Induction of tumor necrosis factor α gene expression by lipoprotein lipase

Genevieve Renier,[1,*] Emil Skamene,[*] Juan B. DeSanctis,[†] and Danuta Radzioch[*]

Department of Experimental Medicine,[*] McGill Centre for the Study of Host Resistance, Montreal General Hospital Research Institute, Montreal, Quebec, Canada H2L-4M1, and Institute of Immunology,[†] Central University of Venezuela, Caracas, Venezuela

Abstract In view of the suppressive effect of tumor necrosis factor α (TNFα) on lipoprotein lipase (LPL) and of the potential proatherogenic effects of these two macrophage secretory products, we have tested the possibility that LPL could modulate the production of TNFα. Treatment of macrophages with lipoprotein lipase induced tumor necrosis factor α gene expression and protein secretion. Maximal increase of TNFα mRNA levels occurred after a 3-h treatment with 200 ng/ml LPL. An additive effect of interferon γ (IFNγ) was observed on LPL-induced TNFα mRNA expression. De novo mRNA synthesis was required for induction of TNFα mRNA by LPL as no induction was observed when macrophages were pretreated with actinomycin D before adding LPL. We further established that LPL induced the transcription of the TNFα gene in macrophages. We also found that LPL caused the nuclear migration of one member of the NFkB family that appears to bind to a site in the murine TNFα gene promoter. Furthermore, we demonstrated that the treatment of macrophages with LPL increased the stability of TNFα mRNA. These results show that the TNFα gene induction in response to LPL involves both transcriptional activation and the enhancement of TNFα mRNA stability. Overall, our data demonstrate a new role for LPL, that of modulating macrophage TNFα gene expression. This effect may represent one of the mechanisms by which LPL may favor the development of atherosclerosis.—**Renier, G., E. Skamene, J. B. DeSanctis, and D. Radzioch.** Induction of tumor necrosis factor α gene expression by lipoprotein lipase. *J. Lipid Res.* 1994. **35:** 271–278.

Supplementary key words gene expression • macrophage

TNFα is an important secretory product of macrophages directly involved in inflammatory reactions after a variety of bacterial infections (1). It has been well established that TNFα gene expression is tightly controlled both at the transcriptional and posttranscriptional levels (2, 3). Both the TNFα promoter and 3' untranslated region are involved in the response to lipopolysaccharide (LPS), the former at the transcriptional level and the latter at the translational level, leading to a synergistic effect on protein synthesis. LPS-mediated transcriptional activation of the TNFα gene has been shown to involve kB-type enhancers (2). In addition to LPS effects on transcriptional activation, cell stimulation with LPS is associated with a release from translational blockage (3). These data indicate that LPS is a very powerful TNFα inducer as it augments both the transcription and the stability of the TNFα messenger. The rapid down-regulation of TNFα receptor expression (4) and the release of several secretory products, such as prostaglandin E_2 (5), protect macrophages from the harmful effects of TNFα.

Lipoprotein lipase (LPL) is a key enzyme in the metabolism of lipoproteins (6). It is constitutively expressed by macrophages (7) and synthesized by the parenchymal cells of various tissues (8–12).

The demonstration of a suppressive effect of TNFα on LPL production (13) and the possibility that both macrophage cytokines may play a role in the development of atherosclerosis (14, 15) led us to investigate the possible effect of LPL on the regulation of TNFα mRNA gene expression in macrophages. Our results demonstrate that LPL can induce TNFα mRNA expression and that its effect involves transcriptional events as well as posttranscriptional modifications. These observations underline the importance of LPL in macrophage biology and define a novel mechanism of regulation of TNFα production.

MATERIALS AND METHODS

Reagents

Fetal bovine serum (FBS) was purchased from Hyclone Lab. (Logan, UT). Dulbecco's minimal essential medium (DMEM) was obtained from ICN Biochemicals Inc., Costa Mesa, CA, and supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine (ICN, Biochemicals Inc.) and penicillin-streptomycin (Flow, McLean,

Abbreviations: LPL, lipoprotein lipase; TNFα, tumor necrosis factor α; IFNγ, interferon γ; LPS, lipopolysaccharide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

[1]To whom correspondence and reprint requests should be addressed.

VA). Actinomycin D (Dact), phospholipase A_2 (PLA_2) (200–400 U/mg protein) were purchased from Sigma Chemical Co. (St. Louis, MO). Recombinant murine IFNγ was obtained from Amgen Biologicals (Thousand Oaks, CA).

Purification of LPL

LPL was isolated from human postheparin plasma. The enzyme was purified as previously described (16) using two column steps of heparin-Sepharose affinity chromatography and elution with 2 M NaCl. Purity of the protein was tested by Western blot analysis as previously described (14).

Macrophages

Peritoneal exudate macrophages were harvested 4 days after C57BL/6 mice were injected intraperitoneally with 1 ml of 1% thioglycolate medium. For our studies on the stability of TNFα mRNA, run-on and DNA-binding protein assays, we used the ANA-1 macrophage line, established by infection of the normal bone marrow of C57BL/6 mice with the J2 recombinant retrovirus as previously described (17). Either macrophage lines or freshly harvested peritoneal macrophages were cultured in DMEM containing 10% FBS and treated for different time periods with the appropriate agents.

RNA extraction

Ten million macrophages were plated in 35-mm plastic petri dishes (Falcon, Lincoln Park, NJ). After the treatment of macrophages with activating agents, macrophages were lysed with guanidine isothiocyanate. Total RNA was purified by centrifugation through a cesium chloride gradient as previously described in detail by Chirgwin et al. (18).

Northern blot analysis

Fifteen μg of total RNA was separated on a 1.2% agarose gel containing 2.2 M formaldehyde as previously described (19). The blots were prehybridized for 18 h in prehybridization buffer. The mRNA expression was analyzed by hybridization with [^{32}P]dCTP (sp act ca. 3000 Ci/mmol, Amersham Corp., Arlington Heights, IL), labeled purified TNFα, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) DNA inserts. Hybridization was detected by autoradiography with Kodak X-Omat-AR films (Rochester, NY). RNA expression was quantified by high resolution optical densitometry (SciScan 5000, USB).

Nuclear run-on assay

Nuclear run-on experiments were performed as described by Greenberg and Ziff (20). α-^{32}P UTP-labeled RNA (2×10^6 cpm/ml) was hybridized to TNFα, IL-1β, GAPDH, and pBR322 DNA linearized probes spotted on nitrocellulose.

DNA binding assays

The isolation of nuclei was performed as previously described (21). Briefly, 5×10^7 ANA-1 cells were collected, washed with cold PBS, and lysed in 1 ml ice-cold buffer A (15 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES, 0.1 mM EDTA, 1 mM DTT, 2 μg/ml aprotinin, 0.1% PMSF, and 0.5% Nonidet P-40). After 10 min incubation on ice, lysed cells were centrifuged and the nuclei were washed with buffer A without Nonidet P-40. The nuclei were then lysed in a buffer containing 2 M KCl, 25 mM HEPES, 0.1 mM EDTA, and 1 mM DTT. After a 15-min incubation period, a dialysis buffer (25 mM HEPES, 1 mM DTT, 0.1% PMSF, 2 μg/ml aprotinin, 0.1 mM EDTA, 11% glycerol) was added to the nuclei preparation. Nuclei were collected by centrifugation for 20 min at 13000 *g*. Aliquots (50 μl) of the supernatants were frozen at −70°C and protein concentration was determined.

DNA retardation (mobility shift) electrophoresis assays were performed as previously described by Fried and Crothers (22). Briefly, 5-μg nuclear extracts were incubated for 15 min in the presence of 5 × binding buffer (125 mM HEPES, pH 7.5, 50% glycerol, 250 mM NaCl 0.25% Nonidet P-40, 5 mM DTT). End-labeled double-stranded consensus sequences of the TNFα promoter NFkB-enhancing element (10,000 cpm per sample) were then added to the samples for 30 min. Samples were then analyzed on a 4% nondenaturing polyacrylamide gel, containing 0.01% Nonidet P-40.

DNA probes

The cDNA probe for murine tumor necrosis factor (TNF) was kindly provided by Dr. A. Cerami (Rockefeller University, NY). The murine GAPDH probe was prepared in our laboratory by polymerase chain amplification. cDNA was obtained from total RNA using a reverse transcription reaction. Two synthetic primers were used to enzymatically amplify a 456-bp of the GAPDH cDNA. The GAPDH probe was subsequently purified on a low melting agarose gel. For Northern blot analysis, purified DNA inserts were labeled with α-[^{32}P]dCTP using a nick translation DNA labeling kit (Boehringer-Mannheim). A 42-mer double-stranded oligonucleotide (5'-GATCCAAGGGGACTTTCCATGGATCCAAGGGGACTTTCCATG-3'; (5'-GATCCATGGAAAGTCCCCTTGGATCCATGGAAAGTCCCCTTG-3') containing the consensus sequence for the NFkB enhancer of the murine TNFα gene promoter was synthesized with the aid of an automated DNA synthesizer. After annealing, a double-stranded oligonucleotide was labeled with γ-[^{32}P]ATP using the Boehringer-Mannheim 5'-end-labeling kit.

Determination of TNFα protein content

A double-sandwich ELISA was used to determine the quantity of TNFα in the supernatants of macrophages as

described in detail by Sheehan, Ruddle, and Schreiber (23). Hamster monoclonal antibody to murine TNFα was purchased from Genzyme (Boston, MA), and rabbit polyclonal anti-murine TNFα was prepared and purified by standard procedures. Briefly, 2 μg per well of a monoclonal antibody against TNFα was absorbed in 96-well plates and incubated in the presence of different dilutions of the test sample or with the murine recombinant TNFα standards (Genzyme). After washing, the polyclonal antibody to TNFα was added. Anti-rabbit IgG peroxidase was added to the wells and incubated for 1 h. The peroxidase reaction was developed by adding peroxidase substrate and analyzed in an automated plate reader (Dynatech, Chantilly, VA).

Determination of protein concentrations

Total protein content was estimated according to the Bradford method (24) using a colorimetric assay (Bio-Rad, Mississauga, ONT).

Determination of endotoxin concentrations

The endotoxin content of all media and LPL preparations was determined by a quantitative limulus amebocyte lysate assay (Whittaker, Walkersville, MA). The endotoxin content in the LPL preparations and in the media was found to be lower than 0.007 ng/ml and 0.012 ng/ml, respectively.

RESULTS

The effects of LPL on the TNFα mRNA expression were studied in fresh peritoneal murine macrophages harvested from C57BL/6 mice and in the ANA-1 macrophage line derived from the bone marrow of C57BL/6 mice.

Treatment of macrophages with LPL (5–500 ng/ml) significantly augmented, in a dose-dependent manner, the expression of TNFα in macrophages (**Fig. 1**). The maximal effect was observed at the dose of 200 ng/ml LPL. The same RNAs were also tested for GAPDH mRNA expression. No modulation of the expression of this gene was observed after LPL treatment of the cells (Fig. 1). Exposure of macrophages to 500 ng/ml LPL and PLA_2 increased the TNFα mRNA expression to the same extent without affecting IL-1β and GAPDH expression (**Fig. 2**). These results indicated that the expression of TNFα mRNA was selectively augmented in macrophages following LPL treatment.

To investigate the kinetics of the effect of LPL on TNFα mRNA induction, ANA-1 cells were treated with LPL for 1, 3, 6, 18, or 24 h and the expression of TNFα mRNA in response to LPL was measured. As shown in **Fig. 3,** a peak in TNFα mRNA expression occurred between 3 and 6 h after LPL treatment leading to a gradual return to the basal level. Similar kinetics were observed when the cells were exposed to IFNγ alone or to a combination of IFNγ and LPL. An additive effect of LPL and IFNγ on TNFα mRNA expression was observed. Similar results were observed when LPL and IFNγ were cultured with murine peritoneal macrophages isolated from C57BL/6 mice (data not shown). Recovery of enhanced amounts of TNFα from the culture media reflected the increase in TNFα mRNA expression in response to LPL. Macrophage treatment with IFNγ plus LPL enhanced the TNFα secretion even further (**Fig. 4**).

To establish whether or not the TNFα mRNA induction by LPL requires de novo RNA synthesis, we exam-

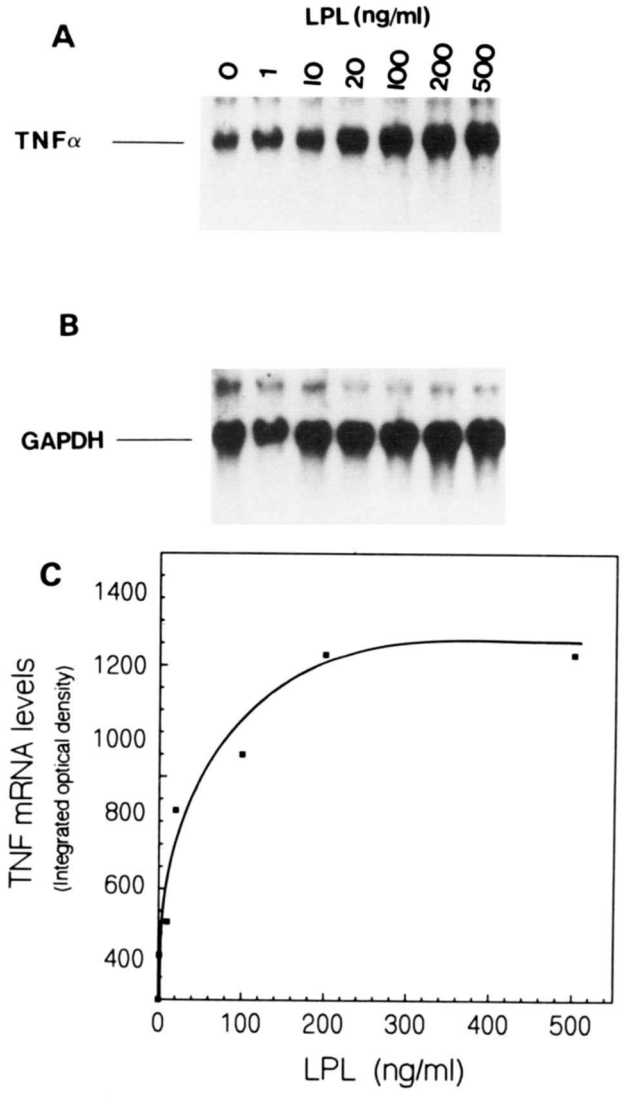

Fig. 1. Effect of LPL on the induction of TNFα mRNA expression. ANA-1 cells were cultured for 3 h with increasing concentrations of LPL (0–500 ng/ml). Total RNAs were extracted and analyzed by Northern blot for TNFα mRNA (A) and GAPDH mRNA expression (B). mRNA levels normalized to the levels of GAPDH mRNA are presented in panel C.

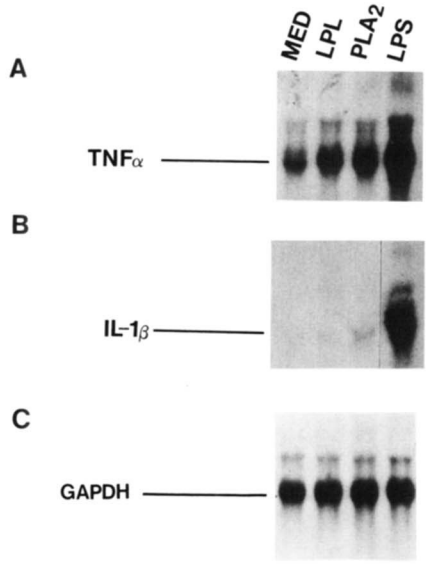

Fig. 2. Effect of LPL (500 ng/ml) or PLA_2 (500 ng/ml) on TNFα mRNA expression. Total RNAs were analyzed by Northern blot for TNFα mRNA (A), IL-1β (B), and GAPDH (C) mRNA expression after a 3-h treatment of ANA-1 cells with either stimulus. Med, medium.

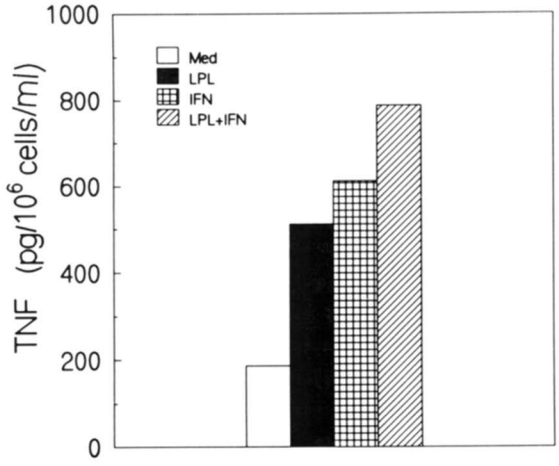

Fig. 4. TNFα secretion by ANA-1 cells after 24 h exposure to medium (Med), 100 ng/ml LPL (LPL), 100 U/ml IFNγ (IFN), or 100 ng/ml LPL in combination with 100 U/ml IFNγ (LPL+IFN). After the incubation period, supernatants were collected, centrifuged, and tested for TNFα protein content by ELISA. TNFα secretion is expressed in pg of protein released by 10^6 cells per ml.

ined the effect of LPL on TNFα mRNA expression in cells treated with actinomycin D. No increase in TNFα mRNA was observed when actinomycin D was added to macrophages prior to the addition of LPL (**Fig. 5**), suggesting that LPL-induced TNFα gene augmentation requires active RNA synthesis.

To confirm that the increase in the level of TNFα mRNA was a direct consequence of augmented transcriptional activity in macrophages stimulated with LPL, we performed nuclear run-on assays. As shown in **Fig. 6,** the transcription of TNFα gene was augmented over levels obtained in untreated macrophages: 1.64-fold in response to LPL, 2.59-fold after treatment with IFNγ, 2.19-fold in response to a combination of IFNγ and LPL, 4.93-fold in response to LPS. None of the treatments had any effect on the transcription rate of the macrophage IL-1β and GAPDH genes. Under similar experimental conditions, the background hybridization of the transcripts with pBR322 was undetectable.

We also tested whether or not treatment of macrophages with LPL could induce changes at the level of TNFα gene promoter binding proteins. We found that LPL caused, in a time-dependent manner, the nuclear migration of one member of the NFkB family, which ap-

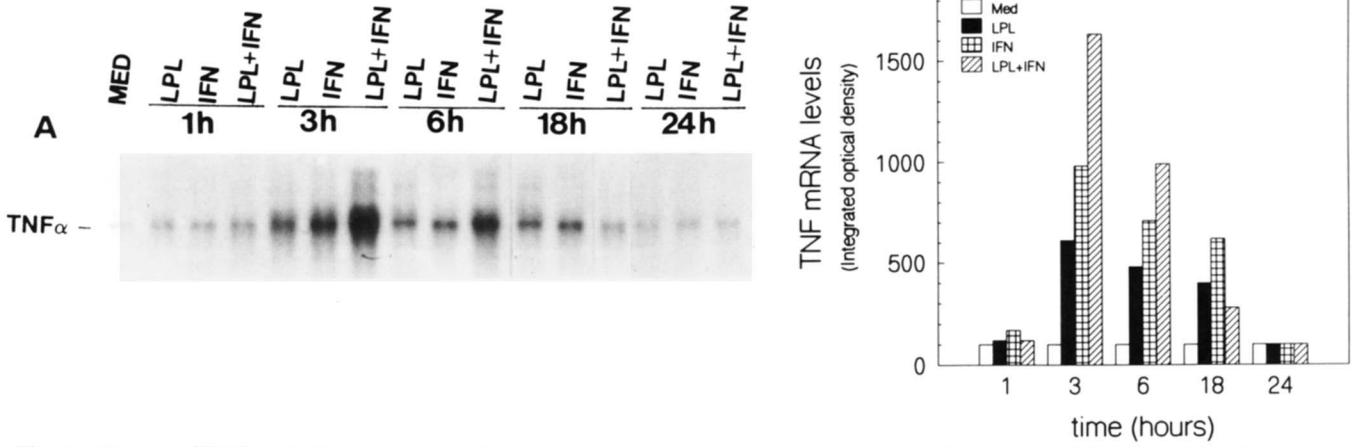

Fig. 3. Kinetics of TNFα mRNA induction by LPL in macrophages. ANA-1 cells were cultured for 1, 3, 6, 18, and 24 h in medium (Med) or in medium containing 100 ng/ml LPL (LPL), 100 U/ml IFNγ (IFN), or 100 ng/ml LPL in combination with 100 U/ml IFNγ (LPL+IFN). Total RNAs were isolated and were assessed by Northern blot analysis for TNFα mRNA expression (A). mRNA levels normalized to the levels of GAPDH mRNA expression in each sample are presented in panel B.

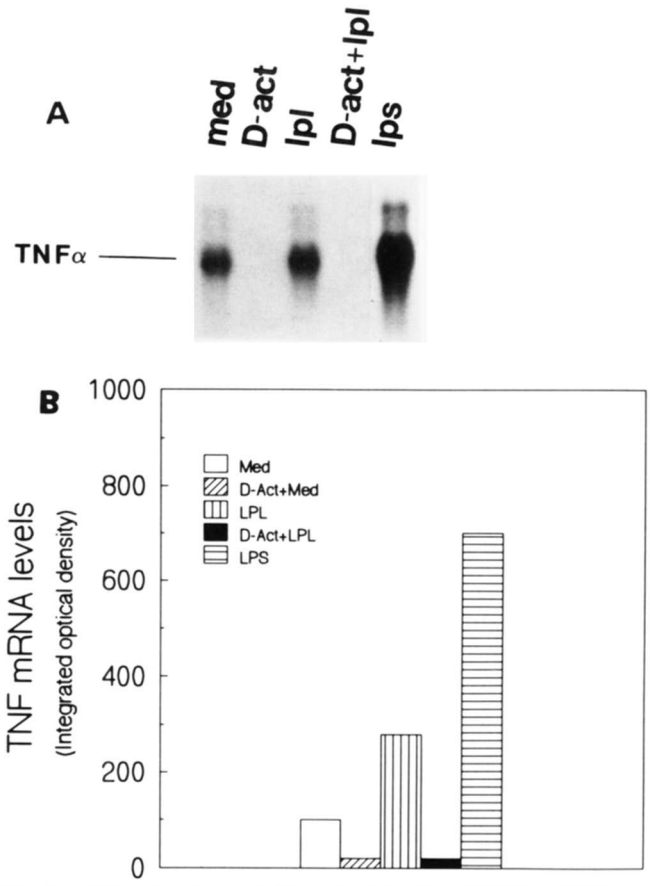

Fig. 5. Effect of actinomycin D on the induction of TNFα mRNA expression by LPL. ANA-1 cells were treated with 5 μg/ml actinomycin D (D-act) or medium (Med) for 60 min prior to the addition of 100 ng/ml LPL (LPL) for 3 h. A 3-h treatment with LPS (100 ng/ml) was used as a positive control for the induction of TNFα. At the end of the incubation period, RNAs were extracted and mRNA levels were analyzed by Northern blot for TNFα mRNA expression (A). mRNA levels normalized to the levels of GAPDH mRNA expression in each sample are presented in panel B.

pears to bind to a site in the TNFα gene promoter (**Fig. 7**). The level of enhancement in the binding of nuclear proteins extracted from macrophages stimulated with LPL was consistently lower than that observed in macrophages stimulated with 1 μg/ml of LPS, used as a positive control.

Although these observations were indicative of transcriptional activation taking place in response to stimulation of macrophages with LPL, they did not preclude the possibility that the mRNA half-life was affected as well. To investigate this possibility, we measured the half-life of TNFα mRNAs in untreated and LPL-treated macrophages after the addition of D-actinomycin. As shown in **Fig. 8,** the rate of decay of TNFα mRNA was slower in LPL-treated cells than in the controls (TNFα mRNA half-life: 22 ± 2 min vs. 13 ± 1 min). No significant difference in the half-life of TNFα mRNA in IFNγ- or IFNγ+LPL-treated cells was observed as compared to the controls (TNFα mRNA half-life: IFNγ-treated cells: 13 ± 1 min; IFNγ+LPL treated cells: 17 ± 2 min; untreated cells: 13 ± 1 min).

DISCUSSION

Macrophages and monocytes secrete TNFα in response to bacterial infection or treatment with bacterial cell wall products, such as LPS (25). IFNγ was shown to enhance TNFα mRNA expression in macrophages (26) while TNFα protein secretion was not detectable or detected only at very low levels (<50 U/10^6 cells/ml). Interestingly, it has been shown that TNFα inhibits LPL activity in macrophages (13) and LPL mRNA expression and secretion in adipocytes (27).

To investigate whether or not LPL can modulate macrophage gene expression, we have tested the effect of LPL on TNFα mRNA expression and protein secretion. We found that LPL alone or in association with IFNγ may act as a potent inducer of macrophage TNFα expression.

We have shown that the LPL effect was not due to any endotoxin contamination in the LPL preparation (level <0.007 ng/10^6 cells/ml) and that LPL treatment did not augment other genes inducible by LPS, such as IL-1.

The demonstration that PLA_2 can also induce TNFα mRNA expression supports previous evidence that PLA_2 is directly involved in the transcriptional activation of the TNFα gene (28) and that lipases other than LPL may modulate TNFα gene expression.

Incubation of macrophages with LPL results in a dose-dependent TNFα mRNA induction. An increase in the level of TNFα mRNA expression was observed from 10 to 200 ng/ml of LPL. As the constitutive mass of LPL released from murine macrophages and ANA-1 cells is ap-

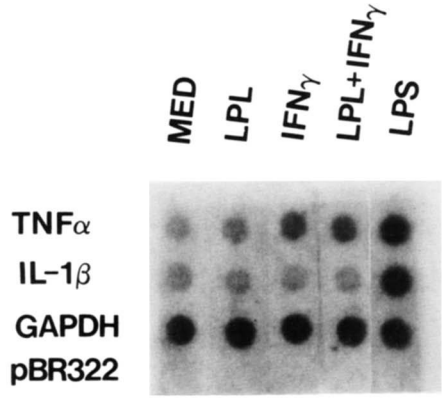

Fig. 6. Effect of LPL on the transcription of TNFα gene in the ANA-1 macrophage line. ANA-1 cells were treated for 3 h with 100 ng/ml LPL, 100 U IFNγ, 100 ng/ml LPL+100 U IFNγ, or 100 ng/ml LPS. Nuclei were isolated, and transcriptional activity was determined in run-on assays. pBR322-Linearized plasmid DNA was used as a control for nonspecific hybridization. MED, medium.

proximately 50 ng/ml per 24 h and as it may be further increased by exposure of the cells to interleukin-2 (G. Renier, unpublished observations), it is possible that LPL may modulate the TNFα gene expression under both physiological and pathophysiological conditions.

The enhancing effect of LPL on TNFα mRNA expression increases with time and maximal induction is observed after 3 h of treatment with LPL.

Our results indicate that the augmentation of TNFα gene expression at this specific time point takes place at the transcriptional and posttranscriptional levels. Our data support the hypothesis that LPL induces the TNFα gene at multiple levels. First, we have found that in the presence of actinomycin D, no induction of TNFα gene expression occurred in LPL-treated macrophages. Therefore, de novo mRNA synthesis is required in macrophages stimulated with LPL for TNFα mRNA induction to occur. Second, run-on experiments indicate that LPL induces the transcriptional activation of TNFα mRNA. These results demonstrate that, in LPL-treated macrophages, the observed increase in TNFα mRNA expression is at least partly due to an increase in the TNFα gene transcriptional activity. Finally, when untreated and LPL-treated macrophages were tested for binding to the NFkB consensus sequence of the TNFα promoter, a clear augmentation of protein binding was observed in LPL-treated macrophages as compared to untreated macrophages. These data suggest the involvement of the NFkB-type enhancer element in LPL-mediated transcriptional activation of the TNFα gene.

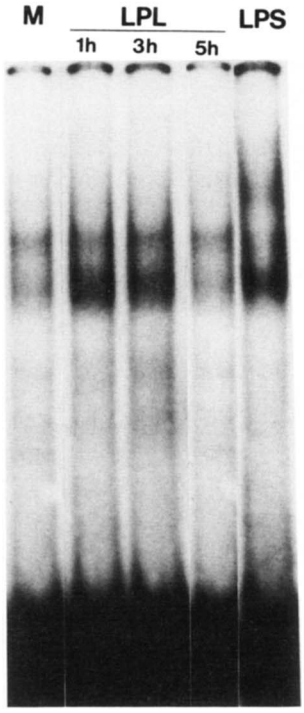

Fig. 7. Binding of nuclear proteins extracted from untreated (M) or LPL-treated cells (LPL) to the regulatory NFkb sequence of the murine TNFα gene promoter. ANA-1 cells were treated for 1, 3, and 5 h with 100 ng/ml LPL. The nuclear proteins isolated from untreated, LPL-treated, and LPS-treated (positive control) were incubated with double-stranded NFkB regulatory element of the TNFα gene. Retardation was assessed by gel electrophoresis in 4% polyacrylamide gels.

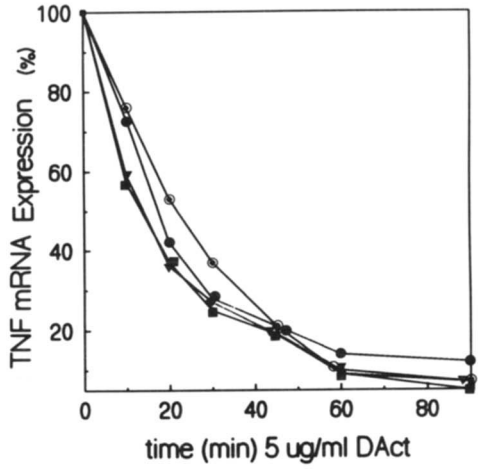

Fig. 8. Evaluation of the half-life of TNFα mRNA in ANA-1 macrophages cultured for 3 h in medium (Med) (■), in the presence of 1000 ng/ml LPL (LPL) (⊙), 100 U/ml IFNγ (IFN) (▼), or 1000 ng/ml LPL in combination with 100 U/ml IFNγ (LPL+IFN) (●). Levels of TNFα mRNA expression after 10, 20, 30, 45, 60, and 90 min of actinomycin D treatment were calculated and plotted as percent expression compared to 100% of TNFα mRNA extracted from cells treated with appropriate stimuli or from untreated macrophages prior the addition of actinomycin D.

The increase in the steady-state level of TNFα mRNA expression may be the result of augmented transcription of the gene and/or to an increase in TNFα mRNA stability. Evaluation of TNFα mRNA stability in actinomycin D-treated macrophages demonstrated increased stability of LPL-induced TNFα mRNA as compared to the stability of constitutive TNFα mRNA expression. While LPL and IFNγ both increase the steady-state levels of TNFα mRNA, they appear to exert their effect by different mechanisms. Indeed, while both transcriptional and post-transcriptional mechanisms are responsible for the increase in the steady-state levels of TNFα mRNA in LPL-treated macrophages, IFNγ treatment led to an increase in the transcriptional activity without affecting TNFα mRNA stability. Despite these differences, we speculate that LPL may play a role similar to that of IFNγ, acting as a priming stimulus for macrophage TNFα production.

Several mechanisms may be proposed which may account for the observed effect of LPL on TNFα gene expression. LPL has been shown to bind to cell-surface heparan sulfate proteoglycans (29, 30). It can therefore be proposed that LPL may act through LPL-binding protein(s) on the macrophage surface. Alternatively, LPL internalization could occur via a nonspecific receptor-mediated pathway, through the formation of endocytic

vesicles. Finally, LPL could act through the LDL receptor expressed on the macrophage surface (31).

The biological significance of our observation with regard to atherogenesis is uncertain. LPL has been shown to be expressed in the atherosclerotic plaque (32). It is widely believed that LPL may favor the development of atherosclerosis by promoting the formation of foam cells in the arterial wall (33, 34). It is tempting to speculate that LPL might also favor the atherosclerotic process by inducing the release of TNFα in the artery wall. TNFα has been detected and localized in the atheroma (15). A combination of the TNFα local vascular clotting and inflammatory effects might account for its potential pro-atherogenic effects. The mechanisms responsible for the induction of TNFα expression during atherogenesis are unknown. Both macrophage and T-lymphocyte secretory products, such as IL-1 and IFNγ, may be involved in the regulation of expression of this cytokine. We propose that LPL may represent one of these factors.

In conclusion, our study demonstrates a stimulatory effect of LPL on macrophage TNFα expression, suggesting a role of LPL as an autocrine mediator of macrophage activation, possibly involved in feedback regulation of the expression of the TNFα gene.

Manuscript received 21 April 1993 and in revised form 5 August 1993.

REFERENCES

1. Beutler, B., and A. Cerami. 1989. The biology of cachectin/TNF: a primary mediator of the host response. *Annu. Rev. Immunol.* **7:** 625–655.
2. Shakhov, A. N., M. A. Collart, P. Vassali, S. A. Nedospasov, and C. V. Jongeneel. 1990. kB-Type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor α gene in primary macrophages. *J. Exp. Med.* **171:** 35–47.
3. Han, J., T. Brown, and B. Beutler. 1990. Endotoxin-responsive sequences control cachectin/TNF biosynthesis at the translational level. *J. Exp. Med.* **171:** 465–475.
4. Ding, A. H., E. Sanchez, S. Srimal, and C. F. Nathan. 1989. Macrophages rapidly internalize their tumor necrosis factor receptors in response to bacterial lipopolysaccharide. *J. Biol. Chem.* **264:** 3924–3929.
5. Kunkel, S. L., M. Spengler, M. May, R. Spengler, J. Larrick, and D. Remick. 1988. Prostaglandin E_2 regulates macrophage-derived tumor necrosis factor gene expression. *J. Biol. Chem.* **263:** 5380–5384.
6. Eckel, R. H. 1989. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N. Engl. J. Med.* **320:** 1060–1068.
7. Khoo, J. C., E. M. Mahoney, and J. L. Witztum. 1981. Secretion of lipoprotein lipase by macrophages in culture. *J. Biol. Chem.* **256:** 7105–7108.
8. Eckel, R. H. 1987. Adipose tissue lipoprotein lipase. *In* Lipoprotein Lipase. J. Borensztajn, editor. Evener, Chicago, IL. 79–132.
9. Borensztajn, J. 1987. Heart and skeletal muscle lipoprotein lipase. *In* Lipoprotein Lipase. J. Borensztajn, editor. Evener, Chicago, IL. 133–148.
10. Scow, R. H., and S. S. Chernick. 1987. Role of lipoprotein lipase during lactation. *In* Lipoprotein Lipase. J. Borensztajn, editor. Evener, Chicago, IL. 149–186.
11. Okabe, T., H. Yorifugi, T. Murase, and F. Takaku. 1984. Pulmonary macrophages: a major source of lipoprotein lipase in the lung. *Biochem. Biophys. Res. Commun.* **125:** 273–278.
12. Eckel, R. H., and R. J. Robbins. 1984. Lipoprotein lipase is produced, regulated and functional in rat brain. *Proc. Natl. Acad. Sci. USA.* **81:** 7604–7607.
13. Friedman, G., T. Chajek-Shaul, R. Gallily, O. Stein, E. Shiloni, J. Etienne, and Y. Stein. 1988. Modulation of lipoprotein lipase activity in mouse peritoneal macrophages by recombinant human tumor necrosis factor. *Biochim. Biophys. Acta.* **963:** 201–207.
14. Renier, 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. *Arterioscler. Thromb.* **13:** 190–196.
15. Barath, P., M. C. Fishbein, J. Cao, J. Berenson, R. H. Helfant, and J. S. Forrester. 1990. Detection and localization of tumor necrosis factor in human atheroma. *Am. J. Cardiol.* **65:** 297–302.
16. Fredrickson, D. S., J. L. Goldstein, and M. S. Brown. 1978. The familial hyperlipoproteinemias. *In* The Metabolic Basis of Inherited Disease. J. B. Standbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill, New York. 604–655.
17. Cox, G., L. Gandino, E. Blasi, D. Radzioch, B. J. Mathieson, and L. Varesio. 1989. Heterogeneity of hematopoietic cells immortalized with v/myc/v-raf recombinant retrovirus infection of bone marrow or fetal liver. *J. Natl. Cancer Inst.* **81:** 1492–1496.
18. Chirgwin, J. M., A. E. Przybyla, R. S. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acids from sources enriched in ribonucleases. *Biochemistry.* **18:** 5294–5299.
19. Thomas, P. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA.* **77:** 5201–5205.
20. Greenberg, M. E., and E. B. Ziff. 1984. Stimulation of 3T3 cells induces transcription of c-fos protooncogene. *Nature (London).* **311:** 433–437.
21. Han, J., B. Beutler, and G. Huez. 1991. Complex regulation of tumor necrosis factor mRNA turnover in lipopolysaccharide activated macrophages. *Biochim. Biophys. Acta.* **1090:** 22–28.
22. Fried, M., and D. M. Crothers. 1981. Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. *Nucleic Acids Res.* **9:** 6505–6525.
23. Sheehan, K. C. F., N. H. Ruddle, and R. D. Schreiber. 1989. Generation and characterization of hamster monoclonal antibodies that neutralize murine tumor necrosis factors. *J. Immunol.* **142:** 3884–3893.
24. Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
25. Beutler, B. 1991. Cachectin/tumor necrosis factor and lymphotoxin. *In* Peptide Growth Factors and Their Receptor II. M. B. Sporn and A. B. Roberts, editors. Springer-Verlag, Heidelberg. 39–70.
26. Beutler, B., N. Krochin, W. Milsark, C. Luedke, and A. Cerami. 1986. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science.* **232:** 977–979.

27. Mackay, A. G., J. D. Oliver, and M. P. Rogers. 1990. Regulation of lipoprotein lipase activity and mRNA content in rat epididymal adipose tissue in vitro by recombinant tumor necrosis factor. *Biochem. J.* **269:** 123–126.
28. Spriggs, D. R., M. L. Sherman, K. Imamura, C. Rodriguez, G. Robbins, and D. W. Kufe. 1990. Phospholipase A_2 activation and autoinduction of tumor necrosis factor gene expression by tumor necrosis factor. *Cancer Res.* **50:** 7101–7107.
29. Cheng, C. F., G. M. Oosta, A. Bensadoun, and R. D. Rosenfeld. 1981. Binding of lipoprotein lipase to endothelial cells in culture. *J. Biol. Chem.* **256:** 12893–12896.
30. Shimada, K., P. J. Gill, J. E. Silbert, W. H. L. Douglas, and B. L. Fanburg. 1981. Involvement of cell surface heparin sulfate in the binding of lipoprotein lipase to cultured bovine endothelial cells. *J. Clin. Invest.* **68:** 995–1002.
31. Fogelman, A. M., I. Shechter, J. Seager, M. Hokom, J. S. Child, and P. A. Edwards. 1980. Malondialdehyde alteration of low density lipoproteins leads to cholesteryl ester accumulation in human monocyte-macrophage. *Proc. Natl. Acad. Sci. USA.* **77:** 2214–2218.
32. Yla-Herttuala, S., B. A. Lipton, M. E. Rosenfeld, I. J. Goldberg, D. Steinberg, and J. L. Witztum. 1991. Macrophages and smooth muscle cells express lipoprotein lipase in human and rabbit atherosclerotic plaques. *Proc. Natl. Acad. Sci. USA.* **88:** 10143–10147.
33. Fowler, S., H. Shio, and N. J. Haley. 1979. Characterization of lipid-laden aortic cells from cholesterol-fed rabbits. IV. Investigation of macrophage-like properties of aortic cell populations. *Lab. Invest.* **41:** 372–378.
34. Schaffner, T., K. Taylor, E. J. Bartucci, K. Fisher-Dzoga, J. H. Beeson, S. Glagov, and R. W. Wissler. 1980. Arterial foam cells with distinctive immunomorphologic and histochemical features of macrophages. *Am. J. Pathol.* **100:** 57–80.