Hyperalphalipoproteinemia (HALP) is caused by a variety of genetic and environmental factors. Among these, plasma cholesteryl ester transfer protein (CETP) deficiency is the most important and frequent cause of HALP in the Asian populations. CETP facilitates the transfer of cholesteryl ester (CE) from high density lipoprotein (HDL) to apolipoprotein (apo) B-containing lipoproteins, and is a key protein in the reverse cholesterol transport system. The deficiency of CETP causes various abnormalities in the concentration, composition, and function of both HDL and low density lipoprotein (LDL). The significance of CETP in terms of atherosclerosis had been controversial. However, the in vitro evidence showed large CE-rich HDL particles in CETP deficiency are defective in cholesterol efflux. Similarly, scavenger receptor BI (SR-BI) knockout mice show a marked increase in HDL-cholesterol but accelerated atherosclerosis in atherosclerosis-susceptible mice. Recent epidemiological studies in Japanese–Americans and in Omagari area where HALP subjects with the intron 14 splicing defect of CETP gene are markedly frequent, have demonstrated an increased incidence of coronary atherosclerosis in CETP-deficient patients. Thus, CETP deficiency is a state of impaired reverse cholesterol transport which may possibly lead to the development of atherosclerosis. The current review will focus on the molecular mechanisms and atherogenicity of HALP, especially CETP deficiency. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Hyperalphalipoproteinemia; Cholesteryl ester transfer protein deficiency; Reverse cholesterol transport; Atherosclerosis

1. Introduction

Plasma high density lipoprotein (HDL)-cholesterol levels are negatively correlated with the incidence of coronary heart disease, suggesting a role for HDL in preventing the development of atherosclerosis. HDL takes up cholesterol from peripheral tissues and the cholesterol is esterified by lecithin:cholesterol acyltransferase (LCAT). The cholesteryl ester (CE) is transferred by plasma cholesteryl ester transfer protein (CETP) to very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) [1,2]. IDL and LDL are then catabolized via hepatic LDL receptor. The HDL obtains apolipoprotein (apo) E and apo E-containing HDL is taken up by the liver via LDL receptor or remnant receptor. Recent evidence shows that the CE moiety of HDL is selectively taken up by the liver via scavenger receptor class B type I (SR-BI) [3]. The HDL becomes enriched with triglycerides (TG) after the CETP-mediated trans-
fer of CE, is hydrolyzed by hepatic lipase (HL) and gets smaller to take up cholesterol. Thus, HDL serves as a shuttle that transports excess cholesterol from peripheral tissues to the liver for excretion. This pathway was designated as ‘reverse cholesterol transport’ system [4–6]. The HDL particles are heterogeneous and recent literature by in vitro experiments shows that pre-migrating HDL may have a very potent antiatherogenic function [7,8].

The first step of reverse cholesterol transport is the interaction between HDL and peripheral cells such as fibroblasts and macrophages, which is postulated to include aqueous diffusion, lipid-free apolipoprotein membrane microsolubilization, and SR-BI-mediated cholesterol flux [9–12]. Tangier disease is a genetic deficiency of HDL characterized by an absence of plasma HDL and deposition of CEs in the reticulo-endothelial system with splenomegaly and enlargement of tonsils and lymph nodes. HDL-mediated cholesterol efflux and intracellular lipid trafficking and turnover are abnormal in Tangier fibroblasts. Recent studies have demonstrated that Tangier disease is caused by mutations of ATP-binding cassette transporter 1 (ABC1) which may be involved in the efflux of cholesterol from cells [13–15].

Although low levels of serum HDL-cholesterol may lead to the development of atherosclerosis, there has been no consensus as to whether subjects with markedly high serum HDL-cholesterol levels are resistant to atherosclerosis. Hyperalphalipoproteinemia (HALP) is a condition caused by various factors and is also associated with some diseases. Although familial HALP with hypobetalipoproteinemia described by Glueck et al. [16] was reported to be accompanied by longevity due to a low incidence of coronary heart disease, the data were not confirmed. Matsuzawa et al. reported two markedly hyperalphalipoproteinemic patients with premature corneal opacity [17,18], which is a sign of lipid depositions in tissues. One of these patients was accompanied by angina pectoris. Therefore, these reports were the first descriptions suggesting that HALP may be a disorder of ‘reverse cholesterol transport’, which can be accompanied by atherosclerosis. These cases suggested that ‘dysfunctional HDL’ may be a potential mechanism leading to increased atherosclerosis despite high serum HDL-cholesterol levels.

2. Pathogenesis of HALP

HALP is associated with various diseases and caused by a variety of factors (Table 1). Some of these can be explained by mutations of molecules involved in HDL metabolism.

2.1. Primary HALP

The most important cause of primary HALP is a genetic deficiency of CETP, which will be discussed later. Groener et al. reported an HALP family with normal CETP activity [19], and the size and composition of HDL particles of a case with HALP reported by Patsch et al. [20] were normal. Therefore, these cases may not be genetic CETP deficiency. Genetic deficiency of HL can also cause HALP. HL-deficient patients often develop HALP and coronary heart disease [21,22] and their HDL particles are enlarged and rich in TG [23]. In the two HALP cases with corneal opacity [18], HL activity was decreased.

Rader et al. reported a case of familial HALP who presented a markedly increased production rate of apo A-I [24]. The primary sequence of the proband’s apo A-I gene, including the 5′-flanking sequence, was normal in this patient. Thus, the mechanism for the apo A-I overproduction in this case is unknown. Recently, a family with vertical transmission of simultaneous increase in HDL-cholesterol, LDL-cholesterol, apo A-I and apo B has been reported [25]. This condition was designated as familial HALP and hyperbetalipoproteinemia. The affected patients were accompanied by xanthomas and coronary artery disease. Elevated apo A-I levels were due to an increased synthetic rate, while the elevation of LDL-apo B resulted from both an enhanced synthetic rate and a prolonged residence time. Another case was also identified, who showed similar kinetic patterns of apo A-I and apo B. However, the molecular defect of these cases is unknown.

Table 1

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<th>Primary (familial) HALP</th>
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<td>CETP deficiency</td>
<td>Familial HL deficiency</td>
<td>Familial HALP with premature corneal opacity and reduced HL activity</td>
<td>Familial HALP with reduced uptake of HDL by lymphocytes</td>
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<td>Familial HALP due to overproduction of apo A-I</td>
<td>Familial HALP associated with variant apo C-III (Lys58 → Glu)</td>
<td>Familial HALP and hyperbetalipoproteinemia</td>
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Kobayashi et al. reported a case of familial HALP whose lymphocytes showed a reduced uptake of HDL [26]. However, the molecular basis for HALP in this case has not been clarified. A polymorphism due to an A to G transition, 78 base pairs upstream from the transcription start site of human apo A-I gene, was shown to be associated with HALP in women, but not in men [27]. However, Minnich et al. demonstrated in French Canadians that this substitution did not directly confer high HDL-cholesterol levels, but may be in linkage disequilibrium with other sequence polymorphism(s) at this locus in a subset of alleles that raise HDL-cholesterol levels [28]. Von Eckardstein et al. reported a family of HALP with variant apolipoprotein C-III (Lys58 → Glu) [29]. The patients were heterozygous for the mutation and plasma apo C-III concentrations were decreased by 30–40%. Large HDL particles enriched with apo E were observed in the patients, although the mechanism of HALP in this family is unknown.

2.2. Secondary HALP

HALP is also associated with a variety of diseases and factors. Alcohol consumption is accompanied by an increase in serum HDL-cholesterol [30]. However, in heavy alcohol drinkers the high HDL-cholesterol level can turn low when they suffer from liver cirrhosis. Alcohol consumption reduces plasma CETP activity and protein mass [31]. Hirano et al. reported similar findings in chronic heavy alcohol drinkers [32]. Small polydisperse LDL, which is one of the characteristic features of CETP deficiency, was observed in heavy alcohol drinkers with markedly reduced CETP activity. Furthermore, chronic alcohol intake induced changes in both plasma concentration and size of HDL. The size of HDL particles was large in alcohol drinkers. The cessation of alcohol was accompanied by a reduction in serum HDL-cholesterol with a concomitant decrease in HDL particle size. The LDL in chronic alcohol drinkers was poor in CE and rich in TG. These LDL particles had a lower affinity than normal LDL to the LDL receptors of human skin fibroblasts [33]. After cessation of alcohol, the affinity of LDL to LDL receptors was normalized. These changes were in parallel with those of CETP activity and mass, suggesting that alcohol intake may directly affect the plasma CETP level, thereby controlling the concentration and composition of LDL and HDL.

Patients with primary biliary cirrhosis (PBC) are often associated with a marked HALP. The levels of serum total cholesterol, apo A-I, apo C-III and apo E are also elevated [34]. The elevation of HDL-cholesterol is attributed solely to the increase in HDL₂-cholesterol. The HDL₂ particles of the patients are enriched with apo E and larger in size than those of controls. HL activity and mass are decreased in these patients, while CETP activity and mass are elevated. Reduced HL activity may partly contribute to the pathogenesis of HALP in PBC. In the PBC patients associated with apo E2/2 [35], serum HDL-cholesterol level was extremely high (244 and 263 mg/dl). In these patients, the LDL density fraction contained HDL₃-like particles with apo A-I, and HDL₄-like particles with predominant apo E and a trace amount of apo A-I. These HDL₄-like particles were similar to those observed in CETP-deficient patients. Therefore, HL and apo E-mediated HDL removal by the liver may be important in the metabolism of HDL (Fig. 1).

An HALP patient with albumin complexing and elevated IgM level was reported [36]. The patient’s plasma contained a unique complex of albumin with apo A-I. HL was decreased and lipoprotein lipase was increased in this patient, which may result in an increase in HDL₂ with an enrichment of phospholipids and thereby with an increased affinity for albumin present in the very high density lipoprotein (VHDL) fraction. HALP is also caused by corticosteroids, sucrose and insulin, estrogen, hypolipidemic drugs such as HMG-CoA reductase inhibitors and fibrates, phenytoin and chlorinated hydrocarbons (Table 1). Taken together, HALP is a syndrome and the lipoprotein profile and its atherogenicity may differ between each type of HALP.

3. Serum HDL-cholesterol level does not correlate with anti-atherogenicity — implications from transgenic animal models

Patients with genetic HDL deficiency do not always present premature atherosclerosis. Subjects with apo A-I<sub>MILANO</sub> are protected from atherosclerosis, although the level of serum HDL-cholesterol is low [37]. Although this was not due to a relatively increased capacity of mutant apo A-I<sub>MILANO</sub> to recruit membrane cholesterol [38,39], an intravenous infusion of recombinant apo A-I<sub>MILANO</sub>/phospholipid complex to apo E-deficient mice fed a high-cholesterol diet from 20 until 25 weeks induced a reduction of aortic lipid and macrophage content and also inhibited an increase in aortic atherosclerosis [40].

Hypercholesterolemic patients who had been treated with probucol present low levels of HDL-cholesterol. In the patients with familial hypercholesterolemia treated with probucol, the reduction rate of HDL-cholesterol correlated with the decrease in Achilles tendon thickness [18]. This reduction of HDL-cholesterol was accompanied by a decrease in HDL-CE and particle size of HDL, which was due to an enhancement of CETP activity [18,41]. The HDL particles from probucol-treated patients had a more potent antiatherogenic
function than those from control subjects [42]. Furthermore, recent data of transgenic animal models have provided an insight into the physiological function of various molecules involved in remodelling of HDL and their relationship with atherosclerosis.

3.1. Apo A-I and A-II transgenic mice

Transgenic mice overexpressing human apo A-I [43,44] showed an increase in serum HDL-cholesterol, human apo A-I and HDL particle size and were resistant to diet-induced atherosclerosis [45]. However, in apo A-I knockout mice, in which apo A-I was totally deficient and serum HDL-cholesterol was decreased by 80% [46], atherosclerosis was not observed even after feeding an atherogenic diet [47].

In contrast to apo A-I overexpressing mice, transgenic mice overexpressing mouse apo A-II showed accelerated atherosclerosis despite increased serum HDL-cholesterol levels [48,49]. Human apo A-I and apo A-I/A-II transgenic mice were developed in an atherosclerosis-susceptible strain. After feeding an atherogenic diet, the area of atherosclerotic lesions in the apo A-I/A-II transgenic mice was 15-fold greater than in the apo A-I transgenic mice despite similar total cholesterol and HDL-cholesterol levels. Thus, the protein composition of HDL may significantly affect its role in atherogenesis and HDL with apo A-I is more antiatherogenic than HDL with apo A-I/A-II.

3.2. LCAT transgenic mice and rabbits

In transgenic mice overexpressing human LCAT [50], serum total cholesterol and HDL-cholesterol were increased, HDL was enlarged, and enhanced atherosclerosis was observed after feeding an atherogenic diet compared to control mice [51]. The composition and function of LCAT transgenic mouse HDLs were abnormal, and the uptake by the liver of [3H]CE incorporated in HDL was reduced by 41%. These data suggested an ineffective transport of HDL-cholesterol to the liver in LCAT transgenic mice. The transgenic rabbits overexpressing human LCAT were also hyperalphalipoproteinemic and the size of HDL was enlarged similar to LCAT transgenic mice, however these rabbits were resistant to atherosclerosis [52,53]. In the LCAT knockout mice [54], a tendency of reduced atherosclerosis was observed, although the difference was not statistically significant [55]. These discrepancies may derive from species difference and most possibly from the fact that mice are deficient in CETP activity. The lipoprotein metabolism of mice is distinctively different from that of humans.

3.3. HL transgenic mice and rabbits

When transgenic mice overexpressing human HL were fed an atherogenic diet with and without zinc, plasma HDL-cholesterol and HDL size were decreased after zinc induction due to overexpression of HL [56]. Abdominal aortic cholesterol content was also reduced.
in HL transgenic mice. Transgenic rabbits overexpressing HL also showed a marked reduction of plasma HDL and IDL [57], but atherosclerotic lesions in the ascending aorta and the aortic arch were thicker in the transgenic rabbits than in the non-transgenic rabbits [58].

### 3.4. CETP transgenic mouse models

Plasma CETP is a hydrophobic glycoprotein with a $M_r$ of 70–74 kDa [1,2]. Human CETP consists of 476 amino acid residues. Human CETP gene is located in chromosome 16 near the LCAT gene and consists of 16 exons and 15 introns [59]. CETP is synthesized by the liver, spleen, adipose tissues, small intestines, adrenal gland, kidney, heart and skeletal muscles, and by cells including monocyte-derived macrophages, B-lymphocytes, and adipocytes. CETP activity is very low in mice and rats and high in rabbits. In CETP transgenic mice with mouse metallothionein-I promoter [60], the transgene overexpression after zinc induction was detected in the liver and small intestines, which caused a decrease in serum cholesterol, HDL-cholesterol and HDL particle size. The changes in lipoprotein profile were more striking in transgenic mice expressing cynomolgus monkey CETP [61,62], than in those developed by Agellon [60]. In the human CETP transgenic mice [60], plasma HDL-cholesterol levels of zinc-treated male and female transgenic mice fell by 27 and 15%, respectively, on a chow diet. In contrast, in the simian CETP transgenic mice [61,62], plasma HDL-cholesterol levels fell by ~50%. CETP transgenic mice with a CETP minigene linked to the natural flanking sequences of human CETP gene showed a 2.5-fold increase in plasma CETP activity in response to a high-fat, high-cholesterol diet [63]. The CETP transgenic mice mated with mouse metallothionein-I promoter [60], the transgene overexpression after zinc induction was detected in the liver and small intestines, which caused a decrease in serum cholesterol, HDL-cholesterol and HDL particle size. The changes in lipoprotein profile were more striking in transgenic mice expressing cynomolgus monkey CETP [61,62], than in those developed by Agellon [60]. In the human CETP transgenic mice [60], plasma HDL-cholesterol levels of zinc-treated male and female transgenic mice fell by 27 and 15%, respectively, on a chow diet. In contrast, in the simian CETP transgenic mice [61,62], plasma HDL-cholesterol levels fell by ~50%. CETP transgenic mice with a CETP minigene linked to the natural flanking sequences of human CETP gene showed a 2.5-fold increase in plasma CETP activity in response to a high-fat, high-cholesterol diet [63]. The CETP transgenic mice mated with mouse metallothionein-I promoter [60], the transgene overexpression after zinc induction was detected in the liver and small intestines, which caused a decrease in serum cholesterol, HDL-cholesterol and HDL particle size. The changes in lipoprotein profile were more striking in transgenic mice expressing cynomolgus monkey CETP [61,62], than in those developed by Agellon [60]. In the human CETP transgenic mice [60], plasma HDL-cholesterol levels of zinc-treated male and female transgenic mice fell by 27 and 15%, respectively, on a chow diet. In contrast, in the simian CETP transgenic mice [61,62], plasma HDL-cholesterol levels fell by ~50%. CETP transgenic mice with a CETP minigene linked to the natural flanking sequences of human CETP gene showed a 2.5-fold increase in plasma CETP activity in response to a high-fat, high-cholesterol diet [63]. The CETP transgenic mice mated with mouse metallothionein-I promoter [60], the transgene overexpression after zinc induction was detected in the liver and small intestines, which caused a decrease in serum cholesterol, HDL-cholesterol and HDL particle size.

### 3.5. SR-BI transgenic mouse models

The class B scavenger receptor, SR-BI, is an HDL receptor which is involved in the selective uptake of CE from HDL [3]. It is abundantly expressed in the steroidogenic tissues and liver. Adenovirus-mediated hepatic overexpression of SR-BI in mice on both sinusoidal and canalicular surfaces of hepatocytes induced almost total disappearance of plasma HDL and a substantial increase in biliary cholesterol [67]. This effect may be due either to increased hepatic HDL-CE uptake or to enhanced secretion of cholesterol into bile, or both. Liver specific overexpression of SR-BI decreased the levels of VLDL apo B, LDL apo B, and HDL-cholesterol [68]. To examine the expression of SR-BI on atherosclerosis, LDL receptor-deficient mice were fed a high-fat/high-cholesterol diet for 2 or 12 weeks to induce atherosclerotic lesions of different stages [69]. These mice were then injected with a recombinant adenovirus encoding murine SR-BI. Transient hepatic overexpression of SR-BI in mice with both early and advanced lesions decreased atherosclerosis, despite decreased HDL-cholesterol levels. The average HDL-cholesterol levels were significantly correlated with the size of atherosclerotic lesions. Ueda et al. [70] developed SR-BI transgenic mice, which showed lower plasma levels and accelerated clearance of HDL. Furthermore, they examined two lines of SR-BI transgenic mice with high (10-fold increases) and low (2-fold increases) in SR-BI expression in an inbred mouse background hemizygous for a human apo B transgene [71]. The low expression SR-BI/apo B transgenics had more than a 2-fold decrease in the development of diet-induced fatty streak lesions compared to the apo B transgenics, however the high expression SR-BI/apo B transgenics with a marked decrease in HDL-cholesterol showed an atherogenic response similar to that of the apo B transgenics. Thus, the expression of SR-BI was considered to be antiatherogenic under the conditions of moderate SR-BI expression. Furthermore, decreased atherosclerosis has been shown in heterozygous LDL receptor knockout mice expressing SR-BI despite decreased HDL-cholesterol [72]. Therefore, the uptake of CE by liver SR-BI may be a key step which determines the efficiency of reverse cholesterol transport system and possibly the development of atherosclerosis.

In contrast, SR-BI knockout [73] or SR-BI attenuated [74] mice showed a marked increase in serum cholesterol, especially HDL-cholesterol, while serum apo A-I level was not changed. The size of apo A-I-containing lipoproteins was much enlarged. These studies indicate that SR-BI may have crucial roles in determining serum HDL-cholesterol levels and HDL
size as well as in controlling cholesterol concentrations in bile. Regarding the atherosclerosis in SR-BI knockout mice, virtually no detectable lesions were observed at relatively young age (4–7 weeks), however a significant lesion developed in the SR-BI/apo E double knockout mice in the aortic root and coronary arteries [75]. Moreover, increased LDL cholesterol and atherosclerosis were demonstrated in LDL receptor-deficient mice with attenuated expression of scavenger receptor BI, which also showed a slight increase in HDL-cholesterol [76]. The effect of attenuated SR-BI expression was partly due to increased LDL cholesterol levels in this model. Taken together, the data on SR-BI overexpression and knockout mice suggest that upregulation of SR-BI could have therapeutic potential for the treatment of atherosclerosis despite low HDL-cholesterol levels. It is also suggested that reduced expression of hepatic SR-BI may be proatherogenic despite high HDL-cholesterol levels, since reverse cholesterol transport is impaired in this condition.

3.6. Pitfalls of transgenic animal models

These animal models have provided a very good tool for assessing the physiological roles of molecules. However, there are difficulties in drawing conclusions on the atherogenicity of each molecule from studies using animal models such as mice, of which the lipoprotein metabolism is quite different from that of humans. One has to assess the species differences in lipoprotein metabolism. Furthermore, these studies may establish a notion that serum levels of HDL-cholesterol do not necessarily correlate with the degree of protection from atherosclerosis. It may be crucial to assess the efficiency of reverse cholesterol transport system irrespective of serum HDL-cholesterol levels, since HDL particles become ‘dysfunctional’ due to the impairment of this system.

4. HALP due to plasma CETP deficiency

HALP patients due to plasma CETP deficiency have been reported mostly from Japan. There are some reports of CETP deficiency from German, Caucasian and Asian populations.

4.1. Gene defects in CETP deficiency

Concerning the gene defects in CETP deficiency, a G-to-A mutation in the 5′-splice donor site of intron 14 was initially identified in Japanese CETP-deficient subjects [77–79]. This mutation is common in the Japanese HALP subjects and in the general population (heterozygotes: about 1–2%) [79,80]. A missense mutation (442D:G) in the exon 15 was later reported [81]. Although the patients were heterozygous for this defect, they had a 3-fold increase in plasma HDL-cholesterol and markedly decreased plasma CETP activity and mass, suggesting that the 442D:G mutation may have dominant effects on CETP and HDL in vivo. This was confirmed in vitro by expressing wild-type and mutant proteins in COS cells. The frequency of heterozygotes for the 442 D:G mutation is also high in the Japanese general population and HALP subjects [82,83].

A nonsense CETP mutation in exon 10 which causes premature stop codon was reported [84]. Another nonsense mutation in exon 6 (G181X) has recently been identified, which causes a total absence of CETP activity [85]. This mutation is also common, although the frequency in HALP subjects is not so high compared with those of intron 14 splicing defect and 442 D:G mutation. Moreover, another defect in the intron 10 splice donor site of CETP gene has been identified, which causes exon 10 skipping, resulting in abnormal downstream splice site selection [86]. Although most of CETP-deficient subjects were found in the Asian population, novel mutations have recently been identified in HALP subjects from the German, Caucasian and Asian populations [87–90]. Fig. 2 summarizes the site of CETP gene mutations reported so far.

4.2. Abnormal plasma lipoproteins in CETP deficiency

Several procedures have been developed to determine CETP activity [91,92]. For example, the CE portion of donor lipoproteins (HDL₃) is radiolabeled with [¹⁴C] or [³H]cholesterol, and unlabeled LDL or the d < 1.063 g/ml fraction is used as a source of CETP followed by incubation at 37°C. After adding donor and acceptor lipoproteins to the assay buffer, a small amount of plasma or a d > 1.21 g/ml plasma fraction is added as a source of CETP followed by incubation at 37°C. To further evaluate the CETP level, CETP mass assays are performed [93,94].

In CETP-deficient patients [95–97], serum total cholesterol levels are moderately elevated, while serum HDL-cholesterol is remarkably increased to 3–6-fold. Serum apo A-I, C-III and E are also markedly increased, while apo B is normal or slightly decreased. The increase in HDL-cholesterol is due to the increase in HDL₂-cholesterol, while HDL₅-cholesterol is not increased. The HDL of the patients is more enriched with CE and poorer in TG than that of controls, while CE content is decreased and TG is increased in the VLDL and LDL fraction. Cholesterol level is normal or slightly high in the LDL fraction, which contains apo E-rich HDLs with a slow α-mobility in agarose gel electrophoresis in addition to apo B-containing LDL [98]. These HDLc-like particles have a higher affinity than normal LDL to the LDL receptor.
In native polyacrylamide gradient gel electrophoresis, the LDL particles in CETP deficiency are small and heterogeneous (polydisperse LDL) and HDL particles are extremely large [97]. By equilibrium density gradient ultracentrifugation, the LDL of CETP-deficient patients comprises a group of heterogeneous lipoprotein particles distributed in a wide density range without a prominent peak, while the LDL from normal controls consists of a homogeneous group of lipoprotein particles distributed in a narrower density range with a single sharp peak [99]. The LDL in each subfraction derived from patients is poor in CE and enriched with TG. In native polyacrylamide gradient gel electrophoresis, each subfraction of control LDL contains only one species of homogeneous LDL particles that decreases in size with an increase in the density. In contrast, each subfraction of patients’ LDL contains two species of LDL particles; smaller LDL particles are found in addition to those which are identical to the normal control LDL particles observed in the corresponding subfractions. The IDL of the patients is also composed of two species of lipoproteins. Thus, two metabolic pathways may exist in the process of mature LDL formation [99]. VLDL is secreted by the liver as two species of lipoprotein particles different in size, and each type of VLDL is successively metabolized to LDL through IDL by a separate pathway. Various modulations might be involved in producing cholesterol-rich LDL, which possesses a high affinity for LDL receptors. The hydrolysis of TG by lipoprotein lipase and HL is crucial for this process. Furthermore, CETP plays an important role in converting small TG-rich LDL particles to large CE-rich homogeneous LDL particles by transferring CE from HDL.

As mentioned earlier, HALP is caused by various genetic or acquired abnormalities in lipoprotein metabolism. The differences in lipoprotein profiles are remarkable between CETP deficiency and the other types of HALP. Fig. 3 demonstrates a native polyacrylamide gradient gel electrophoretic pattern of the three HALP cases. One was a homozygous CETP deficiency whose HDL particles were extremely large (HDLc or HDL1) and LDL particles were polydisperse; the second HALP case was a Caucasian patient with a marked increase in HDL2-cholesterol whose plasma CETP ac-
tivity was normal; the third case was also a Caucasian HALP subject whose CETP activity was normal and the increase in HDL-cholesterol was attributed solely to the increment of HDL₃-cholesterol. The LDL of the latter two patients was monodisperse and the size of HDL particles was much smaller than that of CETP deficiency (HDL₂ size in the second case and HDL₃ size in the third case, respectively). It can be speculated that the HALP of the third case may result from an overproduction of apo A-I. This type of HALP may be accompanied by an increased number of small HDL particles and could be anti-atherogenic.

4.3. Impaired function of lipoprotein particles in CETP-deficiency

The HDL₂ particles from normal controls can protect macrophages from cholesterol accumulation when incubated with acetylated LDL, and enhance cholesteryl esterification of macrophages. This finding is supported by the recent report of Fancone et al, showing increased preβ-HDL levels, cholesterol efflux and LCAT-mediated esterification in vivo in mice overexpressing human CETP and human apo A-I transgenes [106]. Furthermore, HDL particles of human CETP transgenic mice had a more efficient capability of cholesterol efflux than those of non-transgenic mice, and the co-expression of both human apo A-I and CETP improved the efficiency of HDL particles in the human apo A-I/CETP transgenic mice when compared with the human apo A-I transgenic mice [107]. Thus, CETP may be essential for remodeling of large HDL particles into small ones with a potent anti-atherogenic function.

5. Is HALP anti-atherogenic or proatherogenic?

5.1. Possible enhancement of atherosclerosis in CETP deficiency

Species with high or moderate levels of CETP activity are susceptible to atherosclerosis and do not develop apo E and CE-rich HDL (HDLc) on a high-cholesterol diet. In contrast, in species with low CETP activity, HDLc appears in response to atherogenic diet, and these species are relatively resistant to atherosclerosis. Quinet et al. [108] demonstrated a positive correlation of the extent of coronary atherosclerosis with LDL-cholesterol level and with plasma CETP concentration in cynomolgus monkeys fed a high-fat, high-cholesterol diet. A CETP transgenic mouse line developed more aortic atherosclerosis after cholesterol feeding compared to non-transgenic mice [61,62]. Intravenous injection of antisense oligodeoxynucleotides against CETP coupled to asialoglycoprotein carrier molecules targeted to the liver was shown to inhibit plasma CETP activity and increase plasma HDL-cholesterol in cholesterol-fed rabbits [109]. Furthermore, the development of aortic atherosclerosis was inhibited in the cholesterol-fed rabbits injected with antisense oligonucleotides compared with the animals injected with sense oligodeoxynucleotides [110]. Thus, CETP was previously hypothesized to be pro-atherogenic in animal models [111]. Kuiven-
hoven et al. recently observed a significant dose-dependent association of Taq B1B2 genotype marker with the progression of atherosclerosis in the placebo group, while this association was abolished by pravastatin treatment [112]. Pravastatin therapy slowed the progression of coronary atherosclerosis in B1B1 carriers (with higher CETP levels), but not in B2B2 carriers (with lower CETP levels). This common DNA variant appeared to predict whether men with coronary artery disease will benefit from treatment with pravastatin to delay the progression of coronary atherosclerosis.

However, corneal arcus was noted in CETP-deficient cases [113] and a relatively high prevalence of cerebrovascular diseases was observed in certain CETP-deficient families. Some of the homozygous and heterozygous subjects with CETP deficiency were accompanied by atherosclerotic cardiovascular diseases such as coronary heart disease [113,114], cerebrovascular disease and arteriosclerosis obliterans [113]. In the HALP subjects whose CETP and HL activities were both reduced, atherosclerotic cardiovascular diseases occurred [113]. The metabolic abnormalities in HALP associated with a combined reduction of CETP and HL is illustrated in Fig. 4. The increased HDL$_2$ particles in CETP-deficient subjects have a reduced capacity for cholesterol efflux [100]. CETP together with HL transforms large HDL particles into small ones, which have a potent antiatherogenic function [105]. Collet et al. reported that the HDL isolated from mice expressing CETP showed a 2–4-fold increase in SR-B1-mediated HDL-CE uptake compared to HDL from mice lacking CETP [115]. The addition of CETP to HDL in cell culture did not lead to increased selective uptake of HDL-CE by cells. However, when human HDL was enriched with TG by incubation with TG-rich lipoproteins in the presence of CETP, then treated with HL, a significant enhancement of HDL-CE uptake was observed. Therefore, the remodelling of human HDL by CETP, involving CE-TG interchange, followed by the action of HL, leads to the enhanced uptake of HDL-CE by SR-B1. As mentioned earlier, SR-B1 knockout mice show a marked increase in HDL-cholesterol and HDL size, however atherosclerosis is accelerated in atherosclerosis-susceptible mice. The enlarged HDL particles in CETP deficiency resemble those observed in SR-B1 knockout mice and these HDL particles in vivo may be ‘dysfunctional’.

Hirano et al. have identified a unique area in the northern part of Japan named Omagari City of Akita Prefecture, where the prevalence of the intron 14 splicing defect is enormously high, reaching 28 times that in the other part of Japan such as Osaka or Tokyo [116]. In Omagari, the prevalence of HALP subjects with serum HDL-cholesterol more than 100 mg/dl was much lower in subjects over 80 years of age than those under 80 years. The frequency of CETP deficiency was also reduced in subjects over 80 years of age compared with those under 80 years. The prevalence of ischemic changes in electrocardiogram was rather increased in subjects whose serum HDL-cholesterol was more than 90 mg/dl compared with those whose HDL-cholesterol was 50–70 mg/dl (Fig. 5). Moreover, ultrasound examination of carotid arteries demonstrated higher atherosclerotic scores in hyperalphalipoproteinemic CETP-deficient subjects than in control CETP-positive subjects (Fushimi, Maruyama et al. unpublished observation). Transesophageal echocardiography also demonstrated an enhancement of atherosclerosis in descending aorta (Fushimi et al., unpublished observation). Therefore, it is speculated that HALP caused by CETP deficiency is not accompanied by longevity, but by atherosclerotic cardiovascular diseases. Zhong et al. have reported that in Japanese–American men living in Hawaii the incidence of CHD was higher in subjects with CETP mutations than in those without mutations, although the difference was significant only in subjects

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**Fig. 4.** Metabolic abnormalities in hyperalphalipoproteinemia (HALP) associated with a combined reduction of cholesteryl ester transfer protein (CETP) and HTGL (Ref. [113]).

![Diagram](image-url)
whose HDL-cholesterol level was between 41 and 60 mg/dl [117]. However, men with increased HDL levels (> 60 mg/dl) had a low risk of coronary artery disease irrespective of the CETP genotype. Moriyama et al reported no significant difference in the prevalence of coronary heart disease between HALP subjects with and without CETP mutations [118]. They showed that HALP subjects (HDL-cholesterol > 80 mg/dl as well as HDL-cholesterol from 60 to 79 mg/dl) appeared to be protected against coronary heart disease irrespective of the presence or absence of CETP deficiency. The reason for the discrepancy between the data of Omagari and Kochi remains to be clarified. The D442G mutation which has a less remarkable effect on CETP activity than the intron 14 splicing defect was predominant in this Kochi population, while the intron 14 splicing defect with a stronger effect on CETP activity was markedly frequent in Omagari area. These differences in the genotype of CETP deficiency might be considered when such a discrepancy is evaluated.

Furthermore, a CETP polymorphism (I405V) was studied in the Honolulu Heart Program cohort to determine the relationship between the polymorphism, CETP and HDL levels, and coronary heart disease [119]. The V/V genotype was associated with lower CETP concentrations than the I/V or I/I genotype. The HDL-cholesterol levels were higher in men with the V/V genotype than in men with the I/V or I/I genotype. However, the increase in HDL was only significant in hypertriglyceridemic men with V/V genotype. Although the prevalence of coronary heart disease was not significantly different among the three genotypes, it was significantly higher among V/V than I/V or I/I subjects. Thus, although the I/I genotype tended to be associated with a lower prevalence of coronary heart disease, the V/V genotype may be associated with higher HDL-cholesterol levels and increased coronary heart disease in hypertriglyceridemic men. As reported recently by Agerholm-Larsen et al., women not treated with hormone replacement therapy, who were heterozygous or homozygous for Val405 and were associated with lower CETP activity and higher HDL-cholesterol level, had a 1.4–2.1-fold increase in the risk of ischemic heart disease, although no significant associations were detected in men [120]. Thus, it was speculated that CETP deficiency may not be anti-atherogenic, but rather proatherogenic in humans. However, a careful prospective study on the atherogenicity of patients with CETP deficiency is necessary to draw conclusions. It is also essential to determine clearly whether the increase in HDL-cholesterol may protect against or rather accelerate atherosclerosis in CETP-deficient patients.

5.2. Atherogenicity of other types of HALP

The atherogenicity of other types of HALP may depend upon the etiology of HALP. For example, HALP due to HL deficiency may be proatherogenic, while that due to overproduction of apo A-I could be anti-atherogenic (such as HALP with predominant increase in HDL₃ as demonstrated in Fig. 3). Fig. 6 illustrates the determinants of atherogenicity in HALP subjects. Its relation to atherogenicity may depend upon the presence or absence of other coronary risk factors and also upon the efficiency of reverse cholesterol transport system. Furthermore, the number of preβ-HDL particles in tissues may determine the atherogenicity, since these particles were shown to be anti-atherogenic and produced by CETP [101], HL [108] and phospholipid transfer protein (PLTP) [111]. Physicians should carefully follow up HALP patients with dysfunctional HDL due to impaired reverse cholesterol transport system to avoid the development of atherosclerosis.

In this review, the current knowledge on the pathogenesis of HALP was presented with special reference to the lipoprotein and gene abnormalities and atherogenicity of CETP deficiency. CETP plays a crucial role in the reverse cholesterol transport system. Patients with CETP deficiency are sometimes accompanied by atherosclerosis and thus CETP deficiency could rather be designated as ‘atherogenic HALP’. To the authors’ current knowledge, the level of serum HDL may not necessarily imply the functional aspects of HDL particles. HALP may be a condition of an impaired reverse cholesterol transport system. The ‘dysfunctional HDL particles’ produced by the impairment of molecules involved in reverse cholesterol transport system may lead to the acceleration of atherosclerosis in humans. For this reason, CETP inhibitors may not be a good tool for the purpose of treatment of atherosclerosis. Taken together, it may be important to establish a
strategy to assess the efficiency of reverse cholesterol transport system rather than merely determining the level of HDL-cholesterol.

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