Abstract—The endothelium interacts extensively with lipids and lipoproteins, but there are very few data regarding the ability of endothelial cells to secrete lipases. In this study, we investigated the ability of endothelial cells to secrete the triglyceride lipase and phospholipase activities characteristic of endothelial lipase (EL), a recently described member of the triglyceride lipase gene family. No lipase activities were detected under basal conditions, but treatment with cytokines significantly stimulated the expression of both activities. Using antibodies to EL, we determined that both activities were primarily a result of this enzyme. In addition to the increase in lipolytic activity, cytokine treatment was demonstrated to substantially upregulate EL protein and EL mRNA in a dose-dependent manner. Cytokines did not change EL mRNA stability. Both new protein synthesis and activation of NF-κB influenced the induction of EL by cytokines, suggesting that multiple pathways contribute to this process. The upregulation of EL by cytokines is in sharp contrast to the downregulation by cytokines of the other two major members of this gene family, lipoprotein lipase and hepatic lipase, and has implications for the physiological role of EL in inflammatory conditions and its potential role in the modulation of lipoprotein metabolism during inflammatory conditions, including atherosclerosis. (Circ Res. 2003; 92:644-650.)

Key Words: lipase ■ inflammation ■ endothelium ■ cytokines ■ nuclear factor-κB

The endothelium interacts with a variety of extracellular lipids in the blood, including triglycerides and phospholipids carried within lipoproteins. Two members of the triglyceride lipase gene family, lipoprotein lipase (LPL) and hepatic lipase (HL), are synthesized by parenchymal cells but function at the endothelial surface, where they are bound to heparan sulfate proteoglycans.1 LPL plays a central role in energy homeostasis and lipoprotein metabolism by hydrolyzing lipoprotein triglycerides in adipose tissue and muscle.1 It is synthesized by underlying adipocytes and myocytes and is transported to the luminal surface of capillary endothelium, where it is active. HL is another member of the gene family that plays a role in lipoprotein metabolism by hydrolyzing triglycerides and phospholipids of remnant lipoproteins and HDL in the liver.2 HL exists on the surface of hepatocytes3 and is also transported to the endothelial surface of the sinusoids, where it interacts with lipoproteins. In addition, HL is found on endothelium in the adrenals and gonads.4

Neither LPL nor HL is synthesized by endothelial cells. Indeed, there has been relatively little evidence that endothelial cells themselves synthesize any enzymes with lipolytic activity. A recent report indicated that human umbilical vein endothelial cells (HUVECs) secreted an enzyme that had the ability to degrade lysolecithin but expressed relatively little phospholipase and no triglyceride lipase activity under the conditions tested.5 We and others recently reported the discovery of a new member of the triglyceride lipase gene family,6,7 and in contrast to the case of LPL or HL, its mRNA was demonstrated in endothelial cells. The enzyme was named endothelial lipase (EL) because of this unique characteristic, although its expression is not restricted to endothelial cells.8 Previous work showed that transient expression of the EL cDNA led to secretion of phospholipase activity,6,7 and we subsequently demonstrated that EL also has significant triglyceride lipase activity.8 Overexpression of human EL in mice significantly reduced HDL cholesterol levels,6 and our recent data show that acute inhibition of EL results in increased HDL cholesterol levels in vivo.10 Therefore, EL expression appears to be an important modulator of HDL metabolism.

EL mRNA has also been shown to be upregulated by inflammatory cytokines in HUVECs.11 Despite these findings, expression of native lipolytic activity by endothelial cells because of secretion of EL has never been reported. In this report, we demonstrate that although endothelial cells do not secrete triglyceride lipase and phospholipase activity

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under basal conditions, stimulation with inflammatory cytokines results in the stimulation of the secretion of these lipase activities, both of which are inhibited by an antibody to EL. Both EL protein and EL mRNA were found to be significantly upregulated by cytokines in HUVECs and two other endothelial cell types. Therefore, endothelial cells express both phospholipase and triglyceride lipase activities in response to cytokines that is a result of upregulation of EL.

**Materials and Methods**

Human dermal microvascular endothelial cells (HDMVECs), human uterine microvascular endothelial cells (HUuMVECs), cell culture medium EGM-2 BulletKit CC-3162 (used for HUVECs), and EGM-2 MV BulletKit CC-3147 (used for MVECs) were purchased from Clonetics. Recombinant human tissue necrosis factor (TNF-α) and recombinant human interleukin (IL)-1β were from R&D Systems. Heparin was purchased from Sigma. Actinomycin D and cycloheximide were purchased from Calbiochem.

**Antibodies**

A rabbit polyclonal antibody against human (h) EL generated to a peptide in the N-terminal region was described elsewhere and is referred to below as the anti-hEL peptide antibody. A second rabbit polyclonal antibody to hEL was raised using an adenosiviral vector encoding hEL6 as follows: 5 × 10^{17} viral particles were injected into a New Zealand White rabbit (Hare-Marland, Princeton, NJ) via the ear vein. Sera were obtained at various intervals for analysis of antibody to hEL. Six months after injection, the rabbit was anesthetized and exsanguinated. Serum was prepared and stored at −80°C. IgG fractions were purified by use of an ImmunoPure IgG (Protein A) purification kit (Pierce). This antibody is referred to below as anti-AdhEL.

**Cell Culture**

HUVECs were isolated from human umbilical cords as described.12 Passages 2 through 5 were used in the experiments. The HUVECs had typical features of cobblestone morphology. HDMVECs and HUuMVECs were cultured as specified by Clonetics.

**Treatment of HUVECs With IL-1β and TNF-α**

HUVECs were seeded into 60-mm culture plates (Falcon) at a density of 1×10^5 cells and treated in serum-free culture medium containing IL-1β (0 to 3 ng/mL) or TNF-α (0 to 10 ng/mL) for 24 hours or with IL-1β (1 ng/mL) or TNF-α (10 ng/mL) for up to 72 hours.13 Thirty minutes before harvest, heparin stock solution (20 mg/mL) was added to bring the final heparin concentration to 0.4 mg/mL. The culture medium was collected, centrifuged at 3000g for 10 minutes to remove cell debris, and stored in 0.5-mL aliquots at −80°C until use.

**Western Blotting**

Culture medium and Laemml sample buffer were mixed 1:1 (vol/vol) and heated at 85°C for 10 minutes. The samples were size-fractionated with SDS-PAGE (precast 10% polyacrylamide gels; FMC) and transferred to Hybond-P (PVDF) membrane (Amersham Pharmacia Biotech). The anti-hEL, peptide antibody antiserum, used as the primary antibody, was diluted 1:3000, and the secondary horseradish peroxidase–conjugated goat anti-rabbit IgG (Jackson Laboratories) was diluted 1:3000 from a 50% glycerol stock (final dilution, 1:6000). Detection was carried out by the ECL protocol (Amersham Pharmacia Biotech) according to the manufacturer’s instructions. Chemiluminescence signals were detected on x-ray films and quantified by densitometry.

**Northern Blotting**

Total cellular RNA was extracted from cells with TRIZol (Gibco BRL) according to the manufacturer’s protocol. RNA quantification was performed by spectrophotometry, and the integrity of RNA was analyzed by electrophoresis of 5 μg of total RNA on a 1.5% agarose gel stained with ethidium bromide. Total RNA (1 to 5 μg/lane) was electrophoresed through a 1.2% agarose gel containing formaldehyde and blotted to a Hybond-N membrane by capillary transfer. Probe synthesis, hybridization, and detection methods for all Northern blots were performed according to Roche Molecular Biochemical specifications for digoxigenin-labeled probes. A pPCR-ScriptTM Amp SK (+) vector containing the hEL polymerase chain reaction (PCR)-amplified 1.5-kb product was linearized with HindIII and used as a template for digoxigenin-labeled riboprobe synthesis; a pGEM-T vector containing the β-actin PCR-amplified 100-bp product was linearized with SpeI and used as a template for digoxigenin-labeled riboprobe synthesis. Membranes were washed twice at room temperature in 2×SSC with 0.1% SDS for 5 minutes, followed by two washes in 0.1×SSC with 0.1% SDS at 68°C for 15 minutes, followed by incubation for 1 hour at 68°C in DIG Easy Hyb granules before immunological detection. Chemiluminescence signals derived from hybridized probes were detected on x-ray films with DIG luminescence detection kits (Roche Molecular Biochemicals) and quantified by densitometry.

**Real-Time PCR**

One microgram of total RNA was converted into cDNA using the SuperScript first-strand synthesis system for reverse-transcription PCR (Roche Molecular Biochemicals). Reverse-transcription PCR was performed in an ABI Prism 7700 Sequence Detector System (PE Applied Biosystems). PCR primers were designed by use of Primer Express software according to the recommendations of Applied Biosystems. The sequences of primers and TaqMan probes for EL and β-actin genes were as follows: the EL gene, 5'-ACAACCTGTGGAAAGAGTTTCG-3' (forward primer), 5'-CGGATGCCCTGATATTCA-3' (reverse primer), and 5'-FAM-CTGTCACACCCCGCAGGCGGA-TAMRA-3' (TaqMan probe); the β-actin gene, 5'-CTTTACCTTGGACGCAAGTACTC-3' (forward primer), 5'-TCGTACATACTCTGCTTGCTGAT-3' (reverse primer), and 5'-FAM-CCACTCTGGCCCTGCTTGCTC-TAMRA-3' (TaqMan probe). The relative amounts of all mRNAs were calculated by the comparative Ct method.14 β-Actin mRNA was used as the invariant control for all studies.

**Immunoprecipitation**

Pooled culture media were incubated with rabbit anti-AdhEL IgG and control rabbit IgG at 4°C for 1 hour. The anti-hEL peptide antibody, although useful for Western blotting, does not immunoprecipitate EL well. Protein A-Sepharose (Gibco BRL) was added to each sample, and they were incubated for an additional hour. After spinning to pellet the Sepharose beads, the supernatant was used for the lipase activity assay. Control media were diluted with a volume of PBS equivalent to that added to the other samples and were incubated similarly.

**Lipase Activity Assays**

Both triglyceride lipase and phospholipase activities were assayed as previously described.9

**Results**

**Triglyceride Lipase and Phospholipase Activity Secreted by Endothelial Cells in Response to Cytokines Is Primarily a Result of Endothelial Lipase**

Triglyceride lipase and phospholipase activities were determined in conditioned media from HUVECs treated with IL-1β and TNF-α and compared with media harvested from a parallel set of cells not treated with cytokines. The levels of both lipase activities under basal conditions were similar to the background for the assays. Both lipase activities were significantly increased after IL-1β or TNF-α treatment (Figure 1). To determine whether these activities were caused by
EL, we performed several experiments. Consistent with our observation that EL triglyceride lipase and phospholipase activities are inhibited by 1 mol/L NaCl (like LPL and unlike HL), we found that the lipase activities in the cytokine-treated HUVEC media were inhibited completely by 1 mol/L NaCl (data not shown). We also tested whether immunoprecipitation with anti-AdhEL antibody to EL affected the lipase activities. Preliminary studies using conditioned media containing human EL showed that anti-AdhEL IgG significantly reduced the triglyceride lipase and phospholipase activities, whereas control rabbit IgG did not affect lipase activity. This antibody did not inhibit LPL and HL enzymatic activities in similar experiments. As shown in Figure 2, this antibody cleared 75% of triglyceride lipase activity and 75% of phospholipase activity from the IL-1β HUVEC conditioned media and 88% of triglyceride lipase activity and 57% of phospholipase activity from the TNF-α-treated HUVEC conditioned media compared with control antibody. This indicates that the triglyceride lipase and phospholipase activities secreted by HUVECs in response to cytokines are caused primarily by EL.

EL Protein Secretion Is Stimulated by Cytokines

EL protein in HUVEC culture medium was detected by Western blotting using a rabbit anti-hEL peptide antibody as previously described. Media were harvested after the addition of heparin to release EL bound to the cell surface. Immunoblotting of media from HUVECs harvested at various time points after the change of media revealed two major EL bands of 68 kDa and 40 kDa (Figure 3A). In untreated HUVECs, EL protein accumulated progressively in the media over 72 hours of incubation. In cells treated with IL-1β (Figure 3B), expression of EL protein was substantially increased compared with untreated cells. The increase in EL protein caused by IL-1β was apparent by 12 hours, and the difference between IL-1β-treated and untreated cells increased over the 72 hours. In cells treated with TNF-α (Figure 3C), a similar response was seen. Thus, both TNF-α and IL-1β treatment resulted in substantial increases in EL protein in the media, consistent with the increase in lipase activities induced by these cytokines.

Cytokine-Induced EL Expression Is Dose Dependent

HUVECs were treated with increasing concentrations of IL-1β, ranging from 1 pg/mL to 3 ng/mL. Dose-dependent increases in secreted EL protein (Figure 4A) and EL mRNA (Figure 4B) were noted. The relative increases in EL protein and mRNA were greater in the middle dose range (30 pg/mL–1 ng/mL) and plateaued at higher concentrations of IL-1β. Increasing concentrations of TNF-α also resulted in dose-dependent increases in EL protein (Figure 4C) and EL mRNA (Figure 4D).

EL contains several of the consensus heparin binding sequences that have been demonstrated in LPL and HL. To determine whether treatment with TNF-α or IL-1β for 24 hours increased the amount of EL protein that remained bound to cells, we removed the medium for analysis, then washed the cells and added fresh medium containing 10 U/mL heparin to dissociate cell surface–bound EL. After 30 minutes of incubation with heparin, the media were harvested and analyzed for EL protein by Western blotting. As above, both the 68-kDa and 40-kDa forms of EL were detected.

**Figure 1.** Effect of treatment of HUVECs with cytokines. Media were collected from control HUVECs not treated with cytokine (open bars) and from parallel cultures treated with IL-1β (1 ng/mL, black bars) or TNF-α (10 ng/mL, gray bars) for 24 hours and assayed for triglyceride lipase (A) and phospholipase (B) activities. Data are mean ± SD, n=6. *P<0.001, significantly different from control. The experiment shown is representative of three separate experiments. The background of both lipase assays is ~2 nmol/mL-hr.

**Figure 2.** Effect of anti-AdhEL antibody on lipase activity in HUVEC conditioned medium. Culture media were collected after incubation of HUVECs with IL-1β (1 ng/mL, black bars) or TNF-α (10 ng/mL, gray bars) for 24 hours. Samples of the media were treated with control or anti-AdhEL IgG, which were subsequently precipitated with protein A-Sepharose. The triglyceride lipase activity (A) and the phospholipase activity (B) of the supernatants were determined and are compared with the activity in control media (samples 1 and 4). Samples 2 and 5 were exposed to anti-AdhEL IgG, and samples 3 and 6 were exposed to control IgG. Data are presented as percentage of the activity in the control media. The data represent the mean of two separate experiments.

**Figure 3.** Immunoblot of media from HUVECs harvested after 72 hours of incubation. In cells treated with IL-1β, EL protein accumulated progressively in the media. In untreated cells, EL protein was present at concentrations of 68 kDa and 40 kDa. In cells treated with IL-1β, expression of EL protein was substantially increased compared with untreated cells. The increase in EL protein was apparent by 12 hours, and the difference between IL-1β–treated and untreated cells increased over the 72 hours. In cells treated with TNF-α, a similar response was seen. Thus, both TNF-α and IL-1β treatment resulted in substantial increases in EL protein in the media, consistent with the increase in lipase activities induced by these cytokines.
abundantly in the preheparin culture medium, but primarily the 68-kDa form of EL was detected in the heparin-released medium and was significantly increased by cytokine treatment. This suggests that the 40-kDa form, which probably results from proteolytic cleavage of the 68-kDa form, may have less affinity for the cell surface. There was a dose-dependent increase in the 68-kDa form of EL in both IL-1β–treated and TNF-α–treated cells compared with untreated cells (see online Figure, available in the online data supplement at http://www.circresaha.org).

EL Expression Is Also Upregulated by Cytokines in Microvascular Endothelial Cells

To determine whether other types of endothelial cells also respond to cytokines by upregulating EL expression, we selected two different types of microvascular endothelial cells for additional studies. HDMVECs, HUtMVECs, and HUVECs were treated with IL-1β (1 ng/mL) and TNF-α (10 ng/mL) for 24 hours. The media were analyzed by Western blotting with the anti-hEL peptide antibody. Both IL-1β and TNF-α significantly increased the amount of EL protein in the media of the two types of microvascular endothelial cells (Figure 5A). There was also an increase in EL mRNA for both IL-1β–treated and TNF-α–treated cells (Figure 5B).

Cytokines Do Not Alter EL mRNA Stability

To determine whether cytokines affected the steady-state level of EL mRNA by increasing its stability, we measured EL mRNA using real-time PCR in the presence of actinomycin D (5 μg/mL) in HUVECs. The EL mRNA half-life in HUVECs did not change after treatment with IL-1β (1 ng/mL) or TNF-α (10 ng/mL) (Figure 6). Thus, the cytokine-induced increase in the level of EL mRNAs in endothelial cells was not the result of an increase in the stability of the mRNA. Of note, the use of actinomycin D in this study does not exclude the possibility that it inhibits mRNAs or proteins downstream that influence EL mRNA stability. The cytokine effect is presumably transcriptional, but using EL promoter-reporter gene constructs, we were unable to detect cytokine-responsive elements with up to 3 kb of EL promoter (data not shown).
Induction of EL Expression by Cytokines Is Partly Dependent on Protein Synthesis

To determine whether the increase in EL mRNA required protein synthesis, we pretreated HUVECs with the protein synthesis inhibitor cycloheximide at a concentration of 5 μg/mL. Cycloheximide alone had little effect on EL mRNA abundance at this dose. However, pretreatment with cycloheximide markedly blunted the ability of cytokines to induce EL mRNA expression (Figure 7A), indicating that the effect of cytokines on EL in HUVECs depends at least partly on new protein synthesis. VCAM-1 mRNA induced by cytokines was moderately decreased after cycloheximide treatment (Figure 7B), which is consistent with a previous report.15

NF-κB Pathway Is Involved in the Induction of EL mRNA by Cytokines

To define further the signaling pathway(s) responsible for upregulation of EL mRNA by cytokines, we treated HUVECs with agents that antagonize the transcriptional effects of cytokines on gene expression: SN50 (an NF-κB pathway inhibitor),16 SB 20358 (a p38 MARK–specific inhibitor),17 PD 98059 (a p42/44 MARK–specific inhibitor),18 GF 109203X (a PKC inhibitor),19 and D609 (a PC-PLC–activity inhibitor).20 None of these compounds except SN50 had any effect on the induction of EL expression by cytokines. At 50 μg/mL for 24 h, SN50 diminished but did not abolish EL mRNA expression induced by cytokines (Figure 8A). The upregulation of VCAM-1 mRNA, which is known to be

Figure 5. Effect of cytokines on the expression of EL by HDM-VECs, HUVECs, and HUtMVECs. HDMVECs, HUVECs, and HUtMVECs were cultured for 24 hours in the absence (Unt) or presence of cytokines (IL-1β, 1 ng/mL or TNF-α, 10 ng/mL). Media were collected and subjected to Western blotting (A). RNA was extracted and subjected to Northern blotting (B).

Figure 6. Effect of cytokines on EL mRNA half-life. HUVECs were cultured for 24 hours in the absence or presence of cytokines (IL-1β, 1 ng/mL or TNF-α, 10 ng/mL) before addition of actinomycin D (5 μg/mL), after which EL was detected at the indicated times by real-time PCR. The corrected data were then plotted as a fraction of the 0-hour value against time. Results represent the mean±SD from 3 independent experiments.

Figure 7. Effect of protein-synthesis inhibition on regulation of EL mRNA by cytokines in HUVECs. Confluent HUVECs were pretreated with vehicle or cycloheximide (CHX, 5 μg/mL) for 60 minutes and then treated for 24 hours in the absence (Unt) or presence of cytokines (IL-1β, 1 ng/mL or TNF-α, 10 ng/mL), after which EL (A) and VCAM-1 mRNA (B) were detected. Data are presented as the fold change compared with the untreated group. Results represent the mean±SD from 3 independent experiments.

Figure 8. Effect of SN50 treatment on EL mRNA regulation by cytokines. HUVECs were cultured for 24 hours in the absence (Unt) or presence of cytokines (IL-1β, 1 ng/mL or TNF-α, 10 ng/mL) with or without SN50, after which EL (A) and VCAM-1 mRNA (B) were detected. Data are presented as the fold change compared with the untreated group. Results represent the mean±SD from 3 independent experiments.
upregulated via the NF-κB pathway induced by cytokines, was abolished after SN50 treatment (Figure 8B).

Discussion

This report is the first to document that endothelial cells secrete both triglyceride lipase and phospholipase activities in response to the inflammatory cytokines IL-1β and TNF-α. Both lipolytic activities were specifically inhibited by antibody to endothelial lipase. Western blotting confirmed the cytokine-stimulated secretion of EL protein. This regulation appears to be at least in part at the level of mRNA abundance, because EL mRNA levels were increased, consistent with a recent report.11 These results indicate that endothelial cells secrete both triglyceride lipase and phospholipase activity in response to cytokines and that EL is the major source of both lipase activities.

We explored various mechanisms that might be responsible for the effect of IL-1β and TNF-α on the induction of the EL mRNA expression. New protein synthesis was necessary for effective upregulation of EL mRNA by cytokines. Cytokines had little effect on EL mRNA half-life, which suggests that they probably increase the rate of transcription of the EL gene. Therefore, we examined the effects of agents known to modulate transcriptional responses of IL-1β and TNF-α. Of these agents, only SN50, an inhibitor of the NF-κB pathway, diminished upregulation of EL mRNA expression by cytokines, suggesting that this pathway might be involved. NF-κB proteins are known to play a central role in endothelial cell activation by cytokines. This pathway is rapidly activated by cytokines, with a peak at 30 minutes. Although the kinetics of EL induction in endothelial cells by cytokines is not congruent with the kinetics of change of NF-κB, we speculate that NF-κB might be involved in initiating the effect of cytokines, perhaps via induction of other factors that ultimately mediate the upregulation of EL. Also, a short exposure (2 hours) of endothelial cells to cytokines was sufficient to elicit the late induction of EL mRNA (unpublished data). In experiments not presented here, we examined the effect of cytokines on reporter gene expression under the control of 3 kb of the 5′ flanking sequence of the EL gene (which contains two consensus NF-κB binding sites) in transient transfection assays of HUVECs. No significant change of reporter gene expression was observed. The lack of effect of cytokines on this construct suggests that cytokines exert their transcriptional effects on EL, either directly or indirectly, through DNA elements outside the cloned 3 kb of 5′ flanking sequence.

The upregulation of EL by cytokines is in distinct contrast to the two other major members of this gene family, LPL and HL, both of which are downregulated by inflammatory cytokines. LPL activity in vivo is reduced in inflammatory conditions.1,21 and inflammatory cytokines downregulate LPL expression in vitro.21 LPL hydrolyzes triglycerides in triglyceride-rich lipoproteins, generating free fatty acids for energy use by skeletal and cardiac muscle and for conversion to triglyceride and storage by adipocytes, thus playing an important role in energy homeostasis and catabolism of triglyceride-rich lipoproteins.22 Macrophages also express LPL, the activity of which may provide a source of fatty acids for energy utilization.23 The downregulation of LPL by inflammatory cytokines is thought to be at least one explanation for the hypertriglyceridemia associated with inflammatory conditions.24 Although there is less information about HL, administration of lipopolysaccharide and IL-1β to hamsters also resulted in marked downregulation of HL expression.25 HL hydrolyzes triglycerides in remnant lipoproteins, and its downregulation in inflammation could also contribute to elevated triglycerides in inflammation. During acute inflammation, energy needs are increased. It is possible that EL is upregulated in endothelial cells at local sites of inflammation as a way of generating fatty acids derived from lipoproteins to be utilized by local tissues (including possibly the endothelial cells themselves) as an alternative energy source. Endothelial cells abundantly express CD36, which directly binds lipoproteins26 and has an important role in cellular uptake of fatty acids.27,28 Furthermore, other tissues that express EL, such as the thyroid, the gonads, and even macrophages,29 might utilize fatty acids generated from lipoprotein triglycerides and phospholipids by EL for energy. Therefore, it is reasonable that, under certain systemic inflammatory conditions, the coordinated downregulation of LPL and HL and upregulation of EL is a result of a physiological need to direct lipoprotein-derived fatty acids away from skeletal muscle, adipose tissue, and liver and toward local sites of inflammation at which EL is being expressed.

Acute inflammatory states, such as sepsis, are associated with profoundly reduced HDL cholesterol levels.30,31 Furthermore, chronic inflammatory states, such as rheumatoid arthritis and systemic lupus, are also associated with reduced levels of HDL cholesterol.32 As noted above, HL, which has a known role in HDL metabolism, is downregulated by inflammation,25 an effect that would normally be expected to increase, not decrease, HDL cholesterol levels. We previously demonstrated that overexpression of EL in mice markedly reduced plasma levels of HDL cholesterol and apolipoprotein A-I.17 The results presented here suggest the possibility that inflammation-induced upregulation of EL could be a causal factor in the reduced HDL cholesterol levels seen in inflammatory conditions. Atherosclerosis itself is increasingly recognized as a chronic inflammatory condition.33 Epidemiological studies have indicated that systemic markers of inflammation are associated with coronary events.34 Pathological studies have provided substantial evidence for inflammation within the atherosclerotic plaque.35 Our demonstration that inflammatory cytokines upregulate the endothelial expression of EL suggests that EL could be upregulated at the site of atherosclerotic lesions. It is intriguing to speculate that the inflammation associated with atherosclerosis, by upregulating local EL expression, may itself influence systemic lipoprotein metabolism.

In summary, endothelial cells secrete both triglyceride lipase and phospholipase activities in response to cytokines that is primarily a result of upregulation of EL. Both new protein synthesis and the NF-κB pathway mediate induction of EL expression. The upregulation of EL by cytokines is in sharp contrast to downregulation of the other two major members of this gene family, LPL and HL, and has implica-
tions for the physiological role of EL in inflammatory conditions and its potential role in the modulation of lipoprotein metabolism during inflammatory conditions, including atherosclerosis.

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References


Endothelial Cells Secrete Triglyceride Lipase and Phospholipase Activities in Response to Cytokines as a Result of Endothelial Lipase
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Figure legends

**Online Figure 1. Effect of cytokine dose on EL bound to the cell surface.** HUVECs were cultured in the presence of increasing concentrations of cytokines for 24 hours. Media were removed, monolayers were washed, and fresh media containing 10 units/mL of heparin were added. After an additional 30-minute incubation, these media were harvested and analyzed for EL protein using Western blotting. Panel A shows EL in IL-1β-treated cells; lanes 1 to 9 correspond to IL-1β concentrations of 0, 1 pg, 3 pg, 10 pg, 30 pg, 100 pg, 300 pg, 1 ng, 3 ng per mL medium, respectively. Panel B shows similar data for TNF-α-treated cells; lanes 1 to 8 correspond to TNF-α concentrations of 0, 10 pg, 30 pg, 100 pg, 300 pg, 1 ng, 3 ng, 10 ng per mL medium, respectively.
Online Figure 1

A

68kDa

40kDa

1 2 3 4 5 6 7 8 9

B

68kDa

40kDa

1 2 3 4 5 6 7 8