

Lipopolysaccharide Regulation of Lipoprotein Lipase Expression in Murine Macrophages†

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The enzyme lipoprotein lipase is expressed in a number of cell types and plays a central role in lipid metabolism. Multiple factors regulate its expression in a tissue-specific manner. In murine macrophages, lipopolysaccharide inhibits lipoprotein lipase enzyme activity. The current work examines this process in the established J774 macrophage line and primary peritoneal macrophages from endotoxin-sensitive (C3HeB/Fej) and endotoxin-resistant (C3H/Hej) murine strains. Lipopolysaccharide inhibition of macrophage lipoprotein lipase occurred at the enzyme and mRNA levels in a time- and concentration-dependent manner. Cells from endotoxin-resistant animals maintained their expression of lipoprotein lipase following treatment with lipopolysaccharide. Results of gel retention assays showed that lipopolysaccharide treatment of the J774 macrophages altered the level of nuclear proteins recognizing and binding the lipoprotein lipase promoter DNA. Nuclear extracts from resting J774 cells contained proteins which bound specifically to the octamer motif and to the CAAT box within the lipoprotein lipase promoter. Exposure of the J774 cells to lipopolysaccharide for 16 h increased the level of protein-octamer DNA complexes. Similar responses were obtained in endotoxin-sensitive, but not endotoxin-resistant, primary macrophages following in vitro treatment with lipopolysaccharide. This finding suggests that transcriptional events may contribute to the lipopolysaccharide regulation of macrophage lipoprotein lipase expression.

During gram-negative bacteremia, lipopolysaccharide initiates a cascade of events within the macrophage, ultimately leading to septic shock in the host animal. Of particular importance is the induction of transcription for the cytokine genes encoding tumor necrosis factor (TNF), interleukin 1, and interleukin 6 (1, 5, 27). In contrast, resting macrophages constitutively produce lipoprotein lipase (EC 3.1.1.34), the enzyme responsible for the hydrolysis of triglycerides into free fatty acids (2–4, 7, 14, 20, 21, 29, 42). Following exposure to lipopolysaccharide, the macrophage synthesis of lipoprotein lipase is reduced; this inhibition appears to be independent of autocrine feedback loops involving macrophage-derived cytokines (4, 12a, 34, 38, 44). Thus, the lipopolysaccharide regulation of macrophage lipoprotein lipase gene expression is direct and appears to be reciprocal to that of the cytokine genes.

The murine and human lipoprotein lipase gene promoters have recently been cloned and characterized (12, 15, 16, 23, 28, 46). The immediate 5' flanking region of the gene exhibits greater than 80% conservation between the species, suggesting the presence of important *cis*-acting regulatory elements (15). Most notable are the presence of a perfect octamer motif (ATTGTCAT) at bp –46 and an imperfect copy of its associated heptamer motif (CTtATGA) at bp –80. While these sequence elements were originally identified in the promoter of the immunoglobulin μ gene, they have since been localized within multiple transcriptional regulatory domains (17). These sites are recognized by octamer-binding proteins belonging to

the POU family of transcriptional regulators (39). Octamer-binding factors have been observed to exert both positive and negative effects on gene transcription (11, 39). Proteins recognizing the lipoprotein lipase promoter octamer have been detected in nuclear extracts from adipose tissues and cell lines, but such studies have not been performed with macrophages (10, 23, 33).

The current work examines lipopolysaccharide regulation of murine lipoprotein lipase expression in an established macrophage cell line (J774) and primary peritoneal macrophages. Comparison between endotoxin-responsive (C3HeB/Fej; *Lps*^s) and endotoxin-hyporesponsive (C3H/Hej; *Lps*^r) murine strains demonstrates the necessity of a functional *Lps* locus for lipoprotein lipase gene regulation (40, 41). We present evidence suggesting that lipopolysaccharide increases the level of macrophage nuclear proteins which recognize the octamer motif within the lipoprotein lipase promoter.

MATERIALS AND METHODS

Cell culture. Reagents were obtained from Sigma Chemical Co. (St. Louis, Mo.) unless otherwise noted. All cells were maintained in Dulbecco's modified Eagle's medium (Gibco-BRL, Gaithersburg, Md.) supplemented with 10% fetal bovine serum (defined; HyClone, Logan, Utah), 1% sodium pyruvate, 100 U of penicillin per ml, and 100 μ g of streptomycin per ml and cultured at 37°C and 7% CO₂. The histiocytic lymphoma cell line J774 (ATCC TIB 67) was passaged every 3 days (35, 36). The C3HeB/Fej and C3H/Hej mice (Jackson Laboratory, Bar Harbor, Maine) were injected with 4% thioglycolate (2 ml intraperitoneally) to elicit peritoneal macrophages. After 4 days, cells were isolated, washed once in phosphate-buffered saline, suspended in medium at 3×10^6 cells per ml, and cultured for 2 h in 35-mm-diameter culture dishes (Corning Glass Works, Corning, N.Y.), and the medium was replaced. The cells were then incubated for 4 days prior to each experiment. Macrophages were treated with lipopolysaccharide (*Escherichia coli* O111:B4; courtesy R. McCallum, Texas A&M University, College Station) at concentrations of 0 to 100 ng/ml for periods up to 24 h (27). Throughout these periods, the cells maintained greater than 95% viability, as

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judged by trypan blue exclusion. Cells were harvested for total RNA, nuclear proteins, or heparin-releasable lipoprotein lipase activity as described below.

RNA analysis. Total cellular RNA was isolated by the method of Chomczynski and Sacchi (8, 19). Northern (RNA) blots were prepared with approximately 10 µg of total RNA per lane (43), transferred to MSI-NT nylon membranes (MSI, Westboro, Mass.), and UV cross-linked (9, 19). The cDNA probes were labeled by the random primer method (actin) (provided courtesy of L. Choy and B. Spiegelman, Dana Farber, Boston, Mass.) (18) or as a single-stranded riboprobe (lipoprotein lipase) (ATCC 63117) (19), using the Promega (Madison, Wis.) Riboprobe Gemini II system. Blots were hybridized overnight at 55°C, washed, and exposed with an intensifying screen for 1 to 7 days at -70°C (9, 19).

Transcriptional analysis. Nuclear run-on experiments were conducted as previously described (22a, 29a). After 0, 4, or 20 h of exposure to 10 ng of lipopolysaccharide per ml, nuclei were isolated from 5×10^7 J774 cells and the nascent RNA chains were labeled with [α -³²P]UTP (Amersham, Arlington Heights, Ill.). A total of 5×10^6 cpm of each labeled RNA was hybridized at 45°C for 72 h with dot blots prepared with 5 µg of the following linearized plasmids: pBluescript (Stratagene, San Diego, Calif.), TNF (provided courtesy of B. Beutler, University of Texas Southwestern, Dallas), actin, and lipoprotein lipase. After washing, the signal intensity of the dot blots was assessed by autoradiographic exposure to XAR5 film (Kodak, Rochester, N.Y.) for 8 days at -70°C with an intensifying screen.

Gel retention analysis. Nuclear extracts were prepared from J774 cells or primary peritoneal macrophages by the method of Dignam et al. (13). Protein concentrations were determined by using the bicinchoninic acid reagent as directed by the manufacturer (Pierce, Rockford, Ill.). All assays were conducted for 15 min at 4°C with 2 to 5 µg of extract protein in a 15-µl volume containing 2 µg of poly(dI-dC) (Pharmacia, Piscataway, N.J.) and approximately 2.5×10^4 cpm of γ -³²P-labeled probe as previously described (23). The DNA probes derived from the murine lipoprotein lipase promoter were (i) a 223-bp *HindIII*-*EcoRI* DNA fragment spanning base pairs -181 to +42 and (ii) oligonucleotide TGAGTCTTATTGGCATATTCACG, spanning base pairs -54 to -29 (the OCT-1 DNA recognition motif is underlined). Two additional oligonucleotides were used in competition studies: (i) CAACCTTATGATTTTATAGCCAATAG GTGAT, spanning base pairs -83 to -54 of the lipoprotein lipase promoter (the 6-of-7-bp heptamer sequence match [CTCATGA] and CAAT box are underlined) and (ii) ATCTCAACAGAGGGGACTTTCAGAGAGCCA, based on the murine immunoglobulin κ light-chain enhancer (the NF- κ B sequence is underlined) (27). All oligonucleotides were synthesized by Ken Jackson, Molecular Biology Core Facility, Oklahoma Center for Molecular Medicine.

Lipoprotein lipase enzyme assay. Following treatment with lipopolysaccharide, cells were incubated for 1 h in fresh medium containing heparin (10 U/ml), and the supernatants were harvested. The lipoprotein lipase enzyme activity was determined by the method of Nilsson-Ehle and Schotz (30) as previously described (19). All data are expressed as means \pm standard deviations and are representative of two or more individual experiments. Values are reported as the nanomoles of triglyceride hydrolyzed per hour per milliliter of conditioned cell supernatant.

Statistical analysis. Data were analyzed by Student's *t* test, using the Sigma-Stat software package (Jandel, San Rafael, Calif.).

RESULTS

Initial studies determined the effect of lipopolysaccharide on lipoprotein lipase expression in the murine histiocytic lymphoma J774 cell line (35, 36). Lipoprotein lipase enzyme activity was assayed in the heparin supernatants of J774 cells treated with increasing concentrations of lipopolysaccharide for 24 h (Table 1). Half-maximal inhibition of enzyme activity was observed with between 0.3 and 1 ng of lipopolysaccharide per ml; maximal inhibition was achieved at 10-fold-higher concentrations. Northern blot analysis was performed with total RNA from J774 cells treated with various concentrations of lipopolysaccharide for periods of 0 to 24 h (Fig. 1). Blots were hybridized successively with probes for lipoprotein lipase and actin. The concentration-dependent effects of lipopolysaccharide were paralleled at the mRNA level (Fig. 1A). At lipopolysaccharide concentrations greater than 0.3 ng/ml for 18 h, the lipoprotein lipase mRNA signal intensity decreased. In the same lanes, the signal for actin remained relatively unchanged (Fig. 1A). Likewise, exposure to 10 ng of lipopolysaccharide per ml resulted in a time-dependent reduction in lipoprotein lipase mRNA (Fig. 1B). After 6 to 8 h, the signal intensity of lipoprotein lipase relative to actin was less than at the earlier time points; further reductions were evident by 24 h. Nuclear run-on experiments were performed to determine if lipopoly-

TABLE 1. Effects of lipopolysaccharide on lipoprotein lipase expression in J774 cells

Lipopolysaccharide (ng/ml)	Lipoprotein lipase activity ^a
0.....	144 \pm 11
0.1.....	108 \pm 24
0.3.....	114 \pm 18
1.....	56 \pm 8*
3.....	32 \pm 4*
10.....	37 \pm 7*
30.....	32 \pm 12*
100.....	33 \pm 3*

^a Mean \pm standard deviation of quadruplicate assays from a single experiment (representative of *n* = 2); expressed as nanomoles of triglyceride hydrolyzed per hour per milliliter of conditioned cell supernatant. Asterisks indicate *P* < 0.001 (Student's *t* test).

saccharide regulated lipoprotein lipase gene expression at the transcriptional level (Fig. 1C). The relative levels of nascent mRNA were compared among J774 cells exposed to lipopolysaccharide (10 ng/ml) for periods of 0, 4, and 20 h. As a control for the assay, transcription of the TNF gene, a lipopolysaccharide-inducible gene, was examined. Previous Northern blot analyses of J774 cells had determined that maximum TNF signal intensity occurred after 4 h of lipopolysaccharide exposure (27). In support of that finding, the nuclear run-on experiment demonstrated an increased TNF signal intensity relative to baseline levels at 4 h. The level of TNF signal fell after 20 h of lipopolysaccharide exposure. In contrast, the signal intensities of the actin and lipoprotein lipase genes in resting J774 cells were no different from that observed with the negative control (pBluescript vector); no changes were observed with lipopolysaccharide treatment. This finding suggests that the transcriptional level of lipoprotein lipase in resting J774 cells falls below the sensitivity level of the nuclear run-on assay. As an alternative measure of lipoprotein lipase transcription, transient transfection experiments were attempted in the J774 cells with previously described lipoprotein lipase promoter-luciferase reporter gene constructs (23) (data not shown). Despite the use of a variety of transfection protocols, expression of the luciferase gene in the macrophage cell line remained low to undetectable, even when controlled by the high-expression Rous sarcoma virus promoter (data not shown). These negative results neither support nor refute the possibility that lipopolysaccharide regulates J774 lipoprotein lipase gene transcription.

Nevertheless, comparable results were obtained with primary peritoneal macrophages obtained from the lipopolysaccharide-sensitive C3HeB/Fej (*Lps*^s) murine strain. The *Lps* locus, responsible for the lipopolysaccharide response, is normal in these mice. Significant inhibition of lipoprotein lipase enzyme activity was observed after an 18-h exposure to lipopolysaccharide concentrations as low as 0.3 ng/ml (Table 2). Northern blot analysis indicated that this effect was time dependent. The lipoprotein lipase mRNA signal intensity was decreased after 24 h but not after 4 h of lipopolysaccharide treatment (Fig. 2). In contrast, peritoneal macrophages derived from the lipopolysaccharide-insensitive murine strain, C3H/HeJ (*Lps*^d), did not exhibit decreased lipoprotein lipase expression under identical conditions. In these mice, a mutation in the *Lps* locus accounts for their defective lipopolysaccharide response (40, 41). Lipoprotein lipase enzyme activity (Table 2) and mRNA signal intensity (Fig. 2) did not show significant reduction after 24 h of exposure to 10 ng of lipopolysaccharide per ml.

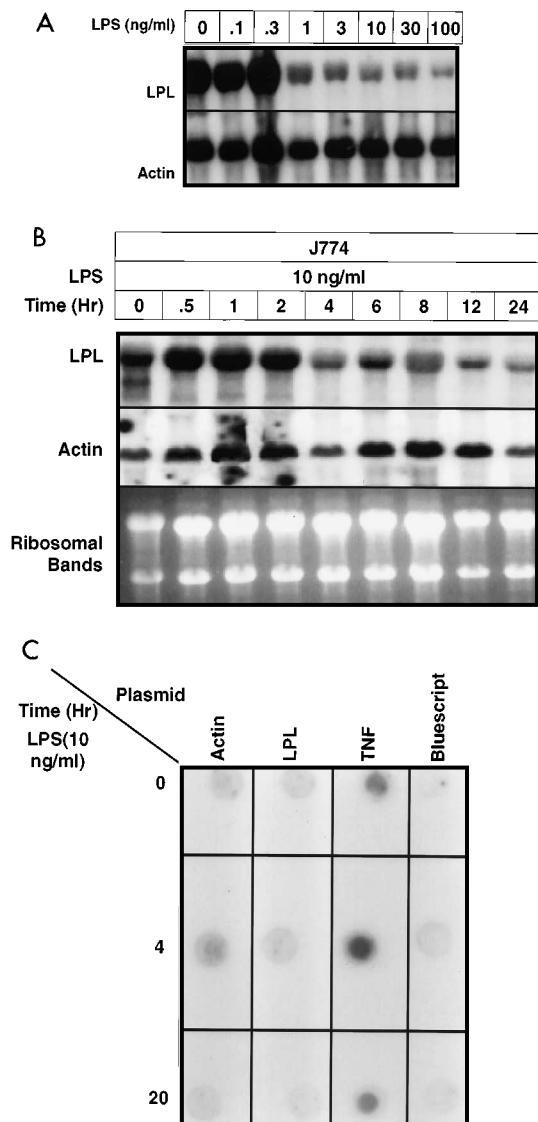


FIG. 1. Lipoprotein lipase mRNA in J774 cells. (A) Concentration dependence. Total RNA was isolated from J774 cells treated for 18 h with 0 to 100 ng of lipopolysaccharide (LPS) per ml and examined on Northern blots successively hybridized with lipoprotein lipase (LPL) and actin cDNA probes. (B) Time dependence. Total RNA was isolated from J774 cells treated with 10 ng of lipopolysaccharide for 0 to 24 h. Blots were probed as in panel A. The ribosomal bands of the Northern blot are shown. (C) Transcription. Nuclei isolated from J774 cells treated with 10 ng of lipopolysaccharide per ml for 0, 4, or 20 h were used in nuclear run-on experiments. The radiolabeled mRNA was hybridized to dot blots prepared with linearized plasmids for TNF, actin, lipoprotein lipase, and pBluescript.

To further explore the mechanisms of lipopolysaccharide inhibition of lipoprotein lipase, nuclear extracts were prepared from untreated J774 cells and examined for the presence of protein factors recognizing the lipoprotein lipase promoter. The locations of the regulatory motifs and putative DNA-binding sites within the immediate 5' flanking region are indicated in Fig. 3A. Gel retention analysis was performed with a *HindIII-EcoRI* DNA fragment spanning positions -181 to +42 of the murine lipoprotein lipase promoter (Fig. 3A). Three major gel retention complexes, designated B₁, B₂, and B₃, were observed with nuclear extracts from untreated J774 cells (no-competitor lane). Of these complexes, the most

TABLE 2. Lipoprotein lipase expression in lipopolysaccharide-exposed primary peritoneal macrophages from endotoxin-sensitive (C3HeB/Fej) and endotoxin-resistant (C3H/Hej) mice

Lipopolysaccharide (ng/ml)	Lipoprotein lipase activity ^a	
	C3HeB/Fej	C3H/Hej
0	31 ± 5	36 ± 10
0.3	13 ± 3*	29 ± 3
10	7 ± 3*	36 ± 5

^a Mean ± standard deviation of five separate datum points within a single experiment (representation of $n = 5$); expressed as nanomoles of triglyceride hydrolyzed per hour per milliliter of conditioned cell supernatant. Asterisks indicate $P < 0.001$ (Student's t test).

prominent was B₂. A 50- to 150-fold molar excess of those restriction fragments which included bp -114 to -16 of the promoter acted as competitors for all three complexes (competitors A, B, C, and D); fragments spanning bp -181 to -115 (E) or bp -15 to +42 (F) failed to compete for any of the mobility-shifted bands. The binding sites were further localized with oligonucleotide probes (Fig. 3B). An oligonucleotide (bp -83 to -54) including the imperfect heptamer motif and CAAT box specifically competed for the B₁ complex in a concentration-dependent manner. Likewise, a second oligonucleotide (bp -54 to -29; equivalent to D in Fig. 3A) containing the octamer motif specifically competed for the B₂ and B₃ complexes. In contrast, equal concentrations of an oligonucleotide based on the unrelated NF- κ B DNA-binding motif had no effect.

The effects of lipopolysaccharide treatment on the gel retention pattern were examined next. Nuclear extracts were prepared from J774 cells treated with lipopolysaccharide at concentrations of 0 to 30 ng/ml for 16 h (Fig. 4). In gel retention analysis using the *HindIII-EcoRI* promoter fragment as the probe, lipopolysaccharide exposure increased the signal intensity of the B₂ and B₃ complexes (Fig. 4A). Since competition analysis had determined that this complex resulted from protein binding to the DNA region spanning the octamer motif, gel retention analysis was conducted with the radiolabeled octamer oligonucleotide as the probe (Fig. 4B). In control J774 extracts, one major (O₁) and one minor (O₂) gel retention band were observed. Consistent with the earlier results, lipo-

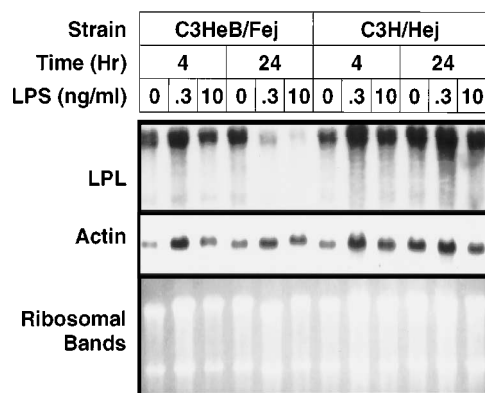


FIG. 2. Lipoprotein lipase mRNA in primary peritoneal macrophages. Total RNA was isolated from primary peritoneal macrophages treated with lipopolysaccharide (LPS) at 0, 0.3, or 10 ng/ml for 4 or 24 h. Macrophages were isolated from endotoxin-sensitive (C3HeB/Fej) and endotoxin-resistant (C3H/Hej) mice. Northern blots were probed successively with lipoprotein lipase (LPL) and actin probes; the ribosomal bands are shown.

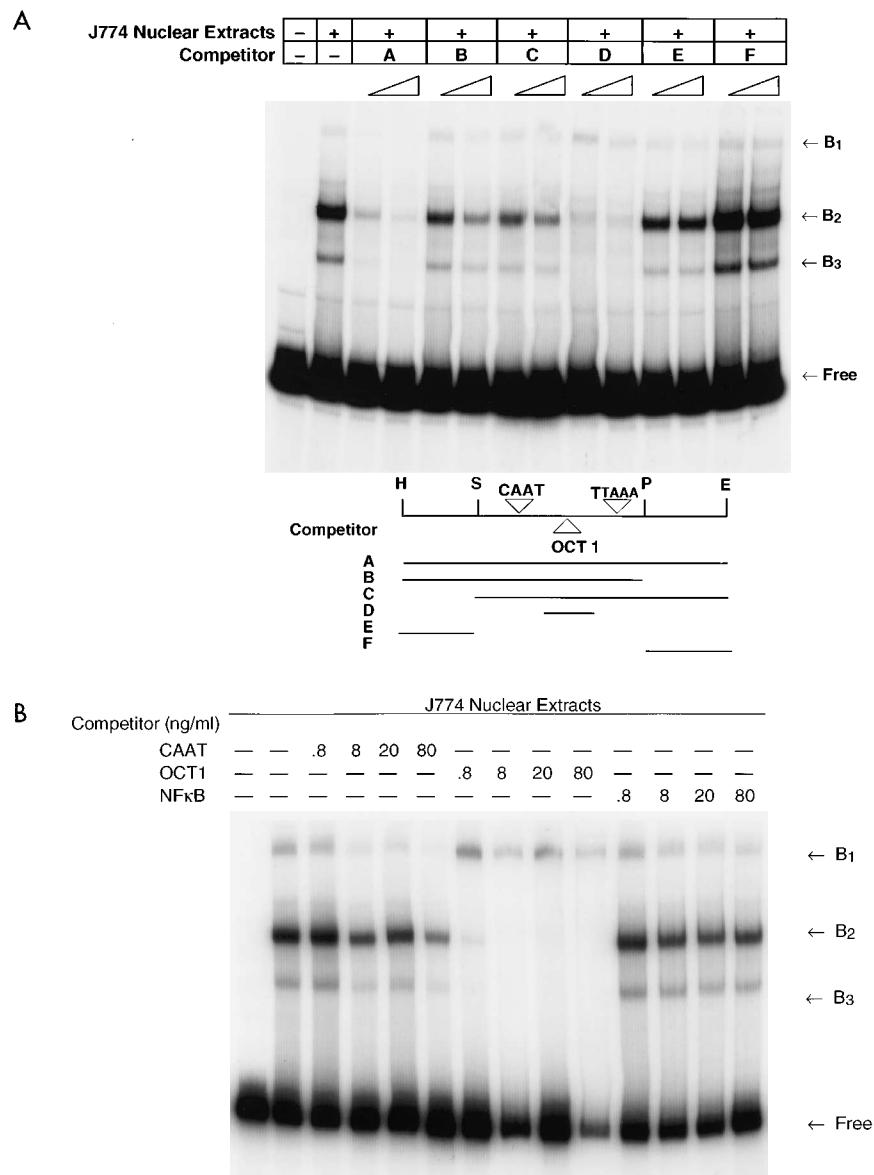


FIG. 3. Gel retention analysis of the lipoprotein lipase promoter: competition studies. Gel mobility shifts were performed with the 223-bp *Hind*III (H)-*Eco*RI (E) fragment encompassing bp -181 to +42; this is shown in panel A along with the location of CAAT box, the octamer motif, and the TATA-like element. (A) Nuclear extracts from untreated J774 cells were incubated in the presence of radiolabeled DNA in the absence and presence of the competitor fragments A through F, added at 50- or 150-fold molar excess relative to the labeled probe. Specific gel mobility shifted complexes are indicated by arrows (B₁ to B₃). (B) Competition experiments were performed with unlabeled oligonucleotides present at 0.8 to 80 ng. The oligonucleotides spanned either the CAAT-heptamer or octamer sequence from the lipoprotein lipase promoter or the unrelated NF-κB motif. All assays were conducted with 5 μg of nuclear extract protein per reaction. S, *Ssp*I; P, *Pvu*II.

polysaccharide exposure increased the signal of the O₁ and O₂ bands. These changes in nuclear protein-binding activity were time dependent and were not detected with J774 extracts harvested within 2 h of lipopolysaccharide treatment (data not shown).

Next, the lipopolysaccharide response of primary peritoneal macrophages was compared with that of J774 cells. Nuclear extracts were prepared from endotoxin-sensitive (C3HeB/Fej) and endotoxin-resistant (C3H/Hej) macrophages following 16 h of exposure to 0.3 ng of lipopolysaccharide per ml (Fig. 5). In gel retention assays using the *Hind*III-*Eco*RI promoter fragment, lipopolysaccharide increased the signal intensity of the B₂ and B₃ complexes in nuclear extracts prepared from endotoxin-sensitive macrophages (Fig. 5A). In contrast, these com-

plexes were decreased under the same conditions in nuclear extracts derived from endotoxin-resistant cells. Likewise, in gel retention assays using the OCT-1 oligonucleotide, lipopolysaccharide exposure increased the O₁ and O₂ complex signal intensity in C3HeB/Fej- but not C3H/Hej-derived nuclear extracts (Fig. 5B).

DISCUSSION

Multiple physiologic mechanisms regulate lipoprotein lipase gene expression in a tissue-specific manner (15). The major sites of lipoprotein lipase synthesis are the adipose tissue, cardiac muscles, and skeletal muscles (15). Following exposure to inflammatory cytokines, adipose tissue expression of lipopro-

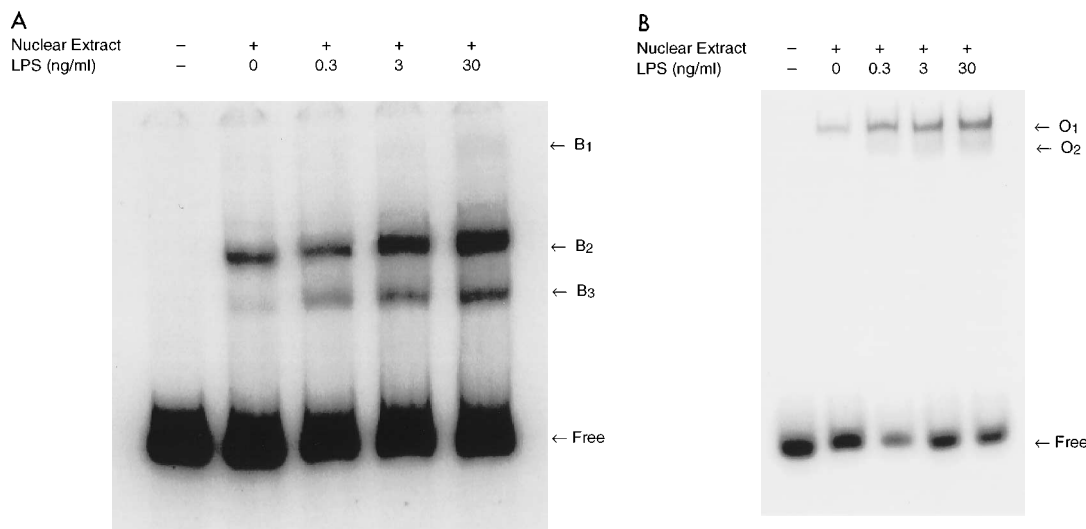


FIG. 4. Gel retention analysis of the lipoprotein lipase promoter: effect of lipopolysaccharide. (A) Gel mobility shifts were performed with the radiolabeled 223-bp *HindIII-EcoRI* fragment and nuclear extracts from J774 cells treated for 16 h with 0 to 30 ng of lipopolysaccharide per ml. Arrows B₁ to B₃ indicate the gel mobility-shifted complexes. (B) Gel mobility shifts were performed with the radiolabeled octamer oligonucleotide and the same J774 nuclear extracts. Arrows O₁ and O₂ indicate the two gel mobility-shifted complexes. All assays were conducted with 5 μ g of nuclear extract protein per reaction.

tein lipase is reduced through transcriptional and posttranscriptional mechanisms (15, 22, 25, 26). However, unlike the case for macrophages, direct exposure of adipocytes to lipopolysaccharide has no effect on lipoprotein lipase expression (26). This systemic inhibition of lipoprotein lipase by TNF, interleukin 1, and interleukin 6 may in part account for the hypertriglyceridemia observed during endotoxic shock. Thus, following lipopolysaccharide activation, macrophages not only decrease their own expression of lipoprotein lipase directly but also reduce that of other tissues indirectly.

Growth factors which regulate macrophage lipoprotein lipase gene expression in a positive and a negative manner have been identified. Differentiation-inducing agents such as phorbol esters and dexamethasone increased human and murine macrophage lipoprotein lipase levels (2–4, 14, 20, 21, 38). Both TNF and interleukin 1 had no effect on lipoprotein lipase in macrophages (34, 44). The cytokine found to inhibit macrophage lipoprotein lipase was gamma interferon (24, 34). It is of interest that in the presence of gamma interferon, macrophages from the endotoxin-resistant C3H/HeJ murine strain were made responsive to lipopolysaccharide and released TNF (1, 6).

Macrophage-specific expression of lipoprotein lipase may underlie the etiology of other diseases, such as atherogenesis (31, 47). Macrophages and foam cells found within atheromas express high levels of lipoprotein lipase, as judged from *in situ* hybridization (31, 45). In murine models, strains with increased susceptibility to atherosclerosis exhibited elevated macrophage expression of lipoprotein lipase relative to nonsusceptible strains (37). Thus, information relating to macrophage expression of lipoprotein lipase may have relevance beyond the field of septic shock.

The current studies suggest that lipopolysaccharide directly regulates murine macrophage lipoprotein lipase gene expression. Only macrophages from endotoxin-sensitive murine strains responded to lipopolysaccharide with decreased lipoprotein lipase enzyme activity and mRNA levels; no effects were observed in macrophages from the endotoxin-resistant C3H/HeJ strain. The lipopolysaccharide effects correlated with changes in the levels of macrophage nuclear proteins which

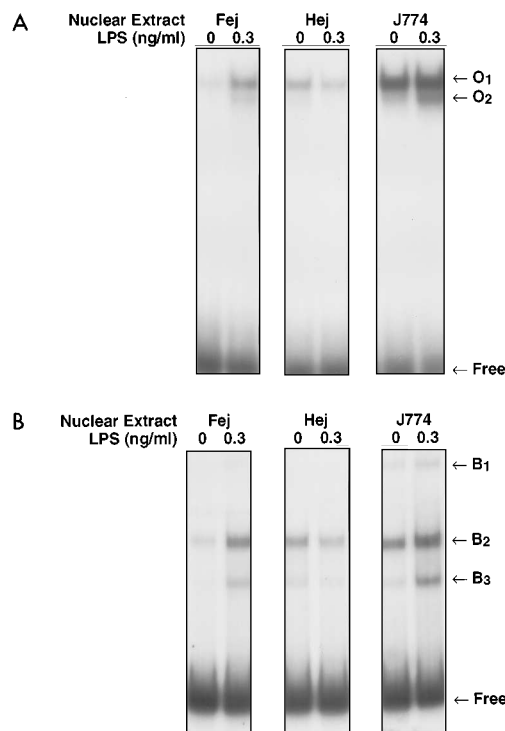


FIG. 5. Gel retention analysis: comparison of J774 cells, endotoxin-sensitive macrophages, and endotoxin-resistant macrophages. (A) Gel mobility shifts were performed with the radiolabeled 223-bp *HindIII-EcoRI* fragment and nuclear extracts from macrophages exposed for 16 h to 0 or 0.3 ng of lipopolysaccharide per ml. Reaction mixtures contained nuclear extract proteins at concentrations of 3.6 μ g for endotoxin-sensitive (Fej) or endotoxin-resistant (Hej) peritoneal macrophages or 1.4 μ g for J774 cells. Arrows B₁ to B₃ indicate the gel mobility-shifted complexes. (B) Gel mobility shifts were performed with the radiolabeled octamer oligonucleotide and 5 μ g of nuclear extract protein from endotoxin-sensitive (Fej) or endotoxin-resistant (Hej) macrophages or J774 macrophages. Arrows O₁ and O₂ indicate the gel mobility-shifted complexes.

bind to the lipoprotein lipase promoter. This finding is consistent with a possible role for transcriptional regulation. However, without the support of nuclear run-on or transfection data, this remains to be proven. A specific protein(s) recognizing the octamer motif located between the CAAT and TATA elements of the promoter was increased following lipopolysaccharide treatment of J774 cells and endotoxin-sensitive primary peritoneal macrophages. In studies on murine adipocytes, the octamer element was essential for lipoprotein lipase promoter function; mutations in this site decreased transcription by 75% (33). While the octamer was first described as a positive regulatory element in the immunoglobulin μ promoter, it has now been found within regulatory regions of many genes (39). The octamer consensus site is recognized by transcriptional regulatory proteins belonging to the POU protein family (11, 39). The active protein complex consists of homo- or heterodimers of the ubiquitous OCT-1 protein and other tissue-specific POU family members (32). Proteins of this family may prove to be involved in the signal transduction pathway mediating the lipopolysaccharide inhibition of macrophage lipoprotein lipase gene expression.

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REFERENCES

- Adi, S., A. S. Pollock, J. K. Shigenaga, A. H. Moser, K. R. Feingold, and C. Grunfeld. 1992. Role of monokines in the metabolic response to endotoxin. Interferon γ restores responsiveness of C3H/Hej mice in vivo. *J. Clin. Invest.* **89**:1603-1609.
- Auwerx, J. H., S. Deeb, J. D. Brunzell, R. Peng, and A. Chait. 1988. Transcriptional activation of the lipoprotein lipase and apolipoprotein E genes accompanies differentiation in some human macrophage-like cell lines. *Biochemistry* **27**:2651-2655.
- Auwerx, J. H., S. Deeb, J. D. Brunzell, G. Wolfbauer, and A. Chait. 1989. Lipoprotein lipase gene expression in THP-1 cells. *Biochemistry* **28**:4563-4567.
- Behr, S. R., and F. B. Kraemer. 1986. Regulation of the secretion of lipoprotein lipase by mouse macrophages. *Biochim. Biophys. Acta* **889**:346-354.
- Beutler, B., N. Krochin, I. Milsark, C. Luedke, and A. Cerami. 1986. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science* **232**:977-979.
- Beutler, B., V. Tkacenko, I. Milsark, N. Krochin, and A. Cerami. 1986. Effect of γ interferon on cachectin expression by mononuclear phagocytes. Reversal of the *lps*^d (endotoxin resistance) phenotype. *J. Exp. Med.* **164**:1791-1796.
- Chait, A., P.-H. Iverius, and J. D. Brunzell. 1982. Lipoprotein lipase secretion by human monocyte-derived macrophages. *J. Clin. Invest.* **69**:490-493.
- Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156-159.
- Church, G., and W. Gilbert. 1984. Genomic sequencing. *Proc. Natl. Acad. Sci. USA* **81**:1991-1995.
- Currie, R. A., and R. H. Eckel. 1992. Characterization of a high affinity octamer transcription factor binding site in the human lipoprotein lipase promoter. *Arch. Biochem. Biophys.* **298**:630-639.
- Dawson, S. J., S. O. Yoon, D. M. Chikaraishi, K. A. Lillycrop, and D. S. Latchman. 1994. The Oct-2 transcription factor represses tyrosine hydroxylase expression via a heptamer TAATGARAT-like motif in the gene promoter. *Nucleic Acids Res.* **22**:1023-1028.
- Deeb, S. S., and R. Peng. 1989. Structure and function of the human lipoprotein lipase gene. *Biochemistry* **28**:4131-4135.
- De Sanctis, I. Blanca, D. Radzioch, and N. E. Bianco. 1994. Lipoprotein lipase expression in natural killer cells and its role in their cytotoxic activity. *Immunology* **83**:232-239.
- Dignam, J. D., R. M. Lebovitz, and R. G. Roeder. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract isolated from mammalian nuclei. *Nucleic Acids Res.* **11**:1475-1489.
- Domin, W. S., A. Chait, and S. S. Deeb. 1991. Transcriptional activation of the lipoprotein lipase gene in macrophages by dexamethasone. *Biochemistry* **30**:2570-2574.
- Enerback, S., and J. M. Gimble. 1993. Lipoprotein lipase gene expression: physiological regulators at the transcriptional and post-transcriptional level. *Biochim. Biophys. Acta* **1169**:107-125.
- Enerback, S., B. G. Ohlsson, L. Samuelsson, and G. Bjursell. 1992. Characterization of the human lipoprotein lipase (LPL) promoter: evidence of two cis-regulatory regions, LP- α and LP- β , of importance for the differentiation-linked induction of the LPL gene during adipogenesis. *Mol. Cell. Biol.* **12**:4622-4633.
- Falkner, F., and H. Zachau. 1984. Correct transcription of an immunoglobulin gene requires an upstream fragment containing conserved sequence elements. *Nature (London)* **310**:71-74.
- Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**:6-13.
- Gimble, J. M., K. Youkhana, X. Hua, H. Bass, K. Medina, M. Sullivan, J. Greenberger, and C.-S. Wang. 1992. Adipogenesis in a myeloid supporting bone marrow stromal cell line. *J. Cell. Biochem.* **50**:73-80.
- Goldman, R. 1990. Control of lipoprotein lipase secretion by macrophages: effects of macrophage differentiation agents. *J. Leukocyte Biol.* **47**:79-86.
- Goldman, R., and O. Sopher. 1989. Control of lipoprotein lipase secretion in mouse macrophages. *Biochim. Biophys. Acta* **1001**:120-126.
- Gouni, I., K. Oka, J. Etienne, and L. Chan. 1993. Endotoxin-induced hypertriglyceridemia is mediated by suppression of lipoprotein lipase at a post-transcriptional level. *J. Lipid Res.* **34**:139-146.
- Hill, M., and R. McCallum. 1991. Altered transcriptional regulation of phosphoenolpyruvate carboxylkinase in rats following endotoxin treatment. *J. Clin. Invest.* **88**:811-816.
- Hua, X., S. Enerback, J. Hudson, K. Youkhana, and J. M. Gimble. 1991. Cloning and characterization of the promoter of the murine lipoprotein lipase-encoding gene: structural and functional analysis. *Gene* **107**:247-258.
- Jonasson, L., G. K. Hansson, G. Bondjers, L. Noe, and J. Etienne. 1990. Interferon-gamma inhibits lipoprotein lipase in human monocyte-derived macrophages. *Biochim. Biophys. Acta* **1053**:43-48.
- Kawakami, M., and A. Cerami. 1981. Studies of endotoxin-induced decrease in lipoprotein lipase activity. *J. Exp. Med.* **154**:631-639.
- Kawakami, M., P. H. Pekala, M. D. Lane, and A. Cerami. 1982. Lipoprotein lipase suppression in 3T3-L1 cells by an endotoxin-induced mediator from exudate cells. *Proc. Natl. Acad. Sci. USA* **79**:912-916.
- Kelly, K., M. R. Hill, K. Youkhana, F. Wanker, and J. M. Gimble. 1994. Dimethyl sulfoxide modulates NF κ B and cytokine activation in lipopolysaccharide-treated murine macrophages. *Infect. Immun.* **62**:3122-3128.
- Kirchgessner, T. G., J.-C. Chuat, C. Heinzman, J. Etienne, S. Guilhot, K. Severson, D. Ameis, C. Pilon, L. D'Auriol, A. Andalibi, M. C. Schotz, F. Galibert, and A. J. Lusis. 1989. Organization of the human lipoprotein lipase gene and evolution of the lipase gene family. *Proc. Natl. Acad. Sci. USA* **86**:9647-9651.
- Mahoney, E. M., J. C. Khoo, and D. Steinberg. 1982. Lipoprotein lipase secretion by human monocytes and rabbit alveolar macrophages in culture. *Proc. Natl. Acad. Sci. USA* **79**:1639-1642.
- McKnight, G. S., and R. D. Palmiter. 1979. Transcriptional regulation of the ovalbumin and conalbumin genes by steroid hormones in chick oviduct. *J. Biol. Chem.* **254**:9050-9058.
- Nilsson-Ehle, P., and M. C. Schotz. 1976. A stable radioactive substrate emulsion for assay of lipoprotein lipase. *J. Lipid Res.* **17**:536-541.
- O'Brien, K. D., D. Gordon, S. Deeb, M. Feruson, and A. Chait. 1992. Lipoprotein lipase is synthesized by macrophage-derived foam cells in human coronary atherosclerotic plaques. *J. Clin. Invest.* **89**:1544-1550.
- Poellinger, L., and R. G. Roeder. 1989. Octamer transcription factors 1 and 2 each bind to two different functional elements in the immunoglobulin heavy-chain promoter. *Mol. Cell. Biol.* **9**:747-756.
- Previato, L., C. L. Parrott, S. Santamarina-Fojo, and H. B. Brewer, Jr. 1991. Transcriptional regulation of the human lipoprotein lipase gene in 3T3-L1 adipocytes. *J. Biol. Chem.* **266**:18958-18963.
- Querfeld, U., J. M. Ong, J. Prehn, J. Carty, B. Saffari, S. C. Jordan, and P. A. Kern. 1990. Effects of cytokines on the production of lipoprotein lipase in cultured human macrophages. *J. Lipid Res.* **31**:1379-1386.
- Ralph, P., M. A. S. Moore, and K. Nilsson. 1976. Lysozyme synthesis by established histiocytic lymphoma cell lines. *J. Exp. Med.* **143**:1528-1532.
- Ralph, P., and I. Nakoinz. 1977. Antibody-dependent killing of erythrocyte and tumor targets by macrophage-related cell lines: enhancement by PPD and LPS. *J. Immunol.* **119**:950-954.
- Rennier, G., E. Skamene, J. B. DeSanctis, and D. Radzioch. 1993. High macrophage lipoprotein lipase expression and secretion are associated in inbred murine strains with susceptibility to atherosclerosis. *Arteriosclerosis*

- Thrombosis **13**:190–196.
38. **Sopher, O., and R. Goldman.** 1987. Bacterial lipopolysaccharide suppresses the expression of lipoprotein lipase in murine macrophages: a process independent of tumor necrosis factor or interleukin 1. *Immunol. Lett.* **15**:261–265.
 39. **Staudt, L. M., and M. J. Leonardo.** 1991. Immunoglobulin gene transcription. *Annu. Rev. Immunol.* **9**:373–398.
 40. **Sultzer, B. M.** 1968. Genetic control of leukocyte responses to endotoxin. *Nature (London)* **219**:1252–1254.
 41. **Sultzer, B. M.** 1969. Genetic factors in leukocyte responses to endotoxin: further studies in mice. *J. Immunol.* **103**:32–38.
 42. **Tajima, S., R. Hayashi, S. Tsuchiya, Y. Miyake, and A. Yamamoto.** 1985. Cells of a human monocytic leukemia cell line (THP-1) synthesize and secrete apolipoprotein E and lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **126**:526–531.
 43. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA* **81**:1991–1995.
 44. **White, J. R., A. Chait, S. J. Klebanoff, S. Deeb, and J. D. Brunzell.** 1988. Bacterial lipopolysaccharide reduces macrophage lipoprotein lipase levels: an effect that is independent of tumor necrosis factor. *J. Lipid Res.* **29**:1379–1385.
 45. **Yla-Herttuala, S., B. A. Lipton, M. E. Rosenfeld, I. J. Goldberg, D. Steinberg, and J. C. Witztum.** 1991. Macrophages and smooth muscle cells express lipoprotein lipase in human and rabbit atherosclerotic lesions. *Proc. Natl. Acad. Sci. USA* **88**:10143–10147.
 46. **Zechner, R., T. C. Newman, E. Steiner, and J. L. Breslow.** 1991. The structure of the mouse lipoprotein lipase gene: a B1 repetitive element is inserted into the 3' untranslated region of the mRNA. *Genomics* **11**:62–76.
 47. **Zilversmit, D. B.** 1973. A proposal linking atherogenesis to the interaction of endothelial lipoprotein lipase with triglyceride-rich lipoproteins. *Circ. Res.* **33**:633–638.