6 VB cells plus 10^6 irradiated KB cells for CTL responses and 10^6 VB cells plus 10^6 irradiated VB cells for LAK responses and were supplemented with 10 ng/ml of either IL-2 or IL-7. Lytic activity was assessed against ^{51}Cr-labeled KB PHA blasts (CTL) or Daudi (LAK) targets at the indicated days.
* Data represent LU per culture.

80 LU, respectively), however, at none of the time points tested did the activity induced by IL-7 approach that of IL-2.

Dose-Response of IL-2 and IL-7 in CTL and LAK Cell Generation. The comparative efficiency of IL-2 and IL-7 in promoting the generation of both CTL and LAK cells was examined by culturing PBMC in MLC or autologous cultures with varying doses of cytokine and testing for lytic activity 12 d later. Table 4 shows that IL-2 enhanced CTL generation when present at concentrations ≥1 ng/ml. In contrast, ≥10 ng/ml of IL-7 was required for a similar effect. However, in terms of total lytic activity generated, IL-7 was at least as effective as IL-2 when present at 10 and 100 ng/ml, although it should be noted that the day of assay (day 12) was more optimal for detection of IL-7 effects than IL-2 effects (Table 3). Notably, both IL-2 and IL-7 enhanced cell recovery to a similar degree in allogeneic cultures. Cells were also stimulated to proliferate by both cytokines in autologous cultures, but to a lesser extent than in allogeneic cultures. The addition of 1 ng/ml or more of either IL-2 or IL-7 to autologous cultures resulted in the development of significant cytotoxic activity against either Daudi (NK-insensitive) or K562 (NK-sensitive) target cells. However, in contrast to the similar effects of these cytokines on CTL induction, IL-2 was far more potent than IL-7 at inducing LAK cells in terms of the maximal amount of lytic activity generated per culture.

Induction of Proliferation by IL-7 Is Independent of IL-2. As IL-2 is a potent inducer of both CTL and LAK cells, the effects of IL-7 on cytolytic cells could conceivably be mediated via the secondary production of IL-2. To address this possibility, we assessed the effects of an IL-2-neutralizing antiserum on the induction of cell proliferation, CTL, and LAK cells by IL-7. The anti-IL-2 serum was a potent inhibitor of the proliferative response in IL-2-supplemented VB anti-KB cultures, causing total ablation of [^{3}H]thymidine incorporation induced by 10 ng/ml of IL-2 (Fig. 3). High concentrations of IL-2 (100 ng/ml) could overcome the inhibitory effects of the anti-IL-2 serum, suggesting the serum itself was not toxic. In contrast, the induction of proliferation by IL-7 was virtually unaffected by the presence of anti-IL-2, implying that under these culture conditions IL-7-mediated proliferation is independent of IL-2.

Effects of Anti-IL-2 on the Induction of CTL and LAK Cells by either IL-2 or IL-7. Fig. 4 shows that the anti-IL-2 serum inhibited substantially, but not totally, the effect of IL-7 on the generation of CTL in MLC, reducing the lytic activity from 88 LU per culture to 8 LU. In contrast, anti-IL-2 had a limited effect on the induction of LAK cell activity by IL-7, reducing LU from 28 to 20. Similar results were obtained if the cultures were assayed at either day 10 or day 14. In control cultures, the anti-IL-2 serum inhibited the IL-2-mediated induction of both LAK cells and CTL to background levels. Interestingly, lytic activity that developed in the absence of exogenously added cytokine was also suppressed by anti-IL-2, suggesting that endogenously produced IL-2 is responsible for such activity in these cultures. In addition, IL-7-mediated induction of CTL and LAK cells was not significantly affected by neutralizing antibodies against either IL-4 or IL-6 (data not shown).

Table 4. *Dose-Response of IL-2 and IL-7 in CTL and LAK Cell Generation*

Culture supplement	CTL		LAK		
	Percent recovery	LU_{25}/culture	Percent recovery	LU_{50}/culture (Daudi targets)	LU_{50}/culture (K562 targets)
ng/ml					
Medium	61	<1	66	<1	4
IL-2					
0.1	78	<1	71	<1	6
1	92	10	83	18	68
10	194	25	178	115	210
100	663	118	296	312	530
IL-7					
0.1	78	<1	64	4	12
1	102	~1	62	12	31
10	319	33	197	28	68
100	424	214	182	55	81

Cultures were established with 10^6 VB effector cells and either 10^6 irradiated KB (CTL) or VB (LAK) stimulator cells and supplemented with the indicated dose of rIL-2 or rIL-7. Lytic activity against ^{51}Cr-labeled KB PHA blasts (CTL), Daudi (LAK), or K562 (LAK) targets was assessed at day 12–14. Values represent mean of two experiments.

Effect of IL-7 in Combination with Other Cytokines. We and others have previously demonstrated regulatory effects of other cytokines on CTL generation and LAK cell induction. For example, cytolytic activity induced by IL-2 is strongly inhibited by the early addition to culture of IL-4 (15–18). Consequently, we assessed the effects of IL-7 in combination with IL-2 and/or IL-4 on the generation of cytolytic cells. The data shown in Table 5 confirm the inhibitory effect of IL-4 on IL-2-promoted generation of CTL and LAK cells. IL-4 also inhibited the induction of LAK cells by IL-7. The inhibitory effects of IL-4 on LAK induction by either IL-2 or IL-7 did not appear to be due to nonspecific cell toxicity or inhibition of cell growth, as there was no consistent correlation between its inhibitory effects on lytic activity and cell recoveries. Furthermore, the development of CTL activity in the presence of IL-7 was not impaired by the addition of IL-4. It should be noted that IL-4 alone had weak activity in promoting CTL, in confirmation of previously published results, although if its addition to

MLC is delayed until day 3 or 4, it has activity equivalent to IL-2 (15). The combination of IL-2 and IL-7 had virtually identical effects on the generation of both populations of cytolytic cells as IL-2 acting alone. The patterns of responsiveness observed at day 14 assay (data not shown) were essentially similar to those observed at day 7 (Table 5). In addition, varying the doses of IL-2 and IL-7 resulted in no evidence for synergy between these two cytokines for either CTL enhancement or LAK cell induction (data not shown).

Characterization of Effector Cells Mediating CTL and LAK Activity. Experiments were performed to compare the cell surface phenotype of cells induced by either IL-2 or IL-7 that mediated lysis of specific CTL targets or LAK cell lysis of Daudi targets. Autologous or allogeneic PBMC were cultured for 9 d in the presence of 20 ng/ml of either IL-2 or IL-7. For CTL cultures, effector cells were separated into $CD4^-$ and $CD4^+$, $CD8^-$ and $CD8^+$, or $CD56^-$ and $CD56^+$ subsets by immunoadsorbent panning and assessed
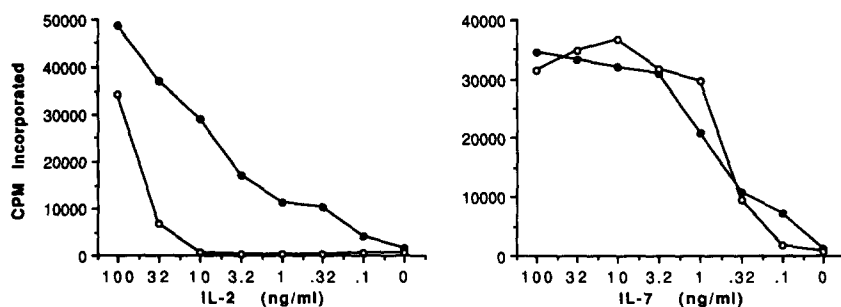

Figure 3. Anti-IL-2 antibodies inhibit IL-2-induced proliferation but not IL-7-induced proliferation in MLC. 10^5 VB cells were cultured with 10^5 irradiated KB cells in 200-μl wells for 4 d in the presence of the indicated amounts of IL-2 or IL-7, with (O) or without (●) a constant concentration of anti-IL-2 serum (1:500). [^{3}H]Thymidine (1 μCi) was added for the last 16 h of culture. Values represent mean of triplicate cultures. Thymidine incorporation by non-cytokine-supplemented cultures was 1,673 cpm and, in the presence of anti-IL-2, was 975 cpm.
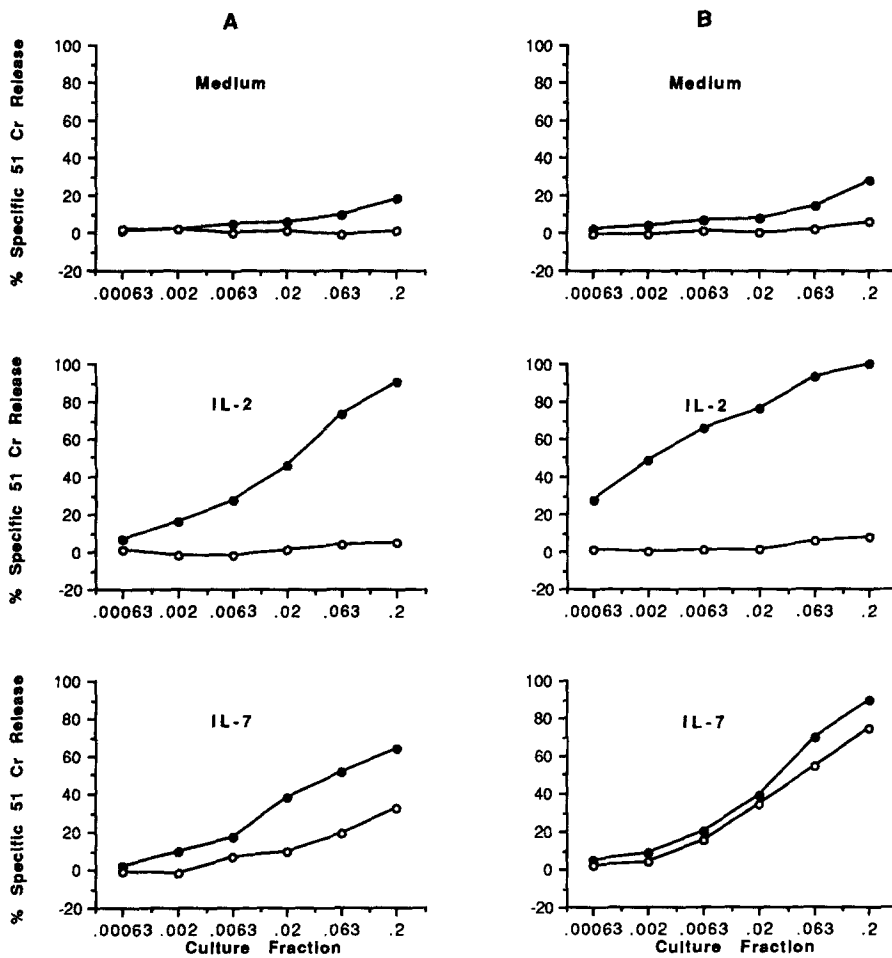
Figure 4. Effect of anti-IL-2 on the generation of CTL and on LAK cell induction. (A) VB anti-KB (CTL) or (B) VB anti-VB (LAK) cultures were supplemented with 10 ng/ml of either IL-2 or IL-7 in the presence (O) or absence (●) of an anti-IL-2 serum. Cultures were assessed at day 7 for lytic activity against either 51Cr-labeled KB blasts or Daudi cells.

for lytic activity against allogeneic targets (Table 6). It should be noted that the lytic activity measured for positively selected cells may be underestimated due to nonquantitative recovery of cells bound to the pan, inhibition of lysis by antibody bound to the cells, or cell damage due to scraping. Among CTL generated in the presence of either IL-2 or IL-7, the vast majority of lytic activity was contained within the CD8+ population with no activity within the CD4+ cells. Among

Table 5. Combinatorial Effects of IL-2, IL-4, and IL-7 on CTL Generation and LAK Cell Induction

Cytokine(s) added	CTL activity*		LAK activity‡	
	Percent recovery	(LU25/culture)	Percent recovery	(LU50/culture)
–	56	3	44	<1
IL-2	156	142	101	350
IL-4	120	10	89	<1
IL-7	142	32	85	17
IL-2 + IL-4	159	56	69	35
IL-2 + IL-7	226	160	177	215
IL-4 + IL-7	145	50	118	~2
IL-2 + IL-4 + IL-7	158	42	130	36

Values represent mean of two experiments.
* VB cells were incubated with irradiated KB cells in the presence of various cytokines (used at 10 ng/ml). Lytic activity against 51Cr-labeled PHA blasts from donor KB was assessed on day 7.
‡ VB cells were incubated with irradiated autologous filler cells and assessed for the ability to lyse 51Cr-labeled Daudi targets on day 7.

Table 6. *Characterization of Effector Cells Mediating CTL Activity*

Stimulus	Effector cells	Percent cells staining for:			Total LU	LU/10⁶ cells
		CD4	CD8	CD56		
IL-2	Unseparated	28	59	19	385	107
	CD4⁻	12	70	ND	250	92
	CD4⁺	98	5.2	ND	<0.1	<0.3
	CD8⁻	66	4.0	ND	16	8.4
	CD8⁺	5.7	98	ND	204	139
	CD56⁻	ND	ND	3.9	500	100
	CD56⁺	ND	ND	99	42	290
IL-7	Unseparated	72	21	2.1	161	38
	CD4⁻	30	54	ND	98	77
	CD4⁺	95	1.0	ND	~0.8	~0.6
	CD8⁻	87	3.0	ND	8.3	2.9
	CD8⁺	6.0	97	ND	63	135
	CD56⁻	ND	ND	0.1	100	27
	CD56⁺	ND	ND	98	4.4	109

PS anti-MH MLC supplemented with either IL-2 or IL-7 were cultured for 9 d before separating into subpopulations of cells by immunoadsorbent panning and assaying for lytic activity against MH targets.

the IL-2-stimulated cells, the CD56⁺ population contained a significant, though small, amount of lytic activity (42 LU), whereas CD56⁺ cells from IL-7-supplemented cultures had very weak lytic activity (4.4 LU).

To examine the cell surface phenotype of LAK cells, effector cells were divided into CD8⁻ and CD8⁺, CD56⁻ and CD56⁺, or γ/δ^- and γ/δ^+ subsets, and assessed for lytic activity against Daudi targets (Table 7). As previously de-

Table 7. *Characterization of Effector Cells Mediating LAK Activity*

Stimulus	Effector cells	Percent cells staining for:			Total LU	LU/10⁶ cells
		CD8	CD56	γ/δ		
IL-2	Unseparated	38	19	6.0	139	147
	CD8⁻	2.6	ND	ND	74	141
	CD8⁺	70	ND	ND	24	165
	CD56⁻	ND	1.2	ND	32	33
	CD56⁺	ND	67	ND	46	320
	γ/δ^-	ND	ND	0.1	139	113
	γ/δ^+	ND	ND	85	5.0	223
IL-7	Unseparated	26	3.5	1.7	16	14
	CD8⁻	1.0	ND	ND	7.6	7.3
	CD8⁺	70	ND	ND	2.4	8.4
	CD56⁻	ND	0.2	ND	4.6	3.8
	CD56⁺	ND	72	ND	3.6	44
	γ/δ^-	ND	ND	0.1	2.8	3.9
	γ/δ^+	ND	ND	44	0.3	6.6

VB PBMC were cultured with irradiated VB filler cells and supplemented with either IL-2 or IL-7. Cells were cultured for 8 d before separating into subpopulations of cells by immunoadsorbent panning and assaying for lytic activity against Daudi targets.

scribed (24–30), LAK effector cells were phenotypically heterogeneous. LAK cells stimulated by either IL-2 or IL-7 were comprised of CD8⁺, as well as CD56⁺ and γ/δ^+ cells. Unlike CTL effector cells, greater lytic activity was found in the CD8⁻ than in the CD8⁺ cells. The most potently lytic cells on a per cell basis were the CD56⁺ cells, which were 10-fold more lytic than their CD56⁻ counterparts. Cells expressing the TCR-γ/δ also exhibited significant lytic activity on a per cell basis, though their overall contribution to the total lytic activity was small due to their low numbers. Thus, the cell surface phenotype of LAK cells induced by IL-2 or IL-7 was similar, with IL-2 inducing 10-fold greater lytic activity in all cell populations tested.

Quantitation of Alloreactive CTL-P and Autologous LAK-P Frequencies in Limiting Dilution Cultures Supplemented with IL-2, IL-4, or IL-7. In these experiments, we wished to examine directly the frequency of cytolytic progenitor cells from PBMC that could respond to various cytokines. To this end, limiting dilution cultures were established to allow estimation of the frequency of CTL-P and LAK-P under conditions where IL-2, IL-4, or IL-7 was used as a culture supplement to promote cellular activation and/or expansion. Limiting numbers of VB-responding cells were cultured in the presence of 10^5 irradiated KB or VB cells and IL-2, IL-4, or IL-7 at 10 ng/ml. After 13 or 14 d, microcultures were assessed for the ability to lyse ⁵¹Cr-labeled KB PHA blasts (allogeneic cultures) or Daudi targets (autologous cultures). As shown in Table 8, nearly equivalent frequencies of CTL-P were detected in allogeneic limiting dilution cultures supplemented with IL-2, IL-4, or IL-7 (1:882 to 1:5,486), with IL-4 activating a slightly lower frequency than either IL-2 or IL-7. In contrast, the frequency of CTL-P detected in noncytokine-supplemented cultures ranged from 1:75,000 to <1:240,000. Fig. 5 shows the percent specific ⁵¹Cr release exhibited by the 24 individual replicate microcultures at each of the responding cell concentrations from experiment 1 (Table 8). Based upon the assumptions that lytic activity depends on both the number of cytolytic cells present in culture and the average lytic ac-

tivity on a per cell basis, an average clone size can be estimated from the data in Fig. 5. The average clone size was calculated by dividing the sum of the percent specific ⁵¹Cr release for the positive wells from each experimental group by the total number of clones per group (calculated from the Poisson equation). The average size of CTL clones in limiting dilution cultures stimulated with IL-4 or IL-7 was similar (11.6 and 10.4% specific lysis). The average clone size calculated for IL-2-supplemented cultures was somewhat greater (19.2% specific lysis).

In contrast to CTL-P, the frequency of LAK-P responsive to the three cytokines differed markedly. IL-2 induced 1:53 to 1:395 PBMC to give rise to cells that could lyse Daudi targets. IL-7 induced fivefold fewer LAK-P than IL-2. IL-4 was the weakest of the cytokines tested in this regard, inducing 220-fold fewer LAK-P than IL-2. Furthermore, only very weak cytolytic activity was demonstrable among cytolytically positive microcultures arising in IL-4 compared with either IL-2 or IL-7 (Fig. 5). Thus, IL-2 and IL-7 induced an equivalent average LAK clone size (13.2 and 12.8% specific ⁵¹Cr release, respectively), which far exceeded the average of the few clones induced by IL-4 (3.2% specific ⁵¹Cr release).

The addition of anti-IL-2 serum to IL-2- and IL-7-stimulated limiting dilution cultures (Table 8, Exp. 3 and 4) had effects similar to those seen in bulk cultures. Anti-IL-2 reduced the frequency of both CTL-P and LAK-P detected in IL-2-supplemented cultures to background (medium alone) levels. In contrast, anti-IL-2 reduced the frequency of CTL-P detected in the presence of IL-7 only two-fold, and had virtually no effect on the frequency of LAK-P responsive to IL-7.

Discussion

Recently it was reported that IL-7, described originally as a pre-B cell growth factor, is also a potent costimulus for the growth of both murine and human CD4⁺ and CD8⁺ T cells (10–13). In the experiments reported here, we investigated the effect of rIL-7 on the generation of CTL and LAK

Table 8. *Frequency of Alloreactive CTL-P and Autologous LAK-P Responsive to Various Cytokines*

Culture supplement	CTL-P				LAK-P			
	1*	2	3	4	1	2	3	4
–	ND	<1:240,000	1:240,000	1:74,963	ND	1:99,834	<1:240,000	1:28,800
IL-2	1:1,691	1:882	1:1,149	1:1,634	1:53	1:58	1:395	1:192
IL-2 + anti-IL-2	ND	ND	<1:240,000	1:26,848	ND	ND	<1:240,000	1:240,000
IL-4	1:2,605	1:5,486	ND	ND	1:9,801	1:14,978	ND	ND
IL-7	1:1,332	1:1,782	1:1,691	1:2,511	1:460	1:319	1:1,325	1:566
IL-7 + anti-IL-2	ND	ND	1:3,184	1:4,614	ND	ND	1:1,132	1:647

Limiting numbers of VB responder cells were cultured with irradiated KB cells (CTL-P) or VB cells (LAK-P) and IL-2, IL-4, or IL-7 at 10 ng/ml. Results are expressed as frequency of precursor cells cytolytic for either KB PHA blasts (CTL-P) or Daudi targets (LAK-P) when tested at day 13 or 14.
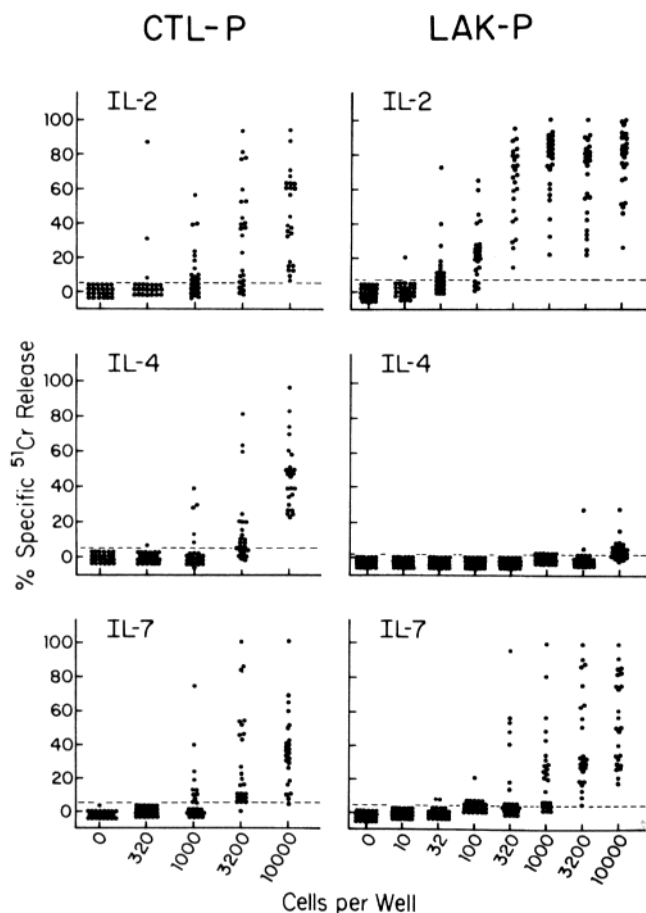* Experiment number.

CTL-P   LAK-P

Figure 5. Cytolytic activity of individual allogeneic (*left*) or autologous (*right*) microcultures supplemented with IL-2, IL-4, or IL-7. Limit dilution cultures were established as described for Table 5. Data show individual percent specific ^{51}Cr release values for 24 replicate CTL cultures (VB anti-KB) or LAK cell cultures (VB anti-VB) at various cell concentrations. The dotted lines indicate the mean plus 3 SD of the ^{51}Cr release by control cultures (feeder cells with cytokines but no responding cells).

cells from human peripheral blood. When IL-7 was added to MLC, it was found to enhance the generation of antigen-specific CTL activity with potency similar to IL-2. Although the two cytokines induced equivalent levels of lytic activity, optimal CTL enhancement by IL-7 occurred at a later time in culture than noted for IL-2 (day 10 vs. 7). IL-7 was also found to induce LAK cells in the absence of overt antigen, but with substantially less potency than IL-2.

Given the known ability of IL-2 to promote T cell growth and differentiation, an obvious and important consideration in the present studies concerns the role of IL-2 as the actual mediator of IL-7 effects. This possibility is particularly relevant in view of the recent demonstration that IL-7 induces the p55 chain of the IL-2R on murine and human T cells (11, 12). To address this issue, an antiserum against human IL-2 was used in culture in conjunction with IL-7. The anti-IL-2 serum had virtually no effect on the enhancement of cell

proliferation induced by IL-7 in MLC, implying that this activity of IL-7 is independent of IL-2. However, the effect of IL-7 on CTL induction in MLC was found to be partially inhibitable by the anti-IL-2 serum, suggesting that IL-2 plays a secondary role in these cultures. This finding is not surprising in view of the fact that endogenous IL-2 is typically produced when T cells are confronted with an antigenic stimulus, as they are in MLC. Indeed, the fact that the anti-IL-2 serum reduced endogenous cell proliferation and both CTL and LAK formation in the absence of added cytokine suggests that low amounts of IL-2 are produced in these cultures. However, assay of culture supernatants for the presence of IL-2 by the CTLL assay (20) failed to reveal higher levels of IL-2 in IL-7-supplemented cultures when compared with nonsupplemented cultures (data not shown). These results suggest that IL-7 does not directly induce IL-2 production. Furthermore, no synergy was observed between exogenous IL-2 and IL-7 in CTL induction (Table 5), and no enhanced response to IL-2 was observed in MLC pre-cultured with IL-7 for 3 d when compared with cells obtained from pre-culture with medium alone (data not shown). In any event, the fact that enhancement of CTL generation by IL-7 was only partially inhibited by the IL-2-neutralizing agent suggests that IL-7 delivers a direct proliferative and/or differentiative signal to at least some T cells. Alternatively, secondary effects of cytokines other than IL-2 may contribute to the results. In this regard, neutralizing antibodies against either IL-4 or IL-6 were unable to inhibit the augmentation of CTL or induction of LAK cells by IL-7 (data not shown).

It is of interest that anti-IL-2 had virtually no effect on cell proliferation in response to IL-7, despite its effects on CTL formation. These results are consistent with the possibility that IL-2 or other cytokines may be responsible for the differentiation and acquisition of cytolytic activity of some IL-7-responsive cells.

In contrast to its effects on CTL generation, IL-7 was shown to induce LAK cells in a largely IL-2-independent manner. IL-7 thus joins IL-5 and IL-6 as cytokines that have recently been shown to augment non–MHC-restricted killing by either NK or LAK cells (31, 32). However, both IL-5 and IL-6, unlike IL-7, appear to be dependent upon either exogenous or endogenous IL-2 for their activity on LAK or NK cells.

Considering the similar activities of IL-2 and IL-7 in promoting the generation of CTL and LAK cells, it was of interest to compare the surface antigen phenotype of the effector cells mediating these two types of cytolysis. CTL generated in the presence of either IL-2 or IL-7 were almost exclusively CD8$^+$ cells, with a small contribution by CD56$^+$ (Leu-19$^+$) cells in IL-2-stimulated cultures and almost negligible contribution by CD56$^+$ cells in IL-7-stimulated cultures. Precursors of LAK cells have been shown to be comprised of several cell types, predominantly CD56$^+$ NK cells and CD56$^+$ T cells (24, 25). More recently, T cells expressing the TCR-γ/δ have been implicated in non–MHC-restricted killing of NK and LAK cell targets (26–28). We found that effector cell populations stimulated by IL-2 or IL-7 and enriched for the expression of CD8 or CD56 or TCR-

γ/δ were all able to mediate lysis of LAK-sensitive Daudi targets. However, similar to previous reports (24, 25), the majority of lytic activity on a per cell basis was found within the CD56$^+$ population. In all the subpopulations of effector cells tested, the lytic activity of IL-7-stimulated cells was $\sim$10-fold less than in IL-2-stimulated cells. Thus, IL-7 appears to act on the same lineages of cytolytic cells as IL-2, though in the case of LAK cells with far less potency.

Limiting dilution analysis in which IL-2, IL-4, or IL-7 were used to promote activation and clonal expansion revealed that these three cytokines stimulated outgrowth of approximately equivalent frequencies of CTL-P in allogeneic cultures. Clone size estimates revealed that IL-2 induced CTL clones with a somewhat greater average lytic activity than either IL-4 or IL-7. Our results confirm and extend those of Paliard et al. (29), who reported similar cloning efficiencies for human T cells in either IL-2 or IL-4. It is of interest that, at the bulk culture level, IL-4 is as effective as IL-2 in promoting CTL generation if added late to culture but is far less effective when added at the initiation of culture (Table 5 and reference 15). The results demonstrating almost equal efficiency of IL-2 and IL-4 in promoting clonal expansion of CTL-P in limiting dilution cultures suggest that there is no requirement for delayed addition of IL-4 when responding cell numbers are limiting. Alternatively, IL-4 may activate an infrequent, inhibitory cell type in bulk cultures that is not represented in limiting dilu-

tion cultures at the cell concentrations used. In contrast to the similar frequencies of CTL-P detected in IL-2-, IL-4-, or IL-7-supplemented cultures, we found IL-2 induced fivefold more LAK-P than IL-7, which in turn induced 34-fold more LAK-P than IL-4. Interestingly, these frequencies of LAK-P responsive to IL-2, IL-4, or IL-7 mirrored the relative efficacy of the three cytokines in the induction of LAK cells in bulk cultures.

Results of limiting dilution experiments performed in the presence of the IL-2 antiserum appeared to mimic those obtained in bulk cultures. Thus, virtually all LAK-P induced by IL-7 were insensitive to the anti-IL-2 reagent. In contrast, approximately half of the CTL-P detected in IL-7-supplemented microcultures were no longer detectable in the presence of the anti-IL-2 serum. These results thus indicate heterogeneity in IL-7-responsive cells at the single cell level in that a fraction of such cells require IL-2 for growth and/or differentiation.

Determination of the role, if any, of endogenously produced IL-7 in the T cell response awaits the development of specific IL-7 inhibitors. Taken together, results presented herein indicate that IL-7 has the capacity to regulate the growth and/or differentiation of cytolytic cell populations from human peripheral blood. As such, this cytokine may be useful, either alone or in combination with IL-2 and/or IL-4, for the in vitro cultivation of immunotherapeutic cell populations.

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