review

Apolipoprotein-mediated removal of cellular cholesterol and phospholipids

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Abstract It is widely believed that high density lipoprotein (HDL) protects against cardiovascular disease by removing excess cholesterol from cells of the artery wall. Recent cell culture studies have provided evidence that a major pathway for removing cholesterol and phospholipids from cells is mediated by the direct interactions of HDL apolipoproteins (apo) with plasma membrane domains. These interactions efficiently clear cells of excess sterol by targeting for removal pools of cholesterol that feed into the cholesteryl ester cycle. The precursors for this pathway in vivo are likely to be lipid-free or lipid-poor apolipoproteins generated either by dissociation from the surface of HDL particles or by de novo synthesis. Fibroblasts from subjects with a severe HDL deficiency syndrome called Tangier disease have a cellular defect that prevents apolipoproteins from removing both cholesterol and phospholipids from cells. This defect is associated with a near absence of plasma HDL, markedly below normal low density lipoprotein (LDL) levels, and the appearance of macrophage foam cells in tissues. Thus, an inability of nascent apoA-I to acquire cellular lipids results in a rapid clearance of apoA-I from the plasma, decreased production and increased clearance of LDL, and sterol deposition in tissue macrophages. Although the molecular properties of this pathway are still poorly understood, these studies imply that the apolipoprotein-mediated pathway for removal of cellular lipids is a major source of plasma cholesterol and phospholipids and plays an important role in clearing excess cholesterol from macrophages in vivo.—**Oram, J.F., and S. Yokoyama.** Apolipoprotein-mediated removal of cellular cholesterol and phospholipids. *J. Lipid Res.* 1996. **37:** 2473–2491.

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Numerous population studies have shown an inverse correlation between risk for cardiovascular disease and plasma HDL levels (1, 2), leading to the widely held view that HDL protects against atherogenesis. This protection may be related to the role HDL plays in ''reverse cholesterol transport'', a pathway by which cholesterol is transported from extrahepatic cells to the liver for excretion from the body (3). It is believed that HDL retards formation of sterol-rich lesions in the artery wall by removing excess cholesterol from cells (4), the first and presumed rate-limiting step of reverse cholesterol transport.

This simple concept for the anti-atherogenic effects of HDL has come under question lately, largely because some human subjects and animal models with a virtual absence of HDL show no symptoms of atherosclerosis (5, 6) while others with above normal plasma HDL levels appear to have enhanced atherogenesis (7, 8). Recent cell culture studies are beginning to shed light on cellular mechanisms that may account for these apparent discrepancies and are generating important new insights into the relevance of HDL-mediated lipid transport to lipoprotein metabolism and atherogenesis. These studies have provided evidence that major precursors for mobilizing cholesterol from cells are HDL apolipoproteins associated with no or very little lipid.

HDL-mediated cholesterol efflux from cells

Many studies have shown that HDL stimulates cholesterol efflux from cultured cells, but how this occurs has been disputed. Aqueous diffusion and apolipoprotein binding models have been proposed as mechanisms by

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; apo, apolipoprotein; LCAT, lecithin:cholesterol acyltransferase; ACAT, acyl-CoA:cholesterol acyltransferase; ER, endoplasmic reticulum; HBP, high density lipoprotein binding protein; SR-BI, scavenger receptor-BI; NCEH, neutral cholesteryl ester hydrolase; PKC, protein kinase C; TD, Tangier disease; CETP, cholesteryl ester transfer protein; CVD, cardiovascular disease; LpA-I, HDL containing apoA-I without apoA-II; LpA-I:A-II, HDL containing both apoA-I and apoA-II; LpA-IV, HDL containing only apoA-IV as protein; γLpE, HDL containing only apoE as protein; PLTP, phospholipid transfer protein; LPL, lipoprotein lipase.

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which HDL removes cellular cholesterol. The aqueous diffusion model is based on studies showing that the phospholipid surface of HDL particles reabsorbs cholesterol that spontaneously desorbs from the plasma membrane of cells and diffuses through the aqueous layer surrounding the cell (9, 10). The apolipoprotein binding model proposes that HDL removes cellular cholesterol through the interaction of HDL apolipoproteins with cell-surface binding sites or receptors (11–17). It is now apparent that HDL can remove cellular cholesterol by both mechanisms, depending on the experimental conditions.

The aqueous diffusion mechanism operates in both directions so that cholesterol molecules are exchanged between cell membranes and HDL particles (9, 10, 18). For net removal of cellular cholesterol to occur, a physicochemical gradient must be established between the cell surface and HDL particles so that more cholesterol molecules are transferred to HDL than in the opposite direction. This gradient depends on the properties of both the cell membrane and cholesterol acceptor particles (9, 18–25). When an acceptor particle has a large capacity to absorb cholesterol, the rate-limiting step for cholesterol removal by this mechanism is desorption from the plasma membrane (9). The cholesterol absorption capacity of HDL, however, can saturate and become rate limiting, especially in the presence of a large source of cell membrane cholesterol. Under these conditions, the addition of the plasma enzyme lecithin:cholesterol acyltransferase (LCAT) improves the acceptor properties of HDL and increases its ability to remove cholesterol from cells (26, 27), presumably because the esterification of cholesterol on the particle surface displaces it into the core and allows surface phospholipids to incorporate more free cholesterol. With many cell types in culture, the rate of desorption of cholesterol from the plasma membrane is relatively slow ($t_{1/2} > 12$ h) (9, 19, 21, 28), and the aqueous diffusion mechanism can be inefficient even in the presence of LCAT at clearing cellular pools of excess cholesterol that are converted to and stored as cytosolic cholesteryl esters (13, 16, 17, 26, 27, 29).

The second mechanism by which HDL promotes cholesterol efflux from cells is mediated largely by its major apolipoprotein, apoA-I, probably after it dissociates from the surface of HDL particles (13–17, 30). Through reversible interactions with cell-surface binding sites, apoA-I not only removes cholesterol and phospholipids directly from the plasma membrane (13–17, 26–35), it also appears to stimulate mobilization of pools of cholesterol that are readily accessible to esterification by acyl-CoA:cholesterol acyltransferase (ACAT) (13, 17, 29, 30, 36, 37), an enzyme localized to the rough endoplasmic reticulum (ER). This process allows HDL to rapidly and efficiently remove excess cholesterol that would otherwise be stored as cholesteryl esters.

Apolipoprotein-mediated cholesterol and phospholipid efflux from cells

Early studies with cultured fibroblasts provided the first hint that HDL could stimulate cholesterol efflux from cells by more than one mechanism. Exposure of fibroblasts to HDL was shown to rapidly deplete cells of cholesterol pools that repressed synthesis of LDL receptors and steroidogenic enzymes and that were accessible to esterification by ACAT (36, 38). These depletions saturated at earlier timepoints and lower HDL concentrations than efflux of radiolabeled cholesterol (36), suggesting that they reflected a process distinct from total cholesterol efflux. Later it was shown that treatment of HDL with proteolytic enzymes such as trypsin to partially digest apolipoproteins completely abolished the ability of HDL to rapidly deplete cells of ACAT-accessible cholesterol but had little effect on HDL-mediated efflux of plasma membrane cholesterol (13, 29, 37). These studies demonstrated that, while HDL lipids stimulate cholesterol efflux from the plasma membrane of fibroblasts, depletion of ACAT-accessible cholesterol is mediated by trypsin-labile HDL apolipoproteins.

Studies with purified apolipoproteins and synthetic peptides have produced direct evidence for a specific apolipoprotein-mediated cholesterol removal pathway in cells. Purified human apoA-I, apoA-II (disulfide-linked homodimer), apoA-IV, and apoE were shown to stimulate cholesterol and phospholipid efflux from a variety of cultured cells, including macrophages, fibroblasts, endothelial cells, and smooth muscle cells (14–17, 26–35, 37, 39–42). This occurs by a saturable process with half-maximal values similar for each of these apolipoproteins (10^{-7}–10^{-8}M) (14–17, 29, 34, 39). Stimulation of cholesterol and phospholipid efflux by apolipoproteins depletes cells of excess cholesteryl esters and forms HDL-like particles in the culture medium (14, 15, 28, 29, 31–34, 40–42).

Removal of cellular lipids has a broad specificity for different exchangeable HDL apolipoproteins. Even apolipophorin III, an insect apolipoprotein, can mediate cholesterol and phospholipid efflux from macrophages (40). Not all apolipoproteins, however, appear to have these lipid transport-stimulating properties, as some of the apoCs and monomeric human apoA-II have little or no activity (14, 28, 39, 40). Based on comparisons among different apolipoproteins, Hara et al. (40) concluded that apolipoproteins require at least four amphipathic helical segments per molecule to stimulate cholesterol efflux from macrophages.

Two separate studies have shown that efflux of both

cholesterol and phospholipids from fibroblasts and macrophages is stimulated by synthetic peptides containing 18 amino-acid amphipathic helices of the type found in HDL apolipoproteins (class A helices) (43). In the study by Mendez et al. (17), peptides containing only one class A helix were almost completely inactive in promoting cholesterol and phospholipid efflux from cells at concentrations below 10^{-5}M. In contrast, peptides containing two tandem repeats of these helices were able to stimulate cholesterol and phospholipid efflux at concentrations below 10^{-6}M. Yancey et al. (35) found that peptides containing one or two amphipathic helices stimulated as much cholesterol efflux from cells as apoA-I, but the half-maximal concentrations required for the single-helix peptides were at least 5- and 20-fold higher than those for the 2-helix peptide and apoA-I, respectively. Despite some differences in apparent efficacies, these studies support the concept that cooperativity between class A amphipathic helices is an important factor in determining the lipid efflux-stimulating activities of apolipoproteins.

Cell-surface apolipoprotein binding sites

Studies from many laboratories have shown that HDL binds with saturable kinetics to the surface of multiple types of cultured cells. The number of these binding sites on fibroblasts, smooth muscle cells, macrophages, and aortic endothelial cells was shown to increase when cells were loaded with cholesterol, leading to the hypothesis that these binding sites may be receptors for HDL that function to facilitate removal of excess cellular cholesterol (11, 12, 44). Cross-competition studies revealed that HDL binding sites on cholesterol-loaded fibroblasts interact with purified apoA-I and synthetic peptides containing two tandem repeats of class A amphipathic helices (17). In contrast, these binding sites do not interact with HDL particles that have been modified by treatment with tetranitromethane (45) or proteases (13) and interact poorly with peptides containing only one amphipathic helix (17). Studies with monoclonal antibodies for apoA-I also suggested that amphipathic helical repeats in apoA-I are responsible for binding of HDL to hepatoma cells (46). Thus, as with lipid efflux, cooperativity between class A amphipathic helices in apolipoproteins is an important factor for establishing high-affinity interactions of HDL with cell-surface binding sites. Because these sites recognize HDL apolipoproteins, they are herein referred to as apolipoprotein binding sites.

Several lines of evidence suggest that the interaction of apolipoprotein with these cholesterol-regulated binding sites is responsible for the removal of cellular lipids by apolipoproteins. First, these sites have similar K_d values for apolipoprotein binding ($\sim 10^{-8}$M) as half-maximal values for stimulation of lipid efflux by apolipoproteins (17, 29). Second, cholesterol loading of cells increases the number of these binding sites (11, 12, 30) in association with increased apolipoprotein-mediated cholesterol and phospholipid efflux (11, 19, 34). Third, treatment of macrophages with the antioxidant drug probucol reduces both cell-surface apoA-I binding and apoA-I-mediated cholesterol efflux (47). Fourth, binding of peptides or apolipoproteins to these sites correlates with removal of lipids from cells (17, 39).

Additional evidence suggests that depletion of ACAT-accessible cholesterol from fibroblasts by HDL may actually be mediated by apolipoproteins that dissociate from HDL particles. Treatment of HDL with increasing concentrations of trypsin abolished the ability of HDL to interact with cell-surface binding sites and to deplete ACAT-accessible cholesterol when less than 20% of its apolipoprotein content was degraded (A.J. Mendez and J.F. Oram, unpublished results), indicating that a small fraction of the most trypsin-labile apolipoproteins in HDL mediate both of these processes. The apoA-I molecules on HDL most sensitive to proteolysis are likely to be less tightly bound to phospholipids (48, 49) and thus more easily dissociable from the particles. This dissociation hypothesis is further supported by studies showing that, after exposing cholesterol-loaded fibroblasts to HDL, the particles that bind reversibly to the cell surface have a several-fold higher ratio of apoA-I to apo A-II than the initial HDL particles (30). As apoA-I is more exchangeable than apoA-II, these results suggest that apoA-I is selectively transferred from HDL particles to cell-surface binding sites. This transfer may be driven by the relative high-affinity of the cell-surface binding sites for apolipoproteins which is at least one order of magnitude higher than that for phospholipid surfaces (50, 51). Because the highest affinity interactions depend on cooperativity between amphipathic helical repeats, these binding sites may be specific for apolipoproteins containing no or very little lipid to block tandem repeats of amphipathic helices. Additional studies are needed to confirm this hypothesis, as it has not been established yet that free apoA-I transfers from HDL to cell-surface binding sites. The above results are also consistent with the possibility that these binding sites preferentially interact with protease-sensitive subfractions of HDL particles enriched with apoA-I.

Not all lipid efflux processes are associated with cell-surface binding of particles or apolipoproteins. Trypsinized HDL, phospholipid vesicles, and phospholipid/triglyceride emulsions stimulate cholesterol efflux from cells despite their lack of interaction with these binding sites (9, 10, 13, 16–26, 28, 29), presumably because these phospholipid-rich particles act as acceptors for cholesterol that desorbs from the plasma membrane.

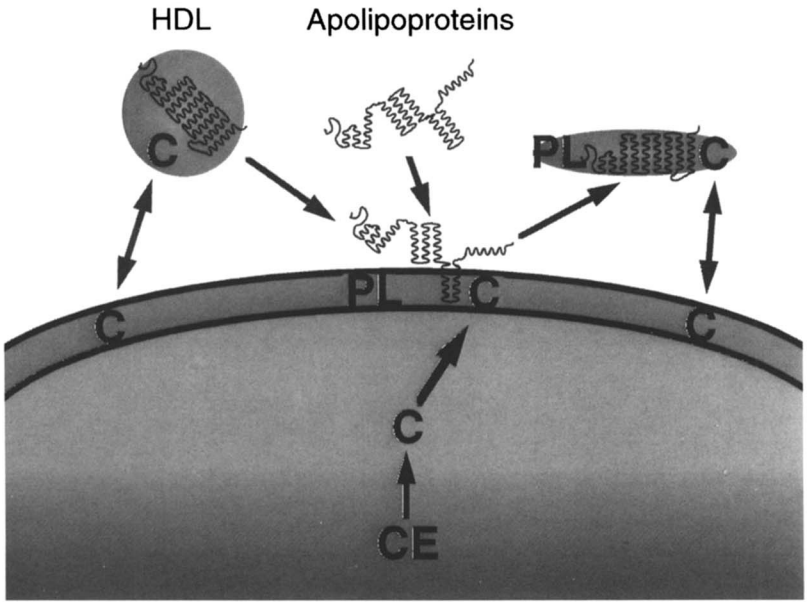

Fig. 1. A model for two mechanisms of HDL-mediated cholesterol efflux from cells. Abbreviations are: C, unesterified cholesterol; CE, esterified cholesterol; PL, phospholipids.

Apolipoproteins and phospholipid-containing particles, however, appear to remove distinct pools of cellular cholesterol when initially incubated with cultured cells. When fibroblasts were exposed to phospholipid vesicles, trypsinized HDL, lipid-free apoA-I, or a peptide containing two amphipathic helices under conditions that caused equivalent amounts of cholesterol efflux, only apoA-I and the synthetic peptide depleted cells of cholesterol available for esterification by ACAT (13, 17). Although free apolipoproteins may promote cholesterol efflux from cells by the aqueous diffusion mechanism after they first pick up cellular phospholipids and become cholesterol acceptors (31, 34, 35), this simple two-step mechanism cannot account for all the cholesterol removed by apolipoprotein–cell interactions. For example, a rat aortic smooth muscle cell line was shown to release very little cholesterol in the presence of apolipoproteins, despite substantial phospholipid efflux to apolipoproteins and cholesterol efflux to HDL particles and phospholipid–triglyceride microemulsions (15, 16, 41). Apolipoproteins appear not to have access to cholesterol in these cells, even though phospholipids are available for removal by apolipoproteins and cholesterol efflux occurs by aqueous diffusion. Therefore, apolipoproteins are targeted to different pools of cellular cholesterol than those desorbing from the plasma membrane and diffusing to phospholipid-containing particles.

Based on these studies, we propose the following model for the two mechanisms of cholesterol efflux from cells (**Fig. 1**). Removal of both cholesterol and phospholipids from cells by purified apolipoproteins is mediated by their interactions with high-affinity binding sites on the cell surface. The cholesterol removed by this process is either the substrate pool for ACAT or is in rapid equilibrium with this pool, thus allowing apolipoproteins to selectively deplete cholesterol available to ACAT and mobilize cholesterol from the cholesteryl ester cycle. HDL particles, however, can remove cholesterol from cells by two mechanisms. First, apolipoproteins can dissociate from HDL particles and interact with cell-surface binding sites, stimulating cholesterol and phospholipid efflux by the same mechanism described above. Second, because HDL contains phospholipids, it is also a good acceptor of cholesterol that desorbs from the plasma membrane and thus can stimulate cholesterol efflux by the aqueous diffusion mechanism. Once apolipoproteins acquire phospholipid, they also may become acceptors for cholesterol that diffuses from the plasma membrane. The aqueous diffusion mechanism can also clear cells of excess cholesterol, although most of the cholesterol initially removed by this mechanism does not appear to be in rapid equilibrium with cholesterol that is substrate for ACAT (13, 17, 29).

The relative contribution of the two mechanisms to HDL-mediated cholesterol efflux depends on the cholesterol and growth state of cells. With cholesterol-depleted and rapidly proliferating cells, the number of apolipoprotein binding sites is at a minimum (11–13, 30) and apolipoprotein-mediated phospholipid and cholesterol efflux is relatively low (13, 34, 52), presumably to limit removal of lipid needed for continual membrane synthesis. Arresting the growth of cells and loading them with cholesterol increases the number of apolipoprotein binding sites and enhances apolipopro-

tein-mediated cholesterol and phospholipid efflux (11–13, 34). Thus, with proliferating cells grown in the presence of serum, the aqueous diffusion mechanism accounts for most of the cholesterol efflux that occurs in the presence of HDL, especially at high concentrations (A.J. Mendez and J.F. Oram, unpublished results). With quiescent and differentiated cells, however, the apolipoprotein-mediated mechanism contributes significantly to the total HDL-mediated cholesterol efflux and may be the major process for clearing cholesteryl esters from cells (29).

The molecular properties of the apolipoprotein binding sites involved in lipid removal are still unknown. Although these sites have features of receptors, it has yet to be shown that receptor proteins are involved in facilitating lipid transport to apolipoproteins. Apolipoprotein-mediated cholesterol efflux is abolished by treatment of macrophages with trypsin and is absent in erythrocytes (27, 53), suggesting that endogenously produced membrane proteins are involved either in promoting apolipoprotein interactions or in other steps of the lipid transport process. Several cell membrane-associated HDL binding proteins have been identified that are candidates for apolipoprotein receptors (54–58), but their actual functions have not been defined. One of these proteins, called HBP, has been cloned and sequenced (59). Its expression is increased by cholesterol loading of cells and its overexpression in cultured cells enhances HDL binding, suggesting that it may function to remove excess cholesterol from cells. However, HBP lacks the classic signal peptides and membrane spanning regions that typify plasma membrane receptors. Recently it was shown that a class of scavenger receptors called SR-BI binds HDL and promotes cellular uptake of HDL lipids (60). As this receptor binds phospholipids and is expressed largely in steroidogenic tissues and the liver, it is more likely that it functions to deliver lipoprotein sterol to tissues rather than to remove lipids from cells through apolipoprotein interactions.

The high-affinity apolipoprotein binding sites on fibroblasts have different properties than apolipoprotein interactions with phospholipids. Studies comparing cellular interactions of apolipoproteins and amphipathic helical peptides have shown that the specificity of cell-surface apolipoprotein binding sites is not a simple function of lipid affinity (17, 61). Yancey et al. (35) also concluded that the total lipid efflux-stimulating activity of apoA-I and amphipathic helical peptides could not be explained by lipid affinity alone, although the ability of synthetic peptides to stimulate cholesterol efflux was in the order of their lipid affinity. Therefore, it is likely that the interaction of amphipathic helices with plasma membrane phospholipids plays some role in apolipoprotein binding, perhaps by "presenting" apolipoproteins in a proper conformation for binding to plasma membrane proteins or receptors. Such interactions at the lipid–protein interface have been postulated for the binding of lipophilic compounds to the multi-drug transporter (62). It is also possible that the binding sites for apolipoproteins are microdomains of the plasma membrane that, because of a unique composition of lipids and proteins, bind apolipoproteins with a higher affinity and different specificity than the bulk of the plasma membrane. Consistent with this idea is a report by Fielding and Fielding (63) that HDL stimulates cholesterol efflux from cellular membrane structures with properties of caveolae, sphingomyelin- and cholesterol-rich invaginations of the plasma membrane containing proteins involved in transmembrane transport and signaling (64). Whether caveolae or other related plasma membrane structures mediate apolipoprotein interactions with cells remains to be determined.

Apolipoprotein-stimulated cholesterol excretory pathway

Exposure of cholesterol-loaded cells to apolipoproteins stimulates efflux of sterols derived from intracellular pools as well as from the plasma membrane (13, 37, 65–71). As a result, stored cholesteryl esters are cleared efficiently from cells (13, 14, 29). This appears to occur because apolipoprotein–cell interactions mobilize free cholesterol away from the cholesteryl ester cycle. Thus cholesteryl esters are hydrolyzed by neutral cholesteryl ester hydrolase (NCEH) but the liberated free cholesterol is no longer available for re-esterification by ACAT, causing a net depletion of cholesteryl esters. Apolipoproteins appear not to have any direct effects on the activities of the ACAT and NCEH enzymes (29, 72).

The mechanisms by which apolipoproteins divert free cholesterol from the cholesteryl ester cycle are unknown. In addition to removing cholesterol from plasma membrane domains that feed into the cholesteryl ester cycle, the interaction of apolipoproteins with cell-surface binding sites may also stimulate steps in the pathway that mobilize sterols from the ER to the plasma membrane. When sterols in cholesterol-loaded cells are radiolabeled with the biosynthetic precursor mevalonolactone, exposure of cells to HDL or apoA-I causes a transient increase in the amount of radiolabeled sterols accessible to cholesterol oxidase treatment of cells (13, 65–68), a procedure that presumably oxidizes cell-surface sterols (73). It has also been shown that efflux of biosynthetically labeled sterols is mediated by plasma apoA-I-containing particles when plasma is added to hepatoma cells (70, 71). As this radiolabeling procedure selectively introduces sterol into the ER, these

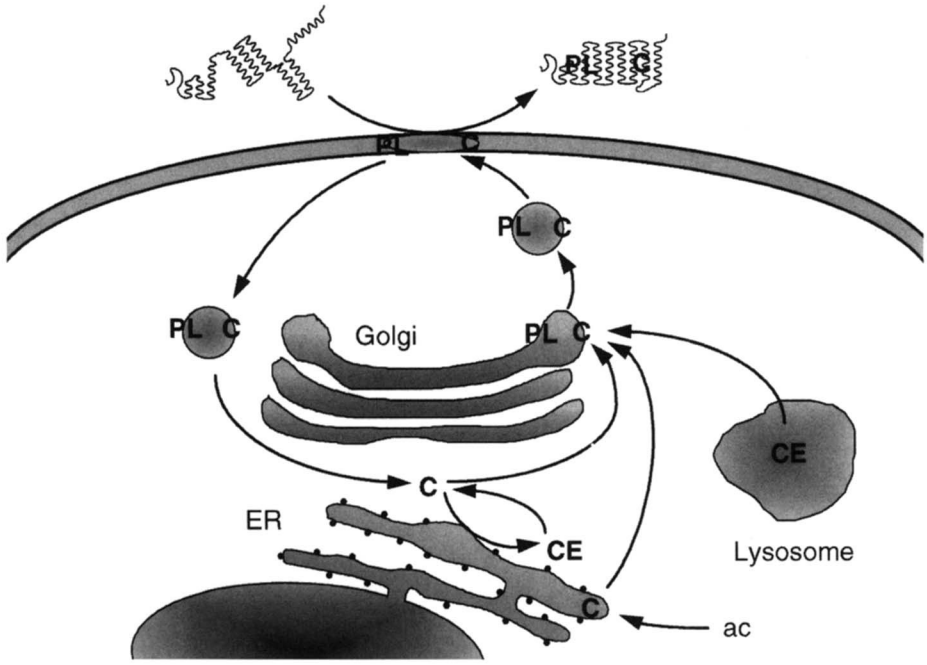

Fig. 2. A model for the intracellular cholesterol transport pathway involved in apolipoprotein-mediated lipid removal. Abbreviations are: C, unesterified cholesterol, CE, esterified cholesterol; PL, phospholipids; ER, endoplasmic reticulum; ac, acetyl units.

studies suggest that apolipoprotein interactions with cells stimulate sterol translocation from the ER to the plasma membrane where the sterol becomes accessible for removal by apolipoproteins.

Cholesterol is recycled between the ER and the plasma membrane by transport pathways that are still poorly understood (74–78). **Figure 2** illustrates a hypothetical model for the transport cycle involved in apolipoprotein-mediated lipid removal. Both the cholesterol and phospholipids removed from cells by long-term (>6 h) incubations with apoA-I appear to pass through the Golgi apparatus, as treatment of cells with inhibitors of Golgi transport (Brefeldin A monensin) blocks the ability of apolipoproteins to stimulate efflux of these lipids from cholesterol-loaded fibroblasts and macrophages (79, 80). It is possible that free cholesterol is transported from the ER and/or lysosomes to the Golgi and assembled in phospholipid-rich vesicles that are subsequently transported to the cell surface. Upon fusion with the plasma membrane, these vesicles may form discrete membrane domains with unique compositions of lipids and proteins, similar to what has been described for caveolae (81). These domains may have only a transient existence in the plasma membrane, and their cholesterol molecules may be recycled back to the ER where they are either esterified by ACAT or reenter the vesicle transport cycle for additional rounds of transport.

The relative rates of the different branches of this pathway may be tightly regulated according to the cellular growth state and cholesterol content. Thus, as cells accumulate cholesterol in excess of that needed for membrane synthesis, the plasma membrane becomes saturated with sterol and more cholesterol is diverted into the ER for esterification, consistent with the "threshold effect" of cholesterol loading observed in macrophages (82). Additional support for this concept stems from studies showing that, as cells are growth arrested and overloaded with cholesterol, a greater fraction of newly synthesized sterols accumulates within cellular compartments accessible to ACAT and resistant to treatment of cells with cholesterol oxidase (13, 67). Cholesterol loading of cells may also alter the properties of transport vesicles so that they bind more apolipoproteins after fusion with the plasma membrane. This would directly target apolipoproteins to pools of free cholesterol that would otherwise be recycled back to the ER for esterification. Thus the reversible binding of apolipoproteins to these membrane domains would add an excretory component to this transport cycle.

This model for a regulated lipid transport cycle is consistent with data showing that cellular binding of HDL apolipoproteins only transiently increases accessibility of pulse-labeled sterols to extracellular cholesterol oxidase (65–68). Although regulation by HDL and its apolipoproteins was not examined, a similar cytoskele-

ton- and Golgi-dependent sterol transport pathway was identified in fibroblasts lacking sterol carrier protein-2 (83). It should be noted, however, that apolipoprotein-induced alterations of the plasma membrane can explain much of the existing data, including enhanced accessibility of sterols to cholesterol oxidase. Also, Golgi transport inhibitors may suppress apolipoprotein-mediated removal of cellular lipids by blocking translocation of proteins that promote cell-surface binding of apolipoproteins or release of plasma membrane lipids, or they may perturb the plasma membrane in other ways. Clearly, additional studies are needed to localize and characterize the cellular compartments involved in the apolipoprotein-mediated lipid removal pathway.

Role of intracellular signals in apolipoprotein-mediated lipid excretion

The interaction of apoA-I with cells activates protein kinase C (PKC), and this is associated with stimulation of protein secretion (84–86) or lipid efflux (37, 41, 87–90), depending on the cell type. With fibroblasts and smooth muscle cells, activation of PKC by acute phorbol ester treatment mimics apoA-I in stimulating translocation of biosynthetically labeled sterol to the plasma membrane (37, 88). Chronic treatment of fibroblasts with phorbol esters, which abolishes PKC activity, eliminates the ability of HDL to stimulate efflux of sterols (37). Li and Yokoyama (41) showed that rat vascular smooth muscle cells had virtually no apoA-I-mediated cholesterol efflux unless activated by treatment with growth factors and phorbol esters. This enhanced cholesterol efflux to apoA-I was reversed by PKC inhibitors and was associated with no changes in cholesterol efflux occurring by aqueous diffusion. These studies suggest that activation of PKC plays a role in the apolipoprotein-mediated cholesterol excretory pathway. Another study, however, showed that down-regulation of PKC by chronic phorbol ester treatment of cholesterol-loaded fibroblasts failed to suppress apoA-I-mediated cholesterol efflux and depletion of ACAT substrate even though efflux of biosynthetically labeled sterol was inhibited (A.J. Mendez, personal communication). Thus PKC may be involved in modulating discrete steps of apolipoprotein-mediated sterol efflux, such as transport of sterols from the ER to the Golgi, or it may play a unique role in modulating transport of biosynthetic sterols in some cell types.

The interaction of HDL with fibroblasts elicits multiple signaling responses that likely involve different agonists within HDL particles. HDL has been shown to stimulate intracellular calcium release (91, 92) and to activate phosphoinositide- and phosphatidylcholine-specific phospholipase C as well as phosphatidylcholine-specific phospholipase D (93). Activation of phosphoinositide-specific phospholipase C has been linked to stimulation of PKC_α and PKC_ϵ isoforms (92). Activation of phosphatidylcholine-specific phospholipase C may be a secondary response to PKC stimulation (93). Acute activation of phospholipase D was most closely associated with apolipoprotein-mediated cholesterol efflux, in that it was increased upon cholesterol loading of cells, it saturated at HDL concentrations similar to those producing near maximum depletion of ACAT-accessible cholesterol, and it also was observed when cells were exposed to apoA-I proteoliposomes (93). These studies indicate that signaling responses to HDL interactions with cells are complex and that additional studies are needed to establish their involvement in lipid transport.

Physiologic relevance of apolipoprotein-mediated lipid removal from cells: lessons from Tangier disease

The best support for the physiologic significance of apolipoprotein-mediated cellular lipid removal comes from studies with fibroblasts from subjects with a genetic HDL deficiency called Tangier disease (TD). Homozygous TD is characterized by a near complete absence of plasma HDL and apoA-I and by massive deposition of cholesteryl esters in tissue macrophages (94–96). The low plasma HDL levels are caused by a rapid clearance of apoA-I and HDL particles (96, 97). The molecular structure and production rates of apoA-I are normal in this disease (98, 99). Recently, Francis Knopp, and Oram (29) reported that fibroblast lines from two unrelated subjects with homozygous TD had a defect in the apolipoprotein-mediated lipid removal pathway. Lipid-poor apoA-I from normal subjects almost completely lacked the ability to remove cholesterol and phospholipids from these TD cells which was associated with a below normal interaction of apoA-I with cell-surface binding sites. In contrast, efflux of radiolabeled cholesterol to trypsinized HDL and efflux of both cholesterol and phospholipids into albumin-containing medium were normal for TD cells, indicating that aqueous diffusion mechanisms of lipid efflux are unimpaired in these cells.

The same lipid transport defect has now been found in at least eight fibroblast lines from unrelated TD homozygotes (29, 100, J.F. Oram, unpublished results) and was shown to occur for other apolipoproteins, including A-II, C-III, and E (100). Moreover, the dimeric amphipathic helical peptide 37pA, which mimics apolipoproteins in stimulating cholesterol efflux and depleting ACAT-accessible cholesterol from normal fibroblasts (17), has almost no effect on these lipid transport processes when the cells are from a homozygous TD patient (**Fig. 3**). These results indicate that the defect in TD cells resides in the apolipoprotein-mediated

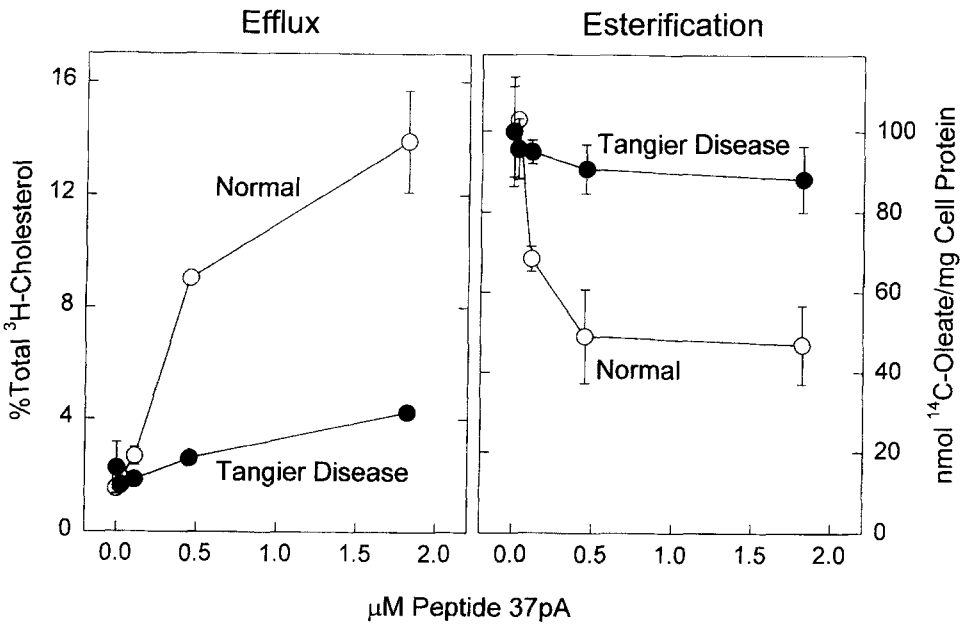

Fig. 3. Effects of synthetic peptide 37pA on cholesterol efflux and esterification by fibroblasts from a normal subject and a patient with homozygous Tangier disease. Fibroblasts were incubated for 72 h with medium containing 10% fetal bovine serum plus [^{3}H]cholesterol to radiolabel cellular pools to equilibrium. Cells were then cholesterol loaded by incubation for 48 h with serum-free medium containing 2 mg/ml albumin plus 30 μg/ml cholesterol. After an overnight equilibrium in serum-free medium containing albumin, cells were incubated with medium containing albumin plus the indicated concentrations of synthetic peptide 37pA ([DWLKAFYDKVAEKLKEAF]$_{3}$P). After 6 h, the medium was collected, cells were pulsed for 1 h at 37°C with [^{14}C]oleate, and medium radioactivity and cellular [^{3}H]cholesterol, [^{3}H]cholesteryl esters, and cholesteryl [^{14}C[oleate were measured. Efflux represents the fraction of total (medium plus cell) [^{3}H]cholesterol appearing in the medium and esterification represent incorporation of [^{14}C]oleate into cholesteryl esters. Each value is the mean ±SD of triplicates.

lipid removal pathway described above. Although different genes may be involved, the defective gene in each TD kindred appears to encode a structural or regulatory protein essential for this lipid removal pathway.

A comparison of different TD and normal fibroblasts lines showed that HDL stimulated cholesterol efflux from growth-arrested, cholesterol-loaded TD fibroblasts to levels 50–70% of normal (28). This is consistent with other studies showing that at least 50% of the HDL-mediated cholesterol efflux from normal fibroblasts occurs by the aqueous diffusion mechanism (13, 28, 29). Both HDL and purified apoA-I were shown to stimulate clearance of cholesteryl esters from growth-arrested, cholesterol-loaded normal fibroblasts. Yet despite significant cholesterol efflux in the presence of HDL, neither HDL nor apoA-I were able to clear cholesteryl esters from TD fibroblasts (29). These findings support the concept that apolipoprotein-mediated lipid removal is much more effective than aqueous diffusion mechanisms in clearing excess cellular cholesterol that feeds into the cholesteryl ester cycle.

Walter et al. (68) and Rogler et al.(69) reported partial defects in the ability of HDL or apoA-I-containing phospholipid vesicles to stimulate efflux of newly synthesized sterols from fibroblast lines obtained from two homozygous TD siblings. In contrast, they found no significant differences between normal and TD fibroblasts in HDL- or vesicle-mediated efflux of cholesterol either associated with the plasma membrane or derived from lysosomal hydrolysis of LDL. Francis, Knopp, and Oram (29) reported, however, that lipid-free apoA-I was unable to remove cellular cholesterol from TD fibroblasts whether the cholesterol tracer was added directly to the plasma membrane or introduced through lysosomal hydrolysis. Although it is possible that these apparent discrepancies reflect different cellular defects in lipid transport among TD patients, they may be related to differences in methodology. Walter, Rogler, and colleagues (68, 69) used phospholid-containing particles that would stimulate radiolabeled cholesterol efflux mostly by the aqueous diffusion mechanism, particularly when the cholesterol tracer is initially associated with the plasma membrane. Efflux of biosynthetically labeled sterol, however, may be more dependent on apolipoprotein–cell interactions, as much of this label originates in the ER. Thus, when cells are exposed to

HDL or apolipoprotein–phospholipid vesicles, the defect in apolipoprotein-mediated sterol removal from TD cells may be more apparent with cells containing biosynthetically labeled sterols. Taken together, all studies with TD fibroblasts so far reported support the concept that the apolipoprotein-mediated lipid removal pathway is defective in TD fibroblasts while the aqueous diffusion mechanism is normal.

Apolipoprotein-mediated lipid transport in vivo

The studies with fibroblasts from TD patients have important implications about the contribution of apolipoprotein-mediated removal of cellular lipids to whole body lipoprotein metabolism. Apparently, a cellular defect that prevents apolipoproteins from removing phospholipids and cholesterol from cells leads to a rapid turnover of apoA-I produced by the liver and a near absence of plasma HDL (94–99). This implies that the apolipoprotein-mediated lipid removal pathway is an absolute requirement for generating even low levels of HDL particles in the blood. This lack of HDL production also appears to have an impact on the LDL pathway, as homozygous TD patients have plasma LDL levels averaging less than 40% normal.

The cell culture studies also imply that apolipoproteins containing very little or no lipid are the precursors for this lipid transport pathway in vivo. The broad specificity of apolipoprotein-mediated lipid removal from cultured cells suggests that more than one exchangeable apolipoprotein may participate in this pathway. As this lipid removal process saturates at apolipoprotein concentrations of 10^{-7}M, less than 1% of the total plasma supply of exchangeable apolipoproteins, only trace amounts of lipid-free or lipid-poor apolipoproteins would be required to stimulate this pathway maximally in vivo, provided they could be regenerated continuously.

Although apolipoproteins completely free of lipid have not been identified in vivo, those containing a relatively small amount of phospholipids (10–40% total weight) are present in plasma and lymph. The best characterized of these is preβ-1 HDL, a small particle containing only apoA-I and some phospholipid (101). This particle has been called "lipid-poor apoA-I" to distinguish it from more phospholipid-rich apoA-I discs and spherical HDL particles containing core lipids. Antibody studies have shown that the apoA-I in preβ-1 particles have different epitopes exposed than apoA-I in HDL discs and spherical particles (102), indicating that these lipid-poor apolipoproteins have a different conformation than those associated with more lipid-rich particles. Lipid-poor apoA-I comprises 5–8% of the plasma apoA-I but is more abundant in the lymph and interstitium (101, 103–107).

When plasma is added to cultured fibroblasts, a large fraction of the cholesterol initially released from cells is associated with lipid-poor apoA-I (101). Based on the model for HDL-mediated lipid removal shown in Fig. 1, there are three mechanisms that may account for the selective transfer of cellular cholesterol into plasma preβ-1 HDL. First, cholesterol may be transported to the apoA-I–phospholipid complexes by the aqueous diffusion mechanism. For this to occur, these minor lipoprotein components would need to have properties that make them much greater acceptors of cholesterol than the bulk of the plasma HDL particles. Second, lipid-free apoA-I may dissociate from the surface of other plasma HDL particles, bind to cell-surface binding sites, and pick up cellular cholesterol and phospholipids to become preβ-1 HDL. This mechanism is supported by studies showing that preβ-1 HDL disappears from plasma with time of incubation in the absence of cells but is retained when fibroblasts are present (108), consistent with cell-mediated regeneration of these particles from other lipoproteins. Moreover, incubation of macrophages with purified apolipoproteins generates particles with properties of preβ-1 HDL (14, 15, 26, 28, 31, 32, 34, 40). Third, preβ-1 HDL may interact directly with cells and remove lipids. At least some of this lipid-poor apoA-I may not contain enough phospholipid to interfere with cellular interactions of amphipathic helical repeats that facilitate lipid removal. The possible involvement of either or both of the last two mechanisms is supported by studies showing that stimulation of cholesterol efflux from cells by purified apoA-I and plasma preβ-1 HDL have some similarities, in that transport of cell-derived cholesterol to both of these forms of apoA-I is reduced when cells are pretreated with proteases and is absent when the cholesterol donors are red blood cells (27, 53, 109).

It is noteworthy that, despite the absence of αHDL, the plasma of TD patients contains lipid-poor preβ-1 HDL particles that can stimulate cholesterol efflux from fibroblasts (110, 111). Some of these particles may have only apoA-II as their major protein (110). As apolipoprotein-mediated removal of cellular lipids is severely impaired in these patients, these findings suggest that a fraction of HDL apolipoproteins can acquire lipids from some other source, presumably circulating lipoproteins.

Although lipid-poor apoA-I may acquire lipids by multiple mechanisms, it is reasonable to assume that many of these particles in plasma and interstitium are the immediate precursors or products of the cellular apolipoprotein-mediated lipid removal pathway. Several studies have provided evidence that most of this apoA-I is generated initially by dissociating from the surface of circulating spherical HDL particles (112–114),

a process that may be facilitated by plasma lipid transfer proteins and enzymes (115–118). Some of the plasma and interstitial lipid-poor apoA-I may represent apolipoproteins newly synthesized by the liver and/or intestine. Organ perfusion and cell culture studies, however, have shown that most apoA-I secreted by hepatocytes is associated with significant amounts of phospholipid and appears as discs or small spherical particles (119–125). This suggests that newly synthesized apoA-I quickly forms phospholipid complexes while within cells and/or after secretion. In the latter case, secreted apoA-I may interact immediately with binding sites on liver cells to pick up lipid by the apolipoprotein-mediated pathway described above. This process may rapidly convert apoA-I secreted by the liver into discs and block further interactions with lipid-removing cell-surface binding sites. Although there are no data characterizing the effects of phospholipid acquisition by apoA-I on its cellular interactions, studies have shown that disc-shaped preβ-2 HDL particles in plasma are not initial acceptors of cellular cholesterol when plasma is added to cultured cells (101), suggesting that these particles do not mediate removal of cellular lipids. The cholesterol in HDL discs may be the preferred substrate for LCAT, making these particles the immediate precursors for spherical HDL. Therefore, by the time a free apoA-I molecule reaches most peripheral cells, it may have already undergone one or more cycles of maturation to spherical HDL and dissociation from the particle surface.

In addition to those containing apoA-I, lipid-poor particles containing apoE (γLpE) (111, 126) and apoA-IV (LpA-IV) (127) have been found in plasma and shown to contribute to the early removal of cellular cholesterol. Little is known about their metabolic pathways, but it is likely that at least some of these particles are also immediate precursors or products of the apolipoprotein-mediated lipid removal pathway. A unique feature of apoE is that it is produced by tissue macrophages as well as the liver (128) and thus can mobilize cholesterol from macrophage-rich tissues without first entering the plasma compartment.

Figure 4A illustrates a possible model for apoA-I-mediated lipid transport between the liver and peripheral tissue. Newly synthesized apoA-I may be secreted by hepatocytes as a lipid-free or lipid-poor apolipoprotein (Step 1). Most of these apoA-I molecules may interact directly with apolipoprotein binding sites on liver cells (Step 2), but some may circulate to peripheral tissues and accumulate in interstitial fluids (Step 3). Through reversible interactions with cell-surface binding sites, lipid-poor apoA-I removes cellular phospholipids and free cholesterol to become disc-shaped particles (Steps 2 and 4). At this stage, amphipathic helices may become masked by lipid interactions, and the discs may lose their ability to interact with cell-surface binding sites, although they are likely to acquire additional free cholesterol that desorbs from the surfaces of cells and lipoproteins. These particles are now committed to the HDL maturation cascade, and the free cholesterol is esterified by LCAT to form the core cholesteryl esters of spherical HDL particles (Step 5). Some of these cholesteryl esters then are transferred to VLDL and LDL by the action of cholesteryl ester transfer protein (CETP) (129) (Step 6) while others are transferred selectively to the liver (Step 7) or steroidogenic tissues (not shown) (130), perhaps after interacting with a receptor specific for these tissues, such as SR-BI (60). The removal of core lipids shrinks the particle and promotes the dissociation of apolipoproteins from the particle surface, generating new precursors for the apolipoprotein-mediated lipid removal pathway (Step 8). A fraction of the lipid-poor apolipoproteins would be cleared from the plasma, probably by the kidney (Step 9).

This model conforms to data from several tracer kinetic studies. Schwartz et al. (132) reported that most of the initial hepatic output of newly synthesized cholesterol in humans appears as free cholesterol associated with plasma HDL, a process that may be facilitated by apolipoprotein interactions with liver cells. Dietschy, Turley, and Spady (133) proposed that biosynthesis of cholesterol by extrahepatic cells accounts for most of the daily supply of cholesterol in humans (~10 mg/kg body weight). To maintain constant amounts of cholesterol in tissues, this cholesterol must be excreted by cells and transported to the liver by the reverse cholesterol transport pathway. Although aqueous diffusion mechanisms may account for some of this transport, it is likely that lipid-poor apolipoproteins play a major role. If the primary source of whole body cholesterol is peripheral tissue, more cholesterol must be transported from extrahepatic tissues to the liver than in the opposite direction. This directional transport may occur because newly synthesized apoA-I molecules are recycled between their free and lipid-bound forms and are used multiple times to mobilize lipids from tissues before being cleared from the plasma. This pathway would also be predicted to modulate plasma LDL levels. In addition to increased LDL production by CETP-mediated transfer of HDL cholesteryl esters (Step 6, Fig. 4A), the delivery of HDL sterols to the liver would act to down-regulate hepatic LDL receptors (Step 7) (134), decreasing LDL clearance from the plasma (Step 10). Thus the apolipoprotein-mediated lipid transport pathway would tend to raise plasma LDL levels.

This model provides a plausible explanation for the clinical features of TD. TD patients produce apoA-I at normal rates, but these molecules are unable to pick up

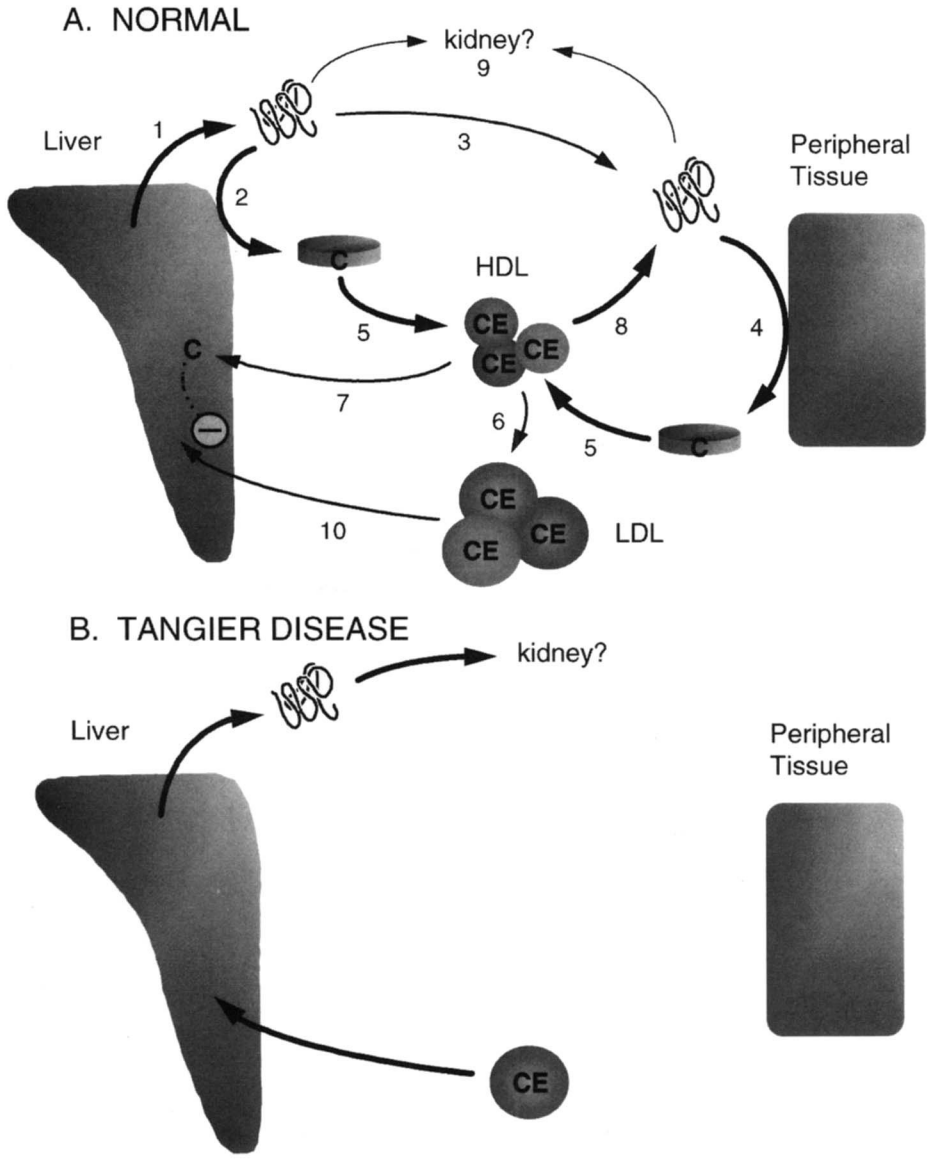

Fig. 4. Model for the apoA-I-mediated cholesterol transport in normal subjects (A) and patients with Tangier disease (B). Abbreviations are: C, unesterified cholesterol; CE, esterified cholesterol. Numbers refer to steps in the pathway discussed in the text.

phospholipid and cholesterol from tissues because of a cellular defect in this process (Fig. 4B). Thus lipid-poor apoA-I is unable to mature into HDL discs and spherical particles and thus is rapidly cleared from the plasma. A faster-than-normal turnover of HDL and its apolipoproteins occurs even when HDL from normal subjects is injected into TD patients (96, 97), probably because the free apoA-I dissociating from these particles cannot pick up tissue lipids to regenerate mature HDL. The one-third normal plasma LDL levels in these patients may reflect both a lack of transfer of HDL sterols to LDL and an inability of the apolipoprotein-mediated lipid transport pathway to deliver tissue-derived cholesterol to the liver and suppress LDL receptors, leading to a greater LDL clearance.

Although cholesterol efflux from all cells would be markedly reduced, the cholesterol content of most cells of the body would be unaffected by a defect in apolipoprotein-mediated lipid removal because it is regulated almost exclusively by the two major sterol delivery pathways: cholesterol biosynthesis and LDL receptor-mediated endocytosis (134). Feedback repression of these pathways limits the amount of cholesterol that accumulates in cells. Also, the aqueous diffusion mechanism of

cholesterol efflux, which is normal in TD cells, may help prevent overaccumulation of cellular cholesterol. Macrophages, however, internalize cholesterol-rich lipoproteins and cell debris by receptor and phagocytotic pathways that are not repressed when cells amass excess cholesterol (135, 136), making these cells dependent on apolipoprotein-mediated cholesterol removal mechanisms to prevent deposition of sterols. This would explain why TD patients accumulate excess sterol in macrophage-rich tissues (96).

Apolipoprotein-mediated lipid transport and cardiovascular disease

Epidemiology studies have shown a strong inverse correlation between plasma HDL levels and risk for cardiovascular disease (CVD) (1, 2), consistent with the concept that HDL protects against atherogenesis. Individual case reports, however, have indicated that not all severe HDL deficiency syndromes are associated with CVD (5), implying that a marked impairment of HDL-mediated cholesterol transport does not necessarily predispose an individual to premature atherosclerosis. These apparent discrepancies can be explained by the concept that apolipoprotein-mediated removal of cellular lipids protects against CVD, but only when atherogenesis is initiated and propagated by other factors. Of particular relevance would be factors that recruit monocytes into the artery wall, induce their differentiation into macrophages, and promote deposition of cholesteryl esters in these and other artery wall cells. Accumulation of sterol-rich lipoproteins such as LDL in the artery wall plays an important role in these atherogenic processes because these particles become both inflammatory signals for monocyte recruitment and sources for excess sterol taken up by macrophages (137).

This concept may also explain why some patients with severe HDL deficiencies have massive deposition of sterols in macrophages of various tissues but do not have symptoms of CVD (5). Macrophages of the reticuloendothelial system probably function largely as components of apoptic pathways for clearing dying and senescent cells and may be responsive to different signals than those involved in atherogenesis. Most of the sterol ingested by these tissue macrophages would be derived from cell membranes rather than circulating lipoproteins. Thus an impaired apolipoprotein-mediated lipid removal pathway could lead to accumulation of cholesterol in tissue macrophages independent of susceptibility to atherosclerosis.

Once atherosclerotic lesion formation is initiated, there are two major aspects of this lipid-transport pathway that could influence lesion progression: *1*) the availability or efficacy of lipid-poor apolipoproteins and *2*) the activity of the cellular pathway for apolipoprotein-mediated lipid removal.

Availability or efficacy of lipid-poor apolipoproteins. Most rare genetic HDL deficiencies caused by a lack of hepatic apolipoprotein production are associated with premature atherosclerosis, consistent with the concept that a lack of HDL apolipoproteins is atherogenic. This is particularly true for patients with impaired production of more than one apolipoprotein, such as those with apoA-I/C-III or apoA-I/C-III/A-IV deficiency syndromes (5, 138). However, some patients with apoA-I deficiency syndromes have no symptoms of CVD (5). Moreover, disruption of the apoA-I gene in mice does not increase atherosclerotic lesion development (6). One hypothesis proposed to explain these findings is based on the apparent redundancy in apolipoprotein-mediated cholesterol removal from cells (111, 126, 127), in that the complete absence of more than one lipid-poor apolipoprotein may be necessary to markedly impair clearance of cholesterol from arterial cells. Indeed, it has been shown that both human and mouse plasma lacking apoA-I-containing particles can still stimulate cholesterol efflux from cultured cells, but in this case most of the cholesterol initially released is associated with γLpE (111, 126). This apolipoprotein redundancy hypothesis may explain the lack of atherosclerosis in some animal and human apoA-I deficiencies, but it is unclear why the absence of both apoA-I and apoC-III in humans would be more atherogenic than the absence of apoA-I alone, when there should be sufficient apoA-IV and apoE to act as back-up removers of cellular cholesterol in both cases. It is just as feasible to assume that the lack of CVD in some of these rare cases of genetic HDL deficiencies reflects resistance to other atherogenic factors in these individuals. Despite the exceptions, these case reports support the conclusion that a reduced availability of apoA-I increases risk for CVD.

In contrast to most other HDL deficiencies, family members with a genetic disorder called apoA-I_{milano} have a remarkable absence of CVD despite low plasma HDL levels (139). This disorder is caused by a mutation in the apoA-I gene that leads to a substitution of cysteine for arginine (140). The resulting covalent dimerization of apoA-I on HDL particles has several metabolic consequences, including an increased dissociation of dimeric and monomeric apoA-I from HDL particles and a slower turnover of the dimeric molecules (141). Could the protective effect of apoA-I_{milano} be related to an increased generation of lipid-free apoA-I?

These rare genetic HDL deficiencies do not contribute significantly to the large population of subjects with low HDL levels and premature atherosclerosis. Metabolic factors that influence production or regeneration of lipid-poor apolipoproteins, however, may be more

common. It is possible that distinct subpopulations of mature HDL particles may be major sources of lipid-poor apoA-I that dissociate from the particle surface. Candidate particles having this property are large apoA-I-containing particles lacking apoA-II (LpA-I) that appear in the HDL_2 subfraction (142). LpA-I particles have a larger fraction of exchangeable apoA-I than particles containing both apoA-I and apoA-II (LpA-I:A-II) (143), and plasma levels of HDL_2 (144) and LpA-I (145, 146) are correlated better with protection against CVD than are other HDL particles. A reduced availability of lipid-poor apoA-I may explain the increased formation of atherosclerotic lesions in transgenic mice overexpressing apoA-II (8, 147). Other metabolic factors may play important roles in regenerating lipid-free apolipoproteins, including plasma enzymes and transfer proteins (e.g., LCAT, CETP, PLTP, LPL) and down-stream receptors (e.g., SR-BI). Subjects with a CETP deficiency have been reported to have CVD despite higher levels of HDL (7), perhaps because the lack of ability to transfer sterol from HDL reduces production of lipid-free apoA-I (Steps 6 and 8, Fig. 4A).

Additional support for the antiatherogenic effects of apoA-I comes from animal model studies showing that an increased availability of apoA-I decreases atherogenesis. Injection of apoA-I into cholesterol-fed rabbits was reported to decrease the number of arterial lesions (148), and transferring a human apoA-I transgene onto several genetic atherogenic backgrounds in mice was shown to reduce lesion formation (149–152). Expression of human apoA-IV in mice was also shown to protect against atherogenesis (153).

Because apoE is produced by tissue macrophages as well as the liver (128), lipid-free apoE may play a unique role in protecting against atherogenesis. Overexpression of apoE in macrophages in transgenic mice has been shown to reduce atherosclerotic lesion formation even with persistent hypercholesterolemia (154, 155). Thus apoE has the potential of playing an autocrine/paracrine role in mobilizing cholesterol from cholesterol-loaded cells in the artery wall. In this fashion, more apoE would be synthesized and secreted by macrophages as they become loaded with cholesterol (156–158). The secreted apoE would then be available to interact with lipid efflux-stimulating binding sites on the cell surface which also increase in number when cells become cholesterol loaded (11, 12). Although this type of secretion/recapture process has not been demonstrated, it would provide a very efficient mechanism for macrophages to regulate excretion of excess cellular cholesterol. Local factors that modulate macrophage apoE production and availability may have important influences on this lipid removal pathway and atherogenesis.

It is also possible that some instances of premature atherosclerosis are caused by alterations in the structural properties of apolipoproteins so as to reduce their efficacy in removing lipids from cells. Both glycation (159, 160) and oxidation (161, 162) of HDL have been shown to markedly reduce the ability of HDL to remove cellular cholesterol, implying that these modifications alter the properties of HDL apolipoproteins so that they lose their cellular lipid transport-stimulating activity. Glycated HDL apolipoproteins have been demonstrated to be present in the plasma of poorly controlled diabetics (163). Could decreased apolipoprotein-mediated lipid removal from tissues contribute to the increased CVD associated with diabetes?

Activity of the cellular apolipoprotein-mediated lipid removal pathway. Studies with TD fibroblasts raise the possibility that many subjects with low plasma HDL levels and CVD have acquired or genetic defects in the cellular pathway involved in apolipoprotein-mediated lipid removal. In these cases, the HDL deficiency would be secondary to an increased turnover of lipid-poor apoA-I because of its inability to pick up cellular lipids, and the premature atherosclerosis would stem from an impaired cholesterol excretory pathway in artery wall cells. These defects would be atherogenic even with high rates of apolipoprotein production. Slightly less than half of the reported cases of homozygous TD between the ages of 35 and 65 have symptoms of CVD, indicating that TD patients have a higher prevalence of CVD than age-matched normolipidemic subjects (164). These findings suggest that the cellular defect in TD, which almost completely abolishes the ability of apoA-I to remove cellular lipids, increases risk for CVD, but only modestly. The lower-than-expected risk for CVD in TD can be explained by the markedly low levels of plasma LDL in these patients (96, 164). As discussed above, LDL may be a potent factor for initiating and propagating lesion formation, conditions required for apoA-I to be antiatherogenic. Considering their very low plasma LDL levels, it is remarkable that nearly half of reported 35- to 65-year-old TD patients have CVD.

In summary, these findings support the hypothesis that apolipoprotein-mediated lipid transport protects against atherogenesis. This protective effect, however, is likely to operate only when atherogenesis is initiated and propagated by other factors. The influence of this pathway on atherogenesis may operate over a wide range of activities. Thus individuals with only a partial impairment of this pathway may have a risk for developing atherosclerosis similar to those with a complete defect because they have higher levels of atherogenic lipoproteins. Thus apolipoprotein-mediated lipid transport would be expected to be a powerful protective factor for CVD among those individuals highly susceptible

to macrophage foam-cell formation in the artery wall. This underscores the importance of characterizing the molecular properties of the apolipoprotein-mediated lipid removal pathway in cells. Identification of the cellular proteins involved would not only lead to the discovery of possible genetic defects that may impair cholesterol transport from cells and enhance atherogenesis but would also pinpoint key steps in the regulation of this pathway. Although more work is needed to confirm the models proposed in this review, the apolipoprotein-mediated lipid removal pathway in cells likely will be an important target for future therapies designed to prevent CVD.

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