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Regulated Expression of Endothelial Cell-Derived Lipase

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A lipoprotein lipase-like gene was recently cloned from endothelial cells. *In vitro* functional experiments have suggested that this endothelial-derived lipase (EDL) has phospholipase activity, and preliminary *in vivo* studies have suggested a role in the regulation of high-density lipoprotein metabolism. To investigate local control of lipase activity and lipid metabolism in the blood vessel wall, we have examined the regulation of EDL expression in cultured human umbilical vein and coronary artery endothelial cells. EDL mRNA levels were upregulated in both cell types by inflammatory cytokines implicated in vascular disease etiology, including TNF-α and IL-1β. In addition, both fluid shear stress and cyclic stretch were found to increase the EDL mRNA levels in these cultured cells. This highly regulated expression of EDL in vascular endothelial cells suggests that this recently identified lipase is intricately involved in modulating vessel wall lipid metabolism and may play a role in vascular diseases such as atherosclerosis.

Key Words: **lipase; cytokine; shear stretch; cyclic stretch; mRNA; gene expression; lipoprotein; atherosclerosis; endothelium; lipids; vascular disease.**

Lipoprotein metabolism is central to the physiology of normal vascular function and the pathophysiology of atherosclerotic vascular disease. Lipoprotein lipase (LPL) and hepatic lipase (HL) play important roles in lipid metabolism by catalyzing the hydrolysis of native lipoproteins. The critical association between lipoprotein concentration and vascular disease, coupled with the central roles of LPL and HL as modulators of lipid levels, suggests an intimate relationship between these enzymes and the vasculature (1, 2). On one hand, overexpression of LPL is associated with reduced vascular disease in animal models (3), and specific LPL variant alleles have been linked to human vascular disease (4). On the other hand, the presence of LPL in the vascular wall, through local gene expression by macrophages, has been implicated in the genesis and progression of atherosclerosis (5). In this context, LPL has been hypothesized to immobilize lipid in the artery wall by serving as a bridge between lipoprotein particles and matrix proteoglycans, and to facilitate the receptor-mediated uptake of lipoproteins by macrophages (6).

Recently, a new member of the lipase gene family has been cloned from endothelial cells, and named endothelial cell-derived lipase (EDL) (7, 8). EDL did not have triglyceride hydrolysis activity, but showed phospholipase activity *in vitro* (7). Interestingly, overexpression of EDL by an adenovirus vector reduced plasma HDL cholesterol concentrations in mice, suggesting that EDL may modulate HDL metabolism (8). The cloning and characterization of EDL provided the first evidence for lipase production directly by vascular endothelial cells. Although both LPL and HL attach to endothelial cells and interact with lipoproteins at the endothelial cell surface, these molecules are not directly expressed by the endothelial cell. Whereas the majority of LPL is bound to the endothelial cell surfaces in muscle and adipose tissue, the majority of HL is bound to endothelial and hepatocyte surfaces in the liver. In contrast to LPL and HL, EDL has the capacity to respond to local alterations in the blood vessel wall, and thus could provide physiological adaptations to energy needs, etc. However, dysregulation of EDL expression in response to disease initiating signals, could promote or support disease processes such as atherosclerosis.

To investigate the parameters of EDL regulation in endothelial cells, we have performed cell culture experiments and measured mRNA levels in endothelial cells undergoing stimulation by disease related stimuli. In these studies we found that EDL mRNA levels are increased in two types of cultured endothelial cells when stimulated by TNF-α and IL-1β. Both of these

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cytokines have been implicated in vascular disease such as atherosclerosis and vascular remodeling. Two forms of mechanical forces felt to be involved in vascular disease were also investigated. Both fluid shear stress and cyclic stretch were found to increase EDL mRNA levels in endothelial cells.

EXPERIMENTAL PROCEDURES

Cell culture. Recombinant human TNF-α was obtained from R and D systems (Minneapolis, MN), and recombinant human IL-1β was kindly provided by Dr. James Topper (Stanford University). Human umbilical vein endothelial cells (HUVEC) and human coronary artery endothelial cells (HCAEC) were obtained from Clonetics, Inc. (San Diego, CA). These cells were cultured under recommended conditions, exposed to IL-1β (10 U/ml), TNF-α (10 ng/ml), mechanical shear stress, or cyclic stretch in the presence of 5% fetal calf serum.

In vitro cyclic stretch. HUVEC were seeded on a sheet of flexible silicone rubber coated with a hydrophilic surface of collagen I (Sigma, St. Louis, MO) and this material horizontally stretched utilizing a specifically engineered device to impart cyclic stretch. After the cells reached confluence, the sheet was fixed on one side of the base plate and attached to a piston, which was driven by the camshaft of a variable speed monitor. Cells were then exposed to an increase of 6% stretch at a frequency of 60 Hz for 6 h. These experiments were performed in duplicate and repeated (three times), and similar results achieved.

Shear stress. To expose endothelial cells to shear stress, confluent monolayers were placed in 5% serum containing medium for 1 h and then exposed to static or flow conditions. Flow was produced by placement of confluent 100-mm culture dishes on a mixing table rotating at 120 rpm for 6–24 h as described previously (9, 10). Compared with well-defined cone-plate viscometer, this technique induced quantitatively similar changes in cell alignment. These experiments were performed in duplicate and repeated (three times), and similar results achieved.

Northern blotting. Total RNA was isolated from HUVEC and HCAEC by the acid-guanidinium phenol–chloroform method. For Northern blot analysis, 20 μg of total RNA was size fractionated on 1.3% agarose gels containing 2.2 M formaldehyde, and transferred to nylon membranes. These membranes were hybridized sequentially with a 514-base pair human EDL cDNA fragment and a human cyclophilin cDNA probe. The probes were labeled with [α-^{32}P]dCTP by random priming (Stratagene, La Jolla, CA), and hybridized at 42°C for 16–24 h in the presence of 48% formamide and 10% dextran sulfate. After hybridization, the membranes were washed at high stringency conditions; 65°C in the presence of 0.2× SSC buffer and 0.5% SDS. Visualization was achieved by exposure to Kodak Biomax MS film (Eastman Kodak, Rochester, NY).

Quantification of the EDL mRNA hybridization signal was achieved by comparison to cyclophilin signals using the AlphaImager 2200 (Alpha Innotech Co., San Leandro, CA).

RESULTS AND DISCUSSION

Because of the implications for vascular disease, lipase activity in the vessel wall has received considerable attention. LPL is localized and functions on the surface of endothelial cells, although LPL gene expression has never been identified in this cell type. LPL activity on the endothelial cell surface is associated with triglyceride metabolism and appears to have a protective effect against vascular disease (3). LPL activity has been identified within diseased vessel wall, and hypothesized to promote atherosclerosis by bridging between lipoprotein particles and matrix proteoglycans, thus retarding lipoprotein efflux from the artery (11). LPL gene expression has been identified in the vessel wall in macrophages, and LPL postulated to facilitate the receptor-mediated uptake of lipoproteins by macrophage. A striking association between macrophage LPL activity and susceptibility to develop atherosclerosis has been documented in inbred mouse lines (5), and high macrophage LPL is considered a potential risk factor for coronary artery disease (12). Given these potential links between lipase expression and activity in the vessel wall and vascular disease, the regulated expression of EDL was investigated.

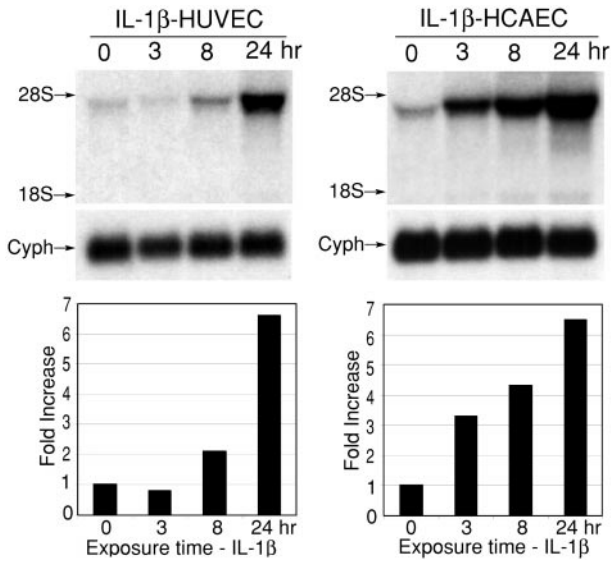

FIG. 1. EDL mRNA levels are increased by IL-1β in cultured endothelial cells. A human EDL cDNA fragment was hybridized to blots containing total RNA isolated from HUVEC and HCAEC. A dominant band observed at 4.4 kilobases reveals a time-dependent increase in EDL mRNA levels in stimulated cells. A maximum 6.6-fold (HUVEC) and 6.5-fold (HCAEC) increase were seen with IL-1β by 24 h. Abbreviations used: Cyph, cyclophilin; HUVEC, human umbilical vein endothelial cells; HCAEC, human coronary artery endothelial cells.

Atherosclerosis is now widely considered to be an inflammatory process (13). Cytokines are thought to have an important role in initiating the expression of a variety of genes that in turn promote cell adhesion and other processes that are required for disease progression. Two cytokines that have received the most attention in this regard are TNF-α and IL-1β. Thus the ability of endothelial cells to regulate EDL mRNA levels in response to these cytokines was investigated. To increase the relevance of these cell culture experiments, two different types of primarily cultured human endothelial cells were used, those derived from umbilical veins (HUVEC) and coronary arteries (HCAEC).

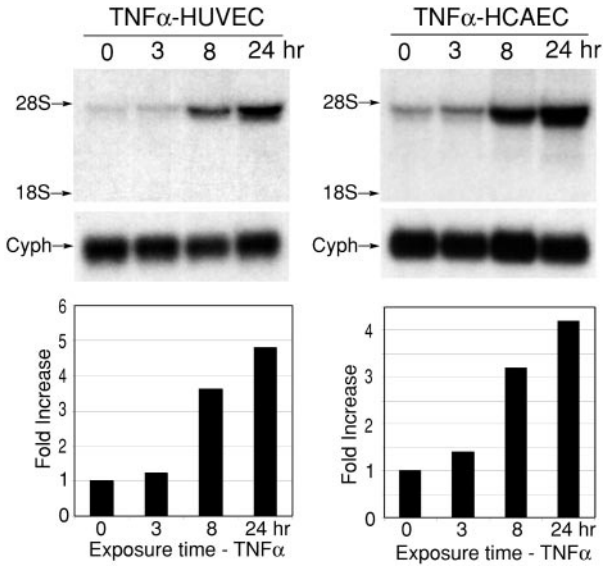

FIG. 2. EDL mRNA expression is induced by TNF-α in cultured endothelial cells. EDL mRNA levels were evaluated in RNA blots by hybridization to a human EDL cDNA probe. Total RNA was isolated from HUVEC and HCAEC. A time-dependent increase in EDL mRNA levels was observed in stimulated cells, with a maximum 4.8-fold (HUVEC) and 4.2-fold (HCAEC) increase seen with TNF-α by 24 h. Abbreviations used: Cyph, cyclophilin; HUVEC, human umbilical vein endothelial cells; HCAEC, human coronary artery endothelial cells.

Confluent monolayers of HUVEC and HCAEC were treated with recombinant IL-1β (10 U/ml) or TNF-α (10 ng/ml), total RNA was extracted and employed for Northern blots which were evaluated for EDL mRNA levels by hybridization (Figs. 1 and 2). These studies revealed a time-dependent increase in the levels of mRNA. For IL-1β, a response was observed after 3 h with the HCAEC, and after 8 h with the HUVEC. After 24 h, there was approximately a 6.5-fold overall increase in EDL levels, when EDL hybridization was normalized to expression of the constitutively active control gene cyclophilin. The increase in EDL mRNA levels was somewhat slower in response to TNF-α, requiring 8 h for a response. After 24 h, there was a 4.8-fold maximal increase for HUVEC and a 4.2-fold maximal response for HCAEC.

Numerous studies have linked physical forces in the vasculature with the pathogenesis of atherosclerosis or cellular processes that are felt to be essential for development of atherosclerosis (14). In particular, cyclic stretch or cyclic strain has been thought to represent the type of mechanical stimulation associated with aging and accentuated in hypertension. Regions of low shear stress have long been identified as areas of early atherosclerotic changes and the signaling pathways activated in endothelial cells by shear stress are currently felt to have an important role in the disease process (14). *In vitro* cell culture models have been designed to mimic both types of these physical forces,

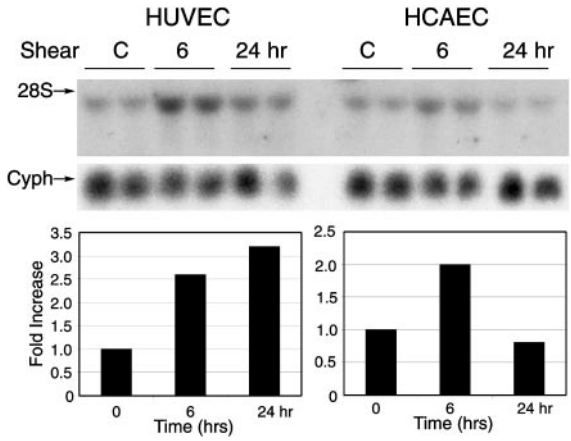

FIG. 3. EDL mRNA levels in endothelial cells are increased under conditions of shear stress. HUVEC and HCAEC were exposed to shear stress (12 dynes/cm^2) at two time points over 24 h and EDL mRNA levels evaluated by Northern blot. EDL message levels were found to be increased by 3.2-fold in HUVEC and by 2.0-fold in HCAEC when EDL signals were compared to those obtained by hybridization evaluating mRNA levels of the constitutively active control gene cyclophilin. Two separate experiments are shown. Abbreviations used: Cyph, cyclophilin; HUVEC, human umbilical vein endothelial cells; HCAEC, human coronary artery endothelial cells.

and were employed here to investigate the regulation of EDL in primary cultured human endothelial cells.

Both HUVEC and HCAEC were employed in these studies, and mRNA levels evaluated after cells had been exposed to the appropriate stimulus (Figs. 3 and 4). The model of shear stress employed in these studies transmitted to the cells 12 dynes/cm^2 of shear, and

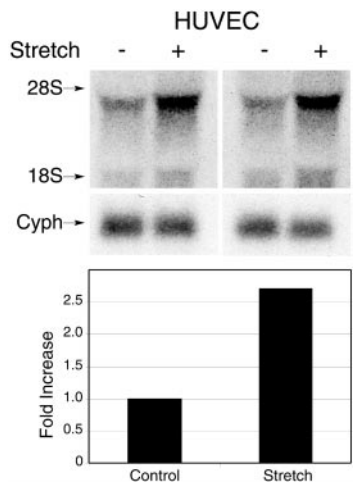

FIG. 4. EDL mRNA levels are increased by cyclic stretch. A human EDL cDNA fragment was hybridized to blots containing total RNA isolated from HUVEC exposed to cyclic stretch for 6 h. A dominant band observed at 4.4 kilobases reveals a 2.7-fold cyclic stretch-related increase in mRNA levels when the EDL signal was normalized to that observed for the constitutively active cyclophilin gene. Two separate experiments are shown. Abbreviations used: Cyph, cyclophilin; HUVEC, human umbilical vein endothelial cells.

endothelial cells were evaluated at time points between 6 and 24 h. Levels of EDL mRNA were increased by 3.2 folds in HUVEC at 24 h and by 2.0-fold in HCAEC at 6 h. A cellular response to shear stress was verified by typical cell alignment along the direction of shear stress at the 24-h time point, although cell alignment was not evident at 6 h. For exposure to cyclic stretch, HUVEC were grown on silicon rubber coated with collagen, subjected to a 6% stretch at a frequency of 60 Hz for 6 h, and mRNA levels subsequently evaluated by Northern blotting. These studies revealed a 2.7-fold increase in EDL mRNA levels over this time period when comparison was made to the constitutively expressed control gene cyclophilin.

These *in vitro* data suggest that EDL expression in endothelial cells is highly regulated *in vivo* by cytokines and physical forces widely felt to be involved in the genesis of vascular disease. In addition, recent immunohistochemistry data has verified that EDL is expressed in the arterial vessel wall endothelium *in vivo* (K. Hirata, unpublished data). Thus EDL is likely to have a significant role in regulating lipid metabolism in the vessel wall. The endothelial expression of EDL provides for local control of gene expression, allowing the vessel to participate in local lipid metabolism. Since the exact function of this novel lipase is poorly understood at this point, it is difficult to predict whether EDL expression will support or protect against the development of vascular disease. It has been reported that overexpression of LPL in apolipoprotein E knockout mice shows protection against atherosclerosis (15). In this context, it is attractive to speculate that EDL may modulate the atherosclerotic process by facilitating cholesterol exchange between lipoproteins and the vessel wall.

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