Effects of cytokines on the production of lipoprotein lipase in cultured human macrophages

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Abstract Macrophages are important cells in the pathogenesis of atherosclerosis because of their tendency to accumulate lipid and become transformed into foam cells. Cultured human monocyte-derived macrophages spontaneously secrete lipoprotein lipase (LPL), and LPL has been linked to increased lipid uptake by these cells. Because secretion of various macrophage products depends on activation by lymphokines, we studied the effects of immunoregulatory lymphokines on LPL secretion by cultured human macrophages. After culturing cells in RPMI 1640 medium with 20% fetal calf serum, recombinant human gamma-interferon (gamma-INF), interleukin-1 (IL-1), and interleukin-2 (IL-2) were added to the medium and LPL secretion was assessed by measuring LPL activity and/or LPL mass in the medium. Gamma-INF suppressed LPL production both when added to freshly plated cultures of human blood monocytes, as well as when added to monocyte/macrophages from mature cultures (day 6) that were producing large amounts of LPL. IL-1 inhibited medium LPL when added to freshly plated cultures, but not when added to mature cultures. On the other hand, IL-2 did not inhibit LPL in freshly plated cultures, but produced a dose-dependent suppression of LPL from mature cultures. None of the cytokines were cytotoxic to macrophages, and cells that were cultured in gamma-INF demonstrated partial recovery from LPL-suppressive doses of the cytokine. After exposure of cells to 50 U/ml of gamma-INF and 50 U/ml of IL-2 for 3 days, LPL mRNA levels, when expressed as LPL/gamma-actin ratios, were 42% and 53% of controls, respectively. Thus, activation of human macrophages in vitro by gamma-INF resulted in a suppression of LPL production. IL-1 and IL-2 had more complex effects on macrophage LPL, which depended on the timing of cytokine addition. —**Querfeld, U., J. M. Ong, J. Prehn, J. Carty, B. Saffari, S. C. Jordan, and P. A. Kern.** Effects of cytokines on the production of lipoprotein lipase in cultured human macrophages. *J. Lipid Res.* 1990. **31:** 1379–1386.

Supplementary key words gamma-interferon • interleukin-1 • interleukin-2

Lipoprotein lipase (LPL) hydrolyzes the triglyceride core of triglyceride-rich lipoproteins into monoacylglycerol and free fatty acids. In addition to providing lipid substrate to muscle and adipose tissue, LPL is secreted by human monocyte-derived macrophages in culture (1, 2), which then leads to increased lipid uptake by these cells (3, 4). Lipid accumulation by macrophages in the arterial wall occurs during transformation into foam cells, which are important components of the atherosclerotic lesion (5, 6). Although this process is incompletely understood, LPL-mediated hydrolysis of lipoproteins may be an important step in foam cell formation and in the development of atherosclerosis (5).

Among the numerous secretory products of the monocyte/macrophage cells are cytokines, such as tumor necrosis factor/cachectin (TNF) (7) and interleukin-1 (IL-1) (8), which are powerful activators of a number of macrophage functions (9). Lymphokines such as interleukin-2 (IL-2) and gamma-interferon (gamma-INF) are secretory products of activated lymphocytes and are known to regulate monocyte/macrophage function as well. However, it is not known whether cytokine-mediated regulation of macrophage function is important in the development of atherosclerosis. There is evidence that various cytokines inhibit adipocyte LPL (10–13), and therefore interfere with adipose lipid uptake and likely contribute to the development of cachexia (14). However, the effects of cytokines on LPL vary with the species and the cell system studied. For example, although TNF inhibits LPL in 3T3-L1 adipocytes, it does not directly inhibit LPL in human adipocyte cultures (15). Furthermore, TNF does not inhibit LPL production or secretion from human monocyte/macrophages (16), yet inhibits LPL in mouse peritoneal macrophages (17). Other cytokines may affect macrophage LPL, however, and the regulation of LPL in macrophages may be different from regulation in adipocytes.

The present studies were performed to investigate the role of several cytokines in the regulation of LPL production by human macrophages. We chose to study two

Abbreviations: LPL, lipoprotein lipase; LPLa, lipoprotein lipase activity; LPLm, lipoprotein lipase mass; TNF, tumor necrosis factor; IL-1, interleukin-1; IL-2, interleukin-2; gamma-INF, gamma-interferon; TCA, trichloroacetic acid.

lymphokines (IL-2, and gamma-INF) produced by activated lymphocytes, and IL-1, a cytokine produced by activated monocyte/macrophages. These studies demonstrated that the addition of gamma-INF to macrophages in vitro resulted in a marked suppression of LPL production by these cells, whereas IL-1 and IL-2 had effects on LPL that depended on when they were added to the cultures.

MATERIALS AND METHODS

Culture of macrophages

Human monocytes were isolated from buffy coats obtained from healthy donors by centrifugation on Ficoll-Hypaque, as described previously (16). Peripheral blood mononuclear cells were plated under sterile conditions in single culture dishes (Falcon Primaria, diameter 60 mm) in RPMI 1640 medium (endotoxin-free) at a density of 10^7 cells per dish. After incubation for 90 min at 37°C in a 5% CO_2–95% humidified air atmosphere, the culture medium and nonadherent cells were removed and the adherent layer of monocytes was rinsed twice with fresh culture medium. Thereafter, the cells were maintained in the incubator in 5 ml of RPMI 1640 medium supplemented with 20% fetal calf serum (standard medium). Cytokines were added to the medium at the times and concentrations indicated. After addition of a cytokine, medium was again changed every 2–3 days, and each medium change included addition of the cytokine at the same concentration. The cytokines were sterile human recombinant gamma-INF (2.7×10^7 units/mg, Genentech, Inc, South San Francisco, CA), IL-1 (10^8 units/mg, Genzyme, Boston, MA), and IL-2 ($>7 \times 10^6$ units/mg, Amgen, Thousand Oaks, CA). To study LPL in the medium, medium was collected (without the addition of heparin) 24 h after a medium change, centrifuged at 4°C to remove nonadherent cells, and immediately frozen at −70°C for the assay of LPL activity (LPLa) and LPL mass (LPLm). Prior to freezing, the proteinase inhibitors EDTA, benzamidine, and phenylmethylsulfonyl fluoride were added at a final concentration of 1 mM to the samples for LPLm determination. To examine cellular LPL, cells were scraped in the presence of 1 ml of extraction buffer, used previously by us for adipocyte LPL (15, 18), containing 0.5% deoxycholate, 0.02% NP-40, 0.73% sucrose, 0.1% albumin, 125 μg/ml heparin, and 25 mM Tris, pH 8.3. This sample was divided into two samples for assay of LPL activity and mass. To the sample for LPL mass, protease inhibitors were immediately added, as described previously.

Assays for LPL

The catalytic activity of LPL (LPLa) was measured as previously described (19), using a [^{3}H]triolein-containing substrate emulsified with lecithin, and containing normal human serum as a source of apolipoprotein C-II. After incubating the sample with substrate for 45 min at 37°C, the reaction was stopped by adding Belfrage-Vaughn extraction mixture (20), and the liberated ^{3}H-labeled free fatty acids were quantified by liquid scintillation counting. Activity was expressed as nEq of free fatty acids generated per minute per ml. The immunoreactive mass of LPL (LPLm) was measured as previously described (18, 21). Briefly, a microtiter plate was coated with affinity-purified chicken anti-LPL antibody, and bovine LPL standards and samples were added in a buffer containing 1 M NaCl, 0.1% Triton X-100, 0.1% albumin, protease inhibitors, and 25 mM Tris-HCl (pH 7.4). After the addition of biotinylated anti-LPL antibody followed by streptavidin-horseradish peroxidase (Bethesda Research Laboratories), the plate was developed and read in an ELISA plate reader at 490 nm.

Extraction of RNA and LPL mRNA quantitation

For the quantitation of LPL mRNA, media were removed from four plates, and the adherent cells were lifted by adding 2 ml of RNA extraction solution (4 M guanidinium thiocyanate, 0.5% SDS, 0.1 M beta-mercaptoethanol, and 25 mM Na citrate, pH 7.0) and scraping with a rubber policeman. RNA was then extracted from macrophages using the guanidinium–phenol–chloroform method of Chomczynski and Sacchi (22). RNA was resolved by electrophoresis on a 2.2 M formaldehyde–1% agarose gel and transferred onto a nylon membrane (Hybond N, Amersham Corp.), or blotted directly onto a nylon membrane using a slot-blot manifold apparatus (Schleicher and Schuell) for slot-blot analysis. The cDNA probes coding for human LPL (23) and gamma-actin (24) were radiolabeled by the random-priming method (25) with alpha-[^{32}P]-dCTP (3000 Ci/mmol; ICN Biomedical, Irvine, CA). The [^{32}P]-cDNA probes (1×10^6 cpm/ml) were hybridized as described previously (26). The blots were washed twice with 2 × SSC (0.3 M sodium citrate, 0.3 M sodium chloride) with 0.1% (w/v) SDS at room temperature, and twice with 0.2 × SSC, 0.1% SDS at 60°C. The blot was dried at room temperature and exposed to Kodak XAR-5 X-ray film at −70°C. Autoradiographic images were quantitated by densitometry. Previous studies demonstrated that there was a linear relationship between the amount of RNA loaded and the LPL mRNA signal.

TCA precipitation and protein assay

To assess the rate of overall protein synthesis, 60-mm plates of confluent macrophages (one plate for each condition) were radiolabeled with 20 μCi of [^{35}S]methionine for 20 min. Cells were then taken up with 2 ml of lysis buffer (0.02 M sodium phosphate (pH 7.5), 0.2 M sodium chloride, 2% (v/v) Triton X-100, 1% (w/v) sodium deoxy-

cholate, 0.2% (w/v) sodium dodecyl sulfate, 5 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride), and 50 μl of this solution was made 10% with trichloroacetic acid at 4°C. The precipitated proteins were then counted. To assess total cellular protein, cells were scraped in 1 ml of lysis buffer and protein was assayed using a Bio-Rad protein assay kit.

RESULTS

Cells were prepared and plated as described in Methods, and assumed a typical monocyte/macrophage morphology when examined by light microscopy. To assess LPL production in the freshly plated cells, medium was removed 24 h after a medium change and LPLa and LPLm were assayed. As shown in **Fig. 1**, a continuous increase in medium LPL was observed over 12 days, and the increase in LPLa paralleled the increase in LPLm. When a high concentration (500 U/ml) of gamma-INF was added to the medium on day 1 in culture, only very small amounts of LPLa and LPLm were detected over the first 12 days of culture (Fig. 1). In spite of the low level of medium LPL in the presence of gamma-INF, cell morphology remained unchanged. The effects of IL-1 and IL-2 on macrophage LPL were studied in a similar manner. Cells were plated and IL-1 (5 U/ml), IL-2 (50 U/ml), and gamma-INF (50 U/ml) were added on day 1. Medium was removed for LPL assay on days 3, 5, and 7, which was 24 h after a medium change. As shown in **Fig. 2**, LPL activity and mass were unaffected by the addition of IL-2. However, both gamma-INF and IL-1 inhibited the appearance of LPLa and LPLm in the medium. At these concentrations, none of the cytokines inhibited LPL on day 3, however, IL-1 and gamma-INF had similar inhibitory effects on day 7 (Fig. 2).

During the first 5 to 10 days of monocyte/macrophage culture, LPL production gradually increases (2). To determine whether cytokines affect LPL differently when added to mature cultures, cells were cultured for 5 to 6 days in standard medium without cytokines, and then changed into medium containing cytokines. The effects of different gamma-INF concentrations on LPL production is shown in **Fig. 3**. LPLm on days 7, 9, and 12 of culture (1, 3, and 6 after the addition of gamma-INF) showed a progressive decrease in a dose-dependent manner. A concentration of 500 U/ml of gamma-INF almost completely

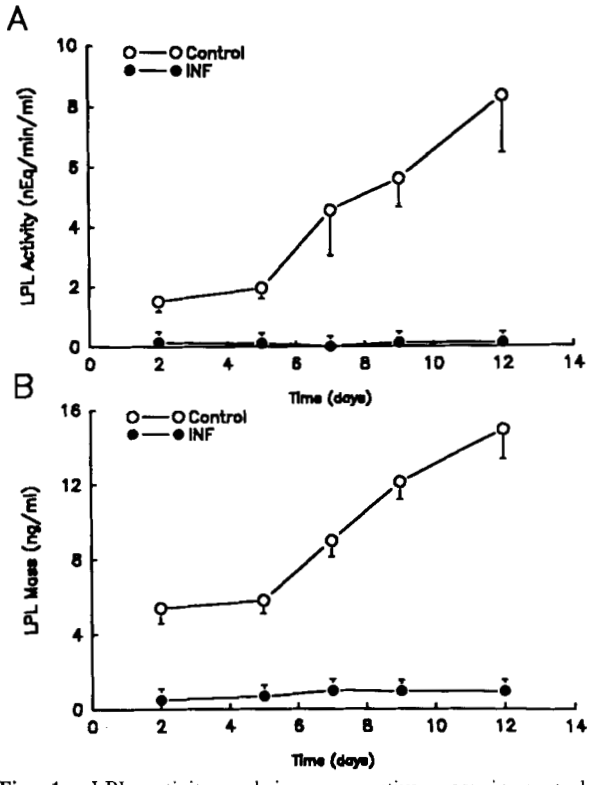

Fig. 1. LPL activity and immunoreactive mass in control and gamma-INF-treated cells. Human monocyte-derived macrophages were prepared and cultured with or without the addition of gamma-INF (500 U/ml) for 12 days in RPMI 1640 medium supplemented with 20% fetal calf serum. Medium was removed the day after a medium change and LPL activity (A) and immunoreactive mass (B) were measured. Data from two experiments were pooled (mean ± SEM).

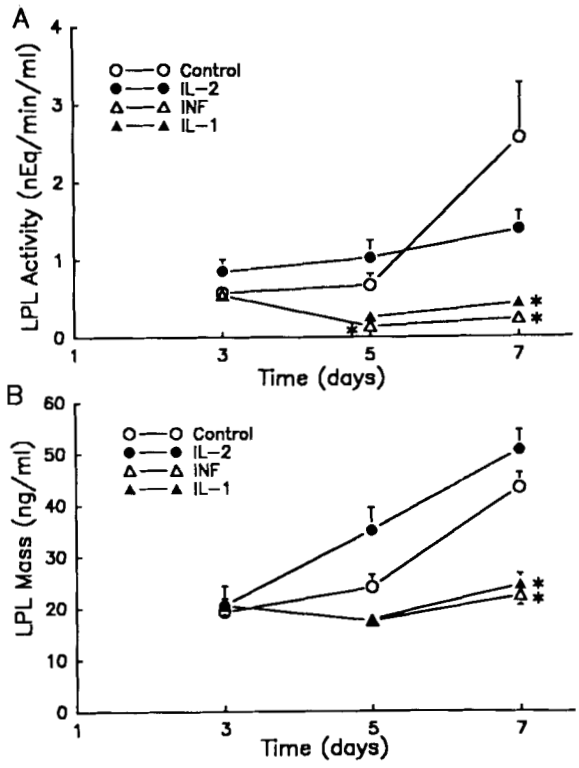

Fig. 2. Effects of cytokines added on day 1. Monocyte/macrophages were prepared and plated as described in Methods, and the indicated cytokine was added on day 1, and medium was changed on days 2, 4, and 6. On the day after medium change, medium was removed and assayed for LPLa (A), and LPLm (B). There were three to four plates of cells for each point. $^{*}P<0.05$ versus control (no cytokines added) using one-way analysis of variance and Bonferroni *t*-test.

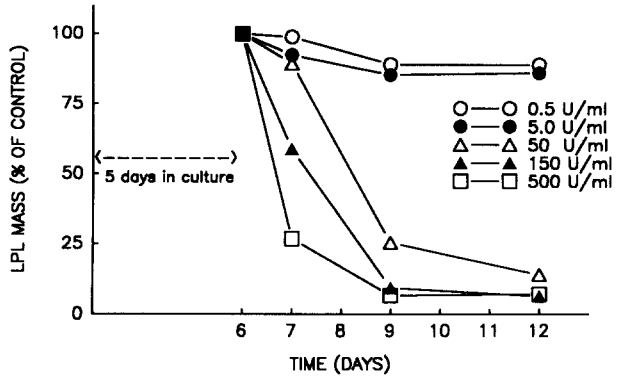

Fig. 3. Dose-dependent effect of gamma-INF on LPL. Human monocyte-derived macrophages were cultured for 5 days, and gamma-INF (0.5 to 500 U/ml) was then added to the medium on day 6. The same concentration of gamma-INF was added during each subsequent medium change, and LPLm was measured on the day after each medium change (days 7, 9, and 12). Control LPLm on day 6 was 10.3 ng/ml. The data from two experiments were pooled.

inhibited medium LPL, and the 50% effective concentration (EC_{50}) was approximately 20 U/ml. To study IL-1 in this manner, cells were cultured for 5 days and then IL-1 (5 U/ml) was added to the medium starting on day 6. In these cultures, LPLa and LPLm were no different from control cultures (data not shown), even though lower concentrations of IL-1 are known to exert potent biological effects on human cells in vitro (27), and the same concentration of IL-1 inhibited LPL when added on day 1. However, when the same human macrophage cultures that were treated with IL-1 were also exposed to IL-2, inhibition of LPL was observed. After culturing cells for 5 days in standard medium, IL-2 (0.5 to 100 U/ml) was added and medium LPL was measured over the ensuing 7 days. As shown in **Fig. 4**, IL-2 yielded a dose-dependent decrease of LPLm on days 7, 9, and 12. On day 12, an IL-2 concentration of 100 U/ml completely inhibited medium LPL, and the EC_{50} for IL-2 was 50 U/ml. As with gamma-INF, cell morphology was unchanged by the addition of IL-2.

To determine whether cellular LPL changed in parallel with medium LPL, cellular LPL was measured 7 days after cells were plated, with cytokines added beginning on day 1. As shown in **Table 1**, very little LPLa was detected in the cells, and the level of LPLm was much less than what was found in the medium. However, the levels of cellular LPL mass were decreased in cells treated with gamma-INF and IL-1, in parallel with the changes in medium LPL. In addition, an aliquot of the cells was assayed for total protein. Also, shown in Table 1, there were no significant differences in cellular protein per dish in the presence or absence of cytokine.

To test the viability of the cell cultures, adherent macrophages were cultured for 12 days, as described above, with the addition of either IL-2 or gamma-INF to the medium on day 6. Cells were then stained with trypan blue, and approximately 90% of both untreated and treated macrophages excluded the dye. To further assess cell viability, [^{35}S]methionine incorporation into human macrophages was examined. Cells that had been cultured in either gamma-INF or IL-2 were pulse-labeled with [^{35}S]methionine, and total cell protein was then precipitated with trichloroacetic acid and counted. The addition of either IL-2 (50 U/ml) or gamma-INF (50 U/ml) to the culture medium had no effect on [^{35}S]methionine incorporation into total protein. In addition, the reversibility of cytokine-mediated inhibition of medium LPL was examined. After measuring LPLm on day 12 of culture, gamma-INF-treated macrophages were washed and cultured in medium containing no cytokines. As shown in **Fig. 5**, partial recovery of normal LPL production was observed after 24 and 48 h.

To examine the mechanism of the suppressive effect of IL-2 and gamma-INF on LPL production, LPL mRNA levels were quantitated. Cells were cultured for 6 days, and then changed to medium containing gamma-INF (50 U/ml) for 3 days. Northern blotting of macrophage total RNA demonstrated LPL transcripts of 3.6 and 3.4 kb (**Fig. 6**), as described elsewhere in other human tissues (23, 26). When compared to control cultures, gamma-INF-treated cultures expressed a lower level of LPL mRNA (Fig. 6). When the data from four experiments were normalized to the message for gamma-actin, the LPL/gamma-actin ratio in cells treated with 50 U/ml gamma-INF was 42% of that in control cells. Similar experiments were carried out to examine the effects of IL-2 on LPL expression when added to cells after 6 days of culture. As shown in **Fig. 7**, IL-2 (50 U/ml) also decreased the expression of LPL in relation to gamma-actin. The mean LPL/gamma-actin ratio in IL-2-treated cells was 53% of control cells.

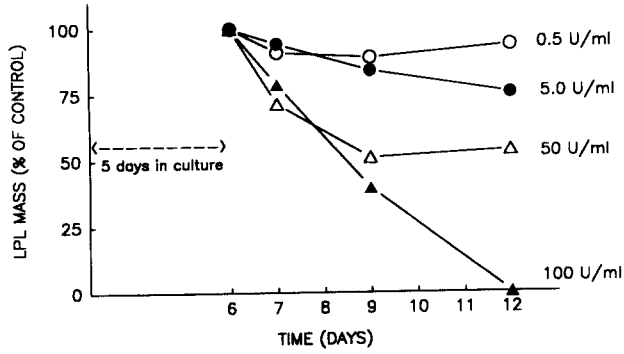

Fig. 4. Dose-dependent effect on IL-2 on LPL. Human monocyte-derived macrophages were cultured for 5 days, followed by the addition of IL-2 in concentrations ranging from 0.5 to 100 U/ml. IL-2 was added with each change of medium, and LPL immunoreactive mass was measured on days 7, 9, and 12 of culturing. Data from two experiments were pooled.

TABLE 1. Distribution of LPL between medium and cells

	LPL Activity		LPL Mass		Total Protein
	Medium[a]	Cells[b]	Medium[c]	Cells[d]	
					mg/plate
Control	2.56	0.04	43.3	6.24	2.78
IL-2	1.39	0.03	50.6	5.92	3.37
Gamma-INF	0.24	ND[e]	22.3	1.33	2.84
IL-1	0.44	0.01	24.4	1.4	2.31

After addition of the indicated cytokine, cells were cultured for 7 days and LPL activity and mass were measured in the medium and the cells. Medium was changed 24 h prior to measurements.
[a]nEq per min per ml.
[b]nEq per min per plate.
[c]ng per ml.
[d]ng per plate.
[e]Not detected.

DISCUSSION

Macrophages are scavenger cells that serve an essential phagocytic function. In keeping with this role as a scavenger, arterial wall macrophages accumulate lipid and become transformed into foam cells. These lipid-filled cells are the earliest findings in the formation of fatty streaks, and thus are important in the development of atherosclerosis (6). Among the many factors that influence macrophage lipid accumulation is lipoprotein lipase, which accelerates the uptake of lipid from triglyceride-rich lipoproteins (3, 4). Because monocyte/macrophages spontaneously secrete LPL in vitro, cell culture of peripheral blood monocytes has been a useful system to study.

Previous studies have suggested that there are a number of differences between the regulation of LPL in macrophages and other LPL-producing cells. Macrophages are not subject to regulation by insulin or insulin-like growth factor-I (28, 29) as are adipocytes (30–32). The effects of recombinant human TNF on LPL have varied depending on the study and the cell system used. 3T3-L1 adipocytes are very sensitive to this cytokine (10, 11), while human adipocyte LPL is unaffected by TNF (15), although LPL in human adipose tissue pieces is inhibited by TNF after long-term treatment of the tissue with insulin and dexamethasone (33). Human macro-

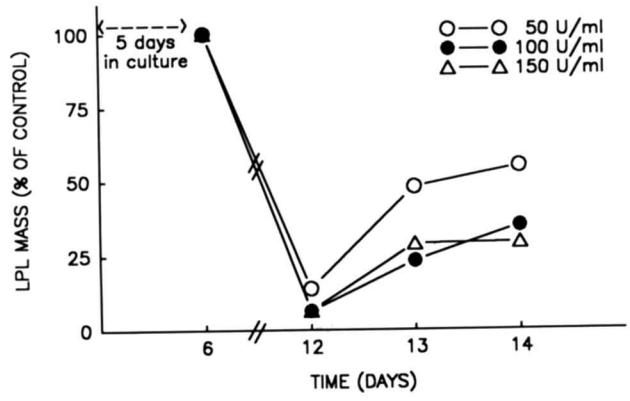

Fig. 5. Reversibility of LPL-suppression by gamma-INF. Human monocyte-derived macrophages were cultured in standard medium for 5 days as described previously, and were then cultured in the same medium containing gamma-INF (50 to 150 U/ml) between days 6 and 12. Beginning on day 12, cells were again cultured in standard medium that did not contain gamma-INF, and LPL mass was measured on days 12, 13, and 14. Data from two experiments were pooled.

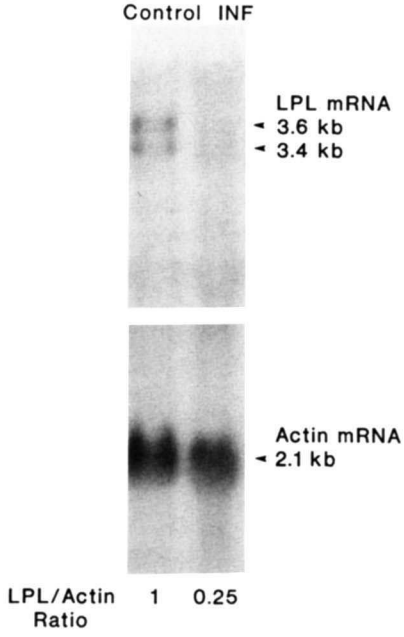

Fig. 6. Effect of gamma-INF on LPL mRNA. Cells were cultured for 6 days in standard medium and then cultured in the presence and absence of 50 U/ml gamma-INF for 3 days. RNA was extracted as described in Methods, and 20 μg of total RNA was added to each lane of the gel. Northern blotting was performed as described in Methods, using the cDNAs to human LPL and gamma-actin.

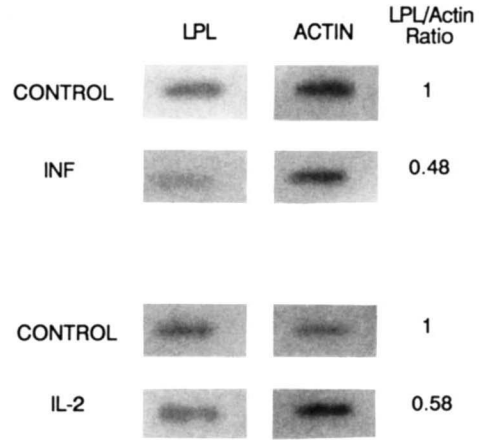

Fig. 7. Effect of gamma-INF and IL-2 on LPL mRNA. Cells were cultured for 6 days in standard medium, followed by 3 days in medium containing either 50 U/ml gamma-INF, 50 U/ml IL-2, or neither cytokine (control). Total RNA (10 μg) was applied to nylon membrane in a slot-blot manifold, and hydridized with the cDNAs for LPL and gamma-actin as described in Methods.

phage LPL does not respond to TNF (16), whereas different studies have demonstrated either no effect (34) or inhibition of LPL production (17) by TNF in thioglycolate-elicited mouse peritoneal macrophages. Previous studies have demonstrated inhibition of 3T3-L1 adipocyte LPL activity by gamma-INF (11) and IL-1 (13). This effect of IL-1 on 3T3-L1 adipocyte LPL activity was not accompanied by a decrease in LPL mRNA levels (35). In the J774.1 macrophage cell line, LPL was not inhibited by IL-1 (36), and one report suggested that IL-2, alpha-INF, and beta-INF had no effect on LPL production by mouse peritoneal macrophages (34). Because a number of cytokines are activators of macrophage functions (37), the present study was undertaken to study the influence of gamma-INF, IL-1, and IL-2 on human macrophage LPL.

Gamma-INF inhibited the production of LPL by human macrophages, whether added to early cultures that were undergoing transformation to macrophages, or to mature cultures that were secreting large amounts of LPL. This effect was dose-dependent, and partially reversible with removal of the cytokine. With both gamma-INF and IL-2, LPL mRNA was reduced along with medium LPL, suggesting that the inhibitory effects occur at the level of transcription or mRNA stabilization. Inhibition of LPL was not due to overall cytotoxicity or inhibition of protein synthesis, as assessed by trypan blue exclusion and [^{35}S]methionine incorporation into protein. On the other hand, the effects of IL-1 and IL-2 depended on when they were added to the maturing cultures of monocyte/macrophages. When added to early cultures, IL-1 inhibited LPL production, whereas IL-2 had no effect. After cells had matured, IL-1 had no effect, whereas IL-2 inhibited LPL in a dose-dependent manner. These effects of IL-1 and IL-2 may be due to differences in cell differentiation. Although IL-1 may not inhibit LPL production in mature cultures, this cytokine may inhibit a critical stage in differentiation that is associated with LPL expression. Conversely, IL-2 may inhibit LPL through a mechanism that requires full differentiation of the macrophages.

Previous studies have identified a number of changes in lipid metabolism as monocytes mature into macrophages. The production of LPL is initially low, and then reaches a maximum at 7 to 14 days after initiation of culture (2). The THP-1 cell, which is a human macrophage cell line, expresses LPL and apoE mRNA when induced to differentiate with phorbol ester (38). In primary cultures of mouse bone marrow macrophages and peritoneal macrophages, LPL secretion increases and decreases in parallel with induction or suppression of differentiation (39). In addition, scavenger receptors are initially present in low numbers on monocyte/macrophages, and then increase in number with differentiation, reaching a maximum at about 7 days (40). Previous studies have generally examined LPL regulation in mature cultures of macrophages, and have not compared effects at different stages of differentiation.

Gamma-INF is secreted as a macrophage-activating lymphokine by sensitized T-lymphocytes. IL-2 was originally described as T-cell growth factor (41). However, normal human monocytes can express receptors for IL-2 on their surface (42), and a direct augmenting effect of recombinant human IL-2 on monocyte/macrophage function has recently been demonstrated in human monocyte cell cultures (43). IL-1 is secreted primarily by macrophages and is an activator of resting T-lymphocytes (9). However, macrophages have receptors for IL-1 and an autocrine effect has been postulated (44).

The inhibition of LPL by these cytokines may be associated with the signal to activate this monocytic cell. Activation of macrophages in vivo by injection of *Corynebacterium parvum* into mice resulted in a >90% inhibition of macrophage LPL activity (34). In addition, conditioned medium from these *C. parvum*-elicited macrophages had a dose-dependent suppressive effect on LPL production by inflammatory macrophages due to a thermolabile factor that is distinct from TNF (34). Furthermore, activation of human macrophages in vitro by bacterial lipopolysaccharide (endotoxin) resulted in a dose-dependent decrease of LPL activity and mRNA levels in macrophages by mediators other than TNF or IL-1 (16, 36). It is possible that some cytokines do not directly inhibit macrophage LPL, but induce the production of some other LPL-inhibitory substance. Alternatively, cytokines such as gamma-INF or IL-2 may be the mediators of lipopolysaccharide-mediated inhibition of LPL production by macrophages. In addition, these results suggest that macrophages constitutively secrete LPL, whereas the lymphokine-driven conversion to the "activated" macrophage results in a decrease in LPL production.

It is not clear what role macrophage LPL plays in human atherosclerosis (5). On the one hand, LPL-mediated triglyceride hydrolysis is consistent with the role of the macrophage as a scavenger, and LPL may assist the macrophage with clearing lipid from the arterial wall. Alternatively, LPL secreted from macrophages may convert very low density lipoproteins and chylomicrons into remnant lipoproteins, which are more atherogenic and are associated with macrophage lipid accumulation. The latter hypothesis is consistent with studies which demonstrate increased macrophage lipid uptake in association with macrophage LPL activity (3, 4). In addition, a recent study showed that lipoproteins that had been treated in vitro with purified LPL were toxic to macrophages and caused lipid accumulation (45).

Several studies suggested that both LPL and cytokines may be important in receptor-mediated cholesterol uptake. When cultured human macrophages were treated with the medium from concanavalin-A-stimulated lymphocytes, they accumulated substantially less cholesteryl ester due to a decrease in receptor-mediated influx (46). In addition, another study demonstrated that lipase-modified LDL was more readily taken up by the LDL receptor and degraded by human monocyte/macrophages, leading to increased cholesterol accumulation (47). Therefore, one may speculate that the inhibition of LPL by cytokines may serve to decrease macrophage lipid accumulation through several mechanisms, including a decrease in LPL-mediated LDL modification. If LPL contributes to foam cell formation in vivo, then regulation of this process by cytokines may be of considerable importance.

We wish to thank the personnel of the Los Angeles Red Cross for helping to supply us with blood for macrophage primary cultures. We also thank Dr. Alan Chait for helpful advice with the cell culture, and Dr. John Goers for supplying the anti-LPL antibodies used in the ELISA. The cDNA for LPL was supplied by Drs. Todd Kirchgessner and Michael Schotz. Grant support: American Heart Association, Greater Los Angeles Affiliate Fellowship Grant (UQ), American Diabetes Association/California Affiliate Fellowship Grant (JMO), NIH Grants DK-32568 (SCJ) and DK-39176 (PAK), The Rebecca Sakai Memorial Fund (SCJ), and Career Development Awards from the NIH (DK-01183, SCJ) and the Juvenile Diabetes Foundation (PAK).

Manuscript received 18 August 1989 and in revised form 4 April 1990.

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