Atherogenic lipoproteins and coronary artery disease: concepts derived from recent advances in cellular and molecular biology

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RECENT ADVANCES have contributed to our understanding of lipoproteins, their metabolism, and their involvement in atherogenesis (for review see refs. 1 to 6). In this review, attention will be focused on specific types of lipoproteins and the mechanisms whereby they may be either atherogenic or antiatherogenic. Human genetic diseases of lipid metabolism and diet-induced changes in lipoproteins have provided unique insights.

Atherosclerotic lesions, as they occur in man or as induced by high-fat, high-cholesterol diets in experimental animals, share many common properties. Narrowing of the vessel lumen is characterized by the deposition of cholesterol in and around cells of the arterial wall in association with cellular proliferation and fibrosis. The cholesterol that accumulates in the arterial wall is derived from plasma lipoproteins; therefore, to understand the mechanisms involved in atherogenesis, it is important to identify the specific lipoproteins responsible for the delivery of cholesterol to the cells and to identify the cell type responsible for the accumulation of the cholesterol. Lipid transport into and out of the vessel wall appears to be a constant and dynamic process. Disease of clinical significance develops when influx and deposition of cholesterol exceeds egress of cholesterol from the arterial wall. The hyperlipidemia that develops in the genetic diseases to be discussed, or that develops with the consumption of high levels of fat and cholesterol, accelerates the lipid deposition and upsets the balance between influx and egress.

Plasma lipoproteins and their metabolism. A brief consideration of the normally occurring major plasma lipoproteins and their metabolism is essential for understanding the role of specific lipoproteins in atherogenesis. Chylomicrons are synthesized by the intestine to transport dietary triglyceride and cholesterol. As they circulate in the plasma compartment, the triglycerides are hydrolyzed by the action of lipoprotein lipase, resulting in the production of cholesterol-enriched chylomicron remnants. The chylomicron remnants are rapidly cleared from the plasma by the liver (figure 1, step 1), and their cholesterol converted to bile acids and excreted or used in membrane and hepatic lipoprotein biosynthesis. Very low-density lipoproteins (VLDLs) are triglyceride-rich particles synthesized by the liver. As the VLDLs circulate, they are acted upon by lipases, which liberates the fatty acids for use as an energy source in various tissues, and are converted to intermediate-density lipoproteins (IDLs). The IDLs are either removed from plasma by the liver (figure 1, step 2) or are converted to low-density lipoproteins (LDLs) by further action of the lipases (figure 1, step 3). By virtue of the removal of triglyceride from the VLDLs, the IDLs and LDLs become enriched in cholesterol. The LDLs are the end product of VLDL catabolism and are the major cholesterol-transporting lipoproteins in the plasma. They are catabolized by both the liver and extrahepatic tissues (figure 1, step 4). High-density lipoproteins (HDLs) are derived from several sources and appear to play a dynamic role in acquiring and redistributing cholesterol among cells (see figure 2, to be discussed below).

Each of the lipoproteins possess one or more protein constituents called apolipoproteins. An important function of certain apolipoproteins is to mediate the binding of the lipoproteins to specific receptors on the surface of cells. Receptor-mediated uptake of lipoproteins by cells is the mechanism by which lipoproteins...
are cleared from the plasma and catabolized by the cells.²,⁴ The two apolipoproteins that have been shown to mediate binding to the lipoprotein receptors are apo-lipoprotein E (apo-E) and apolipoprotein B (apo-B). Apo-E is the protein determinant responsible for receptor-mediated uptake of chylomicron remnants and a subclass of HDL, the HDL-with apo-E. Apo-B is responsible for mediating receptor binding of LDL. Either apo-B or apo-E appears to be involved in mediating uptake of IDL.

Two distinct cell surface receptors are involved in lipoprotein metabolism (figure 1). The apo-B,E(LDL) receptor, as originally described by Goldstein, Brown, and associates,¹,⁷ interacts with either apo-B- or apo-E-containing lipoproteins.² They are present within the liver and most extrahepatic tissues. A second receptor that appears to be unique to the liver is referred to as the chylomicron remnant receptor (a receptor postulated to be the apo-E receptor).³ This receptor interacts with apo-E-containing lipoproteins but not with apo-B-containing LDL.

Atherogenic and antatherogenic lipoproteins. Two genetic diseases of lipoprotein metabolism (type III hyperlipoproteinemia and familial hypercholesterolemia) are associated with accelerated atherosclerosis. Although these relatively uncommon disorders are not responsible for large numbers of patients with coronary artery disease, they provide key insights into the role of specific lipoproteins in atherogenesis. The importance of understanding these disorders lies in the close parallels between the lipoproteins seen in these genetic disorders and those induced by the consumption of diets high in saturated fat and cholesterol. Changes in lipoproteins induced by diet are a major factor responsible for the widespread hypercholesterolemia seen in the U.S. population and the associated high incidence of coronary artery disease.

Type III hyperlipoproteinemia. Type III hyperlipoproteinemia is associated with hypertriglyceridemia and hypercholesterolemia that is characterized by the accumulation in the plasma of cholesterol-rich remnants of chylomicrons and VLDLs or IDLs, which are referred to collectively as β-VLDLs. Patients with type III hyperlipoproteinemia develop accelerated atherosclerosis involving both coronary and peripheral arteries. In considering the potentially atherogenic lipoproteins seen in this disorder, it is important to note that patients with type III hyperlipoproteinemia usually have low levels of LDL and HDL and that the hyperlipidemia is associated exclusively with an elevation in the level of β-VLDL. The underlying genetic defect responsible for the lipoprotein abnormalities is the synthesis of an abnormal form of apo-E, i.e., a form that does not bind normally to either the apo-B,E(LDL) or remnant (apo-E) lipoprotein receptors. A brief description of apo-E and its role in lipoprotein metabolism illustrates how this defect interferes with the catabolism of specific lipoproteins and how these lipoproteins contribute to the development of accelerated atherosclerosis (for a complete discussion of type III hyperlipoproteinemia and how other genetic, environmental, and hormonal factors modulate expression of the disease, see refs. 8 and 9).

The molecular defect in type III hyperlipoproteinemia is caused by the inheritance of genes coding for dysfunctional forms of apo-E. As has been described for other proteins, e.g., hemoglobin, there are multiple alleles in the population that code for apo-E isoforms that differ in protein structure by single amino acid substitutions. The most common isoform of apo-E

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**FIGURE 1.** General scheme of lipoprotein metabolism.

**FIGURE 2.** Role of HDL in acquiring cholesterol from cells containing excess cholesterol and redistributing the cholesterol to cells requiring it for steroid hormone production and membrane biosynthesis [extrahepatic cells, e.g., adrenal, testis, etc., with apo-B,E(LDL) receptors] or to the liver for elimination from the body.
seen in the population is apo-E3 (approximately 60% of the population is homozygous for apo-E3). Type III hyperlipoproteinemia is most often associated with apo-E2 homozygosity. Four variant isoforms of apo-E have been identified in studies of a number of patients with type III hyperlipoproteinemia.\(^{2,4,8,9}\) These variant forms of apo-E exhibit defective binding to lipoprotein receptors, which accounts for the delayed clearance and increased concentration of apo-E-containing, cholesterol-rich lipoproteins in type III patients. The amino acid substitutions that affect receptor binding occur in the midportion of the apo-E molecule and involve substitutions of neutral amino acids for the normally occurring lysine or arginine residues (table 1). It has now been established that the region of the apo-E molecule responsible for mediating apo-B,E(LDL) and remnant (apo-E) receptor binding involves basic amino acids (arginine and lysine) in the vicinity of residues 140 to 160.\(^{2,4,9}\) The description of this ligand binding domain of apo-E has helped to identify the postulated multiple binding sites on the apo-B,E(LDL) receptor, a region enriched in the acidic amino acids glutamic and aspartic acids.\(^{10}\)

The abnormal apo-E, defective in its ability to bind to the lipoprotein receptors, disrupts the normal metabolism of chylomicrons and VLDLs (the β-VLDLs) (figure 1, steps 1 and 2). The impaired receptor-mediated uptake of these lipoproteins results in their accumulation in the plasma. In addition, the presence of the abnormal form of apo-E interferes with the normal formation of LDL from β-VLDL and IDL in type III patients (figure 1, step 3). This could contribute to the low level of LDL seen in patients with type III hyperlipo- proteinemia and could contribute further to the accumulation of β-VLDL in the plasma.\(^{4,8,9}\) The cholesterol-rich remnants of chylomicrons and VLDL (the β-VLDLs) that accumulate must now be cleared from the plasma by an alternative pathway. The alternate route may well be through macrophages (scavenger cells), including macrophages of the arterial wall that participate in foam cell production in atherosclerotic lesions.

Several lines of evidence implicate β-VLDLs in atherogenesis and suggest that macrophages are involved in the process (for review see refs. 2, 3, 5, 6, and 9). Macrophages possess a receptor that interacts specifically with β-VLDL. Both chylomicron and VLDL remnants bind to this receptor and cause massive cholesteryl ester accumulation in the cultured macrophages. In addition, it has been shown that foam cells within the arterial wall possess receptors for β-VLDL.\(^{11}\)

In further support of the postulate that β-VLDL may be atherogenic, animals fed diets high in fat and cholesterol have markedly elevated levels of β-VLDL (chylomicron and VLDL remnants) in their plasma and develop accelerated atherosclerosis.\(^{2,5,6}\) These diet-induced β-VLDLs also cause cholesteryl ester accumulation in macrophages. Furthermore, lipoproteins resembling β-VLDLs are seen in the plasma of humans after consumption of a single high-fat, high-cholesterol meal, and it is reasonable to speculate that these transiently present lipoproteins may contribute to the atherogenic risk seen in populations consuming such diets.\(^{5}\) Dietary intervention could be expected to alter the production of these potentially atherogenic lipoproteins.

Unlike type III hyperlipoproteinemia, in which the accumulation of β-VLDL is caused by the presence of the receptor-defective apo-E, the β-VLDL accumulation seen after fat and cholesterol feeding appears to be secondary to the down-regulation of expression of apo-B,E(LDL) receptors.\(^{2,12}\) Cholesterol feeding decreases the number of apo-B,E(LDL) receptors in the liver, whereas cholesterol feeding does not decrease the expression of remnant (apo-E) receptors.\(^{2}\) Presumably, lipoprotein overproduction induced by the dietary fat and cholesterol, in association with a decrease in apo-B,E(LDL) receptors, exceeds the ability of the remnant receptors to clear the excess particles from the plasma, and both the chylomicron and VLDL remnants accumulate. Thus, either an impaired ability of apo-E-containing lipoproteins to interact with the receptors, as seen in type III hyperlipoproteinemia, or a decreased expression of the apo-B,E(LDL) receptor, as seen in fat and cholesterol feeding in animals, results in accumulation of remnant lipoproteins. Both

**TABLE 1**

Human apo-E variants and their receptor binding activities

<table>
<thead>
<tr>
<th>Substitutions(^{a})</th>
<th>Receptor binding activity relative to apo-E3(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg→Cys</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Arg→Cys</td>
<td>45</td>
</tr>
<tr>
<td>Lys→Gln</td>
<td>40</td>
</tr>
<tr>
<td>Cys→Arg, Arg→Cys</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

\(^a\)The designation of the variants, as listed, refers to the site(s) where their structures vary from that of "normal" apo-E3. For example, the notation Arg→Cys indicates that cysteine is substituted for the normally occurring arginine at residue 158.

\(^b\)Apo-E3 binding activity is set at 100%. The binding activities of the variants associated with type III hyperlipoproteinemia are expressed as a percentage of the "normal" apo-E3 binding to both apo-B,E(LDL) and remnant (apo-E) receptors.
conditions are associated with accelerated atherosclerosis.

A comparison of the changes in HDL in both type III hyperlipoproteinemia and with fat and cholesterol feeding in animals suggests how these lipoproteins may function as protective or antiatherogenic lipoproteins (for review see refs. 2, 5, 6, and 9). The changes include a reduction in the typical HDL, i.e., HDL-without apo-E, and an increase in HDL-with apo-E (HDL, or HDL). These changes appear to reflect the role of HDL in the process referred to as reverse cholesterol transport. Cholesterol deposited in extrahepatic tissues, including cells of the arterial wall, may be acquired by HDL, especially HDL-without apo-E (figure 2). The cholesterol appears to be esterified in the plasma, resulting in an increase in the size of the HDL particles and the acquisition of apo-E. Because apo-E is synthesized by various cells, including macrophages, it is available within the interstitial fluid to become associated with the cholesterol-enriched HDL, i.e., HDL-with apo-E. The presence of the apo-E on these lipoproteins can now target the HDL-with apo-E to cells with either the apo-B,E(LDL) or remnant (apo-E) receptors (figure 2, step 1). Alternatively, it has been postulated that the cholesterol in HDL can be transferred to other lipoproteins (e.g., VLDL or LDL) that are then taken up by receptor-mediated endocytosis (figure 2, step 2). By either process, the HDL can participate in the redistribution of cholesterol from cholesterol-loaded cells to cells requiring cholesterol or to the liver for elimination of cholesterol from the body.

The decreased level of HDL-without apo-E in type III patients (or with fat and cholesterol feeding) could reflect the use of these particles as precursors in the formation of the cholesterol-loaded, HDL-with apo-E. The association of low levels of HDL with an increased risk of coronary artery disease may well be caused by the lack of sufficient quantities of HDL necessary to facilitate the egress of cholesterol from cells of the arterial wall in the face of large quantities of lipoproteins (e.g., \( \beta \)-VLDL), resulting in the deposition of cholesterol within these cells. Studies in vivo and in vitro have shown that high levels of HDL prevent the development of atherosclerosis in dogs and prevent accumulation of cholesteryl ester in macrophages, even when high levels of remnant lipoproteins (\( \beta \)-VLDL) are present.13

Familial hypercholesterolemia. The role of LDLs in the development of accelerated atherosclerosis is dramatically illustrated by studies of patients with familial hypercholesterolemia. As demonstrated by the classic studies of Goldstein and Brown (for review see refs. 2, 7, 12, and 14), patients with this disorder lack or have defective apo-B,E(LDL) receptors (figure 1, step 4; defective receptors prevent normal LDL and IDL uptake and catabolism). Patients homozygous for this disorder often die in the second decade of life from coronary artery disease. It should be kept in mind that these subjects also have abnormally high levels of IDL, resembling VLDL remnants, that could be contributing to the atherogenic process through the mechanisms discussed above.

Not only do LDLs accumulate in the plasma of individuals with familial hypercholesterolemia but also in the plasma of animals fed diets high in fat and cholesterol.5,6 The increase in plasma LDLs in the various animal preparations is very likely secondary to the down-regulation in expression of the apo-B,E(LDL) receptors. The decrease in the number of these receptors would be expected to decrease LDL catabolism and to result in an increase of the plasma concentration. This may well be the mechanism whereby the consumption of high-fat, high-cholesterol diets by humans results in elevated plasma cholesterol levels and predisposes man to an increased risk of developing coronary artery disease.

However, the precise mechanism whereby LDLs are atherogenic is not entirely clear (for review see refs. 3, 5, 6, 12, and 15). High levels of LDLs may in fact lead to endothelial damage (including the possibility of subtle derangement in endothelial cell metabolism) and to an increased flux of LDLs into the arterial wall. The LDLs do accumulate in the arterial wall in association with atherosclerotic lesions, and these lesions are associated with cholesterol accumulation in smooth muscle cells and macrophages. However, at the present time it is difficult to explain how the smooth muscle cells could acquire excess cholesterol, since, at least in tissue culture, the apo-B,E(LDL) receptors are efficiently down-regulated by delivery of cholesterol to these cells and will not accumulate excess cholesterol. Furthermore, based on studies in vitro, macrophages do not accumulate massive amounts of cholesteryl ester after incubation with even high concentrations of normal LDL.

An intriguing hypothesis has been put forth that suggests how macrophages may become loaded with cholesterol in response to high levels of LDL. It has been shown that chemically modified LDL can be recognized by unique receptors on macrophages (referred to as the acetyl LDL receptor or the receptor for chemically modified LDL) (for review see refs. 2, 3, 5, and 15). In the laboratory, LDL modified by acetyl-
lation, acetoacetylation, oxidation, or malondialdehyde modification can be recognized by this receptor and can cause massive accumulation of cholesteryl ester within the macrophages. Such modifications of LDLs could occur in the plasma as the lipoproteins circulate or in the arterial wall as they perfuse through the tissue. In addition, LDL complexed to glycosaminoglycans, such as dextran sulfate, can also be recognized and taken up by macrophages, causing cholesterol accumulation. However, despite the attractiveness of these postulated mechanisms, more data are needed to establish their importance in the pathogenesis of atherosclerosis.

Conclusions. Accelerated atherosclerosis occurs in patients with type III hyperlipoproteinemia and familial hypercholesterolemia. The genetic defects in these disorders focus attention on specific types of lipoproteins as being responsible for the development of accelerated coronary artery disease. The accumulation of chylomicron remnants of intestinal origin and of VLDL or IDL remnants of hepatic origin appears to correlate with coronary disease seen in patients with type III hyperlipoproteinemia. The presence of abnormal apo-E prevents normal receptor-mediated catabolism of these lipoproteins. The type III hyperlipoproteinemic patients also have changes in their HDL that suggest how these lipoproteins participate in the process of reverse cholesterol transport and function as antiatherogenic lipoproteins. Patients with familial hypercholesterolemia have an elevation of plasma LDL (and to a lesser extent an increase in IDL), which is secondary to defective apo-B,E(LDL) receptors that impair normal catabolism. Animals fed diets high in fat and cholesterol have an accumulation of β-VLDL remnants, IDL, and LDL resembling the lipoproteins in patients with both genetic defects.

Attention has been focused on macrophages as playing a key role in atherogenesis and as being at least one of the progenitors of foam cells in arterial lesions. Many studies have highlighted the importance of macrophages, which are presumably derived from circulating monocytes that enter the arterial wall. Such cells within the arterial wall express receptors for both β-VLDL (chylomicron and VLDL remnants) and chemically modified LDL. Thus, in the presence of specific lipoproteins, macrophages are converted to cells with the appearance of foam cells. The precise stimulus that causes monocyte-macrophages to enter specific regions of the arterial wall remains to be determined. They may be responding to the trapping of the abnormal lipoproteins in the matrix of the arterial wall or alterations in endothelial cells. Initially they may be part of a protective, reparative process. However, pathologic changes could result when these cells become excessively overloaded with cholesterol. Certainly in advanced atherosclerotic lesions, cell death and necrosis represent part of the complexity of the lesion and lead to the deposition of extracellular cholesterol and the destruction of the normal architecture of the arterial wall.

This discussion provides insights into the mechanisms whereby the consumption of diets high in saturated fat and cholesterol could predispose humans to an increased risk of coronary artery disease. Such diets could stimulate the production of chylomicron and VLDL remnants and could lead to impaired catabolism of the remnant lipoproteins and LDL by suppression of apo-B,E(LDL) receptors. An NIH Consensus Panel, which reviewed all available data, has reported recently that the plasma cholesterol levels of our population are excessively high by comparison to certain other peoples of the world, that these high levels correlate with our increased incidence of coronary artery disease, and that a reduction of plasma cholesterol levels by diet modification (consumption of a prudent, low-fat, low-cholesterol diet) should be a goal for our entire population. Consideration of the available studies — epidemiologic studies, clinical trials, animal preparations, and metabolic ward studies, including data discussed in this review — support these conclusions. A beneficial effect on the incidence of coronary artery disease can be expected by the population-wide adoption of a prudent, low-fat and low-cholesterol diet.

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