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PLASMA LIPID TRANSFER PROTEINS, HIGH-DENSITY LIPOPROTEINS, AND REVERSE CHOLESTEROL TRANSPORT

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ABSTRACT

Cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) are members of the lipid transfer/lipopolysaccharide binding protein gene family. Recently, the crystal structure of one of the members of the gene family, bactericidal permeability increasing protein, was solved, providing potential insights into the mechanisms of action of CETP and PLTP. These molecules contain intrinsic lipid binding sites and appear to act as carrier proteins that shuttle between lipoproteins to redistribute lipids. The phenotype of human CETP genetic deficiency states and CETP transgenic mice indicates that CETP plays a major role in the catabolism of high-density lipoprotein (HDL) cholesteryl esters and thereby influences the concentration, apolipoprotein content, and size of HDL particles in plasma. PLTP also appears to have an important role in determining HDL levels and speciation. Recent data indicate that genetic CETP deficiency is associated with an excess of coronary heart disease in humans, despite increased HDL levels. Also, CETP expression is anti-atherogenic in many mouse models, even while lowering HDL. These data tend to support the reverse cholesterol transport hypothesis, i.e. that anti-atherogenic properties of HDL are related to its role in reverse cholesterol transport. Recently, another key molecule involved in this pathway was identified, scavenger receptor BI; this mediates the selective uptake of HDL cholesteryl esters in the liver and thus constitutes a pathway of reverse cholesterol transport parallel to that mediated by CETP. Reflecting its role in reverse cholesterol transport, the CETP gene is up-regulated

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in peripheral tissues and liver in response to dietary or endogenous hypercholesterolemia. An analysis of the CETP proximal promoter indicates that it contains sterol regulatory elements highly homologous to those present in 3-hydroxy-3-methylglutaryl-coenzyme A reductase; the CETP gene is transactivated by the binding of SREBP-1 to these elements. A challenge for the future will be the manipulation of components of the reverse cholesterol transport pathway, such as CETP, PLTP, or scavenger receptor BI for therapeutic benefit.

CONTENTS

STRUCTURE AND FUNCTION OF CETP

CETP and PLTP as Members of the Lipid Transfer/LPS Binding Protein Gene Family—Implications for the Structure of CETP

Cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) are members of the lipid transfer/lipopolysaccharide binding protein gene family that also includes the lipopolysaccharide binding protein (LBP) and bactericidal/permeability increasing protein (BPI). For this reason, although they have different physiological functions, they also share striking biochemical similarities. Recent studies have shown that all four proteins bind lipopolysaccharide (39; PR Tobias, P Ulevitch, P Kussie, AR Tall, unpublished

observations) and phospholipids (11, 130), in addition to various other lipids. Another similarity is that although they are all soluble proteins, LBP, PLTP, and CETP are associated with plasma high-density lipoproteins (HDL) whereas BPI is found on the membranes of secretory granules of neutrophils. Two olfactory ligand binding protein genes are also members of this family, but their products have not yet been characterized (23).

Given the functional and structural similarities among members of this gene family, the recently solved crystal structure of BPI (11) is important because it will aid in the understanding of the structure-function relationships of plasma lipid transfer proteins.

BPI is an elongated molecule shaped like a boomerang (Figure 1). It has two domains with similar folds, which are shaped like barrels, and a central beta-sheet domain forming an interface between the barrels. Strikingly, in the solved structure of this protein, each barrel was found to have a pocket occupied by a molecule of phosphatidylcholine. It is possible that lipopolysaccharide binds to the same pockets, because in the LBP, phospholipid binding is coupled with release of bound lipopolysaccharide (130).

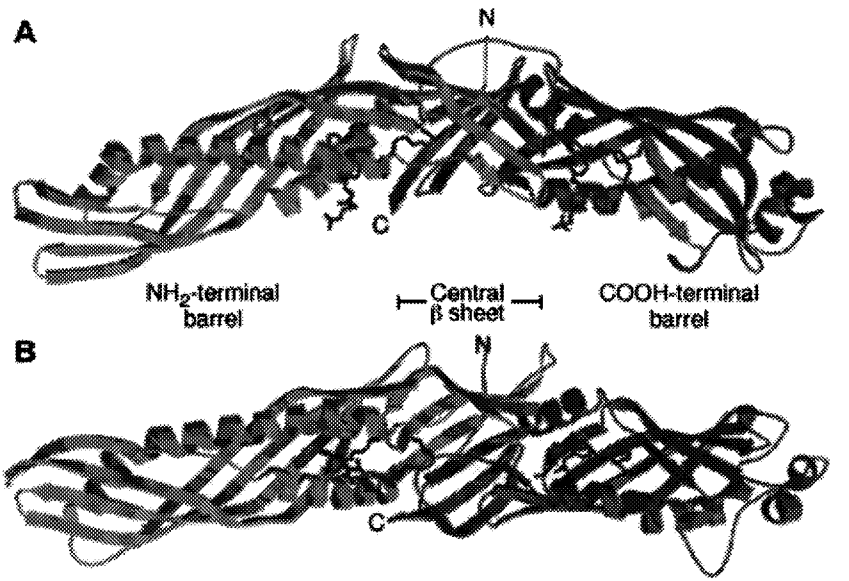


Figure 1 Ribbon diagram of bactericidal/permeability increasing protein from two angles. The NH_2 and COOH terminal domains are shown in *light and dark grey*, respectively. The two phosphatidylcholine molecules are shown as *stick models*. (Reprinted with permission from Reference 11. Copyright, 1997, of the American Association for the Advancement of Science.)

Several findings suggest that despite the relatively low sequence similarity between CETP and BPI (23% identity at the nucleotide and amino acid level), the overall structures of the two proteins are homologous. The proportion of secondary structure elements in CETP determined from circular dichroism measurements is similar (62, 87). The hydrodynamic properties of CETP are consistent with it being elongated in structure. One lysine and one arginine that are conserved in the gene family are required for the lipid transfer function of CETP and for HDL binding (58). Two cysteines that are critical to the function of BPI are also conserved in the gene family.

When the sequences of CETP and BPI are aligned, the C terminus of the 476 amino acid–long CETP extends by 12 extra residues. These residues are predicted to form an amphiphilic helix (124). They also define the epitope of a monoclonal antibody that inhibits the neutral lipid (but not phospholipid) transfer activity of CETP (122). Deletion of residues 470–475 causes a marked decrease of the V_{max} for neutral lipid transfer without influencing significantly the lipoprotein binding affinity of CETP (123). Also, phospholipid transfer activity in this deletion mutant is normal or increased (123). Significantly, the C terminus of BPI ends at the mouth of the lipid binding pocket in the N-terminal domain, which suggests that in CETP, the C-terminal helix could act as a lid at the entrance of this pocket.

The above results could indicate that in the N-terminal domain is the neutral lipid binding pocket and in the C-terminal domain is the phospholipid-binding pocket. Indeed, CETP copurifies with an equimolar amount of phosphatidylcholine (112), binds a maximum of 1.0 mol of cholesteryl ester (CE) (123), and has two distinct lipid binding sites (122). Other data, however, suggest that CE and triglycerides (TG) bind to CETP differently, because a monoclonal antibody and certain chemical inhibitors are reported to inhibit CE and TG transfer differently (26, 32, 84). However, these findings could reflect the larger size of TG over CE molecules and could arise from a greater sensitivity of TG molecules to steric hindrance by these agents. Moreover, CE and TG compete for transfer by CETP (85), which is inconsistent with their presence in independent binding sites.

Mechanism of Neutral Lipid Transfer

CETP can bind a variety of lipids: In addition to CE, TG, and phospholipids, it can bind lipopolysaccharide, cholesterol (21), retinyl esters (132), and esterified 25-hydroxycholesterol (71). Depending on the method of purification, 1.0 mol of CETP copurifies with 1.0 mol of cholesterol, 0.5 mol of TG, and 1.3 mol of phospholipid (21) or 1.0 mol of phosphatidylcholine (112). In addition to a variety of phospholipids (phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, phosphatidic acid), PLTP also transfers cholesterol, diacylglyceride, sphingomyelin, cerebroside, and alpha-tocopherol (64, 86, 98).

CETP, PLTP, and LBP transfer phospholipids but with different specificity. CETP can only exchange phospholipids, whereas PLTP can effect their net mass transfer (115). LBP apparently requires the accessory protein sCD14 to efficiently transfer phospholipids (130). PLTP is responsible for the majority of phospholipid transfer activity in plasma (17), whereas LBP may be playing a dominant role in the transfer of phosphatidylinositol (130).

Despite an earlier controversy, a large body of evidence indicates that lipids carried by CETP are exchanged between lipoprotein substrates by a shuttle (ping-pong) mechanism rather than by the formation of a ternary complex between CETP and donor and acceptor lipoproteins. Lipid transfer kinetic measurements are consistent with the former and not the latter mechanism (10, 21, 26). Furthermore, it is possible to demonstrate that CETP can acquire a labeled substrate lipid from a lipoprotein particle and then donate the labeled lipid to an acceptor lipoprotein (21, 110). As noted above, the crystal structure of BPI confirms that this class of molecules contains intrinsic lipid binding sites, likely involved in the transfer mechanism (11).

Both CETP and PLTP cause the conversion of HDL into larger- and smaller-sized particles (including pre-β HDL) in in vitro experiments. It appears that when the lipid composition of a lipoprotein is altered sufficiently by lipid transfer activity, its apolipoproteins are destabilized. In the case of PLTP, results are consistent with the loss of an apoAI molecule from HDL and the fusion of two remnant particles (72).

The nascent form of HDL is discoidal in shape and contains apoAI, phospholipids, and cholesterol but no CE. Such particles are believed to be the primary acceptors of the tissue cholesterol targeted for eventual delivery to the liver. These particles have pre-β electrophoretic mobility and are observed (along with enlarged particles) in vitro when either CETP or PLTP is incubated with HDL (67, 121). Moreover, increased amounts of pre-β HDL are found in transgenic mice overexpressing either CETP or PLTP, indicating that the transformation of α-HDL into pre-β HDL by lipid transfer proteins occurs in vivo (31, 55). However, the larger-sized HDL particles that appear with prolonged in vitro incubations with either PLTP or CETP are not seen in vivo, indicating they are either artifactual or rapidly removed in vivo. Under physiological conditions, pre-β HDL is converted into α-HDL by lecithyl cholesterol acyl transferase (LCAT) action. Thus, HDL may cycle between α and pre-β forms as it picks up and unloads cholesterol (67).

REGULATION OF CETP GENE EXPRESSION

In humans, CETP mRNA is expressed predominantly in the liver, spleen, and adipose tissue, with lower levels of expression in the small intestine, adrenal gland, kidney, skeletal muscle, and heart (112). Studies conducted on HepG2

cells and transgenic mice expressing human CETP suggest that hepatocytes are the major source of CETP. In rabbits, Kupffer cells also contribute significantly to CETP mRNA in liver (94). However, liver is not the major source of CETP in all mammalian species. For example, in hamsters, adipose tissue, skeletal muscle, and small intestine show the highest levels of CETP expression (60). Adipose tissue appears to be a highly conserved site of CETP expression across species.

In contrast to CETP, human PLTP shows the highest level of expression in the placenta, pancreas, adipose tissue, and lung, with intermediate levels in the liver, kidney, and heart (22, 57). A study of mice showed that PLTP mRNA expression has a similar tissue distribution and revealed brain and testis to show intermediate-level expression (57). Considering the mass of these tissues, the liver, adipose tissue, and lung likely contribute to most of the circulating PLTP mass in both species. PLTP expression in the lung may indicate a role for PLTP in lung function, possibly in the manufacture or delivery of surfactant.

Outside the plasma compartment, CETP can also be found in several interstitial fluids, including follicular and cerebrospinal fluid (6). Evidence suggests that the brain produces and secretes CETP into the cerebrospinal fluid, possibly playing a role in the redistribution of lipids within the central nervous system. CETP has been shown in vitro to play a role in sperm capacitation in follicular fluid (99). Although the mechanism for this is not well understood, it may involve changes in the lipid composition of the sperm plasma membrane as a result of CE transfer activity.

REGULATION OF CETP AND PLTP GENE EXPRESSION BY DIETARY AND ENDOGENOUS HYPERCHOLESTEROLEMIA

In experiments performed on rabbits, Son & Zilversmit showed that CETP plasma activity increases in response to a high-fat, high-cholesterol diet (106). This is largely due to an increase in the production rate and not to a decrease in the catabolism of CETP (82). This increase in CETP activity in response to an atherogenic diet is associated with an increase in hepatic mRNA abundance (96), which in CETP transgenic mice wholly accounts for increased plasma CETP activity (56). Although dietary cholesterol is likely the major factor contributing to this increase, synthetic diets high in saturated fats that lead to hypercholesterolemia also result in a similar increase in plasma CETP activity (68). Diets enriched in *trans*-fatty acids induce a more atherogenic lipoprotein profile: an increase in total plasma-cholesterol and low-density lipoprotein (LDL)-cholesterol and a decrease in HDL-cholesterol (2, 61, 120). Other studies in which mono- and polyunsaturated fats were substituted for saturated fats

showed a decrease in CETP activity as compared with diets high in saturated fats, but these diets failed to raise plasma-cholesterol levels to the same degree as high-saturated fat diets (38, 68). Substitution of one fatty acid for another can alter levels of CETP activity in the plasma, but the degree of the change in CETP activity correlates with the degree of change in the plasma lipoprotein profile (103). Fish oils such as eicosapentanoic acid diminish the increase in plasma-cholesterol by high-fat, high-cholesterol diets, with a decrease in the fold induction of CETP activity (1). Watanabe rabbits, which have a heritable hypercholesterolemia due to a defect in the LDL receptor, also showed a similar increase in CETP activity compared with wild-type rabbits, which suggests that endogenous hypercholesterolemia can also play a role in CETP gene regulation.

Transgenic mice have been made that express the human CETP gene under the regulation of its natural promoter (56). Except for somewhat lower expression in adipose tissue, these mice show a tissue distribution of CETP mRNA similar to that found in humans. This suggests authentic expression of the transgene. In response to a high-fat, high-cholesterol diet, these mice exhibit a marked increase in CETP mRNA in the liver, with an increase in plasma CETP mass and CE transfer activity. In contrast, mice expressing the same transgene under the control of the mouse metallothionein promoter show no change in CETP mRNA or mass in response to a hypercholesterolemic diet. Along with nuclear run-on assays, this indicates that the increase in CETP mass is due to an increase in CETP gene transcription and that the sequence(s) necessary for the increase in response to dietary cholesterol are located in the natural flanking regions. When crossed onto genetic backgrounds deficient in apolipoprotein E (apoE0) or LDL receptor (LDLr0), the mice exhibit a profound increase in hepatic CETP mRNA synthesis, with a corresponding increase in plasma CETP activity (79). When placed on a high-cholesterol diet, these animals exhibit a further increase in hepatic CETP mRNA (2-fold) and CETP plasma activity (3.3-fold). In the transgenic studies there is a strong correlation between plasma-cholesterol levels, plasma CETP levels (Figure 2), and hepatic CETP mRNA (79). That there is no increase in plasma CETP levels in metallothionein promoter-CETP transgenic mice under the same conditions indicates that increased plasma CETP are secondary to increased cholesterol levels. This probably explains the positive correlation between CETP levels and plasma-cholesterol and LDL-cholesterol levels in cross-sectional studies of human populations (38).

Taken together, these data indicate that the mechanism by which CETP gene transcription is regulated by cholesterol is independent of previously characterized receptor-mediated lipoprotein uptake (i.e. dependent on apoE or LDL receptor) and relies upon some unknown cellular sensor of plasma-cholesterol levels. It is possible that cells sense an increase in plasma membrane–free cholesterol mediated by diffusional processes, or an increase in cellular

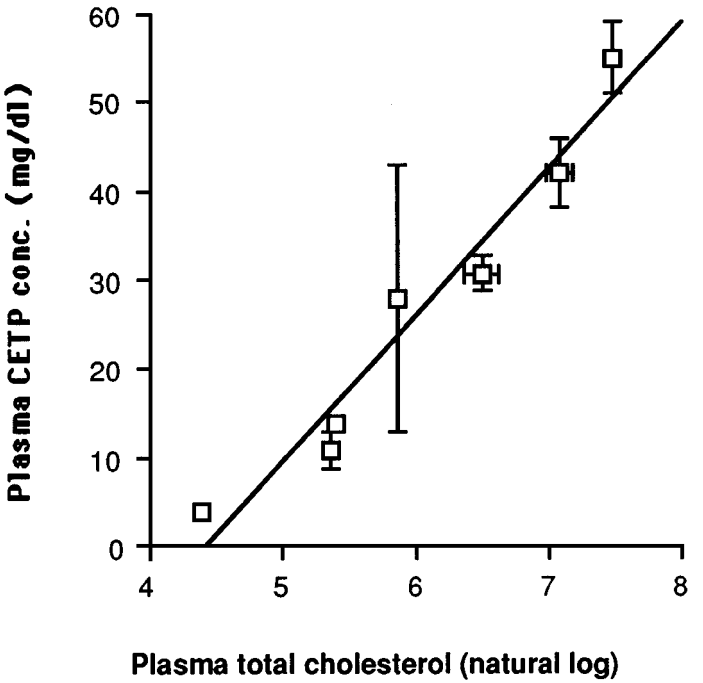


Figure 2 Mean plasma cholesteryl ester transfer protein (CETP) concentration correlated with plasma total cholesterol (expressed as natural log) in CETP transgenic mice with different genetic backgrounds and on different diets. (Reproduced from Reference 79 with permission. Copyright of the American Society of Clinical Investigation.)

cholesterol content due to selective uptake of lipoprotein lipids mediated by scavenger receptor BI (3).

Studies of transgenic mice have attempted to localize sequences in the natural flanking region that are responsive to sterols and play a role in tissue-specific expression of CETP mRNA (90). Deletion of the downstream flank shows no change in the response of the transgene to dietary cholesterol. Deletion of upstream sequences suggests that a 232-bp region between −138 to −370 bp upstream of the transcription start site contains one or more elements responsive to sterols. Another transgenic line containing an additional 200 bp of upstream flank (i.e. −138 bp to −570 bp) showed a greater fold increase in response to dietary cholesterol, indicating that there may be sequences in this region that also contribute to the cholesterol response. However, transgenic lines containing sequences farther upstream showed no greater response to cholesterol.

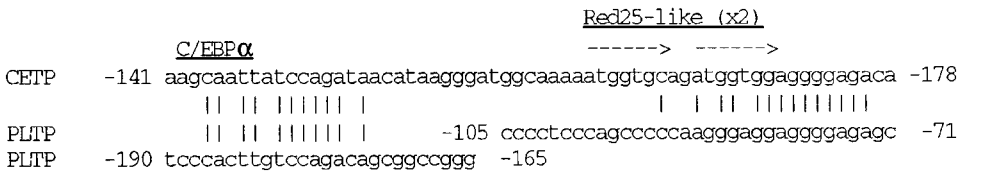


Figure 3 The Red25-like binding element and its vicinity in the promoter of cholesteryl ester transfer protein (CETP) is shown aligned with sequence elements in the proximal −1300 bp of the phospholipid transfer protein (PLTP) promoter with the greatest similarity to this region.

A comparison of the CETP promoter sequence between −138 to −370 bp with promoters of other genes responsive to sterols revealed a number of homologies. Perhaps most striking is a tandem repeat of a sequence with identity to the sterol regulatory element (SRE) in the 3-hydroxy-3-methylglutaryl-coenzyme A reductase promoter responsible for the sterol-mediated regulation of the reductase gene (91) (Figure 3). This element binds at least two proteins: SRE binding protein 1 (SREBP-1) (118) and Red25 (92), which has subsequently been shown to be the transcription factor YinYang-1 (YY-1). Point mutations in the reductase promoter that abolish binding of SREBP-1 to the SRE also abolish the sterol-mediated regulation of that gene.

Oligonucleotides corresponding to this element in the CETP promoter bind SREBP-1 and YY-1 in a specific manner (R Chouinard, A Tall, unpublished results). Furthermore, mutations that altered binding to the reductase promoter caused similar changes in binding in the CETP promoter. Transgenic mice having either the CETP minigene under the control of the natural promoter (NFR) or a promoter containing two point mutations that decrease the binding of SREBP-1a (MUT1) have been crossed with mice expressing the active, nuclear form of SREBP-1a under the control of the phosphoenolpyruvate carboxykinase (PEPCK) promoter (105). Induction of SREBP-1a expression resulting from a low-carbohydrate diet caused a profound induction of plasma CETP activity in SREBP-1/NFR CETP transgenic mice but not in SREBP-1/MUT1 CETP transgenic mice (R Chouinard, A Tall, unpublished results). This shows that SREBP-1 transactivates the CETP promoter in vivo. Point mutagenesis in the tandem repeat SREs of the CETP promoter abolishes this transactivation. However, the MUT1CETP transgenic mice have a normal or increased response to a high-cholesterol diet, indicating that other sequences in the CETP promoter mediate the positive response to dietary cholesterol. It appears that SREBP-1 contributes to basal expression on a chow diet but does not mediate the response to a high-cholesterol diet. The CETP promoter contains both positive and negative SRE, which may allow for a more finely tuned response to conditions

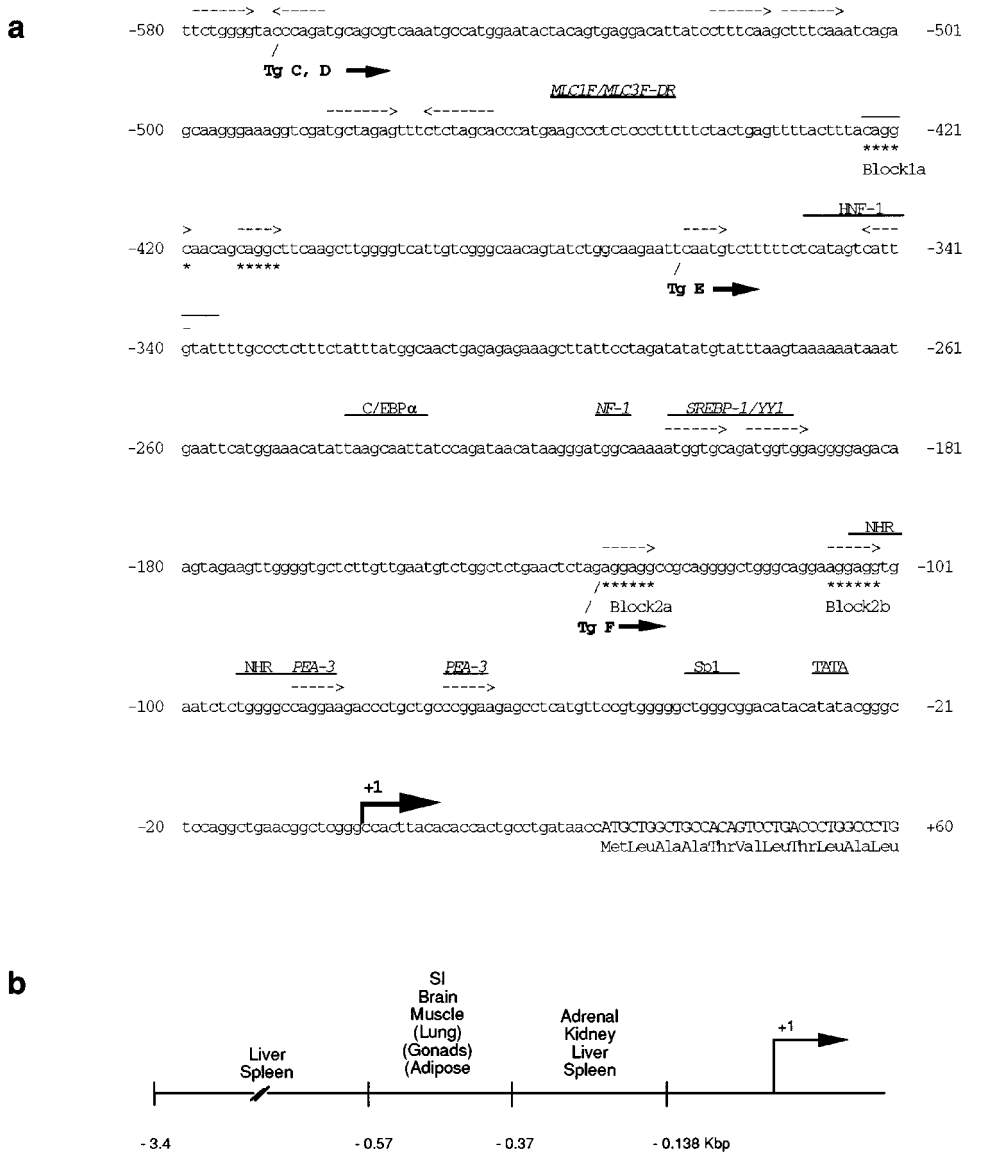
of sterol excess or depletion, or to independent regulation by nonsterol factors regulating SREBP-1.

Other studies have shown regulatory elements in the CETP promoter that are probably not involved in the sterol-mediated regulation of the gene. The CETP promoter contains a consensus binding sequence for the transcription factor CCAAT/enhancer binding protein alpha (C/EBPα) (Figures 3 and 4). Experiments with HepG2 cells have shown that expression of C/EBPα can transactivate a reporter gene under the control of the CETP promoter (5). C/EBPα is expressed in differentiated hepatocytes, but its mRNA decreases rapidly in primary hepatocyte cultures, concomitant with a decrease in levels of CETP mRNA. Therefore, it is likely that the loss of expression of C/EBPα contributes to the very low levels of CETP mRNA expression in HepG2 cells.

The orphan nuclear hormone receptor apolipoprotein AI regulatory protein-1 (ARP-1) binds a site located between -93 and -118 in the CETP promoter (34) (Figure 4). ARP-1 activates transcription of a reporter gene in a construct containing 300 bp of 5′ flank but represses transcription in a construct that contains 636 bp of 5′ flank. This suggests a dual role for this transcription factor, depending on the promoter context. Although minimal CETP promoter-reporter constructs are highly expressed in HepG2 cells, transgenic mice with the CETP gene under the control of the 138-bp promoter immediately upstream of the transcription start site (i.e. -138) showed no expression of CETP above background, indicating that this region is insufficient to support high-level expression of the CETP gene in vivo (90). Indeed, upstream elements out to -3 kb upstream are needed for predominant physiological expression in liver and spleen (90) (Figure 4).

PLTP in the mouse is also responsive to a high-cholesterol diet (57). Mice fed high-fat, high-cholesterol diets showed a significant increase in plasma phospholipid transfer activity, with a corresponding increase in PLTP mRNA in

Figure 4 (*a*) Complete nucleotide sequence between $+60$ and -580 of the human cholesteryl ester transfer protein (CETP) promoter region. Sequence elements experimentally shown to be functional are for a tandem repeat of SREBP-1/YY1, C/EBPα, a nuclear hormone receptor (NHR), and Sp1. (*italics*) Consensus elements for known transcription factors likely to be functional: PEA3, the muscle-specific element MLC1F, the ubiquitous nuclear factor-1 (NF-1), and the hepatocyte nuclear factor-1 (HNF-1). (*arrows*) Direct and inverted repeats; (*asterisks*) sequences found in the proximal promoter or rat, mouse, hamster, and human cholesterol 7a-hydroxylase, human phospholipid transfer protein, and human, rat, and mouse apoE. Tg C, D, E, and F, the 5′ ends of transgenes used to study the in vivo expression of CETP in transgenic mice. (*b*) Distribution of tissue-specific elements in the 3.4 kbp of the human CETP promoter. (*parentheses*) Presence in the tissue of regulatory elements with minor effect. (Reproduced from Reference 90 by permission. Copyright of the American Society for Biochemistry and Molecular Biology.)



the lung. Analysis of the PLTP promoter shows no strong homology to known SREs, even though the gene is up-regulated in response to cholesterol in a manner similar to CETP (Figure 3). This provides additional evidence that the SREs do not mediate the sterol up-regulation of CETP and PLTP gene expression.

In addition to dietary influences, CETP gene expression is also influenced by hormonal and inflammatory stimuli (114). Intraperitoneal injection of NFR-CETP transgenic mice with bacterial lipopolysaccharide (LPS) results in a rapid and profound decrease in hepatic CETP mRNA abundance as well as plasma CETP concentration (78). Corticosteroid administration also decreases CETP mRNA abundance similarly to LPS treatment. CETP transgenic mice that have undergone adrenalectomies fail to decrease hepatic CETP mRNA in response to LPS administration, indicating that adrenal corticosteroid release is required for the LPS response. Interestingly, the decrease in plasma CETP activity in response to LPS is out of proportion to the decrease in plasma CETP mass, and LPS added directly to plasma is a potent inhibitor of CETP activity. Thus, LPS regulates CETP indirectly via corticosteroids at the mRNA level and directly as an inhibitor of CETP in plasma. In hamsters, LPS also down-regulates CETP mRNA in the tissues with the highest CETP expression levels (adipose tissue, heart, and muscle) (40). However, administration of dexamethasone failed to mimic the response to endotoxin, although administration of tumor necrosis factor and interleukin-1 did decrease CETP levels (40). Thus, both corticosteroids and cytokines can contribute to the decrease in CETP gene expression after LPS exposure. The physiological basis for this phenomenon may be that it limits the fall in HDL levels in response to the hypertriglyceridemia induced by LPS. The transfer of LPS to HDL by LBP blunts the cellular response to LPS (107).

Probucol has been shown to increase CETP levels in humans and hamsters (97). In humans treated with probucol, plasma CETP concentration increases significantly in the face of a decrease in CETP mRNA in the peripheral adipose tissue, which suggests that probucol either increases CETP synthesis in another tissue, such as liver, or that it lowers plasma CETP turnover. Studies conducted on hamsters, where adipose tissue is the major organ of synthesis and liver plays only a minor role, showed that probucol treatment increases adipose CETP mRNA levels only in the context of increased dietary cholesterol, which suggests that the probucol effect may be mediated by alterations in a cellular regulatory pool of cholesterol.

Recently, probucol treatment has been shown to increase selective CE uptake in liver and adrenal via the scavenger receptor BI (SRBI) pathway (F Rinninger, AR Tall, unpublished data). This may explain the interaction of probucol and dietary cholesterol treatments. Increased dietary cholesterol increases lipoprotein-cholesterol content, whereas probucol enhances delivery of

this cholesterol to the cellular regulatory cholesterol pool via SRBI. This may alter expression of CETP and other sterol-regulated genes.

CETP ACTIVITY AND INSULIN RESISTANCE

Several studies of obese subjects and of patients with non–insulin dependent diabetes mellitus (NIDDM) have suggested a link between plasma CETP levels and insulin resistance. NIDDM patients, but not their healthy control group, showed a decrease in plasma CETP activity in response to exogenous hyperinsulinemia during a euglycemic clamp (109). The lack of a significant decrease in CETP activity in the normal control subjects may be explained by the fact that they had a lower blood glucose and a lower level of hyperinsulinemia than did their NIDDM counterparts. Plasma CETP activity is also decreased in response to aerobic exercise training (104). This decrease in CETP did not correlate either with changes in lean body mass or with changes in percentage of body fat and occurred even in subjects who did not lose weight. This suggests that the decrease in CETP levels is not simply related to changes in adipose tissue mass. Exercise also reduced the insulin response to a glucose challenge, indicating a decrease in peripheral insulin resistance. Plasma PLTP activity is also reduced during hyperinsulinemic, euglycemic clamp (119). In hamsters, CETP mRNA in adipose tissue is increased during fasting and decreased with feeding (60). These changes could be controlled by insulin responses.

Together, these data suggest that effective insulin action in adipose tissue and muscle may be controlling CETP levels (Figure 5). There could be indirect effects, e.g. insulin activates the lipoprotein lipase gene and this causes enhanced remnant clearance and removal of CETP on remnants. More interesting is the possibility that effective insulin action in adipose tissue and muscle decreases CETP and PLTP gene expression in these tissues (Figure 5). In turn, CETP expression in peripheral tissues could increase the effectiveness of insulin signaling: CETP action on HDL promotes efflux of cholesterol from cellular plasma membranes, potentially improving the effectiveness of insulin signaling by maintaining membrane cholesterol/phospholipid ratio in an appropriate range. Common CETP gene mutations could represent “thrifty” mutations that have an advantage during cycles of starvation but that predispose to insulin resistance during times of plenty.

An alternatively spliced form of the CETP mRNA is detectable in all human tissues (52). Its proportion ranges from 20% to 50% of total CETP mRNA among various tissues and varies by a factor of two during developmental stages (52, 129). Cellular expression of the alternatively spliced mRNA leads to a protein that is poorly secreted and its expression in transgenic mice does

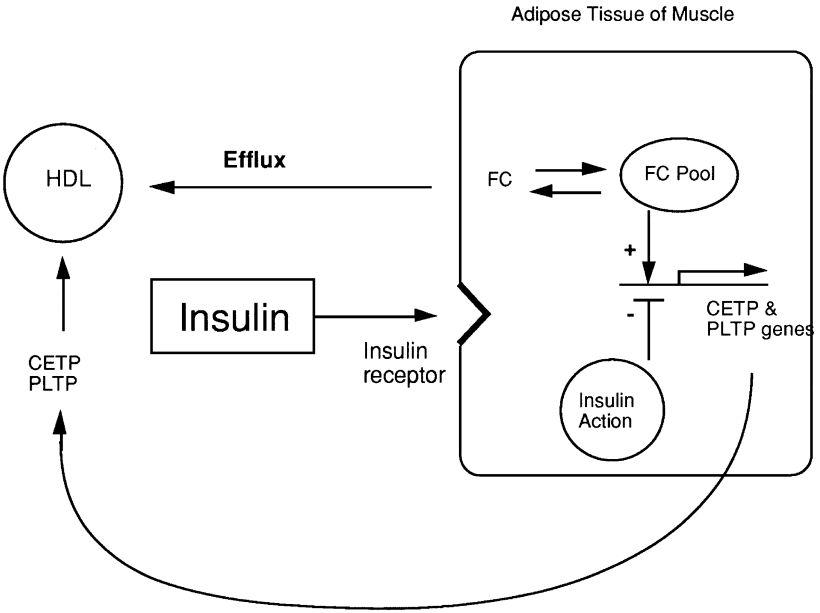


Figure 5 A speculative model of sterol and insulin regulation of the gene expression of lipid transfer proteins in adipose tissue and muscle. Increases in cellular sterol pools increase cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) gene transcription. Increased secretion and concentration of lipid transfer proteins modify high-density lipoproteins (HDL) to stimulate cholesterol efflux from cells. Efective peripheral insulin action decreases CETP and PLTP levels, perhaps as a result of a promoter effect. The proposed feedback loops, acting via HDL, may serve to maintain plasma membrane cholesterol to phopholipid ratio in an optimal range for insulin signaling. FC, Free cholesterol.

not alter the lipoprotein profile (129). The alternative splicing could represent a form of postranscriptional regulation to decrease the expression of CETP. This phenomenon is not observed in other species. It could also represent an ancient thrifty mutation to decrease CETP expression that has become widespread in human populations.

FUNCTION OF CETP DEDUCED FROM TRANSGENIC MOUSE STUDIES

Transgenic technology has enabled the investigation of gene dosage effects on complex metabolic systems. Because mice have negligible plasma CE transfer activity, a comparison of wild-type mice with transgenic mice expressing an exogenous CETP gene demonstrates the contribution of this gene.

Transgenic mouse lines expressing CETP at about human levels under the control of a heterologous promoter (4) or its own natural flanking sequences (56) show significant (20–30%) reductions in plasma HDL with little effect on the total cholesterol content of VLDL and LDL. High levels of simian or human CETP expression in mice cause plasma total cholesterol and apoAI to decrease further and LDL-cholesterol and apoB to increase to moderate levels with increasing CETP levels (74). The increase in LDL-cholesterol and apoB appears to reflect enhanced return of HDL-cholesterol to the liver, resulting in down-regulation of hepatic LDL receptor (59).

In a study of compound transgenic animals generated from a cross between human apoAI transgenic mice and human CETP transgenic mice, it was seen that coexpression of both human genes in the same animal leads to a more pronounced, 50% decrease in HDL-cholesterol levels and a reduction in HDL particle size (42). This indicates that human CETP has an enhanced interaction with HDL containing human, rather than mouse, apoAI (42). Similar experiments with LCAT and PLTP transgenic mice also show an enhanced interaction with HDL containing human rather than mouse apoAI in vivo (55). The basis for this in vivo species specificity is not well understood. Transgenic mice overexpressing human apoAI do not have lipid transfer inhibitory activity toward human CETP on their HDL particles, which suggests that lipid transfer inhibitory protein interaction with apoAI is also species-specific (75).

A dramatic interaction between CETP and hypertriglyceridemia has been demonstrated in transgenic mice made hypertriglyceridemic by expressing apoCIII (41). Although CETP expression reduces HDL levels in transgenic mice, in apoCIII transgenic mice, it decreases HDL levels to a greater extent. When human apoAI, the preferred substrate of human CETP, is also coexpressed, HDL levels decrease by 68% and HDL particle size decreases markedly (41).

When transgenic mice overexpressing human PLTP (to about twofold endogenous levels) are crossed with human apoAI transgenic mice, there is a modest (25%) increase in HDL-cholesterol and apoAI levels (7, 55). There is also a marked (56%) increase of pre-β HDL particles in human PLTP/apoAI transgenic mice (55). These particles may be generated from the interaction of HDL with PLTP, as seen in in vitro experiments (121). A similar result is obtained with dramatic overexpression in mice of human PLTP (to about 13-fold endogenous levels) by the use of an adenovirus vector (29). This treatment also causes an increase of pre-β particles, but HDL-cholesterol levels are decreased by 54% overall, relative to untreated mice, and there is increased HDL lipid (rather than apolipoprotein) uptake by the liver (29). Together, these results could indicate that the increase in pre-β particles caused by a mild increase in PLTP diverts mature HDL toward the nascent HDL pathway but that more

excessive increases saturate this pathway and lead to HDL catabolism through the selective uptake pathway.

More detailed analysis of human CETP transgenic mice indicates that expression of human CETP in human apoAI transgenic mice leads to a twofold increase in pre-β mobility HDL particles and a 1.4- and 2.2-fold increase in the HDL_{3a} and HDL_{3c} fractions, respectively, at the expense of the larger HDL_{2b} population (31). Pre-β HDL is also observed on incubation of plasma from simian CETP transgenic mouse, reflecting both the presence of CETP and the continued activity of hepatic lipase in mouse plasma (83). These changes are likely due to the combined interaction of CETP CE-TG exchange activity with hepatic triglyceride lipase as originally suggested by Deckelbaum et al (24). The exchange of HDL CE with VLDL TG, followed by the hydrolysis of TG, would lead to the formation of smaller, more-dense HDL particles as well as the shedding of apoAI, which would then become a pre-β particle by acquiring other lipids (70).

FUNCTION OF CETP AS DEDUCED FROM CETP MUTATIONS IN HUMAN POPULATIONS

The first CETP mutation to be identified was a splicing defect in intron 14 that caused skipping of exon 14 and a resultant absence in CETP mass and activity in plasma (13). Subsequently, several other missense, nonsense, and splicing mutations in CETP were identified. As with the first mutation, these were found in a Japanese population (see Figure 6). To date, only a few occurrences of CETP mutations have been reported among non-Japanese populations (33, 44).

Mutations that cause CETP deficiency are common in the Japanese population. The D442G mutation has an allelic frequency of about 6–7% in Japan and is characterized by 30% lower CETP levels (111). The intron 14 splicing mutation is a null allele (14) and is carried by another 1–2% of the Japanese

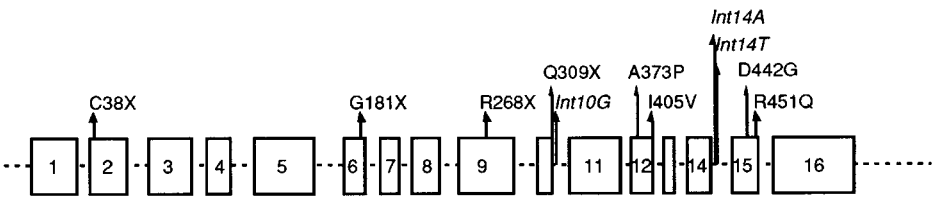


Figure 6 Location of cholesteryl ester transfer protein mutations in human populations: C38X (33); G181X (8); R268; Q309X (37); Intron 10G (101); A373P (33); I405V (33, 66); Int14A (13, 127); Int14T (51); D442G (51); R451Q (33). *Boxes* indicate exons. Mutations (*in italics*) are splicing defects; X, nonsense mutations.

population (51). These two mutations account for about 10% of the variability in HDL-cholesterol in the Japanese population (51).

CETP deficiency is associated with marked hyperalphalipoproteinemia and alterations in the size and composition of HDL and LDL. HDL_2 levels increase two- and sixfold among hetero- and homozygotes; HDL_3 levels remain unchanged. In patients with complete CETP deficiency, HDL is increased in size and enriched in CE. It is predominantly of the form containing both apoAI and apoAII and is enriched in apoCIII and apoE (128). These changes result in delayed catabolism of the apolipoprotein components of HDL (50).

CETP deficiency also alters the TG-to-CE ratio of VLDL and intermediate-density lipoproteins, reflecting their role in neutral lipid exchange mediated by CETP (63). This effect is most prononounced in complete CETP deficiency and is intermediate in partial CETP deficiency.

Complete CETP deficiency causes a small-sized LDL population with low affinity for the LDL receptor (102). Evidently this causes an up-regulation of the LDL receptor because CETP deficiency is characterized by lowered LDL levels, caused by a 50% increase in fractional catabolic rate (49). Despite this decrease, the plasma levels of small-diameter LDL particles may be higher in these patients than in control subjects (100). In patients with only partial CETP deficiency, such changes are not seen in LDL.

When comparing organisms with and without CETP, as in CETP transgenic mice studies and humans homozygous for CETP deficiency (see below), lack of CETP is associated with increased HDL-cholesterol. An inverse relationship between CETP activity and HDL levels is also observed by experimental manipulation of plasma CETP levels by probucol treatment (81), neutralizing antibody injection (27, 35, 133), inhibitor treatment (65), and CETP anti-sense oligodeoxynucleotides injections (108).

CETP Levels and Polymorphisms in Relation to Lipoprotein Levels in Human Populations

Although genetic CETP deficiency is clearly associated with increased HDL-cholesterol levels, an inverse relationship between CETP and HDL-cholesterol has not been consistently demonstrable in cross-sectional studies of human populations. This could be because a multiplicity of factors (some of which are random environmental influences) influence HDL-cholesterol and therefore mask the contribution of CETP. Furthermore, CETP and HDL may interact to different degrees in various tissues, whereas variations in genetic influences on CETP levels may also alter the tissue distribution of CETP. However, at least a quarter of the variations in plasma levels of CETP have been ascribed to genetic causes (66, 80), indicating that mutations (variants) of regulatory sequences are responsible for the expression levels of the protein.

In most studies on CETP polymorphisms, when an allele was found to be associated with increased plasma CETP levels, it was also associated with decreased HDL-cholesterol. Three restriction fragment length polymorphisms at the CETP locus were found to independently alter plasma CETP levels among subjects matched for factors that influence HDL levels (66). In this study, the *Taq* IB (in intron 1), *Msp* I (in intron 8), and I405V (in exon 14) polymorphisms were found to account for ~11%, 17%, and 4%, respectively, of the variance in CETP mass in a healthy Dutch population. One or more underlying mutations in partial linkage disequilibrium with these loci must be responsible for this effect on plasma CETP levels: The haplotype represented by *Taq* I B1-*Msp* I M1-405I alleles is overly represented in the lowest HDL decile, whereas the *Taq* I B2-*Msp* I M2-405V haplotype is enriched in the highest HDL decile (66). The I405V polymorphism is also a neutral mutation that is a marker for an underlying, functionally significant mutation, as the specific activity of CETP with a valine or an isoleucine at residue 405 is not different (15). The frequency distribution of the plasma CETP concentration among men homozygous for the 405V allele is bimodal (but that for the 405I homozygotes is not), which suggests a subpopulation of these men carry a functional mutation(s) in linkage disequilibrium with this polymorphism that affects the expression levels of CETP (15).

Epidemiologic Studies

Using a sib-pair analysis method to determine genetic determinants of HDL-cholesterol levels in healthy, normolipidemic people, Cohen et al found the hepatic triglyceride lipase gene and the apoAI/CIII/AIV locus, but not the CETP gene, to contribute to all the genetic component of the variability in HDL-cholesterol (20). In a study with fewer informative sib-pairs, Bu et al found a linkage between the CETP locus and HDL-cholesterol levels. This population included subjects with hyperlipidemia (16).

It is noteworthy that in hypertriglyceridemic humans, the determinants of plasma HDL-cholesterol were assessed by a stepwise regression analysis to be CETP (15% contribution) and LCAT (10%), lipoprotein lipase and hepatic triglyceride lipase (14% together) activities, and plasma TG levels (5%) (116). It is probable that the phenotypic results of common genetic variation at the CETP locus are moderated by hypertriglyceridemia.

Recent cross-sectional studies have shown that an inverse relation between CETP and HDL-cholesterol exists among hypertriglyceridemic men only (28, 48, 117). Among normotriglyceridemic subjects, CETP is directly related only to lipoprotein lipase activity, and after statistical adjustment for this association, no significant relationship remains between HDL-cholesterol and CETP (28). These observations are consistent with the fact that with normal plasma levels of

CETP, CE transfer activity is limited by the concentration of TG-rich acceptor particles. Only in hypertriglyceridemia does CETP become rate limiting and therefore the determining parameter of HDL-cholesterol. Thus, a threshold effect would exist for a relationship between CETP and HDL. As HDL levels are inversely proportional with TG levels, most cross-sectional studies have failed to see the weaker inverse correlation between HDL and CETP that is present only at high TG levels.

ROLE OF LIPID TRANSFER PROTEINS IN REVERSE CHOLESTEROL TRANSPORT

The primary recipient of tissue-cholesterol may be a phospholipid-rich, discoidal HDL particle with pre-β electrophoretic mobility (Figure 7). As the

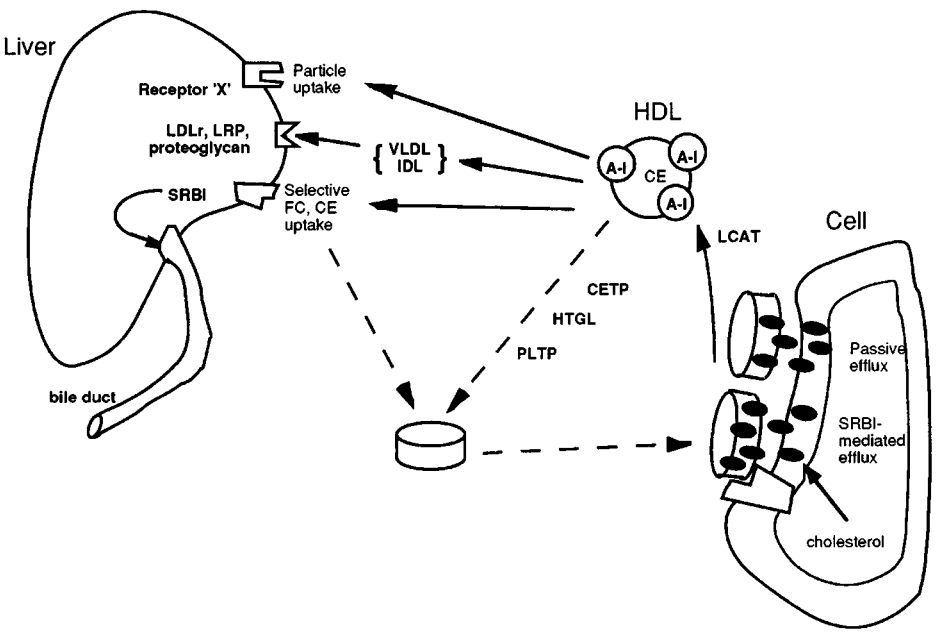


Figure 7 The three pathways of reverse cholesterol transport. (*solid lines*) Cholesterol movement; (*dashed lines*) the regeneration of nascent, phospholipid-rich discoidal high-density lipoproteins (HDL). CE, cholesteryl esters; CETP, cholesteryl ester transfer protein; FC, free cholesterol; HTGL, hepatic triglyceride lipase; IDL, intermediate-density lipoproteins; LCAT, lecithyl cholesterol acyl transferase; LDLr, low-density lipoprotein receptor; LRP, LDL (low-density lipoprotein) receptor-like protein; PLTP, phospholipid transfer protein; SRBI, scavenger receptor BI; VLDL, very-low-density lipoproteins.

cholesterol is esterified by LCAT, this pre-β HDL matures into a spherical α-form which is somewhat less adept at removing cholesterol from cells (67). However, as CETP removes esterified cholesterol out of HDL in exchange for TG and as the latter is hydrolyzed by hepatic triglyceride lipase, small and/or discoidal HDL is regenerated, restarting the cycle. This mechanistic view would imply that CETP participates in the transfer of cholesterol from the peripheral tissues to the liver, via the HDL fraction. Not only does it directly transfer esterified cholesterol to apoB-containing particles for eventual uptake by the liver, but it also remodels HDL back into a form with higher avidity for absorbing cholesterol from peripheral cells (Figure 7).

Phospholipid transfer mediated by PLTP also has a place in the reverse cholesterol pathway. In vitro studies show that PLTP enhances the CE transfer activity of CETP (69) and overexpression of PLTP in transgenic mice increases HDL particle number and especially pre-β HDL (55). As the cholesterol carrying capacity of lipoproteins is a function of their phospholipid content, it can be expected that variations in PLTP levels in plasma would be associated with variations in their capacity to cause cholesterol efflux form tissues. Also, increases in lipoprotein volume caused by neutral lipid transfer would necessitate the delivery of additional surface components, in the form of phospholipids and cholesterol. Thus phospholipid homeostasis is closely linked to cholesterol homeostasis.

Tissue-derived cholesterol on HDL is delivered to the liver by one of several different pathways (Figure 7): CETP-mediated transfer of CEs to TG-rich particles is one of them. LDL receptor–mediated uptake of apoE-containing HDL is another pathway; this requires the formation of large HDL particles with multiple copies of apoE. Selective uptake of HDL CE by the SRBI is a third pathway. Although each pathway may have specialized functions, the redundancy among the reverse cholesterol transport pathways implies that CETP or SRBI deficiency would be only partially disruptive. This would have to be the explanation as to how reverse cholesterol can take place in mice, rats, and humans with complete deficiency of CETP.

Nascent HDL (phospholipid-rich, pre-β particles) may be regenerated 1) by combined action of CETP and hepatic triglyceride lipase; 2) as a result of SRBI extraction of CE and free cholesterol from mature HDL particles; 3) as a by-product of lipolysis of TG-rich particles secreted by the liver or small intestine.

Genetic alterations of CETP levels, in humans and in transgenic mice, have shown that an absence of CETP is associated with a disruption of cholesterol efflux from cell membranes, of cholesterol esterification, and of CE transfer to TG-rich particles. Each of these are important steps in the movement of cholesterol toward the liver.

Many in vitro studies have shown that HDL does mediate cholesterol efflux from cultured macrophage foam cells. HDL from CETP-deficient subjects have a moderate, partial defect in causing cholesterol efflux from cells (25, 53, 88). Such HDL have an increased ratio of HDL_2 to HDL_3 and have a relative deficit in small HDL classes that are preferred acceptors of cholesterol from cellular membranes. Even though partly defective, all HDL particles are able to mediate cellular cholesterol efflux to some extent. Thus, serum from subjects with homozygous CETP deficiency has a 1.5–2.0 fold greater ability to stimulate cellular cholesterol efflux compared to serum from normal subjects. Apparently the massive increase in HDL particle number and size overcomes a moderate qualitative defect in cholesterol efflux per unit of HDL lipid content (A Inazu, AR Tall, G Rothblat, unpublished)

The esterification of HDL-cholesterol by plasma LCAT creates more cholesterol binding capacity on the surface of HDL. Whether the removal of the CEs is necessary for the continuation of the esterification reaction has been a controversial question. Incubation of plasma from CETP transgenic mice shows a 20–40% increase in cholesterol esterification relative to plasma from wild-type animals, and incubated plasma from both normal and CETP-deficient humans reveals a moderate 55% decrease in cholesterol esterification rate in human homozygous genetic CETP deficiency (89). The results suggest that in vivo CETP remodels HDL into a form that acts as a better substrate for the LCAT reaction.

The down-regulation of hepatic LDL receptor in high-expressing CETP transgenic mice implies that increased transfer of HDL CE into the liver results in expansion of a regulatory cholesterol pool (59). Increased reverse cholesterol transport (RCT) of HDL CE from plasma to the liver has been shown in CETP transgenic mice and by neutralization of rabbit CETP with a monoclonal antibody (125).

In an attempt to measure RCT in the whole animal, Osono et al looked at several transgenic lines of mouse expressing varying amounts of simian CETP (93). They measured the effect of increasing expression levels of plasma CETP on the rate of RCT (93). The latter was taken to be the sum of the cholesterol synthesis rate and LDL-cholesterol uptake in the peripheral tissues, having determined that peripheral tissue cholesterol content is at a steady state. In several transgenic lines of mice with varying amounts of HDL, the flux of cholesterol from periphery to liver was unchanged (93). This provided no support that altered HDL levels influenced RCT. This could be because CETP increases the fractional clearance of HDL CE in the liver but does not alter the total flux (or synthesis) of CE in plasma. Further studies are required to understand the regulation of reverse cholesterol transport and its relationship to atherogenesis.

RELATION TO ATHEROSCLEROSIS

Relationship of Altered CETP Levels to Atherosclerosis and Coronary Heart Disease

Since this topic was last reviewed (113), substantial new information has been published (131). Whereas most of the earlier information was correlational in nature (95), the recent data have been based on genetic deficiency or excess of CETP. In CETP transgenic mice, both increased and decreased atherosclerosis have been described, depending on the model employed. Importantly, in a sizable population–based human study, an excess of coronary heart disease (CHD) was found, which suggests an overall protective role of CETP expression in humans (131). Nonetheless, in human CETP deficiency associated with very high HDL levels, there is a low overall prevalence of CHD, and thus the notion of CETP inhibition as a therapeutic strategy (14) is still alive.

ANIMAL STUDIES Because mice have negligible plasma CE transfer activity, human or simian CETP transgenic mice have provided an excellent model for the assessment of the effects of CETP expression on lipoprotein metabolism and atherosclerosis. The most prominent lipoprotein changes in CETP transgenic mice are decreased HDL-cholesterol and apoAI levels and an increased proportion of apoAI in pre-β HDL (31). An early report of severe atherosclerosis in simian CETP transgenic mice with high levels of expression (73) has not been confirmed in subsequent studies using similar models. These studies of CETP transgenic mice have found either a mild excess of fatty streak lesions (43) or no effect on atherosclerosis (30), despite lowered HDL levels.

When cross-bred with other transgenic strains to produce a more human-like lipoprotein profile (i.e. with increased VLDL and/or LDL), or to provide a background with enhanced atherosclerosis susceptibility, the expression of CETP leads to variable modest effects on atherosclerosis. When the CETP transgene is crossed onto apoE or LDL receptor gene knock-out backgrounds, there is a moderate but significant 1.5- to 2.0-fold increase in atherosclerosis (Table 1). However, in the apoE0 background, the effect is highly variable, and in apoE0/apoAI Tg/CETP transgenic mice, there is no significant reversal of the protective effect of apoAI compared with apoE0/apoAI transgenic mice (A Plump, J Breslow, AR Tall, unpublished data).

A salient feature of human dyslipidemia not displayed by apoE0 or LDLr0 models is an increase in TG-rich VLDL levels. ApoCIII transgenic mice have increased VLDL levels, and the effects of CETP expression on HDL levels, size, and formation of pre-β HDL are markedly enhanced in this hypertriglyceridemic model (54). Human apoCIII/CETP transgenic mice have a decrease in aortic fatty streak lesions compared with apoCIII transgenic mice (43).

Table 1 Effect of other genes on the atherogenicity of CETP in transgenic mice[a]

Background	Atherogenicity	Reference
WT	Increased	73
WT	Unchanged	30
apoC-III	Decreased	43
apoC-III/apoA-I	Decreased	43
apoC-III/LDLr0	Unchanged	77
LDLr0	Increased	76
LCAT	Decreased	30
apoE0	Increased	UD

[a]WT, wild type; UD, A Plump, J Breslow, AR Tall, unpublished data.

Recently, a mouse model of the common human condition of combined hyperlipidemia was developed by crossing the apoCIII transgene onto the LDL receptor gene knock-out background. These animals display increased VLDL- and LDL-cholesterol and marked atherosclerosis susceptibility on a Western-type diet. In this setting, CETP expression markedly decreases HDL-cholesterol levels; apoAI levels are also decreased but the plasma concentration of apoAI in pre-β HDL is maintained because this represents a markedly increased proportion of the total HDL (77). This model shows increased atherosclerosis susceptibility as a result of apoCIII overexpression, and the lesions are advanced and complex (77). Despite profound lowering of HDL-cholesterol levels, the CETP transgene has no net effect on atherosclerosis in the combined hyperlipidemia mouse (77). Thus, in the setting of hypertriglyceridemia due to increased VLDL levels, CETP expression is either anti-atherogenic or does not result in the increase in atherosclerosis that might otherwise be expected to result from decreased HDL levels. These results in animals with increased VLDL levels appear to be most relevant to human atherogenesis.

Recently, the interaction of CETP and LCAT transgenes has been examined in mice. Interestingly, LCAT transgenic rabbits show a marked decrease in aortic atherosclerosis (47), confirming the original Glomset hypothesis that LCAT activity would increase RCT and reduce atherosclerosis (36). However, in LCAT transgenic mice the opposite is found, i.e. atherosclerosis is increased (12). Rabbits normally express high levels of CETP activity in plasma whereas mice do not. In CETP/LCAT transgenic mice, atherosclerosis is reduced compared with LCAT transgenic mice (30). These results suggest that CETP modifies the dysfunctional HDL accumulating in LCAT transgenic mice so that the HDL can perform more efficiently in RCT.

HUMAN STUDIES Of approximately 3300 Japanese-American men in the Honolulu Heart Program, about 6% of them were heterozygous for CETP gene mutations, predominantly the D442G missense mutation (131). In this population-based study, heterozygous CETP deficiency resulted in only about a 10% increase in HDL-cholesterol levels, even though mean plasma CETP levels were reduced by about 35%. However, genetic CETP deficiency was associated with a significant ($P < 0.05$) 1.4-fold increase in the relative risk of CHD. When the data were adjusted for other cardiovascular risk factors, the relative risk was 1.6. After adjustment for other risk factors and HDL levels, the relative risk was further increased to 1.7 ($P < 0.01$). This means that in this cohort, a man with genetic CETP deficiency was at 1.7-fold greater risk of CHD compared with an individual matched for other risk factors and HDL levels. Thus, genetic CETP deficiency is an independent risk factor for CHD.

This study was cross-sectional in nature, and the men were elderly. This raises the possibility of a survival effect, i.e. men with CETP deficiency are protected from heart disease and the onset of heart disease is delayed, then increases rapidly in old age. This seems unlikely, as discussed by Zhong et al (131) and as shown by a recent study conducted in the Omagari region of Japan (46). CETP gene mutations are present in about 20% of the region's general population, indicating that CETP deficiency is significantly less prevalent in the elderly compared with those in younger age groups. This means that heterozygous CETP deficiency is not a longevity factor (46). The results in the Honolulu Heart Program cohort are based primarily on the D442G mutation, so the interpretation could be complicated by genetic admixture effects, i.e. D442G could be a marker for other genetic differences related to effects of ethnicity. However, an analysis of the geographic distribution of CETP gene mutations, based on historical information obtained from subjects in the cohort, indicates that the D442G mutation is widespread in different regions of Japan, rendering this interpretation also unlikely (D Sharp, A Tall, unpublished data).

An important finding in the Honolulu Heart Program study was that the relationship between CETP deficiency and CHD was modified by HDL levels (Figure 8). Thus, the excess of CHD in subjects with CETP deficiency was primarily due to increased disease in men with HDL-cholesterol levels between 40 and 60 mg/dl. Men with or without the mutations who had HDL-cholesterol levels >60 mg/dl enjoyed a low prevalence of CHD. A population-based study from Japan has made similar findings: There is an extremely low prevalence of CHD in subjects with CETP gene mutations and HDL-cholesterol levels >80 mg/dl, comparable to that in subjects without mutations (A Inazu, personal communication). A few men with extreme hyperalphalipoproteinemia and CHD have been described (45). Interestingly, these individuals have combined deficiencies of hepatic lipase and CETP. However, despite these cases

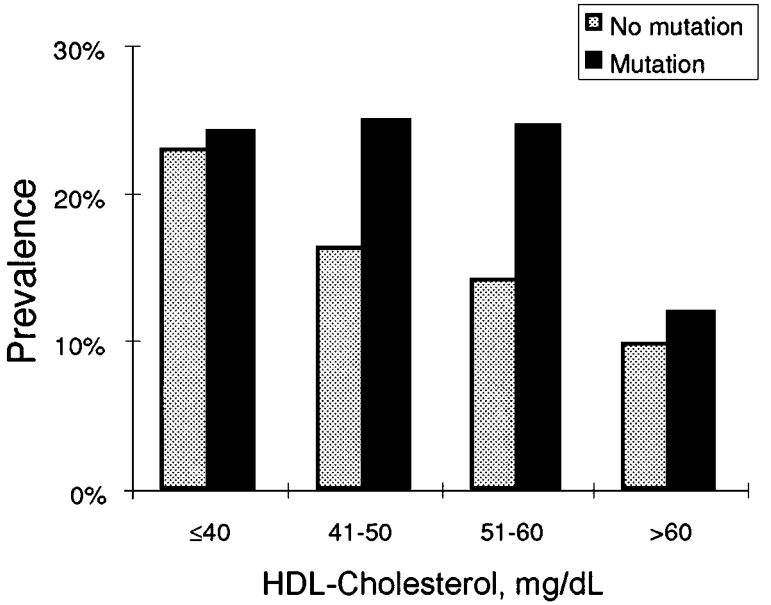


Figure 8 Prevalence of coronary heart disease in the Honolulu Heart Program study of men with and without the cholesteryl ester transfer protein mutations, stratified by plasma high-density lipoproteins (HDL)-cholesterol levels. (Reproduced from Reference 131 by permission. Copyright of The American Society of Clinical Investigation.)

(45), the data still suggest that markedly elevated HDL associated with CETP deficiency is associated with a low prevalence of CHD.

Mechanisms Underlying the Relationships of CETP Expression to Atherogenesis

The recent findings in human genetic CETP deficiency strongly support an anti-atherogenic role of CETP expression in humans and provide substantial support for the RCT hypothesis. For the first time in humans with genetic CETP deficiency, and in many of the CETP transgenic mouse models, we see a dissociation between the general inverse relationship between HDL levels and effects on atherosclerosis. This strongly suggests that the dynamics of cholesterol movement through HDL, i.e. RCT, underlie the anti-atherogenic effects of HDL. Experimental evidence indicates that CETP action on HDL increases the formation of pre-β HDL and in vivo enhances the LCAT reaction (31). Both of these changes would be expected to promote cellular cholesterol efflux. Plasma CETP activity also increases the return of plasma and HDL

CE to the liver (59). Fatty streaks, which are the precursors of atherosclerotic lesions, appear to be initiated by the aggregation of oxidized LDL particles in the vascular intima and their subsequent internalization by macrophages (126). CETP may also attenuate the pathology of atherosclerosis by taking part in the removal of oxidized lipids in LDL. CETP is able to transport oxidized lipids (19), and perhaps for this reason, CETP-deficient patients have been reported to have significantly higher levels of oxidized LDL (18).

A possible protective effect of CETP deficiency on CHD at high HDL levels could indicate anti-atherogenic properties of HDL unrelated to RCT. However, as noted above, serum from homozygous CETP-deficient subjects shows enhanced cholesterol efflux from cultured cells. CETP transgenic mouse plasma does cause less efflux than does wild-type mouse plasma even if it is more effective per unit of HDL mass (9). Furthermore, there are several different pathways of RCT to the liver, which may compensate for the absence of plasma CETP activity. Thus, there could be a complex dose relationship between CETP levels and atherosclerosis susceptibility, where partial deficiency increases risk of CHD and complete deficiency is protective. This indicates that therapeutic strategies aimed at CETP inhibition in humans should be carefully monitored for the levels of effectiveness and should be rapidly reversible. These considerations indicate that CETP inhibitor drugs that are potent ($>50\%$ CETP inhibition in vivo) and result in HDL-cholesterol levels above 60 mg/dl in males may be reasonable candidates for evaluation in human CHD.

CONCLUSIONS AND FUTURE DIRECTIONS

On a basic level, the crystal structure of BPI has provided a model for critical structure-function analysis of the CETP molecule and, more specifically, to test whether homologous lipid binding pockets in CETP have been modified for the binding of neutral lipids and phospholipids in two separate sites. Steady progress has been made in analyzing the CETP promoter and defining SREs. It now appears that the sterol up-regulation involves novel elements and factors. The interesting possibility that peripheral expression of the CETP gene is modified by insulin action, and that there may be opposite effects of sterols and insulin action on peripheral CETP expression, warrants further exploration. This area could provide a connection between regulation of HDL levels and peripheral insulin action.

Although substantial data have accumulated on the relationship of CETP to RCT and atherogenesis, the information indicates complexity. The possibility that CETP inhibitors can be used as therapeutic drugs to increase HDL levels warrants further evaluation. More information on human genetic deficiency states and their relationships to atherosclerosis in younger populations

of different ethnicity would be useful. A challenge will be to attempt to find the significant mutations affecting CETP gene expression underlying associations between CETP gene polymorphisms, CETP, and HDL levels in a variety of ethnic groups. These could provide the key to future epidemiological studies.

A major breakthrough has been the discovery that SRBI mediates the selective uptake of HDL CE in the liver. This constitutes a parallel pathway in RCT to that mediated by CETP, and genetic manipulation of the SRBI pathway will provide another test of the relationship of RCT pathway to atherosclerosis. One suspects that the overall function of this pathway will prove to be anti-atherogenic in many metabolic settings. A major challenge will be to determine if manipulation of the RCT pathway by targeting molecules such as CETP, LCAT, hepatic triglyceride lipase, or SRBI can be used for therapeutic benefit. The complexity of the results obtained to date is somewhat daunting. This could indicate that manipulation of HDL levels by increasing expression of major apoproteins (apoAI) or by decreasing catabolism of major apoproteins (apoAI or apoAII) might be a preferred therapeutic route, especially if this can be achieved while RCT operates at normal or increased level.

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