Induction of tumor necrosis factor α gene expression by lipoprotein lipase requires protein kinase C activation

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Abstract We have previously found that lipoprotein lipase (LPL) induces tumor necrosis factor alpha (TNFα) mRNA expression and TNFα protein production in the ANA-1 macrophage cell line and in resident murine macrophages. The present study was designed to elucidate the intracellular signalling pathways involved in the LPL-induced TNFα gene expression. Treatment of macrophages with two protein kinase C (PKC) inhibitors, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine hydrochloride (H7) and calphostin C, suppressed LPL-induced TNFα mRNA expression and protein production. In contrast, no inhibition of the TNFα mRNA expression occurred when macrophages were exposed to an inhibitor of calmodulin-dependent kinase N-(6-amino-hexyl)-5-chloro-1-naphthalene-sulfonamide hydrochloride (W7). Overnight treatment of ANA-1 cells with 100 ng/ml 4β-phorbol 12β-myristate 13α- acetate (PMA) caused the suppression of both PKC activity and LPL-induced TNFα mRNA expression. We have also found that LPL treatment increased PKC activity in macrophages and induced a translocation of this enzyme from the cytosol to the membrane. Finally, we have demonstrated that H7 inhibited the enhancement of nuclear protein binding to the NFkB consensus sequence in the promoter of the TNFα gene that we observed in LPL-treated macrophages. Moreover, the treatment of macrophages with H7 abolished the stabilization of TNFα mRNA in response to LPL. Overall, these data demonstrate that LPL induces TNFα mRNA expression in a PKC-dependent manner and that the PKC effect involves transcriptional events as well as posttranscriptional modifications.—**Renier, G., M. Olivier, E. Skamene, and D. Radzioch.** Induction of tumor necrosis factor alpha gene expression by lipoprotein lipase requires protein kinase C activation. *J. Lipid Res.* 1994. **35:** 1413–1421.

Supplementary key words macrophage • intracellular signalling pathways

The role of lipoprotein lipase (LPL) as a major enzyme in the catabolism of triglyceride-rich lipoproteins is well known (1). This glycoprotein is secreted by parenchymal cells of various tissues (2–6) and is constitutively expressed by macrophages (7). The importance of LPL in macrophage biology has been recently emphasized by the demonstration of a stimulatory effect of LPL on macrophage tumor necrosis factor alpha (TNFα) expression (8). The augmentation of TNFα gene expression induced by LPL was shown to take place at the transcriptional and posttranscriptional levels (8). The mechanisms, including the intracellular signalling pathways, involved in the observed effect of LPL on TNFα gene expression are unknown.

The Ca^{2+} phospholipid protein kinase, protein kinase C (PKC) is involved in a variety of cellular responses related to differentiation and proliferation (9, 10). PKC-dependent pathways regulate cellular responses in concert with other pathways including those regulated by cAMP-dependent protein kinase and tyrosine protein kinase. Activation of PKC has been reported as a signalling pathway required for the induction of TNFα gene expression by several stimuli (11, 12).

The aim of the present study was to examine the second messenger system involved in mediating LPL-induced TNFα mRNA expression and protein production in macrophages. We report that LPL induces PKC activation in macrophages and that PKC inhibitors abrogate LPL-induced TNFα mRNA and protein production. PKC depletion with prolonged exposure to phorbol-myristate-acetate (PMA) also resulted in an inhibition of LPL-induced TNFα mRNA expression. We conclude, there-

Abbreviations: LPL, lipoprotein lipase; TNFα, tumor necrosis factor alpha; PKC, protein kinase C; H7, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine hydrochloride; W7, N-(6-amino-hexyl)-5-chloro-1-naphthalene-sulfonamide hydrochloride; PMA, 4β-phorbol-12β-myristate-13α-acetate; FBS, fetal bovine serum; LPS, lipopolysaccharide; Dact, actinomycin D; DAG, 1,2-dioleoyl-rac-glycerol; HA1004, N-2-guanidinoethyl)-5-isoquinoline-sulfonamide hydrochloride; GADPH, glyceraldehyde-3-phosphate dehydrogenase.

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fore, that LPL-induced TNFα gene expression requires activation of PKC.

MATERIAL AND METHODS

Reagents

Fetal bovine serum (FBS) was purchased from Hyclone Laboratories (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, VA). H7 (1-(5-isoquinoline sulfonyl)-2-methylpiperazine dihydrochloride), HA1004 (N-2-guanidinoethyl)-5-isoquinolinesulfonamide hydrochloride), W7 (N-6-aminohexyl)-5-chloro-1-naphthalenesulfonamide), lipopolysaccharide (LPS), actinomycin D (Dact), polymyxin B sulfate, 4β-phorbol 12β-myristate 13α- acetate (PMA), and 1, 2-dioleoyl-rac-glycerol (DAG) were purchased from Sigma Chemical Co. (St. Louis, MO). Calphostin C was obtained from Calbiochem (LaJolla, CA). Phosphatidylserine was obtained from Avanti Polar Lipids (Birmingham, AL) and [Ser^{25}]PKC-(19-31) was purchased from Peninsula Laboratories (Belmont, CA).

Purification of LPL

LPL was isolated from human post-heparin plasma. The enzyme was purified as previously described (13) 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). In some experiments, polymyxin B (100 μg/ml) was added to the LPL preparations. This treatment did not modify the effect of LPL on TNFα expression.

Determinations 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. Treatment of macrophages with 7 pg/ml LPS did not induce any TNFα production or any detectable TNFα mRNA expression.

Macrophages

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 (15). The macrophage line was cultured in DMEM containing 10% FBS and treated for different time periods with the appropriate agents.

RNA extraction

Ten million macrophages were plated in 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. (16).

Northern blot analysis

Fifteen μg of total RNA was separated on a 1.2% agarose gel containing 2.2 M formaldehyde as previously described (17). The blots were prehybridized for 18 h in prehybridization buffer. The mRNA expression was analyzed by hybridization with ^{32}P dCTP (sp act approx. 3000 Ci/mmol, Amersham Corp., Arlington Heights, IL) labeled TNFα and glyceraldehyde-3-phosphate dehydrogenase (GADPH) 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).

DNA binding assays

The isolation of nuclei was performed as previously described (18). Briefly, 5 × 10^7 ANA-1 cells were collected, washed with cold PBS, and lysed in 1 ml of 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 a 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. After centrifugation for 20 min at 13000 g, the pellet nuclei were collected. Fifty-μl aliquots of the supernatants were frozen at −70°C and the protein concentration was determined. DNA retardation (mobility shift) electrophoresis assays were performed as previously described by Fried and Crothers (18). 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 sequence of the TNFα gene promoter NFkB enhancing element (10,000 cpm per sample) was then added to the samples and incubated for 30 min. Samples were then analyzed on a 4% non-denaturing polyacrylamide gel, containing 0.01% Nonidet P-40. The specificity of the nuclear protein binding was assessed by incubating the nuclear proteins isolated from LPL-treated macrophages with the labeled DNA probe in presence of 100 molar excess of unlabeled DNA probe.

DNA probes

The cDNA probe for murine tumor necrosis factor (TNFα) 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 GADPH probe was subsequently purified on a low melting agarose gel. For Northern blot analysis, purified DNA inserts were labeled with α-^{32}dCTP using a nick translation DNA labeling kit (Boehringer-Mannheim). A 42-mer double-stranded oligonucleotide (5'-GATCCAAGG GGACTTTCCATGGATCCAAGGGGACTTTCCATG-3'); (5'-GATCCATGGAAAGTCCCCTTGGATCCATGGAAAG TCCCCTTG-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 labelled with γ-^{32}P ATP using the Boehringer Mannheim 5'-end-labeling kit.

Determination of TNFα protein level

A double-sandwich ELISA was used to determine the quantity of TNFα in the culture supernatants of macrophages as described in detail by Sheehan, Ruddle, and Schreiber (19). 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). The bioactivity of the TNFα produced by the ANA-1 cells was assessed using the L929 fibroblastic lytic assay (20).

Determination of protein concentrations

Total protein content was estimated according the Bradford method (21) using a colorimetric assay (Bio-Rad, Mississauga, ONT).

Determination of PKC activity

PKC specific activities in cytosolic and particulate fractions of ANA-1 cells were determined after DE52 chromatography as previously described (22). Briefly, cells were recovered and homogenized (Dounce; 20 strokes) in 4 ml of ice-cold buffer A (pH 7.4) containing 20 mM HEPES, 2 mM $MgCl_2$, 10 mM EGTA, 2 mM EDTA, 2 mM dithiothreitol, and proteinase inhibitors pepstatin (2 μg/ml), leupeptin (2 μg/ml), benzamidine (400 μg/ml), and aprotinin (1 μg/ml). After ultracentrifugation (100,000 *g* for 60 min at 4°C), high-speed supernatants were applied to DE52 columns pre-equilibrated with buffer B (pH 7.4) containing 20 mM HEPES, 2 mM EGTA, 2 mM EDTA, and 2 mM dithiothreitol. The corresponding membrane pellets were solubilized (1 h at 4°C) in buffer A to which was added 1% (wt/vol) Nonidet P-40 and then recentrifuged (100,000 *g* for 60 min at 4°C) before the application of supernatants to DE52 columns. After removal of unbound proteins by washing columns with buffer B, fractions containing PKC were eluted with buffer B containing 0.3 M NaCl. Eluates were assayed using the mixed micelle assay (22) in a final volume of 50 μl by measuring the incorporation of $^{32}P_i$ into the synthetic peptide substrate [Ser^{25}]PKC-(19–31) (Peninsula Laboratories). Maximal activation of PKC was achieved in the presence of 2.25 mM Ca^{2+}, 60 μg/ml phosphatidylserine, and 6 μg/ml DAG. Net activity was determined by subtracting the amount of $^{32}P_i$ incorporated into [Ser^{25}]PKC-(19–31) in the absence of essential cofactors from the maximal apparent activity (obtained in the absence of phosphatidylserine and DAG and in the presence of EGTA (1.2 mM)). Data are expressed as percentages considering the control as 100% activity.

RESULTS

Effect of inhibitors of PKC on LPL-induced TNFα mRNA expression and protein production

The effect of H7 (3–20 μM) on LPL-induced TNFα mRNA expression is shown in **Fig. 1,** panels A and C. Treatment of macrophages with H7 for 1 h prior to the addition of LPL inhibited LPL-induced TNFα mRNA expression in a dose-dependent manner. In contrast, pretreatment of the cells with HA1004 (40 μM) had only a marginal effect on LPL-induced TNFα gene expression (**Fig. 2,** panels A and B).

Exposure of macrophages to a selective kinase C inhibitor, calphostin C (1 μM), prior to the addition of LPL, dramatically inhibited the induction of TNFα gene expression (**Fig. 3,** panels A and B).

We examined next the effect of protein kinase inhibitors on TNFα production. The two PKC inhibitors, H7 and calphostin, totally abrogated the production of TNFα in response to LPL, whereas HA1004 had only a weak effect (**Table 1**).

Effect of calmodulin antagonist, W7, on LPL-induced TNFα mRNA expression

To evaluate the possibility that the Ca^{2+}/calmodulin system could mediate the effect of LPL on TNFα mRNA expression, we used the calmodulin antagonist, W7. Exposure of the cells to W7 (40 μM) prior to the addition of

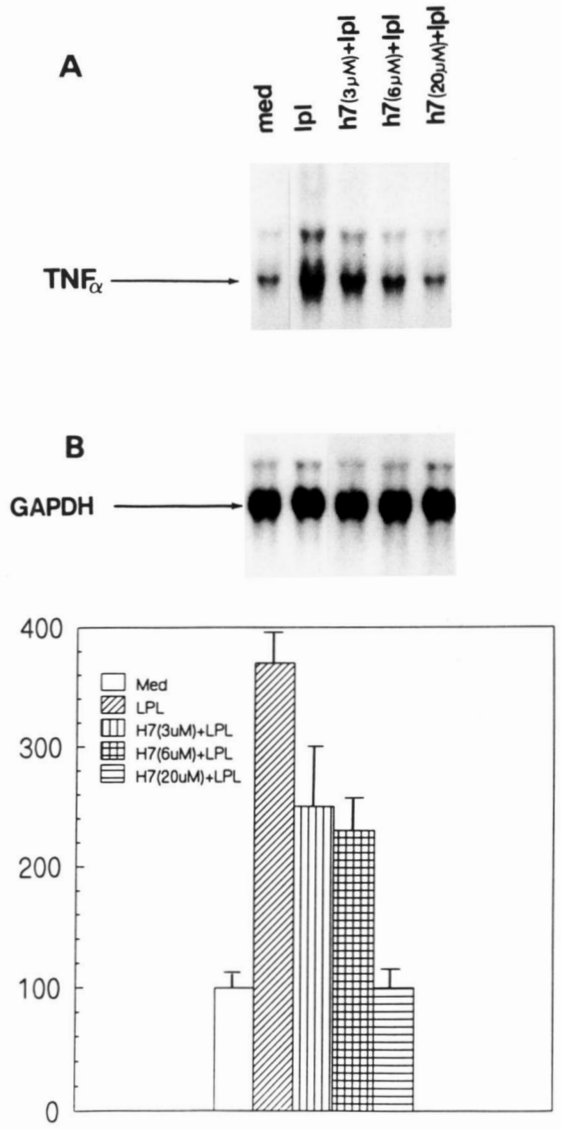

Fig. 1. Effect of H7 on the LPL-induced TNFα mRNA expression. ANA-1 cells were cultured for 1 h with increasing concentrations of H7 (3–20 μM) prior to the addition of LPL (500 ng/ml) for 3 h. Total RNAs were extracted and analyzed by Northern blot for TNFα (panel A) and GADPH mRNA (panel B). mRNA levels normalized to the levels of GADPH mRNA are shown in panel C. Values represent the mean ± SEM of three different experiments.

LPL did not prevent the LPL-induced up-regulation of TNFα gene expression (**Fig. 4,** panels A and B).

Effect of PKC depletion on LPL-induced TNFα mRNA expression

Treating the cells with high concentrations of phorbol esters for extended periods of time, has been reported to result in degradation of PKC and therefore in abrogation of PKC-specific responses. Therefore, we decided to test whether or not PKC depletion by PMA can affect the induction of TNFα mRNA expression by LPL. As shown in **Table 2,** overnight treatment with 100 ng/ml of PMA tremendously reduced total PKC activity in control and LPL-treated cells. Overnight exposure of the cells to 100 ng/ml PMA, resulted in a complete inhibition of the LPL-induced TNFα mRNA expression (**Fig. 5**).

LPL induces PKC translocation in macrophages

As TNFα expression induced by LPL appeared to require an active PKC, we examined the direct effect of LPL on PKC activity. Macrophages were cultured for 60 min in medium alone or in the presence of LPL (500 ng/ml), H7 (20 μm), or H7 in association with LPL. Total PKC activity and PKC activity in cytosolic and particulate fractions were then measured. Our results demonstrated an increased total PKC activity in LPL-treated

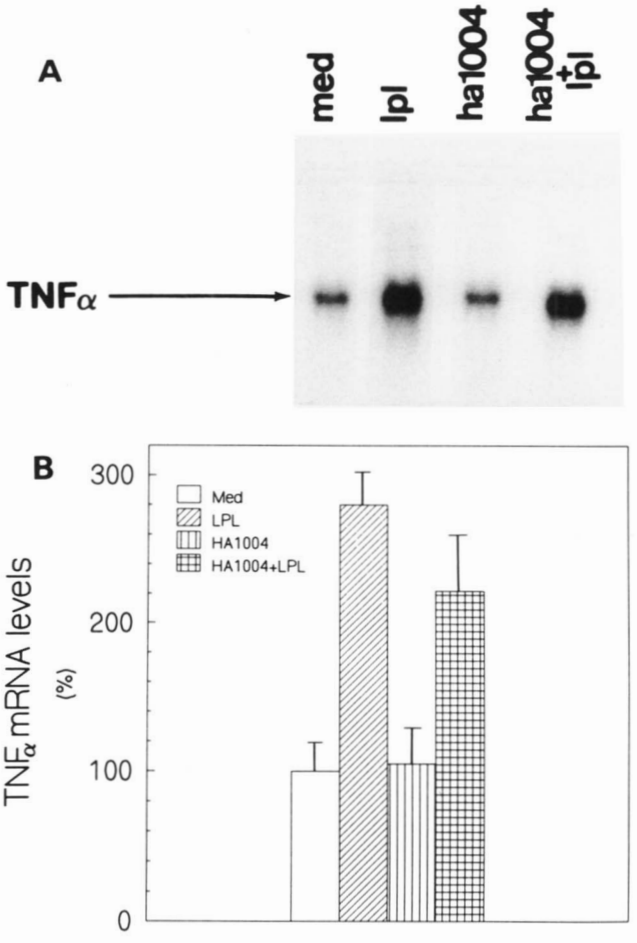

Fig. 2. Effect of HA1004 on the LPL-induced TNFα mRNA expression. ANA-1 cells were cultured for 1 h with 40 μM HA1004 prior to the addition of LPL (500 ng/ml) for 3 h. Total RNAs were analyzed by Northern blot for TNFα mRNA (panel A). mRNA levels normalized to the levels of GADPH mRNA are shown in panel B.

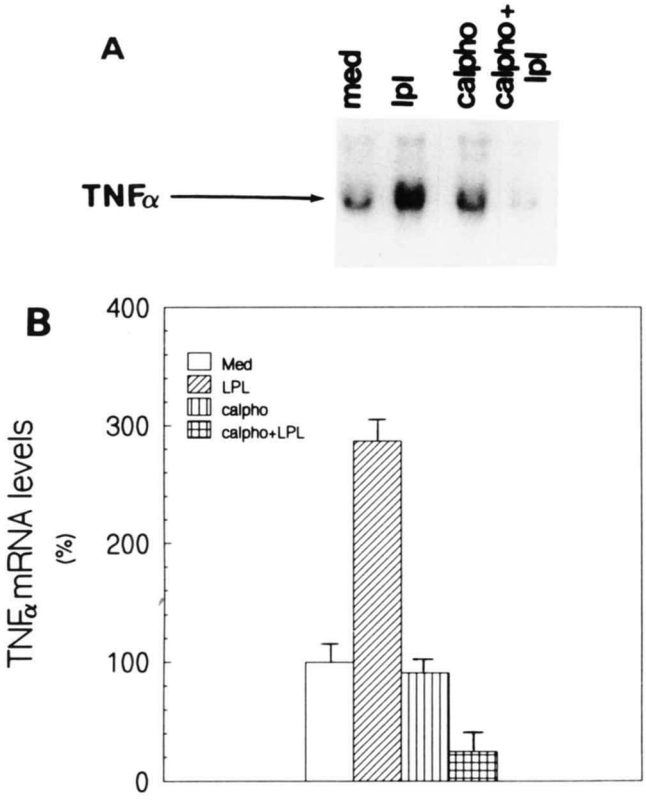

Fig. 3. Effect of calphostin C on the LPL-induced TNFα mRNA expression. ANA-1 cells were cultured for 1 h with 1 μM calphostin C prior to the addition of LPL (500 ng/ml) for 3 h. Total RNAs were analyzed by Northern blot for TNFα mRNA (panel A). mRNA levels normalized to the levels of GADPH mRNA are shown in panel B.

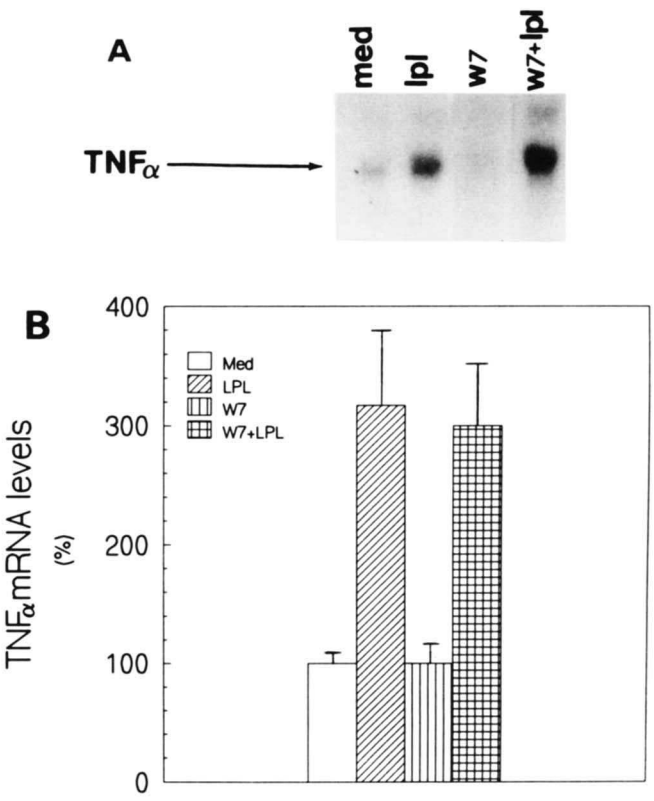

Fig. 4. Effect of W7 on the LPL-induced TNFα mRNA expression. ANA-1 cells were cultured for 1 h with 40 μM W7 prior to the addition of LPL (500 ng/ml) for 3 h. Total RNAs were analyzed by Northern blot for TNFα mRNA (panel A). mRNA levels normalized to the levels of GADPH mRNA are shown in panel B.

cells as compared to untreated cells (**Fig. 6**). The greater increase of PKC activity we detected in the membrane fraction of the LPL-stimulated macrophages as compared to that observed in the cytosolic fraction indicated that LPL induced the translocation of the enzyme from the cytosol to the membrane (Fig. 6).

PKC effect on LPL-induced TNFα gene expression is mediated at transcriptional and posttranscriptional levels

To determine the mechanisms by which PKC activation could contribute to the LPL-induced TNFα gene expression, we tested whether or not pretreatment of macrophages with H7 could abolish the enhancement of nuclear protein binding that we reported in LPL-stimulated mac-

TABLE 1. Effect of PKC inhibitors on TNFα production by ANA-1 cells

Treatment	TNFα Production (units/5 × 10^6 cells/ml)
Medium	60
LPL, 500 ng/ml	120
H7, 20 μM	40
H7 + LPL	40
HA1004, 40 μM	60
HA1004 + LPL	85
Calphostin, 1 μM	65
Calphostin + LPL	45

TABLE 2. PKC depletion in ANA-1 cells by prolonged exposure to PMA

Treatment	PKC Activity (% of control)	
	Cytosol	Membrane
Medium	100 ± 11	100 ± 4
LPL, 500 ng/ml	108 ± 13	119 ± 4
PMA, 100 ng/ml	4 ± 10	70 ± 1
PMA + LPL	0 ± 1	40 ± 2

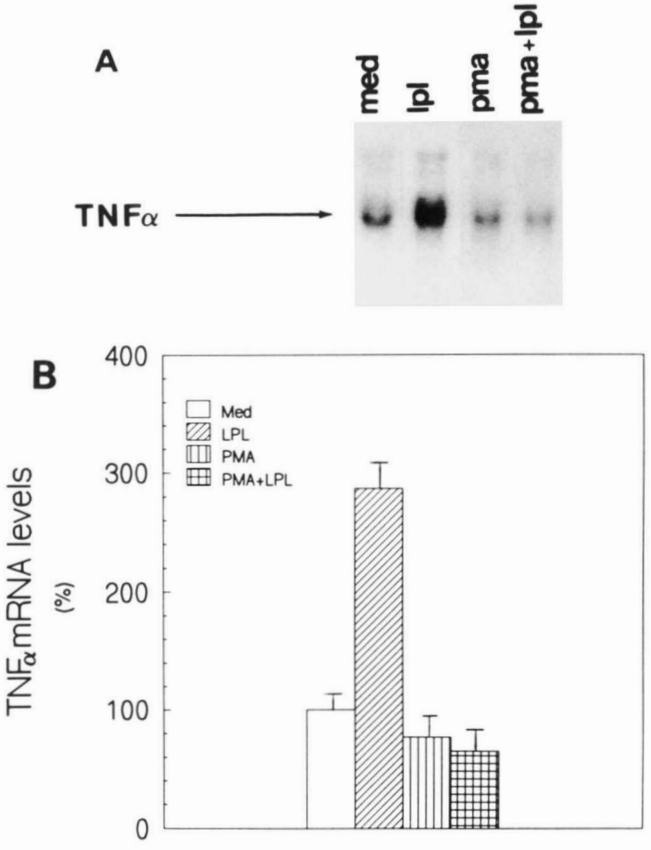

Fig. 5. Effect of PKC depletion on the LPL-induced TNFα mRNA expression. ANA-1 cells were cultured overnight with 100 ng/ml PMA prior to the addition of LPL (500 ng/ml) for 3 h. Total RNAs were analyzed by Northern blot for TNFα mRNA (panel A). mRNA levels normalized to the levels of GADPH mRNA are shown in panel B.

rophages (8). We found that in the presence of H7, the activation of the transcription factor, NFkB, induced by LPL did not occur, resulting in a dramatic reduction of its binding to the NFkB consensus sequences of the murine TNFα gene promotor (**Fig. 7**). The specificity of the protein binding to the NFkB consensus sequence was demonstrated by competition experiments (data not shown). Integrity of the nuclear extracts was shown by silver staining of the samples (data not shown).

We also investigated the possibility that PKC activity could be required for the stabilization of LPL-induced TNFα mRNA. For this purpose, we examined the effect of H7 on TNFα mRNA stability. According to our previous observation (8), the rate of decay of TNFα mRNA was slower in LPL-treated cells than in controls (22 ± 2 min vs. 13 ± 1 min) (**Fig. 8**). When the cells were exposed to H7 prior to the addition of LPL, the half-life of TNFα mRNA was reduced from 22 ± 2 min to 8 ± 2 min (Fig. 8).

DISCUSSION

The present study demonstrates that LPL increases PKC activity in macrophages and that this activation of PKC is required for LPL-induced TNFα gene expression. To our knowledge, this study represents the first report showing that LPL may act as a first messenger and that LPL interaction with macrophages is associated with intracellular signalling events, which represents activation of PKC.

The involvement of second messenger pathways in the control of macrophage function is well known. A number of activities of LPS-treated macrophages are identical to those initiated by PMA, suggesting that LPS-induced functions are mediated by PKC (23). On the other hand, calcium-dependent pathways have been implicated in the control of cytotoxicity by interferon γ (IFNγ) (24) and other lymphokines (25) and, to a lower extent, with the triggering of macrophage activities by LPS. It has been previously shown that inhibitors of PKC can specifically block the signal transduction leading to the expression of TNFα gene by LPS (12).

In order to substantiate the involvement of PKC in mediating TNFα gene expression, we have investigated the role of this second messenger in the control of TNFα gene expression by LPL. Our results demonstrated that H7 inhibited both the LPL-induced TNFα mRNA expression and TNFα protein production in macrophages at concentrations that corresponded to those that inhibit PKC activity in vitro (26). In addition to inhibiting PKC,

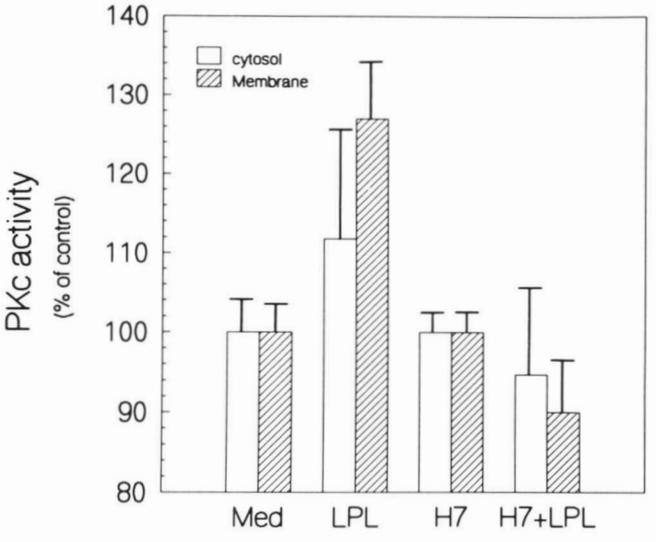

Fig. 6. Effect of LPL on PKC activity in ANA-1 cells. Macrophages were cultured for 1 h with LPL (500 ng/ml), H7 (20 μM), or H7 in association with LPL. PKC activity in cytosolic and particulate fractions were determined.

H7 is also an effective inhibitor of cAMP-dependent (K_i = 3 μM) and cGMP-dependent (K_i = 6 μM) protein kinases. Therefore, to determine what second messenger pathway(s) H7 was inhibiting in our system, we used another PKC inhibitor that has similar affinities for the cyclic nucleotide-dependent protein kinases but reduced affinity for PKC. HA1004 is the weakest PKC inhibitor among the isoquinoline-sulfonamide derivatives (K_i = 40 μM), compared to H7 (K_i = 6 μM). HA1004 had almost no inhibitory effect on the LPL-induced TNFα mRNA expression even when used at the concentration of 40 μM. These results, using two different PKC inhibitors, suggest that inhibition of PKC activation, but not that of cyclic nucleotide-dependent protein kinases, results in the loss of LPL-induced TNFα expression.

Previous studies have reported that PKC but not CaM kinase dependent pathways are involved in the induction of TNFα mRNA by LPS (12). Therefore, we tested the ability of W7, a calmodulin-dependent kinase inhibitor, to suppress the induction of TNFα gene expression by LPL. The inability of W7 to block TNFα mRNA induction in our system provides a clear indication that calmodulin-dependent kinases are not involved in the production of TNFα by LPL. These data further support the concept that TNFα expression is PKC-dependent but not calmodulin kinase-dependent.

It is well known that long term exposure of cells to PMA results in decreased cellular PKC activity (27, 28). Using this approach, we have demonstrated that when PKC activity is decreased by 100% after exposure to PMA, LPL no longer induces TNFα mRNA expression. This data, in addition to the PKC inhibition studies, strongly suggests that LPL-induced TNFα gene expression is mediated via PKC activation.

The definitive proof of the involvement of the PKC-dependent pathway in the induction of TNFα expression by LPL is provided by the determination of macrophage PKC activity in LPL-treated cells. Our results demonstrated that LPL treatment resulted in an induction and translocation of PKC in macrophages. PKC levels remained elevated for 3 h (data not shown). When sustained PKC activation is required as in the case of transcriptional regulation, additional sources of diacylglycerol may be required. Phosphatidylcholine hydrolysis by phospholipase C can liberate large quantities of diacylglycerol relative to phosphatidylinositol (4-5)-biphosphate for extended periods of time, allowing sustained PKC activation. Future studies will address the source of diacylglycerol hydrolyzed in LPL-treated macrophages.

TNFα gene expression is tightly controlled both at the transcriptional and posttranscriptional levels (29, 30). LPL-mediated transcriptional activation of the TNFα has been shown to involve kB-type enhancers (29). LPL in-

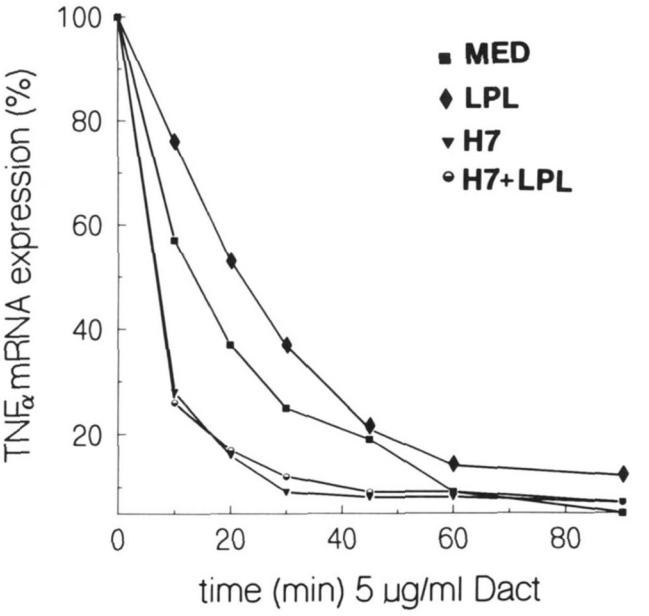

Fig. 8. Determination of the half-life of TNFα mRNA in ANA-1 cells cultured for 3 h in medium (■), in presence of 500 ng/ml LPL (♦), 20 μM H7 (▼) or pretreated for 1 h with H7 prior to the addition of LPL (◒). Levels of TNFα mRNA expression after 10, 20, 30, 45, 60, and 90 min of actinomycin D treatment 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 to the addition of actinomycin D.

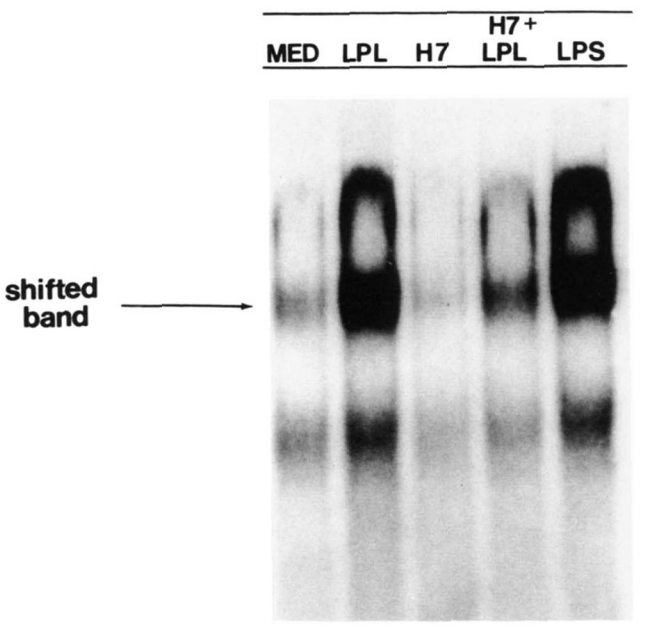

Fig. 7. Binding of nuclear proteins extracted from untreated (Med), LPL (500 ng/ml)-, H7 (20 μM)-, H7 plus LPL- or LPS (1 μg/ml)-treated cells to the regulatory NFkB sequence. ANA-1 cells were treated for 1 h with H7 prior to the addition of LPL. The nuclear proteins were isolated from the cells and incubated with double-stranded NFkB regulatory element of the TNFα gene. Retardation was assessed in 4% PAGE.

duces TNFα gene expression and consequently augments the TNFα mRNA expression. The previous observations that phorbol esters, potent activators of PKC, induce the activation of NFkB (31) and the presence of NFkB responsive elements in the TNFα gene promoter led us to examine the possibility that LPL could induce the transcription of the TNFα gene by activating NFkB via a PKC-dependent mechanism. This hypothesis is supported by data that show that H7 inhibits the LPL-induced nuclear migration of one member of the NFkB family which binds to the regulatory sequence of the murine TNFα gene promoter. We propose, therefore, that direct activation of PKC by LPL may be responsible for the activation and the migration of NFkB to the nucleus where it specifically activates transcription of specific genes such as TNFα gene.

Our data indicated also that the increased stability of LPL-induced TNFα mRNA (8) requires the activation of PKC. These results suggest that the PKC effect on TNFα mRNA expression is mediated at the posttranscriptional level as reported previously by Lieberman, Pitha, and Shin (32).

The present study demonstrates the involvement of PKC in the induction of macrophage TNFα gene expression by LPL. Although the biological significance of this observation with regard to atherogenesis remains uncertain, it is worthwhile to note that several events associated with PKC pathways could play a role in atherogenesis. Among them, processes involved in macrophage activation, differentiation, and metabolism of lipoprotein (33) have been shown to require PKC activation. Further understanding of the role of PKC in biological processes associated with the development of atherosclerosis could bring new insights to the therapeutic approach of this disease.

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