Interleukin 7 Induces Cytokine Secretion and Tumoricidal Activity by Human Peripheral Blood Monocytes

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Summary

Peripheral blood monocytes can be induced by stimuli such as bacterial lipopolysaccharide (LPS) to secrete an array of cytokines. We have studied the effects of interleukin 7 (IL-7) on human peripheral blood mononuclear cells (PBMC) and found that IL-7 is a relatively potent inducer of IL-6 secretion. IL-6 protein levels were determined either by the B9 hybridoma growth factor assay or by enzyme-linked immunosorbent assay, and mRNA for IL-6 was analyzed by Northern hybridization. Detailed examination revealed that, among PBMC, monocytes, rather than lymphocytes, were secreting IL-6 in response to IL-7. In contrast to the low concentrations of IL-7 required to stimulate T cell growth and differentiation (as low as 0.1 ng/ml), relatively high concentrations of IL-7 were necessary to induce IL-6 secretion by monocytes (at least 10 ng/ml). An optimal concentration of IL-7 (100 ng/ml) induced monocytes to secrete 10-fold more IL-6 than an optimal concentration of IL-1β (10 ng/ml), and almost as much as LPS. However, significantly more IL-7 than IL-1β was required to induce detectable levels of IL-6. The kinetics of IL-6 secretion by monocytes were identical in response to IL-7, IL-1β, or LPS, with IL-6 protein detectable in culture supernatants as early as 2 h after the initiation of culture. IL-4 was found to markedly inhibit the ability of IL-7 or LPS to induce IL-6 mRNA and IL-6 secretion. In addition to promoting IL-6 production, IL-7 induced the secretion of immunoreactive IL-1α, IL-1β, and tumor necrosis factor α (TNF-α) by monocytes. IL-7 also induced monocyte/macrophage tumoricidal activity against a human melanoma cell target, an activity that may be related to the secretion of IL-1α, IL-1β, and TNF-α. Finally, we used a whole blood culture system as a bridge to in vivo analysis to demonstrate that IL-7 induces cytokine secretion in the absence of culture medium, fetal calf serum, and adherence to plastic. Our data suggest that IL-7, in addition to regulating lymphocyte growth and differentiation, has potent effects on cells of the monocytic lineage. Thus, IL-7 may be an important mediator in inflammation and in the macrophage immune response to tumors.

A primary function of peripheral blood monocytes is the regulated synthesis and secretion of an array of biologically active molecules including enzymes, plasma proteins, and cytokines. Monocyte-derived cytokines include IL-1α, IL-1β, IL-6, IL-8, and TNF-α, all of which have broad immunoregulatory properties and are central to the host response to infection (1–4). Microbial products such as LPS and peptidoglycan are the most effective inducers of cytokine secretion by monocytes. More recently, cytokines themselves have been demonstrated to regulate monocyte cytokine synthesis (5–13). In particular, IL-1α, IL-1β, TNF-α, TGF-β, IFN-γ, granulocyte/macrophage CSF (GM-CSF)[1], and IL-3 have all been shown to stimulate some aspect of monocyte cytokine secretion, either acting alone or in combination with other stimuli. Conversely, IL-4 has potent antagonistic effects on the induction of monocyte activation, including both cytokine secretion and respiratory burst activity (4, 8, 9, 14–17).

IL-7 is a stromal cell–derived cytokine that has a number of effects on lymphocytes. IL-7 stimulates the growth of pre-B cells, thymocytes, and mature T cells, and enhances the generation of CTL and lymphokine-activated killer cells (18–24). Receptors for IL-7 have also been demonstrated on myeloid cells (25), however, until now, no activity for IL-7 on monocytes/macrophages or neutrophils has been reported. In this study, we found that IL-7 has potent effects on cells of the monocytic lineage. IL-7 stimulated the secretion of cytokines including IL-6, IL-1α, IL-1β, and TNF-α from

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[1] *Abbreviations used in this paper:* GM-CSF, granulocyte/macrophage colony-stimulating factor; LAK, lymphokine-activated killer cell.

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purified human peripheral blood monocytes and induced monocyte/macrophage tumoricidal activity. These results implicate IL-7 as an important regulator of inflammation by inducing the secretion of cytokines that are central to the inflammatory process.

Materials and Methods

Preparation of Cells and Cell Cultures. PBMC were isolated from heparinized blood by centrifugation over Ficoll-Hypaque. Monocytes were enriched by counter current elutriation of PBMC followed by adherence to plastic. Briefly, elutriator enriched monocytes were cultured at 2×10^5 cells in 16-mm wells (3524; Costar, Cambridge, MA) in 1 ml of culture medium. After 90 min of incubation at 37°C, nonadherent cells were removed by gentle washing and replaced with fresh culture medium. The elutriated cells were 90–95% monocytes by microscopic examination of Giemsa-stained cytospin preparations and 80–85% CD14$^+$ by flow cytometry. Culture medium consisted of low endotoxin RPMI 1640 (Whittaker M.A. Bioproducts, Walkersville, MD) supplemented with 10% low endotoxin FCS (Cellect Gold; Flow Laboratories, McLean, VA), 50 U/ml penicillin, 50 μg/ml streptomycin, and 5×10^{-5} M 2-ME.

Cytokines. Cytokine preparations used in this study were selected on the basis of their low endotoxin content. All cytokines contained <1 pg of endotoxin per microgram of protein, except IL-1α which contained 3 pg, as determined by the *Limulus* amebocyte lysate assay (Whittaker M.A. Bioproducts). IL-7 was purified from *Escherichia coli* expressing a human IL-7 cDNA, as described previously (23). IL-7 had a specific activity of 3×10^4 U/μg in the murine pre-B cell assay (18). IL-1α and IL-1β were purified from *E. coli* expressing human IL-1α or IL-1β cDNAs as described previously (26) and had specific activities of 1.9×10^6 and 2.2×10^6 U/μg, respectively, in the thymocyte costimulation assay. IL-4 was purified from the supernatant of yeast cells expressing a human IL-4 cDNA and had a specific activity of 10^4 U/μg in a B cell comitogenesis assay (27, 28). GM-CSF was purified from yeast cells expressing a human GM-CSF cDNA and had a specific activity of 5×10^4 U/μg in a human bone marrow proliferation assay.

Other Reagents. LPS from *Salmonella typhimurium* was purchased from Difco Laboratories Inc. (Detroit, MI) and used at 10 μg/ml.

IL-6, IL-1α, IL-1β, and TNFα Assays. IL-6 bioactivity in culture supernatants was determined using the IL-6–dependent B9 hybridoma growth factor assay (3). Briefly, thrice-washed B9 cells were added to serial dilutions of test supernatants in 0.2 ml of RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% FCS (HyClone Laboratories, Logan, UT) in 96-well flat-bottomed plates (Costar). Samples were assayed in duplicate. After 3 d, cell proliferation was assessed by [^{3}H]thymidine (1 μCi/well) incorporation during a 6-h incubation. One unit of IL-6 is defined as the amount required for half-maximal stimulation of B9 proliferation. IL-6 was also assessed by an ELISA specific for human IL-6. ELISA plates (Corning Glass Works, Corning, NY) were coated overnight at 4°C with 5 μg/ml of a murine mAb against human IL-6. After blocking with a 5% solution of nonfat dry milk in PBS, test supernatants were serially diluted in PBS with 10% goat serum and incubated for 1 h at room temperature. Plates were washed and a rabbit antiserum raised against human IL-6 was added at 1:1,000 dilution in PBS/20% goat serum. After a further hour at room temperature, a horseradish peroxidase coupled goat anti–rabbit Ig (Sigma Chemical Co., St. Louis, MO) at 1:2,000 dilution in PBS/5% nonfat dry milk was added. After washing, the substrate 3,3′,5,5′-tetramethylbenzidine (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was added and absorbance of wells determined 1 h later using a Dynatech ELISA reader. IL-6 concentrations in test samples were determined by comparing titration curves with titrations of a standard human IL-6 preparation (R & D Systems, Minneapolis, MN) using the DeltaSoft 1.8 ELISA analysis program (Biometallics Inc., Princeton, NJ).

IL-1α concentrations in test supernatants were also determined by ELISA. The IL-1α ELISA was identical to the IL-6 ELISA except that the coating mAb and second step rabbit antiserum were specific for human IL-1α instead of IL-6.

TNF-α and IL-1β concentrations were determined using commercial ELISA kits (R & D Systems), as per the manufacturers protocol.

Northern Blot Analysis of IL-6 mRNA. Total cellular RNA was isolated as previously described (29). 10 μg of RNA was separated on 1% agarose/6% formaldehyde gels, transferred to nylon filters (Hybond; Amersham Corp., Arlington Heights, IL) and stained by methylene blue to ensure equal loading in each lane. After prehybridization, the filters were hybridized at 63°C in a buffer containing 5× SSC/10× Denhardt's/50% formamide for 18 h, at which time the filters were washed first in 1× SSC/0.1% SDS, then 0.1× SSC/0.1% SDS, at 68°C. The filters were then subjected to autoradiography.

An antisense RNA transcript was synthesized from a human IL-6 cDNA using ^{32}P-UTP (Amersham Corp.) and T3 RNA polymerase (Promega Biotec, Madison, WI) and was used for probing IL-6 mRNA from Northern blots.

Monocyte-Macrophage-Mediated Tumoricidal Assay. The ability of monocytes/macrophages to lyse A375 human melanoma cells was assessed as previously described (30, 31). Target A375 cells were labeled for 24 h with [^{3}H]thymidine and added to 24 h monocyte/macrophage cultures. After 24 h, culture supernatants were removed and replaced with fresh medium and then cultured for an additional 48 h. The cultures were then washed twice with medium and cells lysed with 0.1% NP-40 in PBS. Lysed cells were then harvested for liquid scintillation (β) counting. Percent cytotoxicity was calculated from the following formula: percent cytotoxicity = 100× [1−(cpm test/cpm control)]; where cpm control represents counts per minute of target cells cultured with untreated monocytes, and cpm test represents counts per minute of target cells cultured with cytokine-treated monocytes.

Whole Blood Cultures. Heparinized whole blood was cultured in polypropylene tubes (Falcon 2005; Becton Dickinson & Co., Mountain View, CA) at 2 ml per tube in the presence or absence of various stimuli, as previously described (32, 33). After 24 h of incubation at 37°C in a humidified atmosphere of 5% CO$_2$ in air, plasma was separated by centrifugation at 700 *g* for 10 min and filtered through 0.45-μM millipore filters. Cytokine levels were determined by ELISA, as described above.

Results

IL-7 Stimulates IL-6 Secretion by Human Peripheral Blood Monocytes. Initially, we observed that supernatants from PBMC cultures stimulated with IL-7 for 36 h contained elevated levels of IL-6 compared to control noncytokine supplemented cultures (Table 1). Culture supernatants were assessed for the presence of IL-6 by the ability to cause the proliferation of the IL-6–dependent B9 hybridoma cell line (3). Because PBMC, in particular monocytes, are exquisitely sensitive to endotoxin

Table 1. *IL-7 Induces IL-6 Secretion by Monocytes*

	IL-6					
	Exp. 1			Exp. 2		
Stimulus	PBMC	Monocytes	T cells	PBMC	Monocytes	Lymphocytes
			U/ml			
Medium	19	15	<1	<1	<1	<1
IL-1α	ND	ND	ND	654	894	40
IL-1β	ND	ND	ND	272	276	10
IL-7	3,067	5,654	11	1,895	2,437	49
LPS	ND	ND	ND	1,353	>10,000	107

Monocyte- and lymphocyte-enriched cell populations were isolated from PBMC by elutriation. T cells were further enriched from lymphocytes by E-rosetting and monocytes enriched by plastic adherence, as described in Materials and Methods. PBMC, lymphocytes, and T cells were cultured at 10^6/ml and monocytes at 2 × 10^5/ml in the presence of various stimuli for 36 h. Supernatants were collected and assessed for IL-6 bioactivity in the B9 assay (3).

with regards to IL-6 secretion, all reagents used in this study were selected for their low endotoxin levels. In addition we found that heating IL-7 to 100°C for 30 min totally abolished its ability to induce IL-6 secretion by PBMC, whereas heating of LPS had no effect on its activity (data not shown).

Monocytes, T cells, and B cells have all been shown to secrete IL-6, though monocytes appear to represent the major cellular source in human peripheral blood (3, 34). To determine what cells were being stimulated by IL-7 to secrete IL-6, we separated PBMC into populations enriched for monocytes and T cells (Exp. 1), or monocytes and lymphocytes (Exp. 2), as shown in Table 1. PBMC were subjected to counter current elutriation and divided into lymphocyte- and monocyte-enriched fractions, based upon microscopic examination of Giemsa-stained cytospins. In Exp. 1 the lymphocyte fraction was further enriched for T cells by E-rosetting, and in Exp. 2 the lymphocyte fraction was further depleted of monocytes by two cycles of plastic adherence. The monocyte fraction after further enrichment by plastic adherence contained 90–95% monocytes by examination of Giemsa-stained cytospins and 85–90% CD14$^+$ cells by flow cytometry whereas the lymphocyte fraction contained <1% CD14$^+$ cells. The data in Table 1 demonstrate that after stimulation with IL-7, IL-1α, IL-1β, or LPS, the vast majority of IL-6 was secreted by the monocyte-enriched population, with little activity coming from the lymphocyte or T cell populations. However, T cells did secrete high levels of IL-6 after 5 d of incubation with the combined stimulus of PMA plus PHA, as previously reported (reference 34; data not shown), thus confirming the capability of T cells to secrete IL-6. Table 1 also indicates that LPS was the most potent stimulus for monocyte IL-6 production, followed by IL-7, IL-1α, and IL-1β.

IL-7 Stimulates IL-6 mRNA in Monocytes. To examine the effects of IL-7 on IL-6 gene expression, we assessed IL-6 mRNA levels by Northern analysis after culturing monocytes with various stimuli for 4 h. Fig. 1 demonstrates that IL-7, IL-1α, IL-1β, and LPS all stimulated significant IL-6 mRNA accumulation in monocytes, whereas in cells cultured in medium alone, IL-6 mRNA was not detectable. In agreement with our IL-6 secretion data (Table 1), the level of IL-6 mRNA expression induced by IL-7 was greater than that induced by IL-1β, though slightly less than that induced by LPS.

Dose-Response of IL-7 Induction of IL-6 Secretion. IL-7 and IL-1β were compared for their ability to induce IL-6 secre-

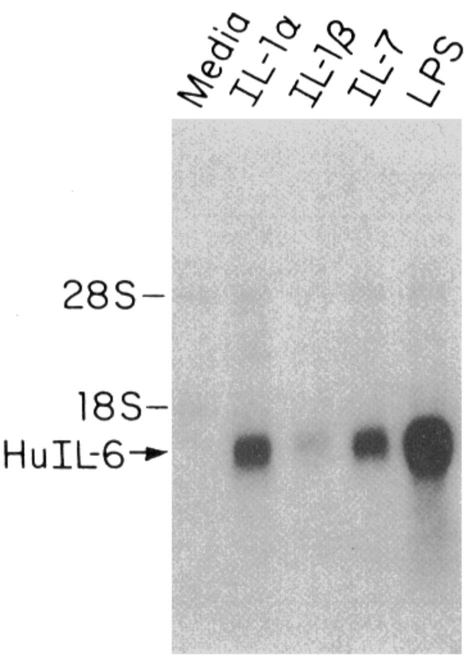

Figure 1. IL-7 induces IL-6 mRNA in monocytes. Monocytes were cultured with medium alone, IL-1α (100 ng/ml), IL-1β (100 ng/ml), IL-7 (100 ng/ml), or LPS (10 μg/ml). After 4 h RNA was extracted and Northern analysis was performed with an IL-6 antisense RNA transcript probe.

tion by monocytes. IL-6 levels were assessed by either the B9 hybridoma growth factor assay (Fig. 2 *A*) or by ELISA (Fig. 2 *B*). IL-7 induced significant IL-6 production when used at concentrations of 10 ng/ml or greater, though optimal IL-7 activity was observed at 100 ng/ml. In contrast, IL-1β had a significant effect on IL-6 secretion at 0.1 ng/ml, in agreement with previously published data (7). However, optimal concentrations of IL-7 induced ~10-fold higher levels of IL-6 than optimal concentrations of IL-1β (Fig. 2). Identical results were obtained when IL-6 was assayed by either B9 assay or by ELISA.

Monocytes Are Less Sensitive to IL-7 than Are T Lymphocytes. We have previously shown that IL-7 costimulates the proliferation of human T cells and enhances CTL generation at concentrations as low as 0.1 ng/ml (21, 23). To directly compare the concentrations of IL-7 required to stimulate T cells versus monocytes, we used the same preparation of IL-7 and compared its ability to either induce IL-6 secretion by monocytes or enhance CTL generation in human MLC (Table 2). As shown above (Fig. 2), 10 ng/ml or more of IL-7 was required for the induction of IL-6 secretion and the optimal effect was seen at 100 ng/ml. In contrast, as little as 0.1 ng/ml of IL-7 could enhance CTL generation and maximal effects were seen at 10 ng/ml (Table 2). Thus, CTL precursors appear to display a ~100-fold greater sensitivity to IL-7 than monocytes.

Table 2. *Difference in Sensitivity of CTL Precursors and Monocytes to IL-7 Stimulation*

IL-7	CTL activity (percent lysis*)	Monocyte secretion of IL-6‡
ng/ml		*U/ml*
0	0.3	<1
0.1	8.9	<1
1	30	<1
10	61	1,920
100	47	5,923
1,000	ND	7,867

* MLC were supplemented with the indicated concentration of IL-7 for 7 d and assayed for the ability to lyse specific PHA blasts from the stimulator donor, as previously described (23). Data represent lytic activity at a 0.2 culture fraction.

‡ Counter current elutriator-purified monocytes were incubated with the indicated concentration of IL-7 and supernatants collected after 36 h and assayed for IL-6 bioactivity.

Kinetics of IL-6 Induction by IL-7. Monocytes were stimulated with optimal concentrations of IL-7, IL-1β, or LPS and supernatants were collected at various time points and assayed for IL-6 activity. As shown in Fig. 3, low levels of IL-6 were detectable in the supernatants of IL-7, IL-1β, or LPS-stimulated monocytes as early as 2 h after the initiation of culture. IL-6 was not detected in nonstimulated cultures at any time point (data not shown). All three stimuli showed similar kinetic profiles of IL-6 induction with optimal levels of IL-6 being detected within 24 h. Again IL-7 was found to be a more potent inducer of IL-6 secretion than IL-1β.

IL-4 Inhibits the Ability of IL-7 to Induce IL-6 Secretion and IL-6 mRNA. IL-4 has been reported to inhibit both monocyte function and cytokine secretion induced by mitogenic stimuli such as LPS (4, 8, 9, 14–17). We initially observed that the addition of IL-4 to PBMC cultures inhibited the ability

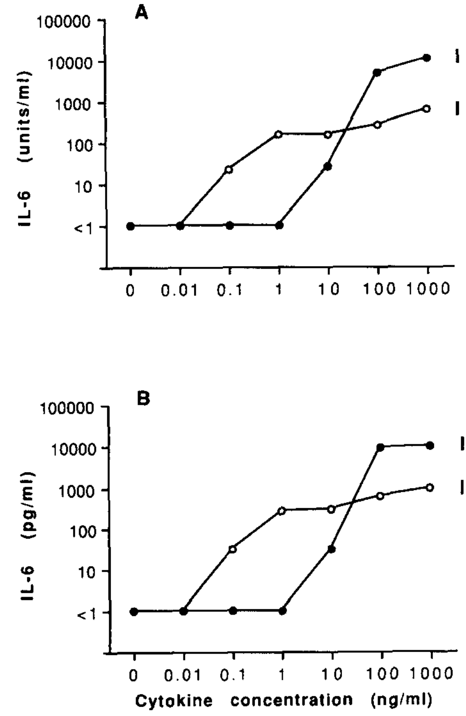

Figure 2. Dose-response of IL-7 induced IL-6 secretion. Monocyte cultures were established with various concentrations of IL-7 or IL-1β and supernatants collected after 36 h. IL-6 levels were detected either by (*A*) the B9 hybridoma growth factor assay, or (*B*) IL-6 ELISA.

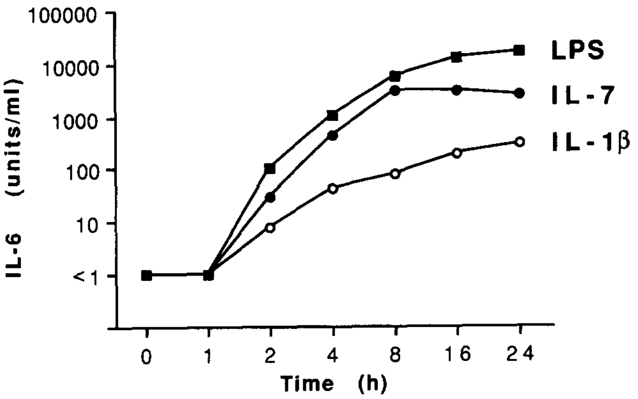

Figure 3. Kinetics of IL-6 production by monocytes. Monocytes were stimulated with IL-7 (100 ng/ml), IL-1β (100 ng/ml), or LPS (10 μg/ml) for various time periods and supernatants collected and assayed for IL-6 bioactivity in the B9 assay.

Table 3. *IL-4 Inhibits the Ability of IL-7 and LPS to Induce Monocytes to Secrete IL-6*

	IL-6			
	Exp. 1		Exp. 2	
Stimulus	– IL-4	+ IL-4	– IL-4	+ IL-4
	U/ml			
Medium	<1	<1	<1	<1
IL-7	19,220	3,665	2,437	106
LPS	16,343	6,176	10,143	1,502

Monocytes were cultured with IL-7 (100 ng/ml) or LPS (10 μg/ml) either in the presence or in the absence of IL-4 (50 ng/ml in Exp. 1 and 100 ng/ml in Exp. 2). Supernatants were collected after 36 h and assayed for IL-6 bioactivity.

of IL-7 to induce lymphokine-killer cell (LAK) activity (23). To determine whether IL-4 could also inhibit the production of IL-6, we cultured purified monocytes with IL-7 or LPS, either alone or in the presence of IL-4. IL-4 treatment alone was unable to induce IL-6 secretion by monocytes, however IL-4 was found to markedly inhibit the ability of either IL-7 or LPS to promote IL-6 secretion (mean of 88% and 74% inhibition, respectively) (Table 3). Suppression of IL-7-induced IL-6 secretion could be seen at concentrations of IL-4 as low as 0.1 ng/ml (data not shown). The inhibitory effects of IL-4 on IL-6 secretion were also apparent at the mRNA level (Fig. 4). The addition of IL-4 to monocytes stimulated with either IL-7 or LPS significantly downregulated IL-6 mRNA.

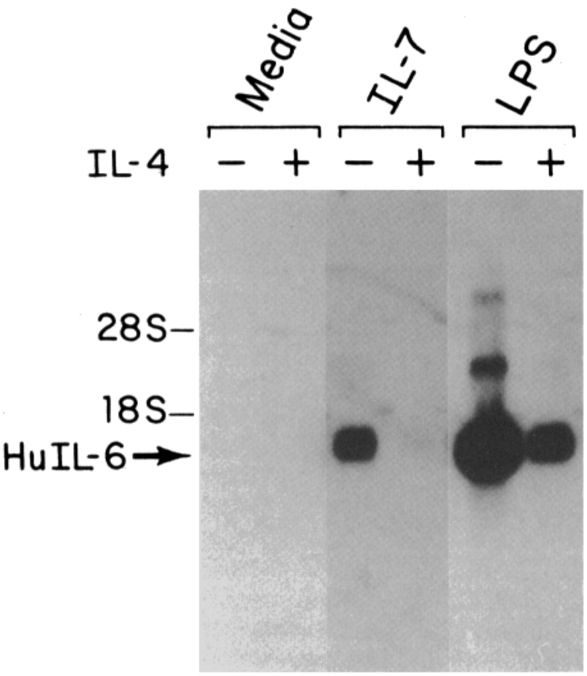

Figure 4. IL-4 inhibits the ability of IL-7 and LPS to induce IL-6 mRNA in monocytes. Monocytes were cultured with medium, IL-7 (100 ng/ml), or LPS (10 μg/ml) either with (+) or without (–) IL-4 (100 ng/ml). After 4 h RNA was extracted and Northern analysis was performed with an IL-6 antisense RNA transcript probe.

IL-7 induces IL-1α, IL-1β, and TNF-α Secretion by Monocytes. We next investigated the ability of IL-7 to induce the secretion of other cytokines by monocytes, namely IL-1α, IL-1β, and TNF-α. Monocytes were cultured for 36 h with various concentrations of IL-7 or 10 μg/ml of LPS, and supernatants were collected and assessed for the presence of IL-1α, IL-1β, IL-6, and TNF-α by cytokine-specific ELISAs. Supernatants from monocytes cultured with medium alone or up to 10 ng/ml of IL-7 contained nondetectable levels of all four monokines (Table 4). However, IL-7 at 100 ng/ml or greater induced significant secretion of IL-1α, IL-1β, IL-6, and TNF-α by monocytes. Optimal concentrations of IL-7 induced >100-fold increases in production of all three cytokines.

IL-7 Induces Monocyte/Macrophage Tumoricidal Activity. Monocytes can be stimulated in vitro by LPS or certain cytokines, such as GM-CSF, to become cytotoxic for selected tumor cell lines (30, 31). As a result, we assessed the ability of IL-7 to promote monocyte/macrophage tumoricidal activity against the A375 human melanoma cell line. Table 5 demonstrates that IL-7 significantly enhanced monocyte/macrophage tumoricidal activity, inducing a mean of 43% cytotoxicity in three experiments compared to 55% and 63% with GM-CSF and LPS-cultured monocytes, respectively.

IL-7 Induces IL-6 Secretion in Whole Blood Cultures. Recently, the ability of LPS to induce IL-6 and TNF-α in whole blood cultures was described (32, 33). This culture system more closely resembles in vivo conditions than isolated PBMC or monocytes and avoids any effects that may be attributed to cell preparation, culture medium, FCS or adherence to plastic. Therefore, we assessed the ability of cytokines, namely IL-7

Table 4. *IL-7 Induces Monocytes to Secrete IL-1α, IL-1β, and TNF-α in Addition to IL-6*

	Cytokine production			
IL-7	IL-1α	IL-1β	IL-6	TNF-α
ng/ml		*pg/ml*		
0	<1	<5	<3	<2
0.1	<1	<5	<3	<2
1	<1	<5	<3	<2
10	<1	<5	<3	10
100	202	1,572	8,964	1,050
1,000	527	2,586	12,714	1,170
LPS	372	2,860	11,606	2,370

Monocytes were incubated with the indicated concentration of rIL-7 or LPS (10 μg/ml) and supernatants collected after 36 h. Culture supernatants were assayed for IL-1α, IL-1β, IL-6, and TNF-α by specific ELISAs.

Table 5. *IL-7 Induces Monocyte/Macrophage Tumoricidal Activity*

Stimulus	Percent cytotoxicity		
	Exp. 1	Exp. 2	Exp. 3
IL-7	31.4 ± 1.5	60.3 ± 2.5	37.6 ± 3.3
GM-CSF	75.2 ± 6.5	49.3 ± 3.4	40.5 ± 1.9
LPS	69.9 ± 6.0	58.4 ± 1.9	60.4 ± 4.3

IL-7 (100 ng/ml) was compared to GM-CSF (100 ng/ml) and LPS (10 μg/ml) for its ability to induce monocyte/macrophage tumoricidal activity as described in Materials and Methods. Data represent percent cytoxicity of monocytes cultured in cytokine or LPS as compared to monocytes cultured in medium alone and are mean ± SEM of six replicate cultures. CPM for A375 cells cultured with no monocytes and A375 cells cultured with nonstimulated monocytes were: Exp. 1, 19,286 and 18,903; Exp. 2, 34,655 and 28,119; Exp. 3, 39,303 and 31,491.

and IL-1β, to stimulate IL-1α, IL-1β, IL-6, and TNF-α production in whole blood cultures. As shown in Table 6, cytokines were not detected in whole blood cultured for 24 h in the absence of a stimulus. As previously reported, LPS was a potent stimulus for cytokine secretion in whole blood cultures. In addition, high levels of IL-6 were detected in the plasma from whole blood stimulated with IL-7 or IL-1β. However, in contrast to purified monocyte cultures where IL-7 was almost as potent a stimulus as LPS, IL-7 was a relatively poor stimulus for cytokine production in whole blood. IL-7 also induced IL-1α and IL-1β secretion in whole blood, though TNF-α levels were only marginally above background.

Discussion

Interleukin 7 has a number of biological effects on lymphocytes and lymphocyte precursors, including stimulating the growth of pre-B cells, thymocytes, and mature T cells (18–24). In the experiments reported herein, we demonstrate that purified rIL-7 also has potent effects on human peripheral blood monocytes. When IL-7 was added to PBMC or purified monocytes, it was found to stimulate the secretion of high levels of IL-6, IL-1α, IL-1β, and TNF-α. In addition, IL-7 was a strong inducer of monocyte/macrophage–mediated lysis of the A375 human melanoma cell line.

Table 6. *Production of Cytokines in Whole Blood Cultures*

Stimulus	Cytokine production			
	IL-1α	IL-1β	IL-6	TNF-α
		pg/ml		
Medium	<1	<5	<3	<2
IL-1β	<1	–	432	5
IL-7	110	209	801	9
LPS	11,100	7,542	9,954	6,870

Whole blood cultures were supplemented with IL-1β (100 ng/ml), IL-7 (100 ng/ml), or LPS (10 μg/ml). After 24 h, plasma was isolated and assessed for the presence of IL-1α, IL-1β, IL-6, and TNF-α by specific ELISAs.

The activity of IL-7 on monocyte cytokine secretion resembles that of bacterial endotoxin, though we believe that our results are not due to endotoxin contamination of our cytokine preparations for the following reasons. First, our cytokines are extensively purified and particular preparations selected on the basis of their low endotoxin content (usually <1 pg per μg of protein). Second, heating of IL-7 totally ablated its ability to stimulate IL-6 secretion and IL-6 mRNA, whereas heating of LPS had no effect on its activity on monocytes (data not shown). Finally, IL-7, at concentrations up to 1 μg/ml, was unable to induce IL-6 secretion by the THP-1 monocytic cell line whereas LPS is a potent inducer of IL-6 secretion by these cells (data not shown).

Dose-response studies revealed that relatively high concentrations of IL-7 (10 ng/ml or greater) were required to stimulate IL-6 production by monocytes (Fig. 2). In contrast, we have previously shown that far lower concentrations of IL-7 (0.1–1 ng/ml) will enhance human T cell proliferation and the generation of CTL in MLC (21, 23). The relatively high concentrations of IL-7 required to stimulate monocytes compared to T cells might be explained on the basis of the particular receptors used by the different cell types. Both low and high affinity binding of IL-7 to its receptor have been described (25). Alternatively, there may be differences in the post receptor signaling pathways used by monocytes and T cells. In support of this concept, we have recently shown that IL-7 induces macrophage inflammatory protein-1β mRNA accumulation in human monocytes but not in purified T cells (S. F. Ziegler, T. W. Tough, T. L. Franklin, R. J. Armitage, K. H. Grabstein, and M. R. Alderson, manuscript submitted for publication).

IL-7, in addition to promoting IL-6 production, induced the secretion of IL-1α, IL-1β, and TNF-α by peripheral blood monocytes. Thus, IL-7 may have a very broad range of biological activities on the immune system, both directly and via the cascade of cytokines that it induces. At this time it is not clear whether the effect of IL-7 on the production of IL-6 by monocytes is direct or mediated via a cytokine cascade. Conceivably, IL-7 could function by inducing another cytokine, for example IL-1, which could then in turn induce IL-6 secretion. However, we believe that this is unlikely since the kinetics of induction of IL-6 secretion by IL-7 and IL-1β are identical and because IL-7 is a more potent inducer of IL-6 secretion than either IL-1α or IL-1β. We are currently investigating whether any of the effects of IL-7 on monocytes are mediated via a cytokine cascade using neutralizing antibodies against IL-1α, IL-1β, and TNF-α.

Previous studies have demonstrated that IL-4 inhibits IL-1, IL-6, and TNF gene expression and protein secretion induced by mitogenic stimuli such as LPS (4, 8, 9, 14–17). In this paper we extend these previous findings to show that IL-4 also inhibits the ability of IL-7 to induce monocyte cytokine synthesis at both the mRNA and protein levels. Thus, our data suggest that IL-4 may be an important regulator of inflammation induced by stimuli other than Gram-negative bacteria. We have previously demonstrated that IL-4 is a potent inhibitor of the induction of LAK cells by either IL-2 or IL-7 and CTL generation by IL-2 (23, 27). Collectively, these data suggest that IL-4 plays a central role in down regulating multiple facets of the immune response. Whether the ability of IL-4 to downregulate LAK cell induction and CTL generation is linked to its ability to inhibit cytokine secretion by monocytes remains to be elucidated.

Incubation of monocytes with IL-7 resulted in the activation of tumoricidal activity against the A375 melanoma cell line (Table 5). Monocyte/macrophage tumoricidal activity is thought to be mediated, at least in part, by the secretion of soluble mediators such as TNF-α and IL-1 (35–37), though cell-cell contact may be involved (38, 39). Therefore, the ability of IL-7 to induce monocyte/macrophage tumoricidal activity correlates well with its ability to induce IL-1α, IL-1β, and TNF-α secretion by monocytes (Table 4). As such, IL-7 joins GM-CSF as a cytokine that may be potentially useful for activating monocytes in vivo to become lytic against certain tumors.

In an attempt to more closely mimick the complexity of in vivo conditions, we used the recently described whole blood culture system (32, 33) and found that both IL-7 and IL-1β induced IL-6 secretion that was detectable in the plasma within 24 h of stimulation. Thus, monocytes derived from circulation, exposed to either IL-1 or IL-7 in vivo, could conceivably contribute to the elevated levels of serum IL-6 observed after various challenges to the immune system (40–42).

Whether IL-7 plays a role in the inflammatory immune response in vivo via the production of IL-6, IL-1α, IL-1β, and TNF-α remains to be elucidated. However, our monocyte data generated in vitro and results using whole blood cultures suggest this is a distinct possibility, providing that monocytes are exposed to the appropriate concentration of IL-7 in vivo. Localized environments in vivo, such as the bone marrow or thymus where IL-7 appears to be constitutively produced (18), may provide the appropriate IL-7 concentrations. Thus, inhibitors of the effects of IL-7 on monocytes, such as IL-4 or the naturally occurring soluble version of the IL-7 receptor (43), may prove to be useful therapeutically to regulate inflammatory immune responses.

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