

SYNERGY BETWEEN RECOMBINANT INTERLEUKIN 2 (rIL 2) AND IL 2-DEPLETED LYMPHOKINE-CONTAINING SUPERNATANTS IN FACILITATING ALLOGENEIC HUMAN CYTOLYTIC T LYMPHOCYTE RESPONSES IN VITRO

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Supernatants from human mixed leukocyte cultures or lectin-depleted supernatants from cultures of PHA-activated human peripheral blood leukocytes were depleted of IL 2 by passage over an anti-human rIL 2 immunoadsorbent column. The column eluates were concentrated, dialyzed, and tested for their ability to synergize with human rIL 2 in facilitating human cytolytic T lymphocyte (CTL) responses to allogeneic, uv-irradiated HT144 melanoma cells in vitro. CTL were generated in the presence of 1×10^{-4} M hydrocortisone sodium succinate in order to minimize the generation of nonspecific lymphokine-activated killer (LAK) cells. IL 2-depleted lymphokine-containing supernatant (LKS), alone or in the presence of ≤ 1 U/ml rIL 2 did not stimulate significant CTL responses. Recombinant IL 2 at >2 U/ml stimulated weak CTL responses in the absence of LKS. However, strong synergistic CTL responses were observed when both IL 2-depleted LKS and >2 U/ml rIL 2 were added to the cultures. CTL generated in these cultures could be distinguished from nonspecific LAK cells on the basis of their i) specificity, ii) T3 phenotype, and iii) kinetics of generation. Nevertheless, rIL 2 and IL 2-depleted LKS were sometimes observed to synergize in facilitating the generation of nonspecific LAK cells as well as the generation of specific CTL. When the times at which rIL 2 and IL 2-depleted LKS were added to the cultures were varied, IL 2 was found to be required early in CTL responses, whereas the synergistic factor(s) in LKS seemed to act later. Recombinant human interferon- γ was unable to replace LKS in synergizing with rIL 2 to elicit CTL responses. In summary, these experiments suggest that LKS contains a late-acting factor(s), antigenically distinct from IL 2, which synergizes with IL 2 in facilitating human CTL responses.

Cytolytic T lymphocytes (CTL)¹ are believed to play a major role in the host defense against viral infections (1, 2) and possibly against neoplasia (3, 4). In addition, it

appears that CTL may mediate at least some forms of homograft rejection (5, 6) and certain immunopathologic processes (7–9). Thus, an understanding of the molecular mechanisms involved in the generation of CTL responses may lead to the development of methods by which CTL responses can be usefully modulated in the treatment of various disease states.

Substantial evidence (reviewed in Reference 10) suggests that at least three distinct types of cell populations play a role in the generation of CTL responses: CTL precursors, helper T cells, and Ia⁺ accessory cells, including dendritic cells and Ia⁺ macrophages. Lytically inactive CTL precursors are triggered to proliferate and differentiate into mature, lytically active CTL as a result of the receipt of two types of signals, both of which are essential for activation (10–12). Signal one results from the interaction of CTL precursors with specific antigen, whereas signal two results from their interaction with nonspecific helper factors. One such nonspecific factor that appears to play an essential role in CTL responses is interleukin 2 (IL 2) (13–15). However, recent evidence from several laboratories suggests that an additional factor(s), sometimes referred to as T cell differentiation factor, may synergize with IL 2 or act in place of added IL 2 in facilitating CTL responses. Murine models in which such a factor has been demonstrated include i) the generation of lectin-induced polyclonal CTL responses (16–18), ii) the generation of antigen-induced specific CTL responses (19–23), and iii) the induction of cytotoxic activity in CTL hybridomas (24, 25). In contrast to the substantial literature on synergy between IL 2 and an additional factor(s) in eliciting murine CTL responses, relatively little has been published regarding the existence of similar synergistic interactions in the generation of human CTL. In this report we demonstrate synergy between recombinant human IL 2 (rIL 2) and IL 2-depleted lymphokine-containing supernatants (LKS) in facilitating allogeneic human CTL responses to ultraviolet (uv)-irradiated melanoma cells in vitro. The synergistic factor(s) is antigenically distinct from IL 2 and appears to act later than IL 2 in the CTL response.

MATERIALS AND METHODS

Media and reagents. Tissue culture medium (TCM) was a 1:1 mixture of RPMI 1640 and Dulbecco's modified Eagle's medium, supplemented with 5×10^{-5} M 2-mercaptoethanol, 0.1 mM nonessential amino acids, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μ g/ml streptomycin (GIBCO Laboratories, Grand Island, NY), and 5% human AB serum (M. A. Bioproducts, Walkersville, MD). Hydrocortisone sodium succinate (HC) was from Sigma Chemical Co., St. Louis, MO, and phytohemagglutinin-P (PHA-P) from Difco Laboratories, Detroit, MI. Purified human rIL 2 was generously donated by

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¹ Abbreviations used in this paper: CTL, cytolytic T lymphocyte; HC, hydrocortisone sodium succinate; LAK, lymphokine-activated killer; LKS, lymphokine-containing supernatant; MLTC, mixed leukocyte-tumor culture; PBMC, peripheral blood mononuclear cell; rIFN- α A, recombinant human interferon- α A; rIFN- γ , recombinant human interferon- γ ; rIL 2, recombinant human IL 2; TCM, tissue culture medium; T_hMF, CTL maturation factor; uv, ultraviolet.

Dr. C. Harvey, Department of Bioseparations Research, Hoffmann-La Roche, Inc. The rIL 2 was greater than 98% pure as assessed by SDS-polyacrylamide gel electrophoresis and had a specific activity of 2×10^7 U IL 2 activity/mg protein. When the rIL 2 was tested for endotoxin contamination by the Limulus amoebocyte lysate assay, no endotoxin was detected, indicating that the maximum possible endotoxin contamination was <0.1 ng endotoxin/ 3.6×10^5 U IL 2. Purified human recombinant interferon- γ (rIFN- γ) and purified human recombinant interferon- α A (rIFN- α A) were donated by Dr. M. Brunda, Department of Experimental Virology and Oncology, Hoffmann-La Roche, Inc.

Cell lines. Human melanoma lines HT144 and RPMI-7951 were obtained from American Type Culture Collection, Rockville, MD, and were maintained by weekly passage in TCM with 5% AB serum. The erythroleukemia line K562 was passed in TCM with 5% fetal bovine serum.

Production of LKS. Mixed leukocyte culture (MLC) supernatants were produced as described (26). In brief, 4×10^6 human peripheral blood mononuclear cells (PBMC) were incubated with 4 to 6×10^6 γ -irradiated (2000 rad) allogeneic PBMC in 2 ml TCM with 1% AB serum. After the cultures were incubated for 48 hr at 37°C, the culture supernatants were harvested. The supernatants were depleted of IL 2 as described below, concentrated 5- to 10-fold by ultrafiltration through an Amicon PM-10 membrane (Amicon Corp., Lexington, MA), dialyzed against TCM, and sterilized by filtration through a 0.2- μ m Millex filter (Millipore Corp., Bedford, MA). The supernatants were then stored at -20°C until used.

PHA-induced LKS were produced by incubation of human PBMC at a density of 2×10^6 cells/ml with 0.1% PHA-P in serum-free TCM. Culture supernatants were harvested after incubation of the cultures for 48 hr at 37°C. The supernatants were concentrated 10-fold by Amicon ultrafiltration and then depleted of PHA by ammonium sulfate precipitation (27). Sufficient crystalline ammonium sulfate (Schwarz-Mann, Cambridge, MA) was added to the LKS to give a final concentration of 50% saturation. The LKS was then held on ice for 1 hr, and the precipitate was removed by centrifugation at $10,000 \times G$ for 10 min at 4°C. Additional crystalline ammonium sulfate was added to the supernatant to give a final concentration of 85% saturation. The mixture was again incubated on ice and then centrifuged as described above. The precipitate was redissolved in Hanks' balanced salt solution (HBSS), and the resulting solution was dialyzed overnight against HBSS. The lectin-depleted, dialyzed LKS was then depleted of IL 2 as described below, filter sterilized, and stored at -20°C until used.

Depletion of IL 2 from LKS. LKS were depleted of IL 2 by use of an anti-rIL 2 immunoadsorbent column as follows. Neutralizing anti-rIL 2 antiserum prepared by immunizing rabbits with purified human rIL 2 was kindly provided by Dr. P. Osheroff, Department of Molecular Genetics, Hoffmann-La Roche, Inc. Anti-rIL 2 IgG was prepared by passage of 2 ml of antiserum through a column of protein A-Sepharose (Pharmacia, Piscataway, NJ) and elution of the bound IgG with 0.1 M citric acid, pH 3.5. The eluate was dialyzed against phosphate-buffered saline, pH 7.2 (PBS), and concentrated to approximately 1 ml by Amicon ultrafiltration. The anti-rIL 2 IgG was then coupled to Affi-Gel 10 resin (Bio-Rad, Richmond, CA) for construction of an anti-rIL 2 immunoadsorbent column. One milliliter of packed resin, which had been washed with isopropyl alcohol and then with distilled water, was mixed with an equal volume of anti-rIL 2 IgG, 12.8 mg/ml in PBS, and the mixture was tumbled for 5 hr at 4°C. To block any remaining reactive sites, 0.2 ml of 1 M ethanolic HCl, pH 8.0, was then added, and the mixture was tumbled for an additional hour at 4°C. Subsequently, the resin was poured into a 1 x 5-cm glass column (Bio-Rad), and any unbound protein was washed out with 0.15 M NaCl, 0.1 M NaHCO₃, pH 8.0. Finally, the column was equilibrated with PBS. This coupling procedure resulted in the binding of approximately 90% of the IgG to the resin. To deplete LKS of IL 2 activity, the supernatants were passed through the column at a flow rate of 2 ml/hr. This procedure routinely removed greater than 98% of the IL 2 activity from the LKS (see Table I). To regenerate the column for repetitive use, bound IL 2 was eluted with 0.2 M acetic acid, 0.15 M NaCl, and the column was then re-equilibrated with PBS.

Sensitization of lymphocytes in mixed leukocyte-tumor cultures (MLTC). PBMC were isolated from the blood of normal human donors as described (28). Accessory cells were depleted from the PBMC either by plastic adherence or by treatment of the PBMC with L-leucine methyl ester (Sigma). Removal of plastic-adherent cells was performed by incubation of 40 to 70×10^6 PBMC in 20 ml TCM with 5% AB serum in Corning 25110 tissue culture flasks for 1 hr at 37°C. Nonadherent cells were transferred to a fresh flask and incubated for another hour at 37°C before use. Treatment of PBMC with 5 mM L-leucine methyl ester was performed exactly as described by Thiele et al. (29). Removal of accessory cells from PBMC by plastic

adherence reduced the percentage of esterase-positive cells (Sigma; Kit No. 180-B) from 20-26% to 4-6%, whereas treatment with L-leucine methyl ester reduced the number of esterase-positive cells to less than 0.5%. Nevertheless, both types of accessory cell-depleted PBMC populations responded similarly in these experiments. Cytolytic lymphocytes were generated in MLTC in 24-well tissue culture plates (Costar, Cambridge, MA) by incubation of 1.5×10^6 accessory cell-depleted PBMC with 1×10^5 allogeneic uv-irradiated (960 μ W/cm² for 5 min) melanoma cells or 5×10^4 allogeneic γ -irradiated (10,000 rad) melanoma cells in 1.5 ml TCM with 5% AB serum. HC at a final concentration of 10^{-4} M was included in the cultures to suppress the generation of nonspecific lymphokine-activated killer (LAK) cells (26). Effectors were harvested from the MLTC after 5 or 6 days, unless indicated otherwise.

Cytolytic assays. Cell-mediated lysis of melanoma cells (28) and K562 (26) was measured in overnight ⁵¹Cr release assays as described. The percent specific ⁵¹Cr release was calculated as $[(e - c) / (100 - c)] \times 100$, where e is the percentage of ⁵¹Cr released from target cells incubated with lymphocytes and c is the percentage of ⁵¹Cr released from target cells incubated alone. Spontaneous ⁵¹Cr release in these experiments varied from 30 to 38% for melanoma targets and from 16 to 31% for K562. In every experiment, each effector population was assayed for lytic activity at three to four effector to target ratios. One lytic unit was defined to be the number of effectors required to cause 30% specific ⁵¹Cr release from the targets. Lytic units were calculated as described (28).

Treatment of effector cells with OKT3 and complement. T3⁺ cells were depleted from effector populations by incubation of effector cells with the monoclonal anti-T cell antibody OKT3 (Ortho Pharmaceutical Corp., Raritan, NJ) for 15 min at 37°C followed by rabbit complement (Pel-Freeze Biologicals, Rogers, AR) for 60 min at 37°C as described (28).

IL 2 bioassays. Supernatants were assayed for IL 2 content on the basis of their ability to stimulate the proliferation of an IL 2-dependent murine T cell line (30). Units of IL 2 activity were expressed relative to the BRMP human IL 2 reference standard obtained from Dr. Gary Thurman, Biological Resources Branch, NCI-Frederick Cancer Research Facility.

RESULTS

Synergy between rIL 2 and IL 2-depleted LKS in promoting cytolytic lymphocyte responses. In preliminary experiments, we observed that crude LKS were more effective than rIL 2 in facilitating CTL responses of accessory cell-depleted human PBMC to uv-irradiated allogeneic melanoma cells (data not shown). These observations suggested that the LKS might contain a factor(s) distinct from IL 2 that played a role in the generation of human CTL responses. To study this possibility in more detail, LKS were depleted of IL 2 by passage over an anti-human rIL 2 immunoadsorbent column. This routinely removed greater than 98% of the IL 2 activity from these supernatants (Table I). We then investigated whether these IL 2-depleted LKS could synergize with rIL 2 to facilitate human CTL responses in vitro (Table II).

In these experiments, the specificity of the effectors that were generated was examined by measuring the lysis both of cells of the melanoma line used for sensitization

TABLE I
Depletion of IL 2 from LKS by passage over an anti-rIL 2 immunoadsorbent column^a

LKS Lot No.	Total IL 2 Activity (units)	
	Before anti-rIL 2 column	After anti-rIL 2 column
199	25,540	<100
258	30,712	<50

^a Two lots of PHA-induced LKS were depleted of IL 2 by passage over an anti-rIL 2 immunoadsorbent column as described in *Materials and Methods*. Three milliliters of No. 199 LKS, which had been concentrated 50-fold by the combined use of Amicon ultrafiltration and ammonium sulfate precipitation (see *Materials and Methods*), were applied to the column. The final volume of IL 2-depleted No. 199 LKS was 14 ml. Four milliliters of 125-fold concentrated No. 258 LKS were applied to the column, and the final volume of IL 2-depleted LKS was 17 ml.

TABLE II
Synergy between IL 2 and IL 2-depleted LKS in facilitating allogeneic human CTL responses to UV-irradiated melanoma cells in vitro

Expt.	Contents of Cultures					Viable Cells per Culture × 10 ⁻⁶	% Specific ⁵¹ Cr Release from ^c				Lytic Units per Culture ^e	
	PBMC ^a	HT144 _{uv}	10 ⁻⁴ M HC	rIL 2 ^b	LKS		HT144		K562		HT144	K562
							24:1 ^d	8:1	24:1	8:1		
1	+		+			0.8	5 ± 2	-4 ± 1	1 ± 1	0 ± 1	1	0
	+		+	+	R + S ^f	1.2	10 ± 2	6 ± 1	6 ± 1	4 ± 1	3	2
	+	+	+			0.7	4 ± 3	0 ± 3	2 ± 1	2 ± 1	1	1
	+	+	+	+		0.9	20 ± 3	6 ± 1	3 ± 1	1 ± 1	4	1
	+	+	+		R + S	0.9	3 ± 2	-2 ± 1	1 ± 1	0 ± 1	1	0
	+	+	+	+	R + S	1.5	40 ± 1	25 ± 3	8 ± 1	5 ± 1	25	3
	+	+	+	+	Control ^g	1.1	10 ± 2	3 ± 3	3 ± 2	0 ± 1	3	1
	+			+		2.0	38 ± 6	25 ± 1	58 ± 1	38 ± 5	31	67
							2/1 ^d	0.7/1	18/1	6/1		
	2	+		+			0.6	6 ± 4	2 ± 5	-4 ± 1	-2 ± 1	11
+			+	+	R + PHA ^h	1.3	14 ± 3	9 ± 2	14 ± 2	9 ± 2	58	7
+		+	+			0.5	11 ± 2	-3 ± 4	3 ± 1	2 ± 1	15	1
+		+	+	+		1.3	31 ± 4	14 ± 3	2 ± 2	1 ± 1	137	1
+		+	+		R + PHA	0.7	13 ± 3	9 ± 2	2 ± 2	2 ± 1	28	1
+		+	+	+	R + PHA	1.9	56 ± 3	38 ± 3	12 ± 2	7 ± 2	760	8
+		+	+	+	Control ^g	1.2	10 ± 4	-2 ± 6	3 ± 1	1 ± 1	36	1
+				+		1.6	27 ± 1	8 ± 4	58 ± 3	32 ± 2	140	53

^a PBMC were depleted of accessory cells by treatment with L-leucine methyl ester in Expt. 1 and by plastic adherence in Expt. 2.

^b The concentration of rIL 2 was 10 U/ml in Expt. 1 and 6.7 U/ml in Expt. 2.

^c All values for percent specific ⁵¹Cr release represent the means ± 1 SEM of triplicate determinations. The spontaneous ⁵¹Cr release from HT144 targets was 33% and 38% in Expts. 1 and 2, respectively. The spontaneous ⁵¹Cr release from K562 was 18% in Expt. 1 and 31% in Expt. 2.

^d Effector to target ratio in the lytic assay.

^e One lytic unit was defined to be the number of effector cells required to cause 30% specific ⁵¹Cr release from the targets (28).

^f R = responder PBMC; S = stimulator PBMC. IL 2-depleted LKS used in Expt. 1 was from MLC (R + S) or from control cultures containing PBMC from a single donor incubated alone. In this experiment 0.2 ml of experimental or control IL 2-depleted LKS, which was 10-fold concentrated relative to the original culture supernatant, was added per well.

^g IL 2-depleted LKS used in Expt. 2 was from cultures of PHA-activated PBMC or from control cultures that contained PBMC from a single donor incubated alone and to which PHA was added at the end of the culture period. Twenty microliters of experimental or control IL 2-depleted LKS, which was 10.7-fold concentrated relative to the original culture supernatant, were added per well. In other wells (data not shown), addition of fourfold more or fourfold less control supernatant to cultures containing PBMC, uv-irradiated HT144 cells, and rIL 2 likewise failed to enhance the CTL response to a level above that seen in cultures that received rIL 2 without LKS.

and of cells of the erythroleukemia line K562. K562 cells lack HLA and Ia-like antigens (31) but are highly susceptible to lysis by the nonspecific lytic effectors, which have been called anomalous killers (26, 32) or LAK cells (33). We previously reported that the generation of such nonspecific effector cells can be selectively suppressed by addition of HC at concentrations that have little effect on CTL responses (26). In the experiments shown in Table II, when effectors were from control cultures in which LAK cells had been generated by incubation of PBMC with rIL 2 in the absence of HC, substantial lysis of both melanoma cells and K562 was observed. In contrast, effectors from cultures containing HC caused little lysis of K562, even though in some cases substantial lysis of melanoma cells was observed (reflecting the lytic activity of specific CTL).

Two types of IL 2-depleted LKS were used in these experiments: LKS derived from human MLC (Table II, Expt. 1) and LKS from cultures of PHA-activated human PBMC (Table II, Expt. 2). PHA-induced LKS were depleted of lectin by ammonium sulfate precipitation (27) before depletion of IL 2. As can be seen from Table II, both types of IL 2-depleted LKS yielded similar results in these experiments. Accessory cell-depleted PBMC in the absence of added helper factors did not make significant CTL responses to uv-irradiated HT144 melanoma cells. Addition of rIL 2 without IL2-depleted LKS resulted in modest CTL responses to the uv-irradiated melanoma cells. On the other hand, addition of IL 2-depleted LKS without rIL 2 did not lead to the generation of significant CTL responses. However, when both rIL 2 and IL 2-depleted LKS were added to cultures of accessory cell-depleted PBMC and uv-irradiated HT144 cells, strong specific CTL responses were generated. These responses were synergis-

tic in the sense that they were greater than would be expected on the basis of addition of CTL responses observed in cultures that received rIL 2 without LKS and cultures that received LKS without rIL 2. The enhanced CTL activity in cultures receiving both rIL 2 and LKS largely reflected an increase in the lytic activity per cell. In the experiments shown in Table II, the number of cells recovered from cultures receiving both rIL 2 and LKS was increased approximately 1.5- to 1.7-fold over the cell number in cultures that received only rIL 2. However, this constituted only a minor component of the observed increase in lytic units per culture, which was 5.5- to 6.2-fold. In contrast to the strong CTL responses generated in cultures containing PBMC, HT144 cells, rIL 2, and LKS, significant CTL responses to HT144 were not generated when rIL 2 and LKS were added to cultures containing PBMC without HT144 cells. However, in certain other experiments (see below), nonspecific LAK responses were generated in such cultures. The two blood donors used in experiments 1 and 2 of Table II differed substantially in the magnitude of the CTL responses that their PBMC made. Although the magnitude of the CTL responses generated in experiment 2 were much greater than in experiment 1, the relative magnitudes of the CTL responses generated in cultures containing both rIL 2 and LKS as compared with cultures containing rIL 2 without LKS and LKS without rIL 2 were similar in both experiments.

A final point demonstrated by the experiments in Table II is that lymphoid cell activation is required for the production of the factor(s) in LKS that synergizes with IL 2 to facilitate CTL responses. In experiment 1, when an equivalent volume of a control supernatant from a culture containing PBMC from only a single donor was sub-

stituted for the MLC-derived LKS, the CTL response generated in cultures containing rIL 2 and control supernatant was no greater than the response in cultures containing rIL 2 without LKS. Likewise, in experiment 2, a control supernatant was generated by incubation of PBMC from a single donor in the absence of mitogen, and PHA was added at the end of the culture period. PHA was then depleted by ammonium sulfate precipitation, and the control supernatant was passed over the anti-rIL 2 column. This control supernatant was likewise unable to synergize with rIL 2 in facilitating CTL responses, suggesting both that the production of the synergistic factor(s) required lymphoid cell activation and that the synergistic activity of PHA-induced LKS was not simply due to residual PHA. The latter conclusion is consistent with the observation that MLC-derived LKS, which contained no PHA, behaved similarly to PHA-induced LKS in these experiments.

The effects of simultaneously varying the concentrations of rIL 2 and IL 2-depleted LKS on the magnitude of the CTL responses generated were examined in three experiments, and the results of a representative experiment are shown in Figure 1. Addition of rIL 2 at concentrations <1 unit/ml resulted in little CTL generation regardless of the amount of LKS added. Concentrations of rIL 2 greater than 2 U/ml appeared to be required for strong CTL responses to be generated in the presence of LKS. Because the largest volume of LKS tested in this experiment contained less than 0.4 U of IL 2 activity, it is evident that the synergistic factor in LKS was distinct from IL 2. In other experiments (not shown), inhibition of CTL generation was observed when the concentration of IL 2-depleted LKS was increased beyond that used in the experiment shown in Figure 1. It is unknown whether this was due to an excess of the synergistic factor or to the effects of a distinct inhibitory component of the LKS that became dominant when LKS was added in large

amounts.

Characterization of effectors generated in the presence of rIL 2 and LKS. The specificity of the effectors generated in these experiments was further examined in reciprocal specificity experiments. Similar results were obtained in one experiment with MLC-derived LKS and in two experiments with PHA-induced LKS, and the results of one of these experiments is shown in Figure 2. In this experiment, uv-irradiated HT144 melanoma cells were used as stimulator cells in some MLTC, and uv-irradiated RPMI-7951 melanoma cells were used in others. Effector cells produced in all cultures were tested for their ability to lyse both ⁵¹Cr-labeled HT144 cells and ⁵¹Cr-labeled RPMI-7951 cells. It was found that effector cells produced in response to HT144 cells in the presence of rIL 2 and IL 2-depleted LKS lysed HT144 cells well but were only weakly active against RPMI-7951 cells. Likewise, effectors generated in the presence of uv-irradiated RPMI-7951 cells, rIL 2, and IL 2-depleted LKS caused substantial lysis of RPMI-7951 targets but little lysis of HT144. Thus, the effectors generated in these experiments demonstrated a high degree of specificity in their lysis of melanoma targets, suggesting that the effectors were in fact specific CTL. In the experiment shown in Figure 2, the specificity of the effectors generated against uv-irradiated melanoma cells in the presence of rIL 2 and LKS was similar to the specificity of the effectors generated against γ -irradiated melanoma cells in rIL 2 without LKS. γ -Irradiated melanoma cells, unlike uv-irradiated melanoma cells, did not require the addition of LKS to elicit strong CTL responses.

To define further the nature of the effector cells generated in these experiments, we examined the sensitivity of the effectors to lysis by treatment with the monoclonal anti-T cell antibody OKT3 and complement. We previ-

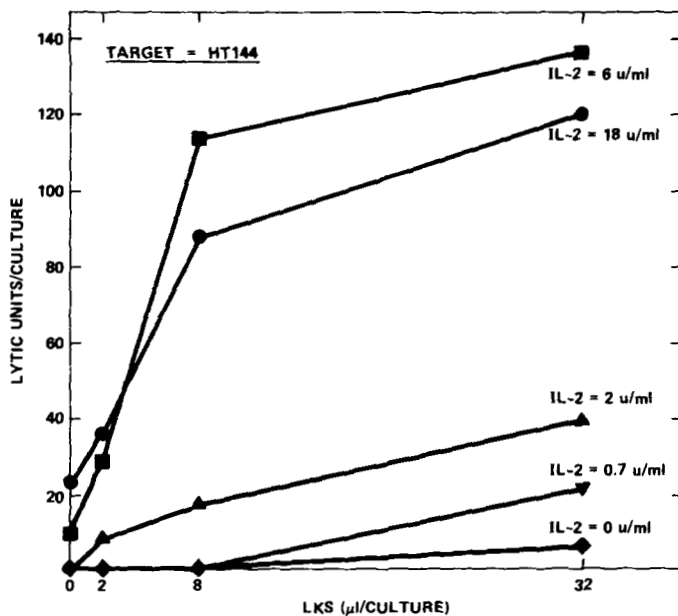


Figure 1. Effects of varying the concentrations of rIL 2 and IL 2-depleted LKS on CTL responses to uv-irradiated HT144 melanoma cells. The LKS used in this experiment was IL 2-depleted No. 199 LKS (cf. Table I), which was 10.7-fold concentrated relative to the original culture supernatant. PBMC were depleted of accessory cells by plastic adherence. All cultures contained 10^{-4} M HC, and the effectors that were generated in these cultures did not cause significant lysis of K562.

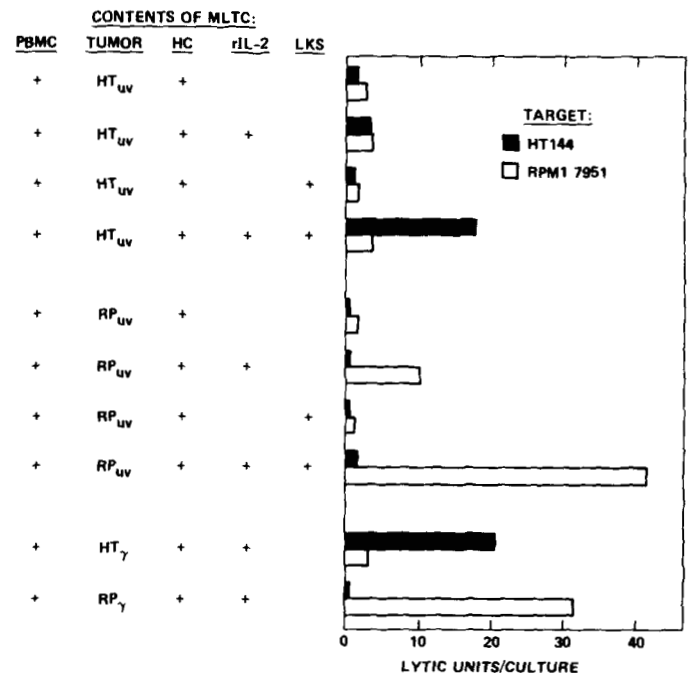


Figure 2. Specificity of effectors generated against uv-irradiated HT144 melanoma cells (HT_{uv}) or RPMI-7951 melanoma cells (RP_{uv}) in the presence of rIL 2 and IL 2-depleted LKS. PHA-induced LKS and plastic-nonadherent PBMC were used in this experiment. For purposes of comparison, the specificity of effectors generated against γ -irradiated HT144 (HT_γ) and RPMI-7951 (RP_γ) in the presence of rIL 2 without LKS is also shown.

ously showed that under the culture conditions that we use to generate effector cells, specific CTL are T3⁺, whereas nonspecific LAK cells (which we previously referred to as anomalous killers) appear to be T3⁻ (26, 28). Similar results have been reported by others (34, 35). The results of an experiment in which MLC-derived LKS was used to facilitate CTL generation are shown in Figure 3. Similar results were obtained in a second experiment with PHA-induced LKS (not shown). In the experiment shown in Figure 3, the incubation of PBMC with both rIL 2 and IL 2-depleted LKS in the absence of HT144 resulted in the generation of nonspecific LAK cells, which lysed K562 to a greater extent than HT144. The lytic activity of these cells was unaffected by treatment with OKT3 and complement even though this treatment lysed approximately 75% of the cells harvested from these cultures. In contrast, incubation of PBMC with both rIL 2 and IL 2-depleted LKS in the presence of uv-irradiated HT144 led to the generation of a mixture of CTL (indicated by the greatly enhanced lysis of HT144 targets) and LAK cells (indicated by the lysis of K562). Treatment of this population of effectors with OKT3 and complement led to a large reduction in the lysis of HT144 but no change in the lysis of K562. Thus, the lysis of HT144 targets in this case was largely due to the action of T3⁺-specific CTL; however, a minor component resulted from the action of T3⁻-nonspecific LAK.

Kinetics of effector generation in the presence of rIL 2 and LKS. The distinction between specific CTL and nonspecific LAK cells generated in these cultures is further illustrated in the kinetics experiment shown in Figure 4. Two such experiments were performed with similar results. In the experiment shown in Figure 4, the CTL response, as detected by lysis of HT144 cells, was observed to peak between days 4 and 6 of culture, whereas

LAK activity, as measured by lysis of K562, was already at a high level on day 3 and gradually declined thereafter. The more rapid kinetics of LAK generation as compared with CTL generation is consistent with previously reported results from other laboratories (32, 33). Moreover, the results shown in Figure 4 demonstrate that the difference in the magnitude of the CTL response generated in the presence of both rIL 2 and IL 2-depleted LKS as compared with the magnitudes of the responses generated in the presence of either rIL 2 alone or LKS alone is not attributable to differences in the kinetics of CTL generation. Finally, the results of the experiments shown in Figures 3 and 4 indicate that rIL 2 and IL 2-depleted LKS are capable of synergizing in facilitating LAK responses as well as in facilitating CTL responses. Whether these two activities are mediated by the same or different cytokines is at present unknown. Synergy between rIL 2 and LKS in the induction of LAK responses was observed only in cultures containing HC. As shown in Table II, in the absence of HC addition of rIL 2 without LKS was sufficient to elicit strong LAK responses, consistent with the previous results of Rosenberg et al. (36).

Effects of delaying the addition of rIL 2 and/or LKS on subsequent CTL responses. In additional experiments, we investigated the effects of varying the times of the addition of rIL 2 and IL 2-depleted LKS to MLTC. If IL 2 and/or the active factor(s) in LKS were required very early in the process of CTL generation, then delaying the addition of rIL 2 and/or LKS by 2 days would be expected to shift the kinetics of CTL generation so that little or no CTL response would be detected 3 days later (cf. Fig. 4), on day 5 of culture. On the other hand, if IL 2 and/or the active factor(s) in LKS were required only after the initial 2 days of culture, delaying the addition of rIL 2 and/or LKS by 2 days should not alter the kinetics of CTL generation, and one would expect to observe a strong CTL response on day 5. Similar results were obtained in four experiments with PHA-induced LKS and in one experiment with MLC-derived LKS. The results of a representative experiment are shown in Figure 5. Addition of both rIL 2 and IL 2-depleted LKS at the initiation of cultures resulted in a strong, synergistic CTL response to uv-irradiated HT144 melanoma cells, as had been observed in previous experiments. However, if IL 2-depleted LKS was added on day 0 and addition of rIL 2 was delayed until day 2, only a small CTL response was observed on day 5. Thus rIL 2 was required early in culture for a strong CTL response to be detected on day 5. This is consistent with the results of Männel et al. (37), who found IL 2 to be required early in the induction of murine CTL responses in vitro. In contrast, when rIL 2 was added at the initiation of culture, addition of IL 2-depleted LKS on day 2 resulted in the generation of a maximum CTL response, as measured on day 5. Thus, in this experiment preincubation of the PBMC with rIL 2 and melanoma cells permitted a strong CTL response to be generated within 3 days after the addition of LKS. In a separate experiment (not shown), a half-maximum CTL response was generated in 2 days after addition of LKS on day 4 to a culture that had received rIL 2 on day 0. The generation of substantial CTL activity within 2 to 3 days after the delayed addition of LKS is clearly faster than a detectable CTL response can be generated in conventional cultures to which rIL 2 and LKS are both added on day 0

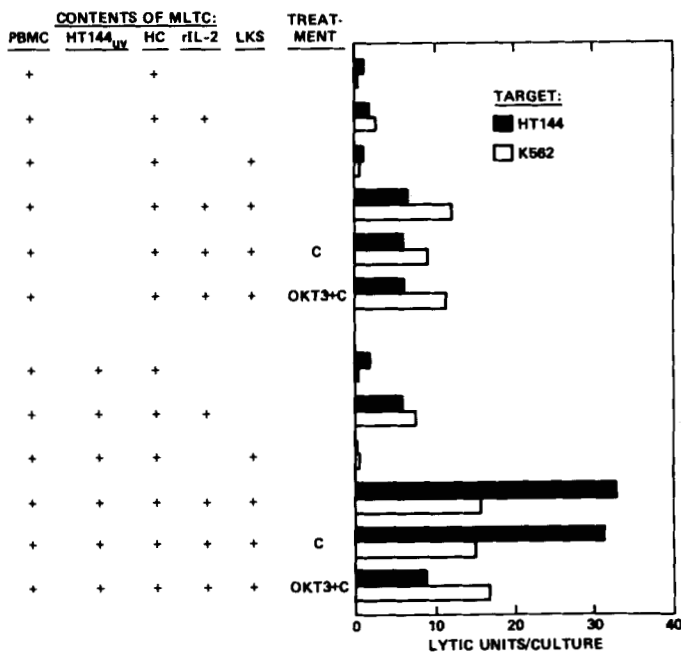


Figure 3. Treatment of effectors generated in the presence of rIL 2 and IL 2-depleted LKS with monoclonal OKT3 and complement (C). Effectors were harvested after 5 days of culture and treated with OKT3 and C or with C alone before assay for lytic activity. Treatment with OKT3 and C reduced the number of viable cells, as determined by trypan blue dye exclusion, by 75 to 78%, whereas treatment with C alone did not affect cell viability. LKS in this experiment was MLC-derived, and the PBMC were depleted of accessory cells by treatment with L-leucine methyl ester.

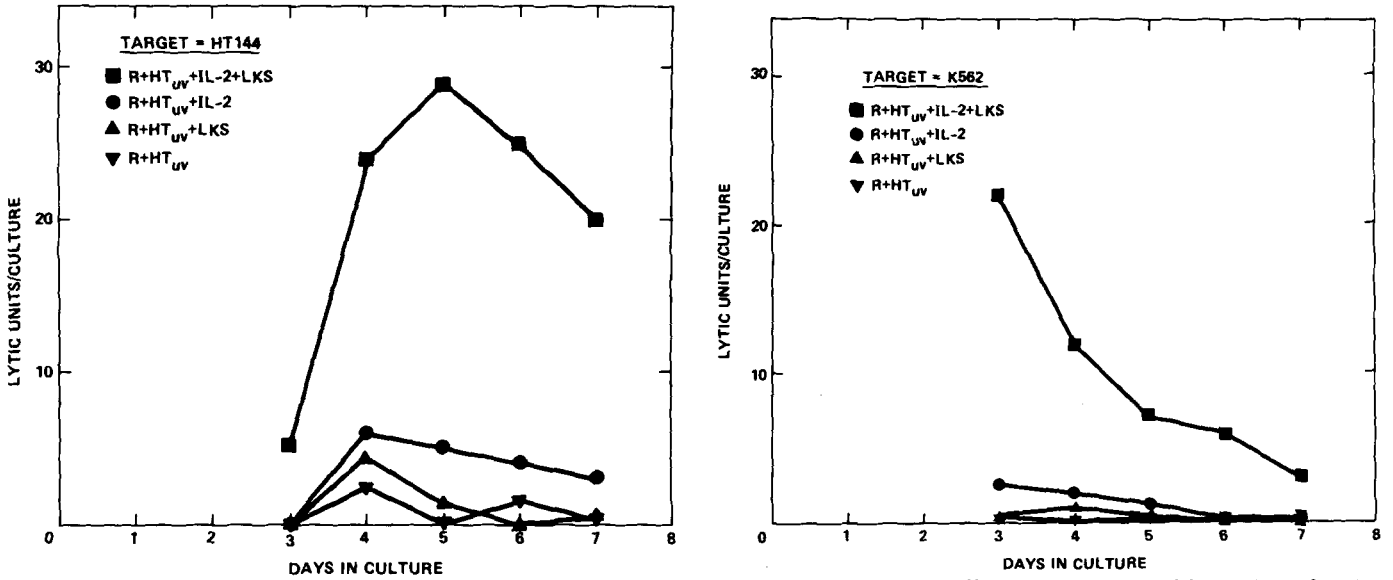


Figure 4. Kinetics of CTL and LAK cell generation in the presence of rIL 2 and IL 2-depleted LKS. Effectors were harvested from MLTC after the indicated number of days in culture and assayed for their ability to lyse HT144 melanoma cells (left) and K562 (right). Lysis of K562 reflects the amount of LAK activity, whereas lysis of HT144 reflects primarily, but not solely, the activity of specific CTL (cf. Fig. 3). All MLTC contained 10^{-4} M HC and uv-irradiated HT144 melanoma cells (HT_{uv}). Responder PBMC (R) were depleted of accessory cells by plastic adherence, and the LKS was from cultures of PHA-activated PBMC.

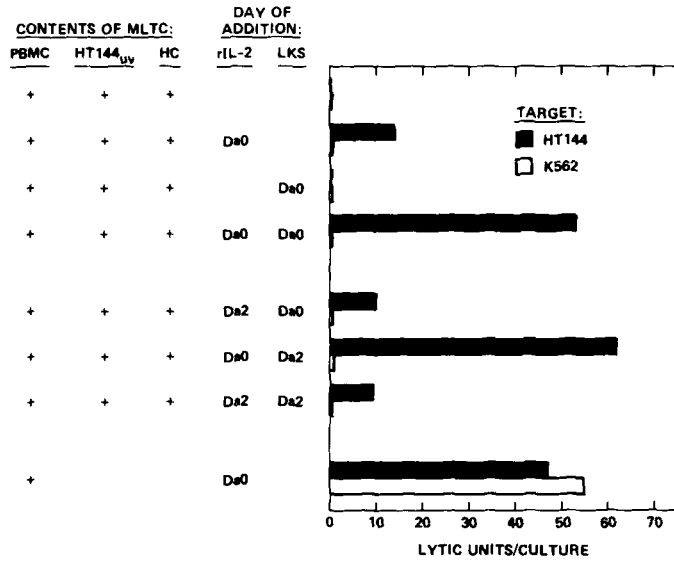


Figure 5. Effects of delayed addition of rIL 2 or IL 2-depleted LKS on the generation of CTL responses to uv-irradiated HT144 melanoma cells. Recombinant IL 2 and IL 2-depleted LKS were added to the MLTC on the indicated days of culture, and effectors were harvested from all cultures and assayed for lytic activity on day 5. In this experiment plastic-nonadherent PBMC and PHA-induced LKS were used.

(cf. Fig. 4). These observations suggest that the synergistic factor(s) in LKS may act at a late stage of the CTL response, perhaps to promote the final maturation of CTL precursors into lytically active CTL. These results further distinguish the synergistic factor(s) in LKS from IL 2.

The active factor in LKS is different from interferon. Farrar et al. (38) have presented evidence suggesting that IFN- γ may play a role in facilitating CTL responses. It was thus important to determine whether the synergistic factor in LKS might be IFN- γ . In two separate experiments, one of which is illustrated in Figure 6, rIFN- γ at a range of concentrations similar to the concentration of interferon activity in the LKS did not synergize with rIL 2 to promote strong CTL responses. This indicates that there are factors other than IFN- γ in the LKS that con-

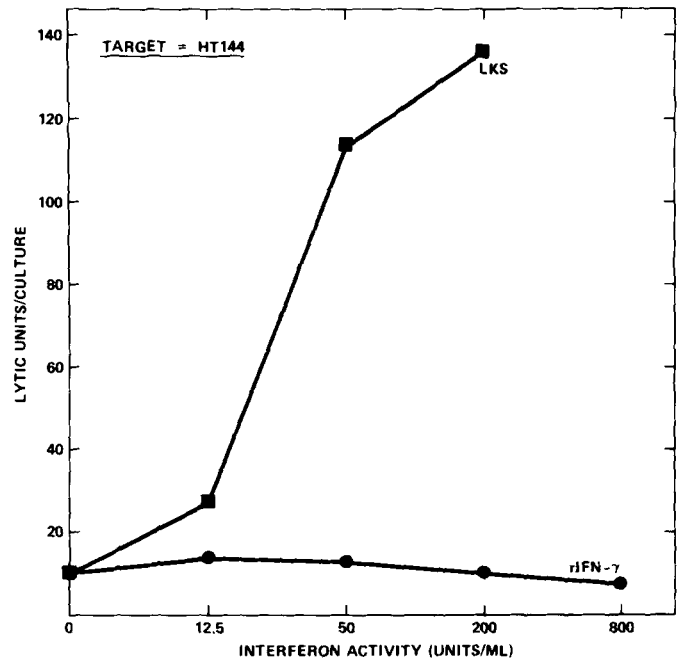


Figure 6. Inability of rIFN- γ to substitute for IL 2-depleted LKS in facilitating IL 2-dependent CTL responses to uv-irradiated HT144 melanoma cells. These data are from the same experiment shown in Figure 1. All cultures for which data are shown in Figure 6 contained 6 U/ml rIL 2 in addition to plastic nonadherent PBMC and uv-irradiated HT144 cells. IL 2-depleted LKS or rIFN- γ were added to the MLTC to give the concentrations of interferon activity indicated on the abscissa. The interferon antiviral activity of the IL 2-depleted LKS and the rIFN- γ was measured in a cytopathic effect inhibition assay by using human amniotic WISH cells and vesicular stomatitis virus (39). Units of interferon activity were expressed relative to the NIH human IFN- γ reference standard Gg 23-901-530.

tribute to the ability of LKS to synergize with rIL 2 in facilitating CTL responses. However, these results do not exclude the possibility that IFN- γ might act in concert with such factors. Interferon- α activity was not detectable in the LKS used in these experiments, and human

rIFN- α A, like human rIFN- γ , did not synergize with rIL 2 to promote strong CTL responses (data not shown).

DISCUSSION

Synergy between IL 2 and a cytokine(s) distinct from IL 2 in facilitating the induction of murine CTL responses in vitro has been described in reports from a number of laboratories (16–19, 22–25). Likewise, in some in vitro models a factor(s) distinct from IL 2 has been reported to act in the absence of added IL 2 to promote the generation of murine CTL (20, 21). In contrast, little has been published regarding the involvement of factors other than IL 2 in the generation of human CTL responses. Okada et al. (40) demonstrated that a human T hybridoma supernatant that lacked IL 2 could facilitate the development of human cytolytic effector cells in cultures containing uv-irradiated human B-lymphoblastoid cells. Likewise, the same investigators (41) reported that when human T hybridoma supernatants were passed over a gel filtration column, fractions containing molecules of 45,000 to 50,000 m.w. but lacking IL 2 could facilitate the generation of cytolytic effectors in this model system. However, the experiments described by these authors lacked specificity controls, and it was unclear whether the presence of uv-irradiated B lymphoblastoid cells in the cultures was actually required for the cytolytic effectors to be generated. Hence it is uncertain whether the cytolytic effectors generated in the experiments of Okada et al. were specific CTL or nonspecific LAK.

In the experiments described in this report we demonstrate that human LKS that had been depleted of IL 2 by passage over an anti-rIL 2 immunoadsorbent column contained a factor(s) that synergized with human rIL 2 in facilitating allogeneic human CTL responses to uv-irradiated melanoma cells in vitro. The CTL generated in our experiments could be distinguished from nonspecific LAK cells on the basis of their specificity (Fig. 2), T3 phenotype (Fig. 3), and kinetics of generation (Fig. 4). Nevertheless, in some experiments (e.g., Figs. 3 and 4) IL 2-depleted LKS was found to synergize with rIL 2 in the induction of nonspecific LAK cells as well as in the induction of specific CTL. In contrast to the generation of specific CTL, which required the presence of allogeneic melanoma cells as well as the addition of cytokines (Table II, Fig. 3), the induction of LAK cells by rIL 2 and IL 2-depleted LKS could occur in cultures to which no mitogen or antigen-bearing stimulator cells had been added (e.g., Fig. 3). Our results with regard to synergy between IL 2 and an additional factor(s) in the induction of nonspecific LAK cells are consistent with those in a recent report by Yang et al. (42) of synergy between IL 2 and a factor(s) distinct from IL 2 in the induction of murine lymphokine-induced cytotoxic cells. Whether the factor that synergizes with IL 2 in the induction of LAK cells is the same as the factor that synergizes in the induction of CTL is unknown; however, this question should be answered during the course of purification of the active factors in IL 2-depleted LKS.

Factors that synergize with IL 2 or act in the absence of added IL 2 to facilitate CTL responses in murine systems have frequently been referred to as T cell differentiation factors. However, factors reported to be active in different model systems have differed in their biologic and physicochemical properties. Moreover, in some cases

more than one factor (in addition to IL 2) have been shown to be required (19, 43), or multiple distinct factors have appeared to possess activity (44). Because the relationship of the factor identified in our experiments to the various factors described in murine models is unknown, we have provisionally called the factor(s) identified in our experiments CTL maturation factor (T_c MF). It is evident that T_c MF is antigenically distinct from IL 2, for T_c MF was identified in LKS that had been depleted of IL 2 by passage over an anti-rIL 2 column (Table I) so that the maximum residual contamination of IL 2 in the LKS was too small to have an appreciable effect in the assay for T_c MF (Fig. 1). T_c MF could be further distinguished from IL 2 on the basis of the time during the CTL response when it was required. IL 2 was required early in the induction of CTL responses, whereas T_c MF appeared to act later (Fig. 5). In this regard, T_c MF appears similar to TCF2 described by Männel et al. (37) in studies on the induction of antigen-specific murine CTL responses, to CTDF described by Hardt et al. (43) in studies on lectin-induced murine CTL responses, and to CCDF described by Yang et al. (42) in studies on the generation of murine lymphokine-induced cytotoxic cells. In contrast, TCF1 (37), RIF (43), CHF (45), and IL 3 (46), as well as IL 2 (37), have all been reported to act early in the generation of CTL responses. The observation that T_c MF acts later than IL 2 in the process of CTL generation also suggests that T_c MF acts by a mechanism different from the induction of endogenous IL 2 production.

In our experiments, rIL 2 in the absence of IL 2-depleted LKS was consistently able to facilitate modest CTL responses to uv-irradiated melanoma cells. Hence it is possible that T_c MF is not essential for CTL responses to occur but enhances weak IL 2-dependent responses. However, IL 2 has been shown to cause lymphoid cells to release other lymphokines, including IFN- γ (47), B cell growth factor (48), and lymphotoxin (49). It is thus possible that the addition of rIL 2 to cultures of PBMC and uv-irradiated melanoma cells may have evoked the endogenous production of a small amount of T_c MF that facilitated the maturation of IL 2-activated CTL precursors. This may also explain prior observations indicating that addition of rIL 2 alone was sufficient to promote strong CTL responses by unfractionated human PBMC to uv-irradiated, allogeneic peripheral blood lymphocytes (36) or melanoma cells (50). Moreover, because the accessory cell-depleted PBMC used in the current experiments were composed of a heterogeneous population of lymphoid cells, we cannot exclude the possibility that T_c MF as detected in these experiments promoted the maturation of CTL indirectly by acting on T helper cells rather than via a direct action on CTL precursors.

A number of other important questions regarding T_c MF also remain to be answered. The cellular source of T_c MF is at present unknown. The T_c MF-containing LKS used in these experiments were from cultures containing mixtures of lymphocytes and monocytes. Although lymphoid cell activation was required for T_c MF production (Table II), T_c MF could have been produced by either activated lymphocytes or monocytes. The physicochemical properties of T_c MF and its relationship to other cytokines likewise remain to be established. Recombinant human IFN- γ lacked T_c MF activity (Fig. 6), suggesting that a factor(s) in the LKS other than IFN- γ is required. Recent

preliminary experiments indicated that recombinant human IL 1 α (51) lacks significant T_CMF activity (Wilson and Gately, unpublished results), but recombinant human IL 1 β has not yet been available to us for testing. Further studies to characterize the physicochemical properties of T_CMF and to define its role in T cell-mediated immune responses are in progress.

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REFERENCES

- Ennis, F. A., M. A. Wells, G. M. Butchko, and P. Albrecht. 1978. Evidence that cytotoxic T cells are part of the host's response to influenza pneumonia. *J. Exp. Med.* 148:1241.
- Lin, Y.-L., and B. A. Askonas. 1981. Biological properties of an influenza A virus-specific killer T cell clone. Inhibition of virus replication in vivo and induction of delayed-type hypersensitivity reactions. *J. Exp. Med.* 154:225.
- Urban, J. L., R. C. Burton, J. M. Holland, M. L. Kripke, and H. Schreiber. 1982. Mechanisms of syngeneic tumor rejection. Susceptibility of host-selected progressor variants to various immunological effector cells. *J. Exp. Med.* 155:557.
- Mills, C. D., and R. J. North. 1983. Expression of passively transferred immunity against an established tumor depends on generation of cytolytic T cells in recipient. Inhibition by suppressor T cells. *J. Exp. Med.* 157:1448.
- Nemlander, A., A. Soots, and P. Häyry. 1984. *In situ* effector pathways of allograft destruction. I. Generation of the "cellular" effector response in the graft and the graft recipient. *Cell. Immunol.* 89:409.
- LeFrancois, L., and M. J. Bevan. 1984. A reexamination of the role of Lyt-2-positive T cells in murine skin graft rejection. *J. Exp. Med.* 159:57.
- Allan, J. E., and P. C. Doherty. 1985. Immune T cells can protect or induce fatal neurological disease in murine lymphocytic choriomeningitis. *Cell. Immunol.* 90:401.
- Guthrie, M., P. A. Lodge, and S. A. Huber. 1984. Cardiac injury in myocarditis induced by coxsackievirus group B, type 3 in BALB/c mice is mediated by Lyt 2⁺ cytolytic lymphocytes. *Cell. Immunol.* 88:558.
- Lin, Z., H. T. Lau, M. A. Hardy, and A.-K. Ng. 1985. Anti-islet of Langerhans cytolytic lymphocytes from spontaneously diabetic BB rats. *Fed. Proc.* 44:606. (Abstr. 1144.)
- Burakoff, S. J., O. Weinberger, A. M. Krensky, and C. S. Reiss. 1984. A molecular analysis of the cytolytic T lymphocyte response. *Adv. Immunol.* 36:45.
- Weinberger, O., S. Herrmann, M. F. Mescher, B. Benacerraf, and S. J. Burakoff. 1981. Antigen-presenting cell function in induction of helper T cells for cytotoxic T-lymphocyte responses: evidence for antigen processing. *Proc. Natl. Acad. Sci. USA* 78:1796.
- Weinberger, O., S. Herrmann, M. F. Mescher, B. Benacerraf, and S. J. Burakoff. 1981. Cellular interactions in the generation of cytolytic T lymphocyte responses. Analysis of the helper T cell pathway. *Eur. J. Immunol.* 11:405.
- Gillis, S., A. E. Gillis, and C. S. Henney. 1981. Monoclonal antibody directed against interleukin 2. I. Inhibition of T lymphocyte mitogenesis and the in vitro differentiation of alloreactive cytolytic T cells. *J. Exp. Med.* 154:983.
- Kern, D. E., S. Gillis, M. Okada, and C. S. Henney. 1981. The role of interleukin-2 (IL-2) in the differentiation of cytotoxic T cells: the effect of monoclonal anti-IL-2 antibody and absorption with IL-2 dependent T cell lines. *J. Immunol.* 127:1323.
- Granelli-Piperno, A., L. Andrus, and E. Reich. 1984. Antibodies to interleukin 2. Effects on immune responses in vitro and in vivo. *J. Exp. Med.* 160:738.
- Raulet, D. H., and M. J. Bevan. 1982. A differentiation factor required for the expression of cytotoxic T-cell function. *Nature* 296:754.
- Wagner, H., C. Hardt, B. T. Rouse, M. Rölinghoff, P. Scheurich, and K. Pfizenmaier. 1982. Dissection of the proliferative and differentiating signals controlling murine cytotoxic T lymphocyte responses. *J. Exp. Med.* 155:1876.
- Kanagawa, O. 1983. Three different signals are required for the induction of cytolytic T lymphocytes from resting precursors. *J. Immunol.* 131:606.
- Falk, W., D. N. Männel, and W. Dröge. 1983. Activation of cytotoxic T lymphocytes requires at least two spleen cell-derived helper factors besides interleukin 2. *J. Immunol.* 130:2214.
- Finke, J. H., J. Scott, S. Gillis, and M. L. Hilfiker. 1983. Generation of alloreactive cytotoxic T lymphocytes: evidence for a differentiation factor distinct from IL-2. *J. Immunol.* 130:763.
- Garman, R. D., and D. P. Fan. 1983. Characterization of helper factors distinct from interleukin 2 necessary for the generation of allospecific cytolytic T lymphocytes. *J. Immunol.* 130:756.
- Duprez, V., R. Maziarz, O. Weinberger, and S. J. Burakoff. 1984. Thymectomized, irradiated, and bone marrow-reconstituted chimeras have normal cytolytic T lymphocyte precursors but a defect in lymphokine production. *J. Immunol.* 132:2185.
- Hayes, R. L., and J. M. D. Plate. 1985. The identification of a unique lymphokine required for the generation of cytolytic T lymphocytes. *Fed. Proc.* 44:951. (Abstr. 3158.)
- Conzelmann, A., P. Corthésy, M. Cianfriglia, A. Silva, and M. Nabholz. 1982. Hybrids between rat lymphoma and mouse T cells with inducible cytolytic activity. *Nature* 298:170.
- Kanagawa, O., and J. M. Chiller. 1985. Lymphokine-mediated induction of cytolytic activity in a T cell hybridoma. *J. Immunol.* 134:397.
- Muul, L. M., and M. K. Gately. 1984. Hydrocortisone suppresses the generation of nonspecific "anomalous" killers but not specific cytolytic T lymphocytes in human mixed lymphocyte-tumor cultures. *J. Immunol.* 132:1202.
- Lotze, M. T., and S. A. Rosenberg. 1981. *In vitro* growth of cytotoxic human lymphocytes. III. The preparation of lectin-free T cell growth factor (TCGF) and an analysis of its activity. *J. Immunol.* 126:2215.
- Gately, M. K., M. Glaser, S. J. Dick, R. W. Mettetal, Jr., and P. L. Kornblith. 1982. In vitro studies on the cell-mediated immune response to human brain tumors. I. Requirement for third-party stimulator lymphocytes in the induction of cell-mediated cytotoxic responses to allogeneic cultured gliomas. *JNCI* 69:1245.
- Thiele, D. L., M. Kurosaka, and P. E. Lipsky. 1983. Phenotype of the accessory cell necessary for mitogen-stimulated T and B cell responses in human peripheral blood: delineation by its sensitivity to the lysosomotropic agent, L-leucine methyl ester. *J. Immunol.* 131:2282.
- Dick, M. D., W. R. Benjamin, T. Masuno, J. J. Farrar, and M. K. Gately. 1984. Differential effects of positive and negative proliferative stimuli on murine cytolytic and helper T-cell clones. *Cell. Immunol.* 86:118.
- Drew, S. I., P. I. Terasaki, R. J. Billing, O. J. Bergh, J. Minowada, and E. Klein. 1977. Group-specific human granulocyte antigens on a chronic myelogenous leukemia cell line with a Philadelphia chromosome marker. *Blood* 49:715.
- Seeley, J. K., and S. H. Golub. 1978. Studies on cytotoxicity generated in human mixed lymphocyte cultures. I. Time course and target spectrum of several distinct concomitant cytotoxic activities. *J. Immunol.* 120:1415.
- Grimm, E. A., A. Mazumder, H. Z. Zhang, and S. A. Rosenberg. 1982. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. *J. Exp. Med.* 155:1823.
- Zarling, J. M., and P. C. Kung. 1980. Monoclonal antibodies which distinguish between human NK cells and cytotoxic T lymphocytes. *Nature* 288:394.
- Platsoucas, C. D., and R. A. Good. 1981. Inhibition of specific cell-mediated cytotoxicity by monoclonal antibodies to human T cell antigens. *Proc. Natl. Acad. Sci. USA* 78:4500.
- Rosenberg, S. A., E. A. Grimm, M. McGrogan, M. Doyle, E. Kawasaki, K. Koths, and D. F. Mark. 1984. Biological activity of recombinant human interleukin-2 produced in *Escherichia coli*. *Science* 223:1410.
- Männel, D. N., W. Falk, and W. Dröge. 1983. Induction of cytotoxic T cell function requires sequential action of three different lymphokines. *J. Immunol.* 130:2508.
- Farrar, W. L., H. M. Johnson, and J. J. Farrar. 1981. Regulation of the production of immune interferon and cytotoxic T lymphocytes by interleukin 2. *J. Immunol.* 126:1120.
- Familleti, P. C., and D. Stremlo. 1985. Production of human gamma interferon from leukocytes cocultured with exogenous cells. *Methods Enzymol.* In press.
- Okada, M., N. Yoshimura, T. Kaieda, Y. Yamamura, and T. Kishimoto. 1981. Establishment and characterization of human T hybrid cells secreting immunoregulatory molecules. *Proc. Natl. Acad. Sci. USA* 78:7717.
- Kaieda, T., M. Okada, N. Yoshimura, S. Kishimoto, Y. Yamamura,

- and T. Kishimoto. 1982. A human helper T cell clone secreting both killer helper factor(s) and T cell-replacing factor(s). *J. Immunol.* 129:46.
42. Yang, S. S., T. R. Malek, M. E. Hargrove, and C.-C. Ting. 1985. Lymphokine-induced cytotoxicity: requirement of two lymphokines for the induction of optimal cytotoxic responses. *J. Immunol.* 134:3912.
43. Hardt, C., T. Diamantstein, and H. Wagner. 1985. Signal requirements for the *in vitro* differentiation of cytotoxic T lymphocytes (CTL): distinct soluble mediators promote preactivation of CTL-precursors, clonal growth and differentiation into cytotoxic effector cells. *Eur. J. Immunol.* 15:472.
44. Wabuke-Bunoti, M. A. N., A. Taku, R. Garman, and D. P. Fan. 1984. Stimulation of anti-influenza cytolytic T lymphocytes by a synthetic peptide of the influenza hemagglutinin can be modulated by at least three independent helper factors. *J. Immunol.* 133:2186.
45. Taku, A., R. D. Garman, M. A. N. Wabuke-Bunoti, J. M. Curtsinger, C. Haarstad, D. P. Fan, V. L. Braciale, and T. J. Braciale. 1984. A helper factor needed for the generation of mouse cytolytic T lymphocytes is made by tumor cell lines, cloned T cells, and spleen cells exposed to a variety of stimuli. *J. Immunol.* 133:502.
46. Curtsinger, J. M., and D. P. Fan. 1984. Interleukin 3 augments the murine primary cytolytic T lymphocyte response to allogeneic tumor cells. *J. Immunol.* 133:267.
47. Handa, K., R. Suzuki, H. Matsui, Y. Shimizu, and K. Kumagai. 1983. Natural killer (NK) cells as a responder to interleukin 2 (IL-2). IL 2-induced interferon- γ production. *J. Immunol.* 130:988.
48. Howard, M., L. Matis, T. R. Malek, E. Shevach, W. Kell, D. Cohen, K. Nakanishi, and W. E. Paul. 1983. Interleukin-2 induces antigen-reactive T cell lines to secrete BCGF-1. *J. Exp. Med.* 158:2024.
49. Svedersky, L. P., G. E. Nedwin, D. V. Goeddel, and M. A. Palladino, Jr. 1985. Interferon- γ enhances induction of lymphotoxin in recombinant interleukin 2-stimulated peripheral blood mononuclear cells. *J. Immunol.* 134:1604.
50. Gately, M. K., J. C. Jenson, and W. R. Benjamin. 1985. Role of cytokines in T- and B-cell-mediated immunity. *Concepts Immunopathol.* In press.
51. March, C. J., B. Mosley, A. Larsen, D. P. Cerretti, G. Braedt, V. Price, S. Gillis, C. S. Henney, S. R. Kronheim, K. Grabstein, P. J. Conlon, T. P. Hopp, and D. Cosman. 1985. Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature* 315:641.