thematic review

Endothelial lipase: a new lipase on the block

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Abstract Endothelial lipase (EL) is a newly described member of the triglyceride lipase gene family. It has a considerable molecular homology with lipoprotein lipase (LPL) (44%) and hepatic lipase (HL) (41%). Unlike LPL and HL, this enzyme is synthesized by endothelial cells and functions at the site where it is synthesized. Furthermore, its tissue distribution is different from that of LPL and HL. As a lipase, EL has primarily phospholipase A1 activity. Animals that overexpress EL showed reduced HDL cholesterol levels. Conversely, animals that are deficient in EL showed a marked elevation in HDL cholesterol levels, suggesting that it plays a physiologic role in HDL metabolism. Unlike LPL and HL, EL is located in the vascular endothelial cells and its expression is highly regulated by cytokines and physical forces, suggesting that it may play a role in the development of atherosclerosis. However, there is only a limited amount of information available about this enzyme. Some of our unpublished data in addition to previously published data support the possibility that the enzyme plays a role in the formation of atherosclerotic lesion.—Choi, S. Y., K-i. Hirata, T. Ishida, T. Quertermous, and A. D. Cooper. **Endothelial lipase: A new lipase on the block. *J. Lipid Res.* 2002.** 43: **1763–1769.**

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Lipid esters provide a means of allowing the storage and transport of a large variety of molecules of nutritional and biologic importance. However, the hydrophobicity of these compounds requires that they be modified before the constituent fatty acids can be transported across membranes or used for energy. Thus, a series of synthases and lipases have evolved for the purpose of assembling and disassembling lipid esters.

Pancreatic lipase was the first triglyceride lipase characterized. When molecular studies revealed its homology to lipoprotein lipase (LPL) and hepatic lipase (HL) (1–4) it might have been assumed that this family would consist of enzymes that hydrolyzed triglycerides as their primary substrate (5, 6). The discovery of pancreatic lipase-related protein 2 further supported this possibility (7). More recently, the discovery of a homologous enzyme that has phosphatidylserine as its primary substrate (8), however, was inconsistent with this notion. The enzyme that is the subject of this review endothelial lipase (EL), also has almost exclusively phospholipase activity (9, 10) and most recently a phosphatidic acid selective phospholipase A1 that was found by a homology search of the phosphatidylserine esterase has been described (11). Thus, the family should be thought of as glycerol-*sn*-1-fatty acid hydrolases. Remarkable about the enzymes is their disparate and relatively organ specific locations, which suggests that they may have evolved to play relatively specific roles in the metabolism of a particular organ. In addition to contribution to lipid metabolism in a particular organ, two of the enzymes, LPL and HL, have been proven to affect lipoprotein levels and distribution among classes. EL has now potentially joined this group.

The presence of EL on the endothelium lining blood vessels in numerous organs, its presence in macrophages, and the report that its transcription is induced by inflammatory signals (12) raises the possibility that this enzyme may play a role in the response of the vascular wall to injury and thus plays a direct role in the formation of atherosclerotic lesions. Although currently there is a limited amount of information about the enzyme, it is likely that a great deal more will be learned in the near future.

CLONING AND GENE STRUCTURE

EL was cloned by two groups using similar methodologies but addressing very different biological questions.

Abbreviations: EL, endothelial lipase; HCAEC, human coronary aorta endothelial cells; HL, hepatic lipase; HUVEC, human umbilical vein endothelial cells; LPL, lipoprotein lipase; oxLDL, oxidized LDL.

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Our group employed suppression subtraction hybridization to characterize the genetic basis for endothelial cell mediated vascular formation (10). Cultured human umbilical vein endothelial cells (HUVEC) were employed in an in vitro model of tube development on Matrigel. The cloning strategy was to isolate genes that were preferentially expressed by tube forming HUVEC compared to growth-arrested HUVEC in monolayers. Approximately 600 partial cDNA fragments isolated through this process were characterized by nucleotide sequencing, and two cDNA fragments isolated appeared to encode a novel LPL-like gene. Upregulation of the expression of this gene during tube formation was verified by RNA blot experiments. Cloning of the primary human transcript was accomplished by screening a HUVEC lambdaphage cDNA library, and the highly homologous murine transcript was characterized through cloning and sequencing cDNAs from a mouse 11-day embryo cDNA library (10).

A second group cloned the gene using differential display methodology seeking to identify genes that were differentially regulated in macrophage like cells by oxidized LDL (oxLDL) (9). Their in vitro model was THP-1 cells that were differentiated by treatment with PMA. Differentiated THP-1 cells were exposed to either control medium or medium containing oxLDL, and RNA isolated for cDNA synthesis and amplification by polymerase chain reaction. Amplified products were fractionated on a gel and those differentially and specifically expressed in the oxLDL condition isolated and characterized by nucleotide sequencing. Completion of cloning the human transcript was accomplished by 5′-rapid amplification of cDNA ends (RACE) methodology and screening of a human placental cDNA library.

Comparison of the primary sequence of the conceptual human and mouse EL orthologs to the primary sequence of other lipases reveals considerable evolutionary conservation of structure, suggesting conservation of function. Human EL was noted to be 44% identical to human LPL and 41% identical to human HL, with considerable conservative substitutions of non-identical residues. The human and mouse EL proteins were predicted to have a signal sequence, and thus to be secreted proteins in keeping with extracellular function of the similar LPL and HL proteins. Cleavage of the leader sequence was predicted to result in 482 amino acid mature human and murine lipase molecules. Alignment with the human LPL and HL amino acid sequences revealed conservation of the catalytic residues serine (Ser 169), aspartic acid (Asp 193) and histidine (His 274), as well as the 10 cysteine residues involved in disulfide bridge formation (13). Similarly, two stretches of hydrophobic amino acids (163–172 and 272–281) that are adjacent to the catalytic serine and histidine respectively are also conserved. Conservation of these residues is critical since they are felt to be important for lipase interaction with the lipid substrate. LPL and HL both possess lid regions that are important for substrate specificity. By analogy with the predicted three-dimensional structures of LPL and HL, EL has a lid region that probably covers a catalytic pocket and serves to confer substrate specificity for EL (14, 15). Since the EL lid region has minimal sequence homology with the LPL and HL lids, it was assumed from the outset that EL would have different substrate specificity from the other mammalian lipases. Despite the significant differences in the lid region, EL showed striking conservation of sequence to LPL and HL in the regions bordering the lid. In addition, alignment with the human LPL sequence indicated conservation of positively charged clusters involved in heparin binding (13). The corresponding clusters in EL include (cluster 1: Arg 327-Lys 329-Arg 330-Lys 333; cluster 2: Arg 312- Lys 313-Arg 315; cluster 3: Gly184- Arg188; and cluster 4: Arg 450- Lys 452-Lys 459). Finally, five potential glycosylation sites are predicted by the presence of the universal acceptor sequence Asn-X-(Thr-Ser) at positions 80, 136, 393, 469, and 491. These glycosylation sites may modulate the heparin binding properties of EL.

There is evidence for at least one alternative EL isoform that results from protein processing. Study of native protein synthesized and secreted by cultured HUVEC and human coronary artery endothelial cells (HCAEC) was conducted with a polyclonal antiserum directed against a peptide encoding amino acids 8–23 of the secreted protein (9). These studies identified immunoreactive proteins of 55 kDa, 68 kDa, and 40 kDa. Since the dominant band of 55 kDa correlated with the predicted molecular mass of the 482 residue mature protein, this species was considered to represent the native non-glycosylated form of the enzyme. The large species was considered to represent the primary glycosylated species. Based on their characterization of the 40 kDa protein, it was concluded that it most likely represented a proteolytic protein product of the native protein. This conclusion was supported by expression experiments in COS cells employing a cDNA construct, thus obviating the possibility of alternative splicing as a potential mechanism. Such a truncated form of the protein would obviously have a very different functional profile from the complete mature protein. Further experiments employing antibodies directed against the carboxyl terminus will be required to validate these observations, and studies employing homogeneous preparations of the truncated protein in functional assays will provide insights into the potential biological functions of this alternatively processed protein.

Diversity of EL proteins is also generated at the genetic level by alterations in transcripts and by alterations in splicing patterns of the transcripts. Evidence for different transcripts was first provided through cloning experiments that were aimed at characterizing EL variants expressed by cytokine activated cultured endothelial cells (12). Through these efforts, a unique alternative 5′-untranslated sequence was identified (Ishida et al., unpublished observations). Although this alternative sequence is similar at the nucleotide level to the previously described 5′-untranslated and the 5′-most portion of translated sequence, it does not encode an open reading frame. A methionine that serves as an internal residue in the original described transcript could serve as an initiating methionine and produce a truncated EL protein by

translation of the alternative transcript. Importantly, the novel variant protein that would be produced in this way would not have a signal sequence, and thus would not be predicted to be secreted from the cell. That this variant protein is produced in vivo, and that it is not secreted has been verified through experiments in cultured cells (Ishida et al., unpublished observations). The alternative 5′ sequence was identified as resulting from a previously unrecognized exon in the human EL locus on chromosome 18q21.1. While the architecture of the EL transcriptional regulatory sequences has not yet been defined, it would seem likely that these two mutually exclusive exons were evolved to allow for the production of both secreted and non-secreted forms of EL and to allow for different patterns of cell-specific constitutive and regulated expression through the use of alternative promoters. Some evidence for this possibility has been provided by reverse transcriptase polymerase chain reaction experiments employing human tissue RNA samples, which have shown that transcripts containing the two alternative exons are expressed at different levels in different tissues. Interestingly, there is no evidence that this alternative promoter exists in the mouse genome. In addition to the complexity introduced by these features of the EL locus, significant use is made of alternative splicing to generate considerable diversity of potential protein products. Predicted EL variants have been identified that are missing various functional portions of the classical lipase molecule, including the lid region as well as some of the critical catalytic amino acid residues. Understanding of the functional roles of these variant molecules will require specific details of the expression patterns and functional profiles of these EL variants.

ENZYMATIC ACTIVITY

The identification of EL as a new lipase family member was somewhat unexpected since its existence had not been predicted by studies of lipid metabolism. Careful evaluation of the function of this new lipase is thus critically important in order to establish its role within the context of the other known lipase molecules. Although alignment of EL with the human LPL and HL amino acid sequences revealed conservation of the catalytic triads, EL has a minimal homology in the lid domain (9, 10). The lid domain of lipases is known to be critical in determining substrate specificity (14–18) as shown with guinea pig phospholipase and rat pancreatic lipase-related protein 2 that have a high molecular homology but show a quite different substrate specificity from human pancreatic lipase (19, 20). These data suggested that the substrate specificity of EL might be different from those of LPL and HL. Thus, we have begun to characterize EL as a lipase using stably transfected COS cells with the human EL cDNA. Using media collected from the transfected cells, we reported that, unlike LPL or HL, EL did not hydrolyze triolein in the presence or absence of apolipoprotein C-II (apoC-II) (10).

Triglyceridase activity of EL was also studied by Jaye et al. (9) and more recently by McCoy et al. (21) using medium from cells infected with recombinant adenoviruses encoding the human EL cDNA. In contrast to the initial report by Jaye and colleagues in which EL has virtually no triglyceridase activity (9), McCoy et al. (21) reported that EL has detectable triglyceridase activity and does not require apoC-II for activity. Unlike LPL, the activity was not inhibited by the presence of high salt (i.e., 1 M). Furthermore, the hydrolytic activity was inhibited by the presence of serum in a dose-dependent manner.

Our laboratory and others reported that EL has a high level of phospholipase A1 activity when radiolabeled phosphatidylcholine was used as a substrate (9, 10). In the recent report of McCoy et al. (21), the ratio of triglyceridase to phospholipase activity of EL was 0.65 compared with ratios of 24.1 for HL and 139.9 for LPL. Thus, EL is primarily a phospholipase with a minimal, if any, physiologically relevant triglyceridase activity in the presence of serum. These data were further confirmed by *in vivo* studies in which mice overexpressing EL appear to have a substantially higher phospholipase A1 activity in post-heparin plasma as compared to wild-type or knockout mice (unpublished observations) and lower HDL levels. EL may account for most of the phospholipase activity in HL deficient patients (Choi, Zambon, and Brunzell, unpublished observations).

Phospholipase A1 enzymes hydrolyze the *sn*-1 fatty acids from phospholipid substrates. Although phosphatidylcholine was used to determine the phospholipase activity of EL (9, 10), there is a distinct possibility that other glycerophospholipids such as phosphatidylserine and phosphatidylethanolamine will also be substrates. It is less likely that sphingolipids will be hydrolyzed by this enzyme. It is not yet known whether there are fatty acid preferences for the enzyme. Thus, further studies are required to identify the substrate profile for this enzyme using different phospholipid emulsions and to identify the serum factors affecting the triglyceridase activity.

TISSUE DISTRIBUTION

EL was identified as a product of endothelial cell gene expression and was present at a high level in embryonic endothelial cells but its level decreased with maturation. In the adult, mRNA was detected in a number of tissues including placenta, lung, liver, testis, thyroid, and ovary (10). Similar results were reported by Jaye et al. (9) using Northern blots.

The tissue-specific expression of EL in the adult is different from that of LPL and HL. LPL is expressed in muscle, adipose, heart, mammary gland, brain, and macrophages. HL is synthesized by the hepatocyte and remains there, adherent to both hepatocytes and endothelial cells or is transported to the endothelial cells in the adrenal glands and ovaries.

Because of the change in the location of expression from the developing vascular tree to specific organs the

question arises as to whether its processing in the adult is similar to HL and LPL where it moves from the parenchymal cell through the endothelial cell to the vascular surface. Because liver is the major organ in lipoprotein metabolism and EL expression was detected at a high level in the liver by Northern blot analysis, we studied the localization of EL in detail. Immunohistochemical analysis of mouse liver sections show that EL is expressed in the endothelial cells but not in the hepatocytes (unpublished observations). Expression of EL by hepatic endothelial cells was further confirmed by in situ hybridization in mouse and rat liver sections (unpublished observations). Thus, this enzyme is different from LPL or HL since EL functions at the site where it is synthesized, whereas both LPL and HL are synthesized at one site and translocated to another site where it functions. The difference in tissue distribution suggests distinct and perhaps nonoverlapping functions for HL and EL despite their similar localization and similar molecular sequences.

REGULATION OF EL EXPRESSION

The groups that cloned EL reported that EL is synthesized by the vascular endothelial cells and one suggested it can regulate the level of plasma HDL. Thus, the regulation of EL expression in response to disease initiating signals such as cytokines or cholesterol feeding was studied.

Atherosclerosis is an inflammatory process (22) and cytokines are thought to have an important role in initiating the expression of a variety of genes that 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 expression of EL by these cytokines in endothelial cells was determined by Quertermous and colleagues (12). When cultured human endothelial cells such as HUVEC and HCAEC were treated with IL-1β or TNFα, there was a significant increase (i.e., 4-fold) in EL mRNA levels. There have been a number of studies suggesting that physical forces in the vasculature are linked to the pathogenesis of atherosclerosis. Shear stress to the endothelial cells at 12 dynes/cm^2 of shear induced a 2-fold increase in HUVEC and HCAEC as measured by Northern blot analysis. Additionally, cyclic stretch increased EL mRNA levels by 2.7-fold. These data together suggest that EL expression in endothelial cells is highly regulated by cytokines and physical forces known to be involved in the development of atherosclerosis.

Plasma cholesterol levels are closely related to the development of cardiovascular disease. One of the mechanisms by which cholesterol level is associated with the development of CAD (coronary artery disease) is through influencing vascular endothelial function. We have tested whether feeding a high cholesterol diet can regulate the expression of EL in mice since EL is synthesized by the vascular endothelial cells. To do this we fed both wild type and apoE-deficient animals with a high fat diet and assessed the presence of EL in the liver and aorta using specific anti-EL antibodies. ApoE-deficient mice have substantially elevated levels of plasma cholesterol due to the impaired clearance of lipoproteins by the liver (23), and thus have been used as one of the animal models of atherosclerosis. Similarly, mice that overexpress apoE are resistant to lesion development upon cholesterol feeding. Our immunohistochemistry data suggest that EL is present in the aorta of wild-type mouse at a low level (unpublished observations) and the level of expression is elevated in apoE knockout mice (unpublished observations).

The regulation of EL expression by cytokines and by cholesterol feeding and the influence of EL on regulating the level of plasma lipoproteins suggest that EL could play an important role in the development of atherosclerotic lesions.

PHYSIOLOGIC ROLE OF EL

At this time the physiologic role of EL is not understood. Its localization, regulation, and the presence of intra and extracellular forms, however, provide some basis for speculation. Its effect on HDL may tempt some speculation regarding a role in lipoprotein metabolism and atherogenesis, but it is still premature to draw any conclusions.

The highest level of mRNA was detected in the thyroid gland (10). This organ has a high metabolic rate and a high rate of blood flow. Since it is constantly active and has no way to store fatty acids, it may use the fatty acids of lipoprotein-associated phospholipids, which are always abundant in the blood even during inter-prandial periods. Similarly the lung, which constantly must synthesize surfactant, has a continuous need for fatty acids, glycerolipids and phospholipids without having a significant storage capacity. Thus, in the lung it could provide both energy and a source of surfactant components (24).

Its abundance in the endothelial cells of the liver raises different possibilities since this organ can easily derive fatty acids from a variety of sources. It is tempting to speculate that it plays a role in removing the excess phospholipid that accumulates on the surface of remnants. Previously, it was postulated that all of this went to form HDL, (25, 26) but no quantitative studies were ever published. This remodeling may facilitate the ability of the remnant particles to acquire apoE, which is most abundant in the liver. Additionally, phospholipid hydrolysis by EL may result in the optimal exposure of apoE on the remnant surface. Consistent with this, Brasaemle et al. (27) previously reported that in vitro treatment of chylomicron remnants with HL resulted in increased exposure of apoE due to the hydrolysis of phospholipids on the particles.

It is not yet clear where the intracellular form of the enzyme is present. Specific antibodies will be necessary to study this. It is, however, tempting to speculate that such a form of EL may help dispose of the 1-acylglycerolphosphatidylcholine generated by the action of the numerous cellular phospholipase A2 enzymes.

Lastly, induction by cytokines and shear stress and elon-

gation (12) may allow it to help deliver fatty acids to areas of endothelial injury as a fuel source.

ROLE IN LIPOPROTEIN METABOLISM

Study of the association of serum lipid levels with atherosclerosis has led to the characterization of HDL level as an inverse correlate of coronary heart disease risk (28–30). Perhaps up to 70% of the variation in HDL cholesterol (HDL-C) levels in humans is genetically determined, but the genes contributing to this variation are incompletely defined. It is generally postulated that variation in plasma HDL-C levels are determined not by the rate at which they are produced, but rather by the rate of catabolism of their components. There are several known molecules that play a role in regulating plasma HDL-C levels (31), including HL (32–35), LPL (36–40), lecithin:cholesterol acyl transferase (LCAT) (41,42), cholesterol ester transfer protein (CETP) (43), and phospholipid transfer protein (PLTP) (44). Intense study over the last few years has focused on the HDL particle in the context of reverse cholesterol transport and anti-oxidant properties, providing significant insights into the mechanism of HDL's antiatherogenic properties. Despite significant insights gained through these studies, there continues to be large gaps in our understanding of HDL metabolism. Further studies are required to better understand how HDL particles may be manipulated with regard to both level and composition to affect the atherosclerotic disease process through therapeutic intervention.

Serum HDL-C levels are regulated in part by members of the lipase enzyme family. The lipases have highly conserved structural domains, and these enzymes function to metabolize triglycerides and phospholipids. Two members of this family, HL and LPL, are involved in the uptake and metabolism of lipids in circulating lipoprotein particles. Both genetic and metabolic studies indicate that HL levels are inversely related to plasma HDL-C concentration (34, 35, 45).

When EL was overexpressed by intravenous injection of adenovirus containing human EL cDNA, there was a significant reduction in plasma HDL levels (9). Recently, our group generated mice overexpressing EL in endothelial cells or lacking EL on the C57 B6 background and analyzed plasma lipoprotein profiles by fast protein liquid chromatography (FPLC) analysis. Mice that overexpress EL showed reduced HDL-C levels (unpublished observations). Conversely, mice that are deficient in EL showed a marked elevation in HDL-C levels. These data suggest that EL may play a physiologic role in HDL metabolism. Further studies are required to determine the mechanisms by which EL mediates HDL metabolism.

LPL acts on triglyceride rich lipoproteins while HL acts on IDL, LDL, and HDL to modify their size and perhaps behavior. Whether EL has a comparable role is open to speculation. It could, for example, play a role in disposing of extra surface phospholipid during lipolysis. Such studies will require the use of mouse models that have more human-like levels of VLDL and LDL.

In addition to their lipolytic properties, it has been postulated that LPL and HL have non-enzymatic effects that may accelerate the binding and perhaps uptake of apoB-containing lipoproteins. Similarly, HL affects selective cholesterol ester transport (46–49). It is reasonable to expect that EL shows at least some of these properties.

POTENTIAL ROLE OF EL IN ATHEROSCLEROSIS

Role of LPL in atherosclerosis was previously discussed in a comprehensive review by Goldberg (50). The anti-atherogenic effect is the result of its function in facilitating plasma lipoprotein uptake. The pro-atherogenic effect is suggested by the presence of LPL in macrophages (51–53) and increased accumulation of atherogenic lipoproteins such as LDL in the vessel wall (54–56). Similarly, studies suggesting pro- and anti-atherogenic roles of HL were previously reviewed by Santamarina-Fojo et al. (57). These data on HL and LPL together raise the possibility that EL may also have pro- and anti-atherogenic roles by affecting plasma lipoprotein metabolism and endothelial functions.

EL can influence the level of plasma HDL levels by remodeling the particles through its phospholipase activity. Our data in transgenic and knockout mice suggest that EL levels are inversely related to the HDL-C levels and plasma phospholipid concentrations. Further studies to look for polymorphism in the EL gene that affect HDL levels may be very revealing and contribute to our understanding of the genetic basis of HDL levels.

It is also possible that EL may influence the metabolism of apoB-containing lipoproteins through a non-enzymatic "bridging function" as shown for LPL and HL. The non-enzymatic action of LPL and HL on plasma lipoprotein metabolism has been studied by many laboratories including ours (58–65), and this action may require interactions of the enzyme with cell surface molecules such as apoE, proteoglycans, and receptors. Our preliminary data show that EL facilitated the uptake of proatherogenic lipoproteins such as LDL and chylomicron remnants by cultured cells (Grosskopf and Choi, unpublished observations). These data suggest that this enzyme may play a role in reducing the plasma levels of apoB-containing lipoproteins and thus plays an anti-atherogenic role. However, EL present in the vascular endothelial cell surface could promote the accumulation of LDL in the artery wall and thus increase the susceptibility to atherogenesis. Taken together these data suggest that, like LPL, EL may also function locally as a pro-atherogenic molecule.

In summary, the unexpected discovery of a new member of the lipase family that has primarily phospholipase A1 activity and is located on the vascular wall where it can contribute to the physiologic function of many organs and participate in vascular pathology has opened a new chapter in "lipaseology". The story, albeit not yet written, is certain to be interesting.

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