Bacterial lipopolysaccharide reduces macrophage lipoprotein lipase levels: an effect that is independent of tumor necrosis factor

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Abstract Human monocyte-derived macrophages secrete lipoprotein lipase (LPL) in culture. The regulation of human macrophage LPL production is poorly understood. Since bacterial lipopolysaccharide (LPS) alters production of several macrophage secretory products, its effect on human monocyte-derived macrophage LPL was tested. LPS treatment produced a dramatic dose-dependent decrease in LPL activity in macrophage-conditioned media. At 100 ng/ml LPS, medium LPL activity dropped by 60%. The effect of LPS on macrophage LPL activity was rapid, was blocked by polymixin B, and was not due to cytotoxicity. LPS lowers (by about 60%) the steady state level of LPL mRNA, suggesting that its effect is exerted at the level of mRNA metabolism. Since LPS stimulates macrophage production of cachectin/tumor necrosis factor (TNF), a potent inhibitor of LPL production by the 3T3-L1 adipocyte-like cell line, it was determined whether TNF reduces macrophage LPL levels. Treatment of human macrophages with up to 1000 U/ml of recombinant human TNF had no effect on macrophage LPL activity. When TNF was added in combination with LPS, no additional effect on LPL activity was observed over that seen with LPS alone. Furthermore, the LPS effect was not blocked by a monoclonal anti-TNF antibody. Thus, bacterial LPS potently decreases macrophage LPL activity and mass independent of an autocrine effect of TNF.—**White, J. R., A. Chait, S. J. Klebanoff, S. Deeb, and J. D. Brunzell.** Bacterial lipopolysaccharide reduces macrophage lipoprotein lipase levels: an effect that is independent of tumor necrosis factor. *J. Lipid Res.* 1988. **29:** 1379–1385.

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Macrophages are thought to play a critical role in the development of atherosclerosis. Early in atherogenesis, circulating blood monocytes adhere to vascular endothelium; adherent monocytes then migrate to the subendothelial regions of the artery wall, where they mature into macrophages (1). Arterial wall macrophages and macrophage-derived foam cells are major cellular components of the atherosclerotic lesion. The transformation of macrophages to foam cells is not well understood, but massive intracellular accumulation of lipid occurs.

Macrophages secrete a variety of biologically active products including coagulation factors, monokines, proteases, and growth factors (2). Macrophages have been shown to secrete lipoprotein lipase (LPL), an enzyme that hydrolyzes the triglyceride (TG) core of TG-rich lipoproteins, producing fatty acids and monoacylglycerols (3–5). Secretion of LPL by macrophages in culture leads to increased lipid accumulation by macrophages (6, 7). Local secretion of LPL by arterial wall macrophages may play a role in macrophage lipid accumulation and foam cell formation.

A primary source of LPL in vivo is the adipocyte. LPL production in adipocytes and adipocyte-like cell lines is regulated by a variety of hormones and pharmacologic agents (8). Some animal species injected with bacterial lipopolysaccharide (LPS) develop massive hypertriglyceridemia (9). This rise in serum TG levels in LPS-treated animals is associated with diminished adipocyte LPL activity (10, 11). Kawakami et al. (10) demonstrated that macrophages from LPS-treated animals secrete a protein that dramatically reduces LPL activity in the 3T3-L1 adipocyte-like cell line. This macrophage secretory product, called cachectin, appears to be the same as tumor necrosis factor (TNF) (12), a previously described monokine. TNF has been purified and recombinant forms have recently become available (13).

In addition to stimulation of TNF production, activation of macrophages by LPS increases production of monocyte-derived growth factor (14), prostaglandin E_2 (15), and several other monokines (16, 17). LPS exposure also strongly inhibits secretion of apolipoprotein E (18) and lysosomal acid hydrolases (19) by macrophages and decreases macrophage scavenger receptor activity (20).

Abbreviations: LPL, lipoprotein lipase; LPS, lipopolysaccharide; TNF, tumor necrosis factor; TG, triglyceride; apo, apolipoprotein.

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These wide-range effects of LPS suggested that macrophage production of LPL might also be regulated by LPS treatment. It was hypothesized that LPS treatment of macrophages would stimulate secretion of TNF (cachectin), and that this monokine would suppress macrophage LPL production by an autocrine effect. It is reported here that bacterial LPS indeed suppresses the appearance of LPL activity in human macrophage-conditioned medium. However, in striking contrast to adipocytes, this effect on macrophages does not appear to be mediated by TNF.

MATERIALS AND METHODS

Materials

Recombinant human TNF was provided by Genentech Inc., South San Francisco, CA. The preparation, assayed (by the supplier) for its cytolytic activity on actinomycin D-treated L929 mouse fibroblast cells, contained 3.6×10^7 U/mg. It was stored at 4°C and diluted in 0.9% sodium chloride containing 0.5% bovine serum albumin to 2×10^4 U/ml immediately prior to use (100 U/ml $\sim 10^{-10}$ M). A murine monoclonal anti-human TNF (TNF-D) antibody provided by Genentech Inc. had a neutralization titer of 2700 U/mg of antibody. Biologic potency of recombinant human TNF and the anti-human TNF antibody was confirmed using the 3T3-L1 cell line (21). Addition of 1000 U/ml of recombinant human TNF to 3T3-L1 cells reduced LPL activity in 3T3-L1-conditioned media from 7.6 ± 0.3 to 0.7 ± 0.4 nmol fatty acid released/ml per min (mean ± SD). Furthermore, addition of anti-TNF antibody (2 μg/ml) blocked the effect of TNF on 3T3-L1 cells, restoring LPL activity in conditioned media to 6.9 ± 0.8 nmol fatty acid released/ml per min (mean ± SD). Lipopolysaccharide from *Escherichia coli* serotype 055:B5 (Sigma, St. Louis, MO) was dissolved in pyrogen-free water (Travenol, Chicago, IL) and stored in aliquots at 4°C. Endotoxin-free RPMI 1640 was obtained from Whittaker MA Bioproducts (Walkersville, MD). Ficoll-Hypaque was purchased from Pharmacia (Piscataway, NJ). Tissue culture supplies, including positively charged polystyrene dishes (Primaria), were purchased from Falcon (Oxnard, CA).

Macrophage preparation

Heparinized blood obtained from fasting, healthy volunteers was layered onto Ficoll-Hypaque and centrifuged at 500 *g* for 30 min at room temperature. Mononuclear cell bands were collected and washed twice with RPMI 1640. Mononuclear cells were plated in serum-free RPMI 1640 at a density of 10^6 monocytes per positively charged 35-mm dish. Four hours after plating, nonadherent cells were removed by washing three times with serum-free RPMI 1640. Adherent cells were then fed with 1 ml per dish of RPMI 1640 supplemented with 20% autologous serum. Cells were incubated at 37°C with 95% air/5% CO_2 and fed every 3 days. Mononuclear cells prepared in this manner morphologically were >95% macrophages at day 6. Experiments were performed 6 to 26 days after plating.

Lipoprotein lipase assay

Lipoprotein lipase activity was measured in macrophage-conditioned medium or in detergent extracts of macrophages. Unless otherwise noted, the conditioned media represent 24-hr collections of macrophage secretory products. Conditioned media were assayed for LPL activity at the end of the collection period. Alternatively, conditioned media were quickly frozen in a dry ice-ethanol bath and stored at −70°C until the day of assay. Macrophage intracellular LPL activity was measured in some experiments. In these experiments, conditioned media were removed, dishes were washed twice with Krebs-Ringer phosphate buffer, and the cell layers were extracted with 0.5 ml per dish of a detergent solution as previously described (22, 23). Following centrifugation of cell extracts, an aliquot of each aqueous phase was assayed for LPL activity.

LPL activity in conditioned media and cell extracts was measured using radiolabeled triolein emulsified with phosphatidylcholine as substrate (22, 23). Lipolytic activity was expressed as nmol of fatty acid released per min by 1 ml of conditioned medium or cell homogenate.

Preparation and analysis of RNA

Total cellular RNA from control and LPS-treated monocyte-derived macrophages (approx. 1×10^7 cells of each) was prepared by guanidinium thiocyanate–CsCl step-gradient centrifugation as previously described (24). The yield was 151 μg and 125 μg from control and LPS-treated cultures, respectively.

Quantitation of LPL-specific mRNA was carried out by dot-blot hybridization according to published procedures (25–27). A radiolabeled (nick translation) human LPL cDNA probe was used to detect specific LPL mRNA. This probe, isolated in our laboratory from a human adipose tissue cDNA library (27), had a length of 1.36 kb corresponding to nucleotides 271–1630 of the sequence reported by Wion et al. (28). As a control, apolipoprotein E mRNA was quantitated using a full length cDNA probe (27), isolated in our laboratory from a human liver cDNA library. The extent of hybridization was determined by densitometric scanning of autoradiographs of hybridization filters [stringency of washing: 0.5 × SSC – 0.1% SDS, 68°C (SSC = 0.15 M NaCl; 0.015 M Na citrate, pH 7.0)] as described previously (27).

RESULTS

LPS produced a dose-dependent, but incomplete, suppression of LPL activity detectable in human macrophage-conditioned medium, which leveled at higher concentrations of LPS (**Fig. 1**). The decrease in the amount of LPL activity in the medium of macrophages by LPS was highly reproducible and was observed in macrophages that had been in culture ranging from 6 to 24 days, during which time macrophage LPL activity is essentially stable (4). LPL activity in macrophage-conditioned media was 72 ± 5% of control at 1 ng/ml LPS (mean ± SEM for macrophages from nine different individuals; $P < 0.001$). LPL activity wsa 40 ± 7% of control at 10 ng/ml LPS (mean ± SEM for macrophages from eight different subjects; $P < 0.001$) and 40 ± 9% of control at 100 ng/ml LPS (mean ± SEM for macrophages from five different subjects; $P < 0.005$). LPS did not alter enzyme activity when added directly to macrophage-conditioned medium, suggesting that direct enzyme inhibition was not involved (data not shown). Intracellular LPL activity was approximately 10% of extracellular LPL activity and decreased slightly with exposure to LPS. Intracellular LPL activity fell from 12.2 ± 3.0 to 9.6 ± 1.6 nmol fatty acid/ml per min with 100 ng/ml of LPS (mean ± SD). LPL activity

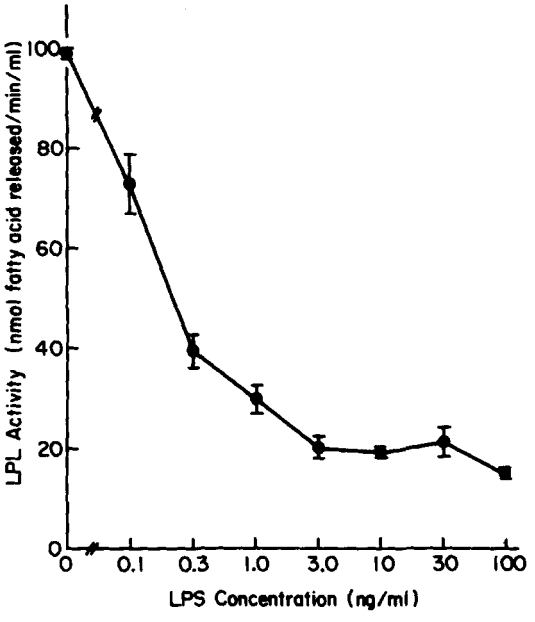

Fig. 1. Effect of LPS on LPL activity in macrophage-conditioned medium. Human monocyte-derived macrophages were placed in RPMI 1640 medium containing 20% autologous serum containing the indicated concentrations of LPS on day 12 of culture. After a 24-hr incubation, media were removed and assayed for LPL activity. Data shown are from a single experiment, which is representative of three experiments (using macrophages obtained from three different subjects). Results represent means ± SD of triplicate dishes.

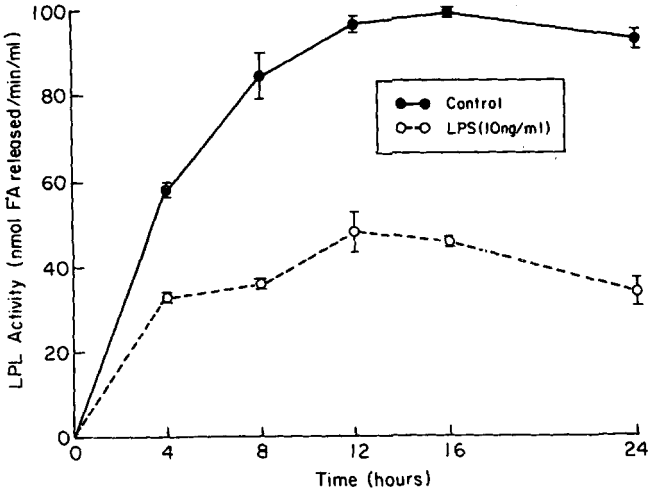

Fig. 2. Time course of LPS effect on macrophage LPL. Media (RPMI 1640/20% autologous serum) were removed from macrophages and assayed for LPL activity on day 12 of culture. Media had been changed 4, 8, 12, 16, or 24 hr before assay. Control dishes (●---●) received media without added LPS, and LPS-treated dishes (○---○) were exposed to media containing LPS (10 ng/ml) for the indicated lengths of time. LPL activity is expressed as nmol of fatty acid released per min by 1 ml of macrophage-conditioned media. Data are shown from a single experiment, which is representative of two experiments. Results represent means ± SD of triplicate dishes.

in macrophage-conditioned medium was markedly decreased as early as 4 hr after exposure to LPS. The inhibition of activity was maximal by 8 hr exposure to LPS, with no further change observed by 24 hr (**Fig. 2**).

Polymixin B is a cationic antibiotic that disrupts bacterial lipopolysaccharide and blocks its biologic effects (29). Polymixin B prevented LPS-mediated suppression of macrophage LPL activity, while polymixin B alone had no effect on LPL activity in macrophage-conditioned media (**Fig. 3**). These results strongly suggest that the decrease in LPL activity was due to LPS or a factor produced in response to LPS treatment.

The decreases in macrophage LPL activity might be explained by cytotoxicity. To address this issue, the ability of LPS-treated macrophages to exclude the dyes erythrosin B and trypan blue was examined. Macrophages pretreated with 1 ng/ml and 10 ng/ml LPS for 24 hr excluded these dyes to the same extent as macrophages not treated with LPS (>95% viable control and LPS-treated cells). In addition, the release of lactate dehydrogenase by LPS-treated macrophages was not greater than that released from non-LPS-treated control cells (85 ± 2.6; 102 ± 24 mU/ml, respectively; mean ± SD, n = 3).

Since LPS stimulates macrophage production of TNF, a potent inhibitor of LPL production by the 3T3-L1 adipocyte-like cell line, it was next determined whether TNF might similarly decrease macrophage LPL levels. Recombinant human TNF in concentrations ranging from 10 to 1000 U/ml showed no effect on LPL activity in

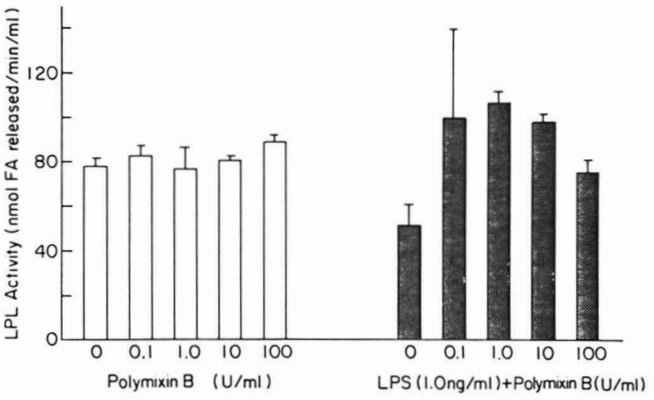

Fig. 3. Polymixin B inhibits the LPS effect. Macrophages were switched to RPMI 1640/20% autologous serum containing the indicated concentrations of polymixin B ± LPS (1.0 ng/ml) on day 11 of culture. Conditioned media were removed 24 hr later and assayed for LPL activity. Data are shown from a single experiment, which is representative of five experiments. Results represent means ± SD of triplicate dishes.

macrophage-conditioned media. In contrast, LPL activity in macrophage-conditioned media decreased by over 80% by LPS treatment in the same experiment (**Fig. 4**). Under a wide variety of conditions, with macrophage exposure to TNF ranging from as short as 6 hr to as long as 7 days, no change in LPL activity was observed. At 10 U/ml TNF, LPL activity in macrophage-conditioned media was 103 ± 5% of control (mean ± SEM for four different subjects; not significant). At 100 U/ml TNF, LPL activity was 109 ± 5% of control (mean ± SEM for four different subjects; not significant), and at 1000 U/ml TNF, LPL activity was 110 ± 9% of control (mean ± SEM for four different subjects; not significant). When various concentrations of TNF were added in combination with a low concentration of LPS (1 ng/ml), no additional suppression of LPL activity was observed over that seen with LPS alone (data not shown).

Additional evidence that the inhibitory effect of LPS on macrophage LPL was not due to production of TNF was obtained using a monoclonal anti-TNF antibody that blocks TNF-mediated effects in other systems (30). This antibody failed to block the LPS inhibitory effect on LPL activity in the medium of macrophages, again suggesting that TNF does not mediate the LPS effect (**Fig. 5**).

To determine whether LPS exerted its effect at the level of mRNA metabolism, the steady state level of LPL mRNA was quantitated by the dot-blot hybridization method. Five and 10 μg of total cellular RNA from control and LPS-treated (100 ng/ml) macrophages were applied to Hybond-N (Amersham) and hybridized to a ^{32}P-labeled human LPL cDNA probe. RNA from the unstimulated THP-1 macrophage-like cell line, which is known not to secrete LPL prior to stimulation with phorbol esters (31), was included as a negative control. Apolipoprotein E mRNA was also measured in the macrophage RNA samples by hydridization to an apoE cDNA probe. Autoradiography of the hybridized RNA preparations demonstrated a decrease in the steady state level of LPL mRNA after treatment with LPS (**Fig. 6**). Densitometric scanning of the autoradiograph demonstrated that the extent of decrease in LPL mRNA (approximately 60%) was similar to that observed for LPL activity in the incubation medium following LPS treatment (52%) (**Table 1**). On the other hand, LPS had no effect on the steady state level of apolipoprotein E mRNA (Fig. 6). Thus, inhibition of the activity of LPL in the medium of human macrophages, and intracellularly by LPS, appears to occur at the level of mRNA.

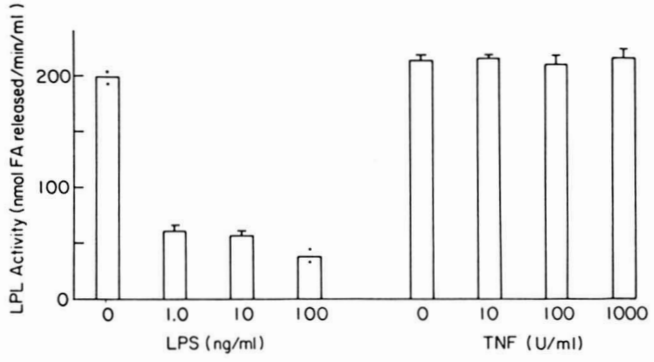

Fig. 4. Lack of TNF effect on secretion of LPL activity. Macrophages were exposed to media (RPMI 1640/20% autologous serum) containing LPS or recombinant human TNF at the indicated concentrations from day 8 to day 11 of cultures. Media were changed every 24 hr during this period. Conditioned media from the final 24-hr period were assayed for LPL activity. Data shown are from a single experiment, which is representative of three experiments (using macrophages obtained from three different subjects). Results represent means ± SD of triplicate dishes (except where indicated).

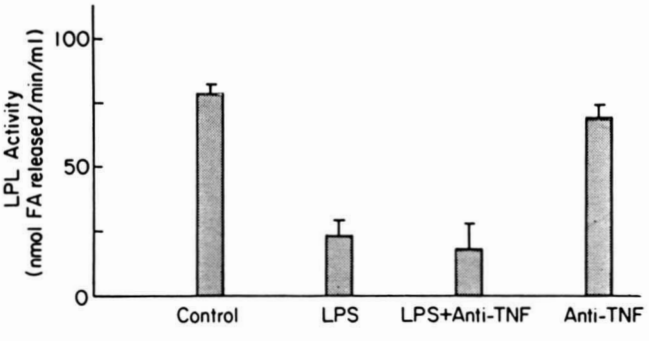

Fig. 5. Anti-TNF antibody does not block the LPS effect. Macrophages were exposed to either LPS (100 ng/ml), monoclonal anti-TNF antibody (2 μg/ml), or both from day 7 to day 8 of culture. Control dishes did not contain added LPS or anti-TNF antibody. Data shown are from a single experiment, which is representative of three experiments. Results represent means of triplicate dishes ± SD.

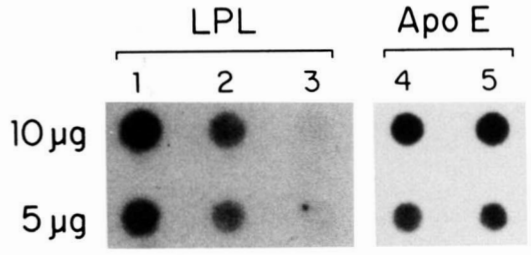

Fig. 6. Inhibition of LPL specific mRNA in LPS-treated human macrophages. Five and 10 µg of total cellular RNA from control, 1, or LPS (100 ng/ml)-stimulated, 2, human macrophages that had been in culture for 7 days, or from the THP-1 macrophage-like cell line that had not been stimulated by phorbol esters, 3, were hybridized with a cDNA probe for LPL. RNAs from control, 4, and LPS-treated macrophages, 5, were also hybridized to an apolipoprotein E cDNA. Autoradiographs of the dot-blots were performed as described in Methods.

DISCUSSION

These studies demonstrate that synthesis and secretion of LPL by human macrophages are dramatically reduced by addition of low concentrations of bacterial LPS. LPL activity in macrophage-conditioned media was significantly reduced by addition of low concentrations of LPS. Addition of LPS directly to cell-free macrophage-conditioned media did not alter LPL activity, indicating that LPS did not affect the assay of LPL activity. The effect of LPS on macrophages was not secondary to cytotoxicity as assessed by dye exclusion and lactate dehydrogenase release.

The decrease in LPL activity was blocked by polymixin B, confirming that the changes were due to LPS or a factor stimulated in response to LPS treatment. The decrease in extracellular activity did not result from intracellular accumulation of enzyme since LPL-specific mRNA levels and LPL activity in the medium were reduced to approximately the same extent by LPS. It is therefore concluded that LPS acts at the level of mRNA metabolism by either decreasing the level of mRNA transcription or mRNA stability.

LPS-treated rodent and human macrophages secrete TNF/cachectin, a protein that potently decreases LPL activity in guinea pig adipocytes (32) and in the 3T3-L1 adipocyte-like cell line (10, 11). This study shows that, in striking contrast to adipocytes, macrophage LPL activity is not altered by TNF. Macrophages exposed to TNF for as long as 7 days at concentrations up to 1000 U/ml did not secrete less LPL activity than non-TNF-treated controls. Addition of TNF plus LPS to macrophages did not drop LPL activity further than the decreases seen with LPS alone. Furthermore, a monoclonal anti-TNF antibody did not prevent LPS suppression of macrophage LPL activity. Thus, it is unlikely that LPS-treated macrophages produced less LPL activity secondary to autocrine TNF effects. The findings in the present study are consistent with a recent report that demonstrated that activated macrophages obtained from the peritoneal cavities of *Corynebacterium parvum*-injected mice secreted much less LPL than did unstimulated or inflammatory macrophages, due to a thermolabile inhibitory factor that was distinct from TNF (33). In addition, a recent report has shown that neither TNF nor interleukin-1 could account for LPS-mediated inhibition of LPL secretion by the J774.1 murine macrophage-like cell line (34).

Other macrophage secretory products can also be modulated by the activation state of the macrophage. In rodent macrophages activated with LPS in vivo, apoE secretion, but not mRNA levels appear to fall at the concentrations of LPS used in the present study (35). However, in the present study, apoE mRNA in human macrophages did not change while LPL mRNA levels fell following treatment with LPS, suggesting that LPS activation of human cells did not result in a generalized reduction of mRNA levels and that the synthesis and secretion of LPL and apoE are not coordinated during macrophage activation by LPS. Further, other macro-

TABLE 1. Effect of LPS on LPL and apoE mRNA levels

Treatment	LPL mRNA		ApoE mRNA	
	Relative Intensity[a]	% of Control	Relative Intensity[a]	% of Control
Macrophage control				
5 µg[b]	258	100	550	100
10 µg	377	100	880	100
Macrophage and LPS				
5 µg	110	43	570	104
10 µg	151	40	850	97
THP-1				
5 µg	26	10		
10 µg	32	8		

[a]Arbitrary units obtained by densitometric scanning of autoradiographs of dot-blot hybridizations.
[b]Micrograms of total cellular RNA hybridized to ^{32}P-labeled cDNA probes.

phage products, such as mitogens, increase during activation by LPS. Therefore, macrophage activation appears to result in variable responses on different macrophage secretory products that may be important in atherogenesis, and suggests that the state of activation of these cells is likely to be important in modulating inflammatory responses to injurious agents that occur during the atherogenic process.

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