Mechanisms for Cellular Cholesterol Transport: Defects and Human Disease

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I. INTRODUCTION

During the past 15 years, an increasing number of gene defects have been identified that underlie cellular cholesterol transport defects in humans. Familial hypercholesterolemia is the prototype of this group of diseases, and the major advances in understanding its pathogenesis and treating the disease have served as a model for studying other disorders. Often, these diseases have helped to pinpoint critical steps of cholesterol trafficking, and the characterization of the defective proteins has provided insights into the molecular machineries operating at these
steps. In the first part of this review, the key processes of cellular cholesterol metabolism are summarized (sect. ii) and general principles of cellular cholesterol transport are introduced (sect. iii). Until now, the progress in understanding intracellular cholesterol movement has largely relied on the characterization of relatively rare single gene diseases, and the description of such disorders constitutes the body of this review (sect. v). In addition, selected other proteins that play key roles in cellular cholesterol movement based on mouse models are discussed (sect. iv). Pharmacological strategies currently used to treat cardiovascular diseases are briefly mentioned because many of them rely on effects on cellular cholesterol trafficking (sect. vi). Finally, because of the organizing potential of cholesterol in the membrane, cellular cholesterol transport processes and their disturbances have implications for a wide variety of human diseases. Selected examples are given in section vii.

The topic of this review, intracellular cholesterol transport, spans into several medical specialties including internal medicine, pediatrics, neurology, and surgery and integrates information from basic sciences such as organic chemistry and biophysics. Comprehensive coverage of the research addressing intracellular cholesterol transport and human disease is not possible within the limits of this review. Typically, correlative evidence for the association of specific genetic variations or protein expression levels with metabolic changes has not been included if a liable causal relationship to cellular cholesterol transport is lacking. I have also aimed to underscore medically relevant aspects of the conditions at the expense of technical subtleties. Importantly, a number of excellent reviews have recently been written on highly popular areas of study, and the reader is referred to these articles for more in-depth information.

II. CELLULAR CHOLESTEROL METABOLISM

A. Cholesterol Biosynthesis

The 27-carbon tetracyclic cholesterol molecule is synthesized from acetate in a series of ~30 enzymatic reactions. The rate-limiting enzyme of the pathway is hydroxymethylglutaryl CoA reductase (HMG-CoAR), which generates mevalonate. This enzyme harbors a conserved sterol-sensing domain (SSD) with five membrane-spanning a-helices that is shared by other proteins implicated in sterol regulation (186). The SSD of HMG-CoAR is critical for the association of the protein with the endoplasmic reticulum (ER) resident protein Insig and regulation of its degradation (208). Production of the first sterol in the cascade, lanosterol, is catalyzed by squalene cyclase. The subsequent ~20 steps constitute the post-lanosterol part of cholesterol biosynthesis in which double bonds are reduced, their positions altered, and methyl groups removed. Importantly, isoprenoid intermediates in the presqualene half of the pathway serve as precursors not only for cholesterol but also for a number of other biomolecules involved in transcription (isopentenyl tRNAs), protein N-glycosylation (dolichol), protein prenylation (farnesyl and geranylgeranyl moieties), and mitochondrial electron transport (ubiquinone).

The ER is the primary site of cholesterol synthesis (189) (see Fig. 1). The first seven enzymes of cholesterol biosynthesis are soluble proteins apart from HMG-CoAR, which is an integral ER membrane protein. HMG-CoAR and some of the other prelanosterol biosynthesis enzymes are also present in peroxisomes (118), but the enzyme directly following HMG-CoAR, mevalonate kinase, is cytosolic (94). The post-lanosterol enzymes are localized to the ER or its extensions, nuclear envelope, or lipid droplets. The significance of this complex subcompartmentalization of the cholesterol biosynthetic pathway remains poorly understood.

B. Transcriptional Regulation of Cholesterol Homeostasis

Due to the low ER cholesterol level, its cholesterol concentration fluctuates much more than that of the plasma membrane upon cholesterol loading (233). The ER sterol levels are sensed by the main cellular cholesterol homeostatic machinery, constituted of membrane-embedded ER proteins (78). In short, the mature SREBP transcription factor is generated in the Golgi complex and translocates to the nucleus to activate genes involved in cholesterol synthesis and uptake. In the presence of ample cholesterol, SREBP transcriptional activity is suppressed by ER retention (Fig. 2). This is mediated by interaction with the SSD protein SCAP, which in turn binds the ER retention protein Insig. Insig also binds the SSD protein HMG-CoAR in a sterol-dependent manner. This binding enhances degradation of the reductase.

Another transcriptional regulatory network is orchestrated by the nuclear hormone receptors liver X receptor (LXR) and peroxisome proliferator activated receptor (PPAR) (Fig. 2). These constitutively nuclear receptors are activated by oxysterols and fatty acids, respectively, and regulate the expression of several key proteins of the cholesterol and fatty acid metabolic pathways (15).

C. Cholesterol Esterification and Ester Hydrolysis

The 3'-OH group of cholesterol can become fattyacylated to form cholesterol esters that can be stored in cytoplasmic lipid droplets (Fig. 1). The enzyme responsible for this activity is acyl-CoA:cholesterol acyltrans-
These are integral proteins of the ER (32). There are two homologs of mammalian cholesterol esterifying enzymes, ACAT1 and ACAT2, that are differentially expressed. ACAT1 is expressed in many tissues and cell types, with the highest levels in macrophages and in the adrenal and sebaceous glands. ACAT2, on the other hand, is more restricted in tissue distribution, being the dominant esterification enzyme.

**FIG. 1.** Cellular cholesterol distribution and key enzymes of cellular cholesterol metabolism. The approximate cholesterol content of the membrane is indicated by shades of gray. The main processes of cholesterol metabolism, key enzymes involved, and their subcellular locations are indicated (see text for details).

**FIG. 2.** Schematic illustration of the transcriptional regulation of cholesterol homeostasis. The sterol-sensing domain (SSD) proteins SCAP and HMG CoAR bind to the ER retention protein Insig in the presence of high cholesterol or lanosterol levels, respectively. Insig binding prevents the transport of SCAP-SREBP complex in COPII-coated vesicles to the Golgi complex where the active SREBP transcription factor is released. Consequently, the transcription of sterol response element-regulated genes is not turned on. Binding of HMG CoAR to Insig leads to its retro-translocation and proteasomal degradation. Conversely, cholesterol depletion promotes the rapid proteasomal degradation of Insig, which is prevented by cholesterol restoration. Oxysterols and fatty acids act in the nucleus to activate LXR and PPAR-dependent gene transcription, respectively. Selected target genes of the transcriptional regulators are indicated.
ifying enzyme in the mouse liver and small intestine (26). Also in adult humans, ACAT2 is localized to hepatocytes and enterocytes (180).

ACAT activity is allosterically activated by high cholesterol levels (32). A conserved His residue located within a long stretch of hydrophobic amino acids may serve as an active site for ACAT catalysis (129). ACAT1 produces cholesterol esters to be stored in lipid droplets in macrophages while ACAT2 serves to esterify cholesterol to be included in apolipoprotein B (apoB)-containing lipoproteins, such as chylomicrons in enterocytes and possibly very-low-density lipoprotein (VLDL) in hepatocytes. However, the functional cooperation of ACAT1 and ACAT2 in the assembly of apoB-containing lipoproteins remains to be established (33). The intracellular localization of the ACAT enzymes has been reported only for ACAT1. In macrophages and fibroblasts, ACAT1 is mostly localized in the ER (197). In addition, macrophage ACAT1 has been found in a region close to the trans-Golgi network and endocytic recycling compartment (113).

Cellular cholesterol undergoes a continuous cycle of esterification and ester hydrolysis even under conditions in which no external cues altering cholesterol balance are introduced. Net breakdown of cholesterol esters in lipid droplets takes place when cellular cholesterol levels fall. The enzyme responsible for the degradation of cholesterol esters in lipid droplets is neutral cholesterol ester hydrolase (nCEH) (Fig. 1). There are a number of nCEH enzymes that differ between cell types. Hormone-sensitive lipase (HSL) is responsible for cholesterol ester breakdown in steroidogenic cells (269), whereas other nCEHs have been cloned from monocyte/macrophages and hepatic cells (74, 75). The mechanisms by which this enzyme activity is regulated is not well understood. In the liver, nCEH activity appears to respond to changes in cholesterol flux through the liver (76). The breakdown of cholesterol esters that enter the cell by uptake of LDL particles is catalyzed by acid lipase in the endosomal compartment (see below).

D. Absorption of Dietary Cholesterol

Dietary cholesterol is absorbed from bile salt micelles with fatty acids and lysophospholipids in the proximal parts of the small intestine. Key proteins involved in dietary cholesterol uptake by the enterocytes have been identified during the past few years. It has become evident that a protein belonging to the SSD proteins, NPC1L1, plays an important role in this process (6). The NPC1L1 protein is localized to the brush-border membrane of enterocytes and is required for intestinal uptake of both cholesterol and plant sterols (phytosterols) (49) (Fig. 3). Recent evidence suggests that this protein is the target of the cholesterol-lowering drug ezetimibe (72, 272).

Whether NPC1L1 functions as a genuine cholesterol transporter promoting cholesterol transfer through the plasma membrane or is indirectly involved in the process is not yet known. In addition, the ABC transporter family half-transporters ABCG5 and ABCG8 (sterolin-1 and steryl-2) constitute a functional heterodimeric unit limiting sterol absorption (81, 245) (Fig. 3). The role of ABCG5 and ABCG8 in dietary cholesterol absorption may not be direct, i.e., inhibition of dietary cholesterol uptake by enterocytes; rather, this transporter stimulates hepatic sterol excretion into the bile (see sect. II E), and thereby modulates the bile-acid/sterol ratio, possibly to promote the secretion of absorbed sterols from the intestinal epithelium back into the gut lumen (101).
E. Cholesterol Metabolism Into Bile Acids, Oxysterols, and Steroid Hormones

1. Bile acid synthesis

Cholesterol cannot be degraded by cells into noncyclic hydrocarbon products. However, hepatocytes can excrete it into the bile to be removed in feces. For this, cholesterol to be removed from extrahepatic tissues needs first to be transported via the circulation to the liver. The process involving cholesterol efflux from extrahepatic tissues and return to the liver for secretion into bile and disposal via the feces is called reverse cholesterol transport (64). Within hepatocytes, cholesterol secretion necessitates its conversion to bile salts. In humans, this conversion accounts for roughly half of the daily elimination of cholesterol from the body. The majority of the rest is secreted into bile as free cholesterol (117). Remarkably, recent evidence suggests that a considerable fraction of cholesterol disposed in the feces may be excreted directly via the intestine, constituting a more direct route for cholesterol removal (120).

Cholesterol can be converted into bile salts via two pathways: the classic or neutral pathway and the alternative or acidic pathway (117). The key regulatory enzyme in the classic pathway is cholesterol 7α-hydroxylase (CYP7A1) and in the alternative pathway sterol 27-hydroxylase (CYP27A). CYP7A1 resides in the ER (19) and CYP27A in mitochondria (168) (Fig. 1). The neutral, CYP7A1 pathway is liver specific. Instead, CYP27A activity is present in almost all tissues, and in extrahepatic tissues its expression is probably related to the production of oxysterols. Oxysterol 7α-hydroxylase (CYP7B1) catalyzes the second step, i.e., conversion of 27-hydroxycholesterol (as well as that of 24 and 25-hydroxycholesterol) to 7α-hydroxylated oxysterols, and is also involved in the regulation of the rate of cholesterol secretion into bile. Yet another important parameter regulating the biliary cholesterol secretion rate is the hydrophobicity of the bile salt. This is governed by the enzyme sterol 12α-hydroxylase (CYP8B1) that controls the ratio of cholic acid and chenodeoxycholic acid synthesis in both the neutral and acidic pathways.

The molecular mechanisms by which cholesterol is secreted into bile have recently been unraveled. The heterodimeric ABC transporter ABCG5/G8 mediates biliary cholesterol secretion at the apical membrane of hepatocytes (82, 274). Importantly, increased G5/G8 expression results in elevated biliary cholesterol secretion, suggesting that the transporter levels, rather than cholesterol delivery to it or biliary acceptors, are rate limiting (273).

2. Hydroxylated sterol derivatives

Cholesterol or its sterol precursors serve as starting material for the production of oxysterols and vitamin D. Oxysterols function as signaling molecules by serving as ligands for LXR transcription factors (15). Furthermore, oxysterols represent a mechanism for export of cholesterol in a more hydrophilic form from other tissues to the liver (169). The most abundant oxysterol in plasma is 27-hydroxycholesterol, which is produced by the mitochondrial sterol 27-hydroxylase (Cyp27a, Fig. 1). 25-Hydroxylase (which in contrast to many of the other enzymes involved in bile acid or oxysterol production is a non-P-450 cytochrome) is localized primarily in the ER (135). Both 25- and 27-hydroxycholesterol are partial LXR agonists, whereas 24(S),25-epoxycholesterol is a strong agonist. 24(S),25-Epoxycholesterol is produced by diversification from the cholesterol biosynthetic pathway (224). Vitamin D is also produced by hydroxylation of a cholesterol biosynthetic precursor, 7-dehydrocholesterol, to produce previtamin D₃. D₃ is further hydroxylated in the kidney and liver to produce active vitamin D (1,25-dihydroxyvitamin D) (95).

3. Steroid hormone synthesis

Cholesterol serves as an obligatory precursor for steroid hormone production in steroidogenic tissues, such as the adrenals, gonads, placenta, and brain. Regardless of the tissue of origin or the steroid hormone to be produced, steroidogenesis is initiated by the cleavage of the cholesterol side chain to produce pregnenolone. The protein catalyzing this reaction, P-450 side chain cleavage (P450scc/CYP11A1) enzyme, is located in the inner mitochondrial membrane (154) (Fig. 1). This enzyme activity is not rate limiting in the process; instead, it is the delivery of cholesterol to the enzyme by steroidogenic acute regulatory protein (STAR), a cytosolic protein with a mitochondrial targeting signal. The precise site of action of STAR is controversial, but recent data provide strong evidence for a function in the outer mitochondrial membrane (21). According to the “cholesterol desorption model” (229), STAR interacts with the mitochondrial outer membrane where it stimulates cholesterol desorption, perhaps through preexisting contact sites between the outer and inner mitochondrial membrane. The import of STAR into mitochondria would therefore remove the protein from its site of action and terminate sterol entry into mitochondria. A candidate protein for the transport of cholesterol from the outer to the inner membrane is peripheral-type benzodiazepine receptor (PB) that is localized in the outer membrane at contact sites with the inner membrane (173).

Steroids made locally in the brain are called neurosteroids. They are multifunctional lipids that mediate their actions, not through classic steroid hormone nuclear receptors, but through ion-gated neurotransmitter receptors (43). In addition to modulation of receptor activity, the functions attributed to specific neurosteroids include...
regulation of myelinization, neuroprotection, and growth of axons and dendrites.

III. OVERVIEW OF INTRACELLULAR CHOLESTEROL TRANSPORT

As described above and illustrated in Figure 1, cholesterol metabolism is compartmentalized into distinct subcellular membranes. Moreover, the cholesterol concentration of cellular membranes is highly variable. The majority of cellular cholesterol resides in the plasma membrane. In addition, endocytic and exocytic membrane trafficking routes connecting the endosomes and the trans-Golgi network to the plasma membrane have cholesterol-enriched domains (216). In contrast, the ER and mitochondria, where many critical enzymatic reactions of cholesterol metabolism take place, are relatively cholesterol poor (Fig. 1). Thus maintenance of cellular cholesterol homeostasis necessitates the transport of cholesterol between subcellular membranes and its exchange with lipoproteins.

Our understanding of cellular cholesterol trafficking is lagging behind the knowledge on protein trafficking. This is largely due to technical limitations in the available methodologies. Monitoring a metabolic conversion or an enzymatic reaction provides an indirect means of tracing cellular cholesterol. In addition, cholesterol movement can be measured by radiolabeling cholesterol or its precursor molecules and following partitioning of the lipid between biochemically resolved subcellular compartments. The cellular distribution of sterols with a free 3'-OH group can be directly visualized in cells by using the cholesterol-binding and membrane-permeabilizing agent filipin. The development of more sophisticated cell biological strategies, e.g., direct visualization of the itineraries of fluorescent sterol derivatives, is providing increasing insight into sterol movement by enabling live cell monitoring.

Due to its hydrophobicity, spontaneous exchange of cholesterol from one membrane to another across the aqueous cytoplasm is slow. In living cells, this movement takes place by a combination of vesicular trafficking and nonvesicular mechanisms (148), such as cytosolic carrier proteins (STAR being the best characterized example; Ref. 154), and most likely via protein-mediated membrane contact sites (170). Assessment of the quantitative contribution of each mechanism is hampered by the possible use of bypass mechanisms upon blocking one route or protein. Membrane trafficking encompasses the selection of cargo into forming vesicles, and cholesterol carrier proteins are known to have targeting information for specific membranes. Yet, especially under conditions where cells are challenged with massive fluxes of cholesterol, such mechanisms seem inefficient (carrier proteins) or may be preoccupied by other cargo (vesicular trafficking). Feasible mechanisms to handle such conditions may be diffusion via membrane contact sites or specialized vesicles. In the following, a brief summary of intracellular cholesterol trafficking pathways is provided. For more in-depth information, the reader is referred to excellent recent reviews (147, 221).

A. Ports of Sterol Entry: Endogenous Synthesis and Lipoprotein Sterol Uptake

Essentially all nucleated cells synthesize cholesterol. Newly synthesized cholesterol leaves the ER rapidly and moves against a steep concentration gradient to reach the plasma membrane (Fig. 4). Secretory transport via the Golgi apparatus plays a minor role in this process (92, 247), while the bulk of sterol can reach the plasma membrane by nonvesicular mechanisms (14). Notably, sterol precursors also traffic between the ER and plasma membrane (123) and can be effluxed to extracellular acceptors (137). Indeed, serum sterol precursor (typically lathoste-
rol) levels are used to estimate the level of body cholesterol synthesis.

On a Western diet, humans synthesize an estimated \(-1\) g of cholesterol/day and ingest \(-400\) mg (85). For intercellular exchange via the interstitial fluid and blood, both pools are incorporated into lipoprotein particles. The major lipoprotein-secreting cells of the body and their sterol sources are summarized in Figure 3.

The majority of cholesterol entering the cells is taken up by receptor-mediated uptake from lipoproteins. The core of lipoprotein particles is composed of triglycerides and cholesterol esters (i.e., fatty acylated cholesterol), while the particle surface is covered by phospholipids and free cholesterol. Low-density lipoproteins (LDL) are internalized via clathrin-coated pits into early endosomes from where the receptor is recycled to the cell surface (145) and the LDL-particle targeted for proteolytic and lipolytic degradation. The enzyme responsible for the hydrolysis of cholesterol esters, lysosomal acid lipase, was recently shown to be present not only in lysosomes as traditionally thought but also in earlier endocytic compartments (231). This suggests that the particle breakdown is initiated rapidly after internalization.

Dietary lipoproteins are packaged into chylomicrons from which fatty acids are lipolyzed to produce cholesterol-sterol-enriched lipoprotein remnant particles. They are rapidly removed from the circulation primarily by the liver in a system involving the LDL receptor (LDLR) and LDLR-related protein (LRP) (102, 271). The latter differs mechanistically from LDLR uptake and is dependent on hepatic secretion of apoE to enrich the remnants with a ligand for receptor-mediated endocytosis (130).

### B. Endosomal Cholesterol Transport

Endocytic circuits harbor substantial amounts of cholesterol that they acquire not only from lipoprotein uptake but apparently also via membrane recycling and nonvesicular equilibration. Endosomal cholesterol may return to the plasma membrane directly or via the Golgi complex, or be transported to the ER (Fig. 4) where it regulates cholesterol homeostasis (Fig. 2). The mechanisms and routes of cholesterol exit from the endocytic circuits remain rather poorly defined. Two proteins, NPC1 and NPC2, are implicated in the process because of disease-causing mutations (34). Moreover, the late endosomal membrane system seems to maintain a delicate cholesterol balance that can be deranged by various cues, such as disturbing the functional cycle of Rab GTPases (98), deleting lysosomal membrane proteins (59), or disturbing the lipid assembly of intraendosomal membranes (116). Hence, some cholesterol-binding proteins such as MLN64 may safeguard the local cholesterol content in functionally important endosomal membrane domains (96). Endosomal cholesterol transport is more comprehensively discussed in several recent reviews (34, 97, 230).

### C. Cholesterol Efflux

The atheroprotective function of high-density lipoprotein (HDL) is thought to be mediated by its ability to transport excess cholesterol from peripheral tissues to the liver for excretion in a process known as reserve cholesterol transport. In this process, cholesterol is released from peripheral cells to poorly lipitated apolipoproteins, in particular apolipoprotein A-I (apoA-I) which, upon further lipidation, is converted to mature HDL particles. The ATP-binding cassette transporter ABCA1 controls the rate-limiting step in HDL assembly by mediating cholesterol and phospholipid efflux from cells to apoA-I, generating nascent pre-\(\beta\)-HDL (270). These particles may serve as substrates for ABCG1-mediated removal of cholesterol to more mature HDL subclasses (HDL\(_{2b}\), HDL\(_{3}\)) (73, 257) (Fig. 4). HDL is remodeled in the plasma, and additional cellular cholesterol may be transferred to the growing particle by scavenger receptor class B type 1 (SR-B1) (276). SR-B1 can also mediate the last step of reverse cholesterol transport, namely, delivery of cholesteryl esters from HDL to the liver without HDL degradation, termed selective cholesteryl ester uptake (119).

The relative contribution of cell surface versus intracellular events in cholesterol efflux and the interplay of the individual receptors in key tissues such as the liver, steroidogenic tissues, and macrophages warrant further investigation. Apparently, ABCA1-mediated lipid efflux to apoA-I takes place from the cell surface, but endocytic circuits play an important role (164). The surface appearance of ABCA1 is in part regulated by endocytic trafficking of the transporter (see sect. ivD). The term retroendocytosis was introduced to describe the internalization of HDL into the cell and subsequent release after lipidation (203), but the concept remains controversial. Recent evidence suggests that SR-B1 can mediate holo-HDL particle uptake followed by HDL resecretion and that this may facilitate cholesterol efflux (172). Whether selective cholesteryl ester uptake also involves holoparticle endocytosis or takes place only at the cell surface remains disputed (see, e.g., Refs. 89, 212).

### D. Intramembrane Cholesterol Mobility

Considering that the exchange of cellular and exogenous cholesterol pools with acceptor/donor lipoproteins takes place in the exoplasmic leaflet of the membrane, a critical parameter regulating this process is the transbilayer movement of cholesterol. Kinetic estimates have
been highly variable, but the available evidence points to a rapid spontaneous flipping of cholesterol between the two bilayer leaflets (225). However, this may not necessarily indicate an equal transbilayer distribution of cholesterol as energy-dependent flippases and the higher affinity of cholesterol for distinct lipid environment(s) probably contributes. At present, the transbilayer distribution of cholesterol is not known.

In model membranes, cholesterol exhibits high affinity towards sphingolipids. This is considered to derive from the favorable packing of the rigid sterol ring structure between the long and saturated acyl chains and sphingosine backbone of sphingolipids (217). The attractive forces involved are weak intermolecular interactions such as van der Waals interactions within the bilayer and hydrogen bonding between lipid head groups and in the water-lipid interphase. The predicted preferential association of cholesterol with sphingolipids forms the basis of the lipid raft concept (215). According to this hypothesis, these lipid interactions described in model systems may also apply in living cells to favor the formation of membrane domains that function in diverse cellular processes. Such lipid assemblies may contribute for instance to signal transduction, membrane transport, intramembrane proteolysis, cell adhesion, and invasion of pathogens. A morphologically identifiable subtype of lipid rafts are caveolae, plasma membrane invaginations formed upon polymerization of the structural protein caveolin in a cholesterol-dependent manner (174).

Obviously, the molecular interaction networks operating in living cells are complex, involving not only interlipid interactions but also lipid-protein and protein-protein interactions. Moreover, the strict requirement of a particular lipid species appears not to apply because in the absence of such species most cells can probably compensate by generating analogous biophysical membrane characteristics using other lipid combinations. In spite of, or perhaps because of, its hypothetical nature, the lipid raft concept has generated much interest and despite, or perhaps because of, its hypothetical nature, the lipid raft concept has generated much interest and has been highly variable, but the available evidence points to a rapid spontaneous flipping of cholesterol between the two bilayer leaflets (225). However, this may not necessarily indicate an equal transbilayer distribution of cholesterol as energy-dependent flippases and the higher affinity of cholesterol for distinct lipid environment(s) probably contributes. At present, the transbilayer distribution of cholesterol is not known.

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IV. CELLULAR CHOLESTEROL TRANSPORT: LESSONS FROM MOUSE STUDIES

Some of the proteins introduced in previous sections are implicated in cellular cholesterol transport, but no human diseases resulting directly from their mutations have been reported. In this section, selected proteins from this category are discussed. The pathophysiological relevance of these proteins is supported by evidence from mouse models. The most popular mouse models for studying atherosclerosis are the LDLR- and apoE-deficient mice. Notably, there are significant differences, both quantitatively and qualitatively, in the cholesterol balance between mouse and human (51). In particular, the high rate of hepatic LDL clearance leads to a low steady-state concentration of cholesterol carried in LDL, and consequently, a less dramatic phenotype when the LDLR is nonfunctional in mice compared with humans. ApoE is a glycoprotein constituent in all lipoproteins except LDL and a principal protein component of chylomicron remnants, very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL), functioning as a ligand for receptors that clear chylomicrons and VLDL remnants (151). ApoE-deficient mice, generated in 1992 by two groups (177, 178), are perhaps the most popular murine model for studying atherosclerosis because of its propensity to spontaneously develop atherosclerotic lesions on a standard diet. Foam cell lesions can be observed already at 10 wk of age and fibrous plaques at 20 wk. A Western-type diet accelerates the process. However, despite inbreeding, the mouse-to-mouse variability remains high (151).

A. Acyl-CoA Cholesterol Acyl Transferases

The deposition of cholesteryl esters in macrophages and smooth muscle cells in the arterial intima is the hallmark of atherosclerosis. Because ACAT is the enzyme catalyzing intracellular cholesterol esterification, its inhibition should reduce atherogenesis. Knock-outs of both ACAT1 and ACAT2 genes have been generated in the mouse and found to produce differential phenotypes, reflecting distinct tissue distributions and biochemical characteristics of the enzymes.

In ACAT1-deficient mice, lack of the enzyme is manifested in macrophages and adrenal cortex, but plasma cholesterol levels, hepatic cholesterol esterification, and intestinal cholesterol absorption are normal (150). ACAT1-deficient mice have been crossed with LDLR- and ApoE-deficient mice. However, atherosclerosis was not significantly inhibited in either model. In fact, when LDLR-deficient mice were substituted with macrophages from ACAT1−/− mice, the development of atherosclerotic lesions was accelerated (62). This was attributed, at least in part, to the increased apoptosis of macrophages due to the toxic effects of increased free cholesterol levels. Instead, ACAT2-deficient mice lack cholesteryl esters in the liver and small intestine (26). They were protected from diet-induced hypercholesterolemia and cholesterol gallstones because of the reduced capacity to obtain cholesterol from the diet. Interestingly, ApoE−/−/ACAT2−/− mice atherosclerotic lesion development was almost completely inhibited (265). The toxic effects of ACAT inhibition may be less severe with partial, pharmacological inhibition of the enzyme. Currently, ACAT inhibi-
bition is a promising avenue of intervention with atherosclerosis development, and clinical trials in patients are underway (133).

B. Scavenger Receptor-Mediated Cholesterol Transport

Scavenger receptors bind chemically modified lipoproteins, in addition to a number of other ligands. Uptake of modified LDL is considered to be an early event in vascular disease, and scavenger receptor function is critical in this context. Class A scavenger receptor (SR-A) was the first receptor shown to bind acetylated LDL (50, 79). Oxidatively modified LDL (oxLDL) is scavenged by both SR-A and the SR-B protein CD36. SR-A and CD36 have been implicated as proatherogenic proteins (121), and CD36 knock-out mice display smaller atherosclerotic lesions (63).

On the other hand, scavenger receptor class B type 1 (SR-B1) is the first characterized cell surface receptor for HDL, with an established antiatherogenic activity (243). SR-B1 is expressed in various tissues, with high levels, e.g., in the liver and steroidogenic cells. SR-B1 mediates the selective uptake of cholesteryl esters from HDL in a process that does not involve net internalization and degradation of the lipoprotein (119). Importantly, SR-B1 appears to be the only molecule responsible for hepatic selective cholesteryl ester uptake from HDL (25, 171). In addition, SR-B1 stimulates bidirectional flux of free cholesterol between cells and HDL (86, 268), and its expression levels correlate with cholesterol efflux to HDL (106). However, the relevance of SR-B1-mediated cholesterol efflux for HDL metabolism in vivo has not been established.

The importance of SR-B1 in HDL metabolism and its antiatherogenic activity has been elucidated by SR-B1 gene manipulation in mice. SR-B1 deficiency results in the accumulation of large HDL particles in the plasma and increased circulating cholesterol levels (191). Importantly, double knock-out mice lacking both SR-A and SR-B1 protein CD36. SR-A and CD36 have been implicated as proatherogenic proteins (121), and CD36 knock-out mice display smaller atherosclerotic lesions (63).

The class E scavenger receptor lectin-like oxidized LDLR (LOX-1) is the major receptor for oxidized LDL in endothelial cells (201) and is also expressed in macrophages and smooth muscle cells (36). Its expression is increased in the endothelium of early atherosclerotic lesions (35), most prominently in arterial bifurcations (37) that are considered as atherosclerosis susceptible sites. Importantly, downregulation of LOX-1 was shown to prevent monocyte adhesion to endothelial cells and apoptosis (126). Moreover, genetic evidence points to the involvement of LOX-1 in human atherosclerotic conditions; a polymorphism was reported to be associated with myocardial infarction (237) and inversely associated with the severity if coronary artery disease (167). Further functional and epidemiological studies are clearly warranted.

C. Caveolin-1 and Cholesterol Transport

Caveolae are a subset of plasma membrane cholesterol-sphingolipid rafts (134, 215), and caveolin-1, the structural protein of caveolae, is one of the first and most abundant molecularly identified cellular cholesterol binding proteins (161, 194, 238). The numerous roles assigned for the caveolin family of proteins (caveolin-1, -2, and -3) in human pathological conditions have recently been comprehensively reviewed in this series (40). Therefore, the present discussion is limited to the most direct and pathophysiologically relevant connections between caveolins and cholesterol transport.

A number of cell culture studies have provided partially contradictory evidence for the role of caveolin-1 in cellular cholesterol trafficking, either as a chaperone complex via the cytoplasm or by regulating cholesterol influx or efflux via plasma membrane caveolae (103). Yet, no abnormalities in plasma cholesterol levels were observed in caveolin-1 null mice (188), and they also showed normal cholesterol absorption (251). However, a caveolin-1/apoE double knock-out mouse was protected from the development of atherosclerosis, despite hypercholesterolemia (66), suggesting that the involvement of caveolin in cholesterol trafficking may become manifested upon a stress situation.

Interestingly, the caveolin-1-deficient mice (that also have low levels of caveolin-2 because of degradation of the protein in the absence of caveolin-1) show reduced adiposity and are protected from diet-induced obesity (188). This phenotype resembles the one found in mice deficient in perilipin, a protein involved in adipocyte lipolysis (236). Indeed, caveolin-1 null mice appear to have a blunted response to lipolytic agonists (40). There is mounting evidence for a link between caveolin-1, perilipin, and protein kinase A in the regulation of lipid droplet turnover (40, 144), but the precise connections remain to be established. Nevertheless, adipocyte lipid droplets
contain high levels of free cholesterol (up to 30% of total cellular cholesterol), and this cholesterol can be readily mobilized (181), suggesting that lipid droplets represent an important station of cholesterol trafficking in these cells.

D. NPC1L1 and Cholesterol Absorption

The critical role of NPC1L1 in dietary cholesterol absorption was revealed by generation of mice lacking the protein. The NPC1L1-deficient mice showed a substantial reduction in intestinal cholesterol and sitosterol absorption (6) and were resistant to diet-induced cholesterol accumulation (48). This phenotype is reminiscent of that produced by the cholesterol absorption inhibitor ezetimibe. Moreover, the drug binds specifically to NPC1L1 (72), strengthening the idea of NPC1L1 involvement in cholesterol absorption and showing that NPC1L1 is a target of the drug. Interestingly, NPC1L1 contains a SSD (47), and recent data suggest that, similarly to some other SSD-containing proteins, its intracellular trafficking is regulated by cholesterol. The predominantly intracellular protein translocated to the cell surface in cholesterol-poor conditions, and this shift was accompanied by increased, ezetimibe-sensitive cholesterol uptake (272). Notably, genetic variation in NPC1L1 was recently associated with reduced cholesterol absorption in a population study (42), reinforcing the medical relevance of this protein.

V. HUMAN SINGLE GENE DISORDERS OF CHOLESTEROL TRANSPORT

In this section, human diseases assigned to defects of cellular cholesterol transport are discussed. The disorders and the genes mutated therein are summarized in Table 1. The emphasis is in the molecular basis of the diseases, but brief descriptions of the prevalence, clinical manifestations, diagnostic principles, and/or therapeutic strategies when applicable are included.

A. Familial Hypercholesterolemia: LDLR Pathway

Familial hypercholesterolemia (FH) is one of the most common inborn errors of metabolism and the most common in the category of monogenic defects of cellular cholesterol processing (80). In most cases, it is an autosomal dominant disorder, with the heterozygous state affecting ~1 in 500 individuals (estimated 10 million worldwide). There is a strong gene dosage effect, and the rare homozygous FH patients exhibit a very severe clinical phenotype. The majority are caused by mutations in the LDLR gene. A rarer, clinically indistinguishable phenotype is caused by mutations in apoB, the ligand for the LDLR. In the latter case, the disease is referred to as familial defective apoB-100.

A third locus underlying autosomal dominant hypercholesterolemia, PCSK9 (proprotein convertase subtilisin kexin 9), was recently identified (2). In addition to an increasing number of patient mutations, there is suggestive evidence for the involvement of PCSK9 gene variants in affecting total and LDL cholesterol levels in the population (210). PCSK9 is a serine protease in the secretory pathway (206) and plays an important role in controlling LDLR levels, but the precise mechanism remains to be resolved. In principle, increased LDL cholesterol in the absence of PCSK9 function could reflect decreased clearance or increased hepatic secretion of apoB-containing lipoproteins. Both possibilities are being investigated (149).

Due to the FH mutations, LDL is not efficiently cleared from the circulation, and the resultant high LDL levels lead to premature atherosclerosis and coronary

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mutant Protein(s)</th>
</tr>
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<tbody>
<tr>
<td>Familial hypercholesterolemias</td>
<td>LDLR, apoB, PCSK9</td>
</tr>
<tr>
<td>Autosomal dominant</td>
<td>ARH</td>
</tr>
<tr>
<td>Autosomal recessive</td>
<td>LAL</td>
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<tr>
<td>Wolman disease, cholesteryl ester storage disease</td>
<td>NPC1, NPC2</td>
</tr>
<tr>
<td>Niemann-Pick type C disease</td>
<td>ABCA1</td>
</tr>
<tr>
<td>Tangier disease</td>
<td>ABCG5, ABCG8</td>
</tr>
<tr>
<td>Sitosterolemia</td>
<td>7-Dehydrocholesterol reductase</td>
</tr>
<tr>
<td>Smith-Lemli-Opitz syndrome</td>
<td>CYP27A</td>
</tr>
<tr>
<td>Cerebrotendinous xanthomatosis</td>
<td>StAR</td>
</tr>
<tr>
<td>Congenital lipid adrenal hyperplasia</td>
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<tr>
<td>Hypobetalipoproteinemias</td>
<td></td>
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<tr>
<td>Abetalipoproteinemia</td>
<td>MTP</td>
</tr>
<tr>
<td>Chylomicron retention disease, Anderson disease</td>
<td>Sar1b</td>
</tr>
<tr>
<td>Familial hypobetalipoproteinemia</td>
<td>ApoB</td>
</tr>
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heart disease. The elevated serum cholesterol concentrations lead to a more than 50% risk of coronary heart disease by age 50 in men and at least 30% in women aged 60 yr (219, 228). Aortic root disease is the most common cardiac manifestation, and by puberty, the patients invariably have some degree of atheroma of the ascending aorta (239). The primary clinical diagnostic criteria are an elevated cholesterol level (LDL-cholesterol in particular, HDL-cholesterol is within the normal range or low), presence of tendon xanthomata in the patient or first degree relative, and a dominant pattern of inheritance of premature heart disease or elevated cholesterol (80). Definite diagnosis can be made by the identification of the mutation involved (for the LDLR, over 700 mutations have been reported so far) (91). Currently, mutations are only detected in 30–50% of patients with a clinical diagnosis. Some of these cases apparently represent mutations in other, unidentified genes.

The treatment of FH has become significantly more effective with the availability of statins (inhibitors of HMG-CoAR) in 1989. Statins can reduce LDL-cholesterol by ~50–60% in homozygous FH patients (141, 142). Combination of a statin with a bile acid sequestrant, fibrate (PPARo agonist that reduces hepatic triglyceride production by increasing fatty acid oxidation), or the cholesterol absorption inhibitor ezetimibe often increases effectiveness of the therapy. In individuals nonresponsive for conventional drug treatment, repeated LDL-apheresis can be performed.

A number of rare cases of an autosomal recessive form of hypercholesterolemia have also been described. All the ~50 cases reported worldwide appear to result from mutations in ARH, a protein involved in the internalization of the LDLR via clathrin-coated pits (71, 222). Failure of the liver to take up and degrade plasma LDL leads to the increased plasma LDL cholesterol levels. The structural features of ARH include a phospho-Tyr binding domain for interaction with the endocytosis signal of the LDLR, a domain for direct interaction with clathrin and a phospholipid-phosphatidylinositol 4,5-bisphosphate-binding domain. Taken together, the data suggest that ARH plays a role in gathering the LDLR into forming endocytic carrier vesicles. Curiously, although the lack of LDLR internalization is evident, e.g., in lymphoblasts and monocyte-derived macrophages, it is not manifested in skin fibroblasts. This may be due to functional redundance with potentially the homologous protein Disabled-2 taking over the function of ARH in fibroblasts (155, 159). Overall, ARH patients appear to respond to lipid-lowering medication more favorably than FH homozygotes, with significant reduction in serum cholesterol by statins, bile acid sequestrants, or their combination (8, 222). The mechanistic reason for this better responsiveness to treatment compared with classic FH is unclear.

B. Wolman Disease and Cholesteryl Ester Storage Disease: Acid Lipase Deficiency

Lysosomal acid lipase (=acid cholesterol ester hydrolase) is essential for the hydrolysis of cholesteryl esters and triglycerides in lysosomes. Mutations in the enzyme give rise to two clinical disorders: the severe infantile-onset Wolman disease and the milder late-onset cholesteryl ester storage disease (CESD) (7, 10). Enzyme activity is more dramatically reduced in Wolman disease, ~200-fold in Wolman disease fibroblasts compared with 50- to 100-fold reduction in cholesterol ester storage disease cells (27).

Wolman disease presents with hepatosplenomegaly, steatorrhea, abdominal distension, adrenal calcification, and failure to thrive during the first weeks of life and usually leads to death before 1 yr of age. In contrast to Wolman disease, CESD is relatively benign. Hypercholesterolemia and accumulation of neutral fats and cholesteryl esters in the arteries predispose affected individuals to atherosclerosis. Massive hepatomegaly and hepatic fibrosis may lead to esophageal varices (11).

Generation of lysosomal acid lipase-deficient mice recapitulated the hepatosplenomegaly but not the shortened life span characteristic to severe acid lipase deficiencies in humans (52). In addition, the mouse model revealed an unanticipated role for the enzyme in adipocyte differentiation, fat mobilization, and development of insulin resistance (53). At present, there is no cure for the disease, but enzyme replacement therapy in the mouse suggests that a similar strategy may be successful in human acid lipase deficiencies (53, 54).

C. Niemann-Pick Type C Disease: Egress of Cholesterol From Lysosomes

Niemann-Pick type C (NPC) disease is characterized by the accumulation of unesterified cholesterol and other lipids, in particular sphingolipids, in late endocytic organelles. This is accompanied by impaired cholesterol esterification in the ER and defective suppression of cholesterol synthesis and LDLR activity (132, 176). The disease belongs to sphingolipid storage diseases in which also Niemann-Pick type A and B diseases are classified, being caused by primary deficiency of acid sphingomyelinase. Clinically NPC disease manifests as a progressive neurodegenerative disorder with visceral involvement, including hepatosplenomegaly, and affecting an estimated 1:150,000 individuals. The vast majority of the cases are caused by mutations in the late endosomal membrane protein NPC1 (30), the rest (5%) being due to mutations in a soluble lysosomal protein called NPC2/HE1 (163). Diagnosis is based on demonstration of decreased esterification of LDL-derived cholesterol in fibroblasts and cellular
sterol/sphingolipid accumulation in late endocytic organelles by using fluorescent probes (104).

NPC1 contains a SSD, similarly to, e.g., SCAP and HMG CoAR (46), but its functional role is not equally well understood. Recent data show that introduction of mutations to the SSD that correspond to activating mutations of SCAP, result in the stimulation of LDL-cholesterol trafficking to the plasma membrane and ER (153). NPC1 and NPC2 are both capable of binding sterol, the former via the SSD, the latter with an incipient cavity that apparently dilates to accommodate the lipid (67, 115, 166). On the basis of genetic evidence, NPC1 and NPC2 are likely to function in the same pathway, but the precise roles of both proteins remain unresolved (220). Interestingly, studies in yeast reveal a role for NPC1 in the recycling of sphingolipids (140), and in human subjects, sphingolipids are more promising than cholesterol as targets for therapies aiming at substrate reduction (122).

Another promising avenue for therapy is the administration of neurosteroids. The rationale is the observed deficiency of neurosteroids in the NPC1 mouse model and the critical role of neurosteroids for several brain functions (83). Furthermore, LXR activation may offer yet another possible option for treatment. This is based on the reduced generation of oxysterols and defective ABCA1-dependent lipid efflux in NPC cells (39, 70).

D. Tangier Disease and Familial HDL Deficiency: Efflux of Cholesterol From Cells

HDLs protect from the development of atherosclerosis, probably primarily by stimulating the removal of cholesterol from atherosclerotic lesion macrophages called foam cells. It is now known that a macrophage plasma membrane protein ABCA1 (ATP binding cassette transporter A1) plays a key role in the efflux of cholesterol to lipid-poor apoA-I that is further lipidated to form HDL (165). The importance of ABCA1 in this process was unraveled by the identification of mutations in this gene as the underlying cause of Tangier disease (20, 24, 196). This rare disease is characterized by HDL deficiency, sterol accumulation in tissue macrophages, and accelerated atherosclerosis. Remarkably, further studies have indicated that a significant fraction (at least 10%) of more common conditions with low circulating HDL levels can be due to heterozygosity for mutations in ABCA1 (41, 68). Heterozygosity for an ABCA1 mutation also predicted the risk of ischemic heart disease in a population study (69).

The detailed molecular events involved in ABCA1-mediated cholesterol efflux from macrophages are still unresolved. It is clear that the protein promotes both cholesterol and phospholipid efflux and that the two processes can be dissociated (235, 259). Close proximity of apoA-I to ABCA1 as evidenced by chemical cross-linking suggests that apoA-I binds to ABCA1 and that an optimal conformation of ABCA1 facilitates the interaction (258). ABCA1 mediates lipid efflux to apoA-I but not efficiently to HDL. Instead, ABCG1 and SR-B1 can mediate further cholesterol removal to more mature forms of HDL (73, 276). ABCA1 appears to release the lipids to the forming nascent HDL on the cell surface, but endocytic circuits contribute to ABCA1-mediated cholesterol efflux from intracellular pools (38, 164). Functional cooperation between ABCA1 and NPC1 is suggested by the defective ABCA1 induction in NPC1-deficient cells (39, 70) and the observation that ABCA1-deficient cells have immobile, lipid-laden late endosomes and altered NPC1 localization (164).

The turnover of ABCA1 is rapid, and its expression levels are highly regulated both at the transcriptional and posttranslational level. ABCA1 gene expression is increased by activation of the heterodimeric transcription factor LXR/RXR, and induction of ABCA1 expression is likely to be critical in the antiatherogenic effects of LXR ligands (241). ABCA1 protein can be proteolytically degraded by a thiol protease calpain, and apolipoprotein binding stabilizes ABCA1 by decreasing this proteolysis (256).

The role of ABCA1 in other tissues where it is present, such as liver and intestine, is being intensively investigated. A direct role in intestinal cholesterol absorption is unlikely based on its reported localization on the basolateral membrane of enterocytes (160). In the liver, ABCA1 promotes biliary cholesterol excretion and may participate in apoB/VLDL secretion (250). Tangier disease patients typically have reduced levels of ApoB-containing lipoproteins (207). However, differences in hepatic apoB production, lipid output, or apoB clearance rates do not seem to explain the phenomenon (4).

Several transgenic and knock-out mouse models to investigate the role of ABCA1 at the whole body level have been generated and recently reviewed (4, 109). It is obvious that the genetic background of the mice greatly influences the phenotype and probably explains many of the discordant findings. However, common to all the ABCA1-deficient homozygous mice is the absence of HDL. Recent mouse studies have uncovered that hepatic ABCA1 contributes to plasma HDL levels (187) and that ABCA1 affects the hepatic phospholipidation and cholesterol acquisition of ApoA-I (278). Importantly, targeted inactivation of ABCA1 in the liver showed that hepatic ABCA1 is critical in maintaining plasma HDL levels (240); direct lipidation of hepatic lipid-poor apoA-I by ABCA1 in the Golgi and at the plasma membrane (143) slows ApoA-I catabolism by the kidney and prolongs its plasma residence time.
E. Sitosterolemia: Intestinal Sterol Absorption

The diet, at least non-Western type, contains typically roughly equal amounts of cholesterol and plant sterols. On the average, ~50% of the cholesterol is absorbed compared with <5% of plant sterols. A rare recessive disorder of sterol absorption, sitosterolemia (also known as phytosterolemia), has helped to shed light on the principles underlying this difference. The disease is caused by mutations in either ABCG5 or ABCG8 (16, 125). Sitosterolemia patients absorb 15–20% of the dietary plant sterols. Interestingly, they also absorb an abnormally high fraction of dietary cholesterol and excrete less cholesterol into the bile than normal subjects. This results in severe hypercholesterolemia in childhood, xanthomas, accelerated atherosclerosis, and premature coronary artery disease (114). Diagnosis is confirmed by showing increased plasma and tissue levels of plant sterols (18, 198). Ezetimibe effectively reduces plasma plant sterol levels in sitosterolemia patients (200). Interestingly, ABCG8 polymorphisms may serve as genetic markers of cholesterol absorption efficiency at the population level (156).

The loss of ABCG5/G8 in humans results in high absorption of plant sterols both in humans (138, 199) and in mice (274). However, the fractional absorption of dietary cholesterol is only moderately increased in humans and not at all in mice (138, 199, 274). This suggests that under conditions of low dietary cholesterol intake, ABCG5/G8 does not have a major influence on the amount of chylomicron cholesterol reaching the liver. The situation may be different under high dietary cholesterol intake as suggested by the upregulation of the transporter in such conditions (16, 190). However, it is evident that ABCG5/G8 not only prevents the movement of the bulk of plant sterols beyond the enterocyte but also facilitates biliary secretion of cholesterol and other neutral sterols. Overexpression of both ABCG5 and ABCG8 leads to a fivefold increase in biliary cholesterol secretion and a ~50% reduction in the absorption of dietary cholesterol (275). Thus the combined role of ABCG5/G8 in hepatic and intestinal sterol balance has to be taken into account when explaining the clinical manifestations of sitosterolemia.

F. Smith-Lemli-Opitz Syndrome and Other Inborn Errors of Cholesterol Synthesis

Until now, seven inherited defects of cholesterol biosynthesis have been described in humans. Although they are, strictly speaking, not primary cholesterol transport defects, they are briefly summarized here because most likely some of their characteristics result from the lack of cholesterol and/or excess sterol precursors in the membrane. According to our recent results, even the immediate precursor of cholesterol, desmosterol, cannot substitute for cholesterol in cellular membranes (249). The reader is referred to excellent reviews and references therein for more comprehensive information on this group of disorders (93, 179). The first disease identified (in 1968), mevalonic aciduria due to a deficiency of mevalonate kinase, is the only disease identified in the premature portion of the cholesterol synthesis pathway (100).

The most common of these diseases, with an estimated incidence of 1/10,000–1/60,000, is Smith-Lemli-Opitz syndrome (SLOS). It is caused by a deficiency of 7-dehydrocholesterol reductase that catalyzes the reduction of 7-dehydrocholesterol to cholesterol in the final reaction of cholesterol synthesis. The clinical spectrum of this disease is amazingly broad, from severe cases with multiple malformations, severe mental retardation, and prenatal/neonatal death to individuals with only minor physical or behavioral problems. Approximately 5% of the mildest patients have normal intelligence.

Desmosterolosis and lathosterolosis are also due to last or second to last steps of cholesterol synthesis and have partial phenotypic overlap with SLOS. However, only a few patients have been described. In general, the earlier defects of postlanosterol cholesterol synthesis [HEM (hydrorps-ectopic calcification-moth eaten) dysplasia, X-linked chondrodysplasia punctata, and CHILD syndrome (congenital hemidysplasia with ichthyosiform erythroderma or nevus and limb defects)] are clinically more severe than the defects in the late steps. Disorganized bone and cartilage structure including dwarfism is typical, possibly related to defective Hedgehog signaling (see below), and most severely affects individuals with HEM dysplasia.

Diagnosis of this group of defects is based on biochemical tests demonstrating elevations of accumulating sterol intermediates. In spite of the fact that the diseases are clearly enzyme deficiencies, their pathogenesis is not well understood. Probably, the lack of cholesterol itself, accumulation of toxic intermediates, abnormal feedback regulation of cholesterol synthesis, and/or abnormal signaling via Hedgehog proteins contribute (93). It has been noted that many of the malformations are consistent with impaired Hedgehog function. Sonic Hedgehog plays a central role in midline patterning and limb development, Indian Hedgehog is important for bone mineralization, and desert Hedgehog is involved in genital development (105). Hedgehog contains a covalently attached cholesterol molecule, and its receptor, Patched, has a SSD analogously to key regulators of cholesterol transport/metabolism. Studies in SLOS and lathosterolosis cells indicate that Hedgehog dysfunction is likely due to decreased sterol levels rather than teratogenic effects of the precursors (44).
The existence of naturally occurring or targeted mouse mutations for most of the disorders will help in studying disease pathogenesis. However, because of the differences between human and mouse cholesterol processing, especially in transplacental and fetal sterol transport, the phenotypes may be strikingly different. For example, desmosterolosis mice exhibit a milder phenotype than that described for human desmosterolosis patients (179, 261).

G. Cerebrotendinous Xanthomatosis and Other Defects of Bile Acid Synthesis

Inborn errors of bile acid metabolism are here discussed only in relation to the enzymes that participate in the early, rate-limiting steps of bile acid synthesis from cholesterol, whereas distal defects in bile acid synthesis and bile acid transporter defects are not included.

Cerebrotendinous xanthomatosis is caused by mutations in CYP27A, the rate-limiting enzyme in the acidic pathway of bile acid synthesis (28). Enzyme deficiency leads to impaired oxidation of the cholesterol side chain in the formation of cholic acid (218). The accumulating metabolite is cholestanol, the 5α-dihydro derivative of cholesterol. Disease diagnosis is made by demonstrating cholestanol in abnormal amounts in the serum and tendon of affected persons. Plasma cholesterol concentrations are low normal. Patients develop multiple abnormalities, including neurological symptoms, cataracts, diarrhea, xanthoma, and atherosclerotic vascular disease (158). The excess production and consequent accumulation of cholestanol appears to play an important role in the disease pathophysiology (152). In addition, the lack of 27-hydroxycholesterol may be relevant. Low levels of this endogenous LXR ligand may stimulate foam cell formation. Moreover, the oxysterol may itself serve as a form of sterol effluxed from cholesterol-laden cells (263). The most effective therapy is supplementation with the missing end product of the pathway, chenodeoxycholic acid (158).

In mice, disruption of sterol 27-hydroxylase gene leads to hepatomegaly and dysregulation of hepatic cholesterol, bile acid, and fatty acid metabolism. However, the mice lack most of the clinical and biochemical abnormalities characteristic to cerebrotendinous xanthomatosis (55, 193). This is probably because in Cyp27−/− mice, classic bile acid biosynthesis is not stimulated as much, and the formed bile alcohols are more efficiently metabolized compared with the human patients (99).

The first human mutations in the enzyme initiating the classical pathway of bile acid synthesis, CYP7A1, have recently been described (183). A family with homozygous mutations of 7α-hydroxylase presented with a phenotype reminiscent of that in familial hypercholesterolemia, with elevated LDL associated with premature atherosclerotic heart disease. In addition, the homozygotes had an increased hepatic cholesterol content, deficient bile acid excretion, and signs of upregulation of the alternative bile acid pathway. Some individuals had hypertriglyceridemia and premature gallstone disease. Importantly, heterozygotes were also hyperlipidemic, indicating a codominant disorder, and suggesting that variations in CYP7A1 gene might contribute to the prevalence of atherogenic hyperlipidemia and cholesterol gallstone disease at the population level (183). Further studies are needed to identify other families with this disorder. Notably, targeted disruption of CYP7A1 in mice yielded a complex phenotype with conflicting results regarding serum lipids (205), whereas mice overexpressing the protein were protected from diet-induced atherosclerosis and gallstone formation (157).

H. Congenital Lipoid Adrenal Hyperplasia: Transport of Cholesterol for Steroidogenesis

Mutations in the mitochondrial cholesterol transporter protein StAR cause congenital lipoid adrenal hyperplasia (lipoid CAH) (128). This rare, recessively inherited and potentially lethal condition results from an almost complete inability to synthesize steroids. The condition is accompanied by the presence of large adrenals containing extremely high levels of cholesterol and cholesterol esters. The patients present with a severe salt-losing syndrome (hyponatremia, hyperkalemia) and failure to thrive that is fatal if not treated in early infancy (227). Mineralocorticoid and glucocorticoid replacement therapy is vital and may allow survival to adulthood.

In addition, all the affected individuals are phenotypic females irrespective of gonadal sex. Deficient fetal testicular steroidogenesis in patients with a 46,XY karyotype results in phenotypically female genitalia (227). This is because androgens are required for normal sexual male differentiation, whereas the fetal ovary does not make steroids during embryonic or early postnatal life. However, at puberty the ovary starts to synthesize steroids in response to gonadotropins. Lipoid CAH 46,XX patients may undergo normal feminization, presumably as a result of StAR-independent steroid synthesis but have anovulation and ovarian cysts (209). A late-onset form of lipid CAH has been described as a result of a heterozygous mutation in the P450scc gene (234).

It has been proposed that the congenital lipid adren al hyperplasia phenotype is the result of two separate events (“two-hit model”): the first hit is the genetic loss of steroidogenesis upon defective transfer of cholesterol to the inner mitochondrial membrane, and the second hit is the secondary interference with cellular processes by the accumulation of steryl esters (22). Star expression is
limited to steroidogenic cells, but the protein belongs to a large family of STAR-related lipid transfer (START)-domain proteins (5). Presumably, widely expressed START-domain proteins may deliver cholesterol to mitochondria in other tissues. The ligands of this protein family also include phospholipids and ceramides, but at least two other proteins, MLN64 and STARD5, bind cholesterol (192, 244).

The STAR knockout mouse recapitulates all the essential features of the corresponding human disease, including female external genitalia, failure to thrive, lipid deposits in the adrenal cortex, and responsiveness to corticosteroid replacement therapy (29, 90).

I. Hypobetalipoproteinemias: Defects of Lipoprotein Assembly

The assembly and secretion of triglyceride-rich lipoproteins require apoB and the ER localized cofactor microsomal triglyceride transfer protein (MTP). Hypobetalipoproteinaemia (i.e., low level of lipoproteins with β-electrophoretic mobility) is a condition characterized by low plasma total cholesterol, low LDL cholesterol, or low apolipoprotein B (apoB) levels. A number of factors, such as illness or strict vegan diet, may result in low apoB/LDL levels. Primary causes include abetalipoproteinemia and chylomicron retention disease, two recessively inherited traits, as well as familial hypobetalipoproteinemia, an autosomally dominantly inherited condition.

Abetalipoproteinemia is due to mutations in MTP (264). MTP is a soluble protein in the ER lumen that transfers neutral lipids (in particular cholesteryl esters and triglycerides) as well as phospholipids to the forming apoB-containing lipoproteins, i.e., chylomicrons in enterocytes and VLDL in hepatocytes (Fig. 3). MTP contains a dimeric complex of protein disulfide isomerase (PDI) together with a unique 97-kDa subunit. Abetalipoproteinemia causing MTP mutations are found in the 97-kDa subunit, and the disulfide isomerase activity of PDI is not required for MTP transfer activity (254). Deficiency of MTP activity leads to a defect in the production of both chylomicrons and VLDL. Clinically it is manifested initially as malabsorption at first postnatal months, developing to fat-soluble vitamin deficiencies, and gradually to neuroretinal and hematological complications. The condition is managed by lipid-poor diet containing essential fatty acids in vegetable oil and supplementation of fat-soluble vitamins (17).

In mice, a homozygous MTP knockout is embryonically lethal (184). Conditional knockouts have been generated and found to mimic aspects of the human condition (31, 185), although evidently plasma apoB48/apoB100 levels in mice are not determined similarly as in humans.

In contrast to abetalipoproteinemia, the majority of familial hypobetalipoproteinemia patients are asymptomatic heterozygote cases due to gene defects, often missense or frameshift mutations, in apoB (204). The frequency of familial hypobetalipoproteinaemia attributed to truncated apoB has been estimated at 1 in 3,000 (262). The individuals are resistant to atherosclerosis and in the homozygous condition resemble abetalipoproteinemia.

Chylomicron retention disease and Anderson disease are caused by mutations in the small GTPase Sar1b (108) (Fig. 3). These conditions are manifested as severe malabsorption and failure to thrive in infancy, with deficiency of fat-soluble vitamins. Affected individuals accumulate chylomicron-like particles in membrane-bound compartments of enterocytes and have selective absence of chylomicrons in blood. All the mutations identified are in SARA2, the protein encoding Sar1b, and all the missense mutations map to the nucleotide binding site of the protein (211). The exchange of GTP for GDP on Sar1 is known to initiate the budding of COPII-coated ER-to-Golgi transport vesicles (9, 202). The finding of Sar1b mutations revealed the requirement for Sar1b in the intracellular transport of chylomicrons. The precise role of COPII in the process remains to be solved: chylomicrons may actually leave the ER in COPII-coated vesicles or COPII may just be needed to recruit additional machinery needed for chylomicrons to mature along the secretory pathway (232, 277).

VI. CELLULAR CHOLESTEROL BALANCE AS A TARGET FOR CARDIOVASCULAR PHARMACOLOGY

Current therapeutic strategies to prevent atherosclerosis are largely based on the use of statins, which inhibit the rate-limiting enzyme of cholesterol biosynthesis, HMG-CoA reductase, and decrease serum LDL cholesterol levels (65). In addition, statins improve endothelial function, e.g., by restoring NO production, enhance the stability of atherosclerotic plaques, and decrease inflammation and oxidative stress (127). The effectiveness of statins in reducing coronary morbidity and mortality is well established. However, the contribution of synthesis versus absorption in whole body cholesterol balance varies between individuals (87) and in persons exhibiting a poor response in cholesterol lowering with statins; therapy with a selective cholesterol absorption inhibitor, ezetimibe, may be more effective. This is based on the idea that in statin nonresponders, de novo cholesterol synthesis is probably low and the contribution of cholesterol absorption for cholesterol levels is more substantial. Importantly, ezetimibe can be used in combination with statin, and coadministration results in an incremental decrease in LDL-cholesterol (13, 56). Ezetimibe targets
NPCL1 in the enterocytes where it reduces the absorption of dietary and biliary cholesterol by preventing its absorption through the intestinal wall (175). It also prevents the absorption of noncholesterol sterols, such as plant sterols, but has no effect on the absorption of fat-soluble vitamins, fatty acids, or bile acids.

Other strategies to inhibit cholesterol absorption are the use of bile acid sequestrants or PPAR-agonists (fibrates) in patients with elevated triglyceride levels (57). The use of plant sterol or stanol-enriched margarine is based on the inhibition of dietary and biliary cholesterol uptake by competing with cholesterol for incorporation into mixed micelles (88).

Among the most promising antiatherogenic approaches under development are agonists of nuclear hormone receptors, in particular LXR, which controls the expression of key regulators of cholesterol transport such as the ABC transporters ABCA1, G1, G4, G5, G8, the ApoCI/CII/CIV gene cluster, as well as lipoprotein-modifying enzymes, e.g., lipoprotein lipase, cholesteryl ester transfer protein, and phospholipid transfer protein (241). Evidence for LXR agonist-induced stimulation of reverse cholesterol transport from macrophages to feces was recently provided using several mouse models (162). In addition, ACAT inhibitors are being evaluated in clinical trials as potential compounds inhibiting foam cell formation and atherosclerotic lesion development (226). Other lipid-lowering agents under development including cholesteryl ester transfer protein (CETP) inhibitors, microsomal triglyceride transfer protein (MTP) inhibitors, ileal bile acid transport (IBAT) inhibitors, and dual PPAR- and γ-agonists, are discussed in more detail elsewhere (60, 111).

Conspicuously, a number of compounds developed for other purposes have adverse effects on cellular cholesterol trafficking that may be related to their potential side effects. These include psychopharmacaca such as the tricyclic antidepressant imipramine that inhibits intracellular cholesterol transport in a manner mimicking the Niemann-Pick type C phenotype (124, 246) and the antifungal agents nystatin and amphotericin B that are known to disrupt caveolae (194). Finally, some compounds affecting cholesterol metabolism, such as the late-stage cholesterol biosynthesis inhibitor triparanol, have severe teratogenic effects resulting in a phenotype that shares characteristics with inborn errors of postlanosterol cholesterol biosynthesis. The triparanol-induced limb abnormalities were shown to originate from altered signaling via the sterol-bound morphogen Hedgehog (77).

VII. CHOLESTEROL-SPHINGOLIPID RAFTS: IMPLICATIONS FOR HUMAN DISEASE

The medical importance of lipid rafts in the pathogenesis of diseases is now well appreciated (213). The cholesterol dependence of rafts is typically addressed by pharmacological cholesterol depletion. However, instead of decreased cholesterol levels, the vast majority of diseases with intracellular cholesterol transport problems lead to cholesterol deposition or subcellular cholesterol imbalance. The preferred subcellular site(s) of the deposition vary as probably do the compensatory changes taking place in other lipids and proteins. Notably, the intracellular cholesterol imbalance will probably not manifest only at the initial site of the defect. For instance, a defect in cholesterol biosynthesis may lead to endosomal sterol deposition (260). Interestingly, connections between the maintenance of cholesterol-sphingolipid rafts and intracellular cholesterol transport are also starting to emerge.

It has been proposed that cholesterol-sphingolipidoses may lead to the jamming of rafts in the endolysosomal compartments (131, 214). In NPC fibroblasts, newly hydrolyzed LDL-cholesterol is indeed incorporated more avidly into detergent-resistant membranes than in control fibroblasts (136). Interestingly, recent evidence suggests that NPC cells with massive cholesterol deposition, such as primary hepatocytes from the NPC mouse, also have excess cholesterol in the plasma membrane (248). Moreover, this inhibits raft-dependent signaling as evidenced by defective insulin receptor activation and its restoration upon plasma membrane cholesterol depletion (248). Thus, in a cholesterol transport defect, excess cholesterol is clearly detrimental to raft-dependent signaling.

For understanding raft-dependent human diseases, the site(s) of altered cholesterol concentration, whether in the circulation or at the cellular level, e.g., in the plasma membrane, endosomes, or lipid droplets, is clearly important. This is exemplified by the multitude of findings concerning cholesterol levels and proteolysis of amyloid precursor protein (APP), a critical process in the development of Alzheimer’s disease (AD). Initially, a link between cholesterol and AD was established by the finding that the allele ε4 of apoE predisposes to an early onset of AD (45). ApoE plays an important role in cholesterol balance and regenerative processes in the brain (146, 252). Later on, genetic variants of a number of other key players of cholesterol transport, including ABCA1, ABCA2, and ACAT, have been reported as modifiers of AD risk (112, 139, 266). Epidemiological evidence shows that treatment of hypercholesterolemic individuals with statins lowers the incidence of AD (12, 107, 267). However, the most commonly used statins do not penetrate the blood-brain barrier efficiently (223), and subjects treated with statins do not show major changes in brain membrane cholesterol (61). It is therefore possible that the beneficial effects of statins in AD are not due to their effects on neuronal rafts.

Nevertheless, the neuronal cholesterol content and distribution clearly have an effect on amyloid peptide...
generation. Recent data suggest that in fact loss of cholesterol in neuronal membranes enhances amyloid peptide generation. Moreover, inhibition of intracellular cholesterol transport by compound U18666A, which mimics the NPC phenotype (cholesterol deposition in late endocytic organelles and impaired cholesterol esterification), leads to a reduction in both secreted and neuronal Aβ species (195). Likewise, inhibition of ACAT was associated with a prominent reduction in Aβ generation, and cholesteryl ester levels directly correlated with Aβ production (182). On the other hand, APP processing and the generated Aβ species apparently affect cellular cholesterol and sphingomyelin metabolism (84). At present, it is not possible to form a complete picture of the functionally relevant causal relations. However, the findings undoubtedly urge us to investigate the basic principles of cholesterol transport and exchange in neuronal and glial cells.

VIII. CONCLUSIONS AND PERSPECTIVES

During the past decade, we have undoubtedly witnessed the “single gene/protein era” of intracellular cholesterol transport. The molecular link between STAR and congenital lipid adrenal hyperplasia was discovered more than 10 years ago, and during the years to come, an increasing number of proteins mutated in monogenic human diseases have been identified. A major breakthrough in the field was the identification of ABCA1 as the Tangier disease gene. This has played an important contribution in the development of new pharmacological antiatherogenic strategies and underscored the fundamental importance of ABC transporters in cholesterol trafficking. On the other hand, the identification of NPC1L1 as the target of ezetimibe serves as an example of how basic research findings may help to validate the use of existing pharmacological regimens.

The leap from the defective protein to the next steps, solving the molecular structures and uncovering protein interaction networks, has been successfully initiated in a number of cases but will probably be more challenging with this group of gene products than with some others. In experimental strategies, the lipophilic nature of the proteins will continue to do tricks on us, but the advancement of tools, such as genome-wide RNAi approaches for finding functionally linked proteins and tissue-specific knockouts of proteins of interest, will increase capacity and precision. Transcriptional regulators as major switches for groups of proteins in the same or related pathways will play an important role in attempts to orchestrate the cellular cholesterol transport machineries.

From the pathophysiological point of view, the spectrum of affected tissues is crucial and at times unexpected, considering the available knowledge at the cellular level. Genotype/phenotype correlations are often not simple to make, and disease characteristics cannot be deduced from the expression pattern of a protein. Rather, symptoms are likely to appear from those tissues in which compensatory mechanisms or parallel pathways fail. An important challenge for the future is to understand how subtle alterations in cholesterol transport and metabolism, perhaps over the span of decades, contribute to the pathogenesis of chronic diseases in the population. It seems evident that besides, and sometimes instead of, few major loci underlying genetic predisposition to cellular cholesterol disturbances, we need to search for multiple variants with modest effects. This necessitates large-scale genetic and environmental epidemiology in human populations. In parallel, we need to develop experimental strategies to study cholesterol trafficking in model organisms and to bridge the gap between cell analyses and in vivo tissue biology.

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