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LIPOPROTEIN METABOLISM IN THE MACROPHAGE: Implications for Cholesterol Deposition in Atherosclerosis[1]

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CONTENTS

[1]Abbreviations used: ACAT, acyl-CoA:cholesterol acyltransferase; apo, apoprotein; FH, familial hypercholesterolemia; HDL, high density lipoprotein; HDL$_c$, a cholesterol-induced form of HDL containing apoprotein E in addition to apoprotein A-I; IDL, intermediate density lipoproteins; LDL, low density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; β-VLDL, β-migrating very low density lipoproteins; WHHL rabbit, Watanabe Heritable Hyperlipidemic rabbit.

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PERSPECTIVES AND SUMMARY

Atherosclerotic plaques are filled with scavenger cells that have ingested large amounts of cholesterol and have become so stuffed with cholesteryl ester that they are converted into foam cells (1, 2). Most of these foam cells arise either from resident macrophages of the artery wall or from blood monocytes that enter the wall at sites of endothelial damage. Macrophages ingest and degrade cholesterol-carrying plasma lipoproteins that have leaked through damaged endothelium and penetrated into the tissue of the wall. When macrophages take up more lipoprotein cholesterol than they can excrete, the cholesterol is stored in the cytoplasm in the form of cholesteryl ester droplets. These droplets give the cytoplasm a foamy appearance in the electron microscope, thus accounting for the term foam cell.

The atherosclerotic plaque is a complicated structure. In addition to cholesterol-filled macrophages, the structure contains large numbers of proliferating smooth muscle cells and a large amount of extracellular material that includes sulfated glycosaminoglycans, collagen, fibrin, and cholesterol (3). Some of the smooth muscle cells contain cholesteryl ester droplets that resemble those of macrophage foam cells. In order to unravel such a complicated structure, in recent years scientists have begun to study the specialized properties of each of the cell types that comprise the lesion. For example, endothelial cells and smooth muscle cells were propagated in vitro, and their analyses identified several distinctive properties that might contribute to the initiation of atherosclerosis (reviewed in 3).

The macrophage, too, has come under study. Extensive investigations over the past five years disclosed that macrophages, isolated from the peritoneal cavity of mice and from the blood of man, possess mechanisms that allow them to take up and digest cholesterol-containing lipoproteins, to store the sterol, and to excrete it in large amounts when conditions permit (4–8). These mechanisms differ from those in other cell types, such as cultured fibroblasts and smooth muscle cells. Awareness of these special mechanisms for lipoprotein uptake made possible the conversion of macrophages into foam cells in vitro (4, 8). These studies shed new light on the possible mechanism for foam cell formation in vivo.

The uptake of lipoprotein-bound cholesterol in macrophages occurs through the process of receptor-mediated endocytosis (4–7). The initial event is the binding of the lipoprotein to a cell surface receptor. Although macrophages express few receptors for normal plasma lipoproteins, they exhibit abundant receptors for lipoproteins that have been altered by chemical derivitization (4) or by complexing with other molecules (5, 7). In addition, macrophages have receptors for at least one type of abnormal lipoprotein that accumulates spontaneously in plasma in hyperlipidemic states (6).

HDL present in the medium to produce a lipoprotein called HDL_C. When injected intravenously into animals, HDL_C is taken up rapidly by lipoprotein receptors on the surface of hepatocytes (11, 12). Thus, apo E may be synthesized by cholesterol-loaded macrophages in order to target the secreted cholesterol to the liver, thereby facilitating "reverse cholesterol transport" (14).

In this article, we review studies carried out over the last five years that have led to these new insights into the mechanisms for cholesterol uptake, storage, and excretion by macrophages. While the data were obtained almost exclusively from in vitro systems, they have important implications for macrophage function in the body and suggest how macrophages might go awry during the formation of foam cells in the atherosclerotic plaque.

UPTAKE OF LIPOPROTEIN-BOUND CHOLESTEROL BY MACROPHAGES

Macrophages can take up large amounts of cholesterol by two mechanisms: (*a*) by phagocytosis of whole cells or fragments of membranes containing cholesterol; or (*b*) by receptor-mediated endocytosis of plasma lipoproteins either in solution or complexed in insoluble form with other tissue constituents. The factors governing phagocytosis were discussed elsewhere (15). In this section we review the various systems for receptor-mediated endocytosis of cholesterol-containing lipoproteins.

The initial studies on receptor-mediated endocytosis of lipoproteins by macrophages, reported in 1979 by Goldstein et al (4), were carried out to resolve a paradox that emerged from studies of the LDL receptor. LDL receptors are present on a variety of nonmacrophage cells grown in tissue culture or taken directly from the body. The LDL receptors mediate the uptake and degradation of LDL by body cells and hence are an important determinant of the plasma LDL-cholesterol level (11). Subjects with homozygous familial hypercholesterolemia have a genetically determined total or near total deficiency of LDL receptors. Plasma LDL cannot penetrate into their cells with normal efficiency, and as a result the plasma LDL level rises. Despite their deficiency of LDL receptors, subjects with homozygous familial hypercholesterolemia nevertheless accumulate LDL-derived cholesteryl esters in macrophage foam cells at several sites in the body, notably in the arterial wall, causing atheromas, and in tendons, causing xanthomas (16). This clinical observation suggested that macrophages have some alternative mechanism for taking up LDL-cholesterol distinct from the LDL receptor. However, in vitro tissue macrophages take up native LDL at extremely slow rates and do not accumulate excessive cholesteryl esters, even when exposed to high concentrations of LDL for prolonged

Most of the cholesterol in plasma lipoproteins is in the form of cholesteryl esters. Macrophages process these esters in a series of sequential reactions that take place in two cellular compartments (8, 9). Immediately after they enter the macrophage via receptor-mediated endocytosis, lipoprotein-bound cholesteryl esters are delivered to lysosomes (first cellular compartment) where they are hydrolyzed by an acid lipase. The liberated cholesterol crosses the lysosomal membrane and enters the cytoplasm (second cellular compartment) where it is re-esterified by a microsomal enzyme and stored in the cytoplasm as cholesteryl ester droplets.

The two-compartment pathway allows quantitative assay of the cellular uptake of cholesterol-rich lipoproteins without the need for radiolabeled lipoproteins. When incubated in the usual medium containing normal serum, macrophages do not take up lipoproteins at a high rate, and hence they do not synthesize cholesteryl esters (4, 8). Thus, when [^{14}C]oleate is added to the culture medium, the cells do not incorporate it into cholesteryl [^{14}C]oleate. However, when the cells are presented with a lipoprotein that they can ingest, cholesterol is liberated and then re-esterified, and this leads to a 100- to 200-fold increase in the rate of incorporation of [^{14}C]oleate into cholesteryl [^{14}C]oleate (4, 8). All of the cholesterol-rich lipoproteins that enter macrophages were found to enhance cholesteryl ester synthesis in this fashion and hence stimulation of cholesteryl [^{14}C]oleate synthesis is used as a functional assay to measure lipoprotein uptake (4–8).

The cholesteryl esters stored in the cytoplasm of macrophage foam cells undergo a continual cycle of hydrolysis and re-esterification (9). Hydrolysis is mediated by a nonlysosomal esterase distinct from the lysosomal acid lipase. Re-esterification is mediated by a membrane-bound enzyme that transfers a fatty acid from fatty acyl coenzyme A to cholesterol. When the extracellular fluid contains a substance, such as high density lipoprotein (HDL), that is capable of binding cholesterol, the free cholesterol is not re-esterified or stored, but is excreted from the cell. When no cholesterol acceptor is available, the free cholesterol is re-esterified for storage, and the cycle of hydrolysis and re-esterification continues (9).

If macrophages metabolize lipoprotein cholesterol in the body as they do in tissue culture, then the cholesterol that they excrete may have two metabolic fates: (*a*) some of it may be transported directly to the liver where it is excreted from the body (the so called "reverse cholesterol transport") (10); and (*b*) some of it may be transferred to other lipoproteins, such as low density lipoprotein (LDL), that deliver it both to liver and to extrahepatic tissues for use in the synthesis of new plasma membranes and steroid hormones (11, 12). When macrophages excrete cholesterol, they simultaneously synthesize and secrete large amounts of apoprotein E (13, 14), a component of plasma lipoproteins that binds avidly to lipoprotein receptors. Secreted apo E and secreted cholesterol may associate with the

incubated continuously with acetyl-LDL take up so much cholesterol that they are converted into foam cells in vitro (4, 8; see below).

In contrast to its apparently universal expression in macrophages, the acetyl-LDL receptor is generally absent from nonmacrophage cells, including cultured human fibroblasts, cultured human and bovine smooth muscle cells, freshly isolated human lymphocytes, human lymphoblasts, mouse Y-1 adrenal cells, and Chinese hamster ovary cells (4, 18). The one exception is cultured bovine endothelial cells, which express a small number of acetyl-LDL receptors and degrade ^{125}I-acetyl-LDL at 6% of the rate of resident mouse peritoneal macrophages (30). Endothelial cells are known to share other properties with macrophages, such as the presence of lipoprotein lipase (31, 32) and the ability to present antigens to T lymphocytes in an immunogenic form (30).

In contrast to tissue macrophages, which express acetyl-LDL receptors but virtually no LDL receptors, monocytes freshly isolated from the blood of normal subjects express receptors for both native LDL and acetyl-LDL (4, 18, 22, 23, 33, 34). After 5 days of culture in vitro, the activity of the acetyl-LDL receptor increases by as much as 20-fold and markedly exceeds (by more than 10-fold) the activity of the LDL receptor (33, 34). Cultured malignant macrophages such as J774 cells (25) and IC21 cells (24) express low levels of LDL receptors and high levels of acetyl-LDL receptors. Monocytes cultured from the blood of subjects with the homozygous form of familial hypercholesterolemia display normal acetyl-LDL receptor activity despite their genetic deficiency of receptors for native LDL (18, 34).

Figure 1 demonstrates the all-or-none difference in the ability of cultured human fibroblasts and mouse peritoneal macrophages to take up and degrade ^{125}I-acetyl-LDL and ^{125}I-LDL. This difference between acetyl-LDL receptors and LDL receptors is one of the most striking biologic differences between macrophage and nonmacrophage cells and implies an important role for the acetyl-LDL receptor in macrophage function in vivo.

LIGAND SPECIFICITY OF THE ACETYL-LDL RECEPTOR Acetylation of LDL removes positive charges from the ϵ-amino groups of lysine and thereby converts a weakly anionic lipoprotein into a strongly anionic one (35). The acetyl-LDL loses its ability to bind to the classic LDL receptor of nonmacrophage cells, but it remains precipitable by antibodies to native LDL (35). The enhanced net negative charge of acetyl-LDL is responsible for its binding to the macrophage acetyl-LDL receptor (4). Other chemical modifications that abolish positive lysine residues and increase LDL's net negative charge also convert the lipoprotein into a ligand for the acetyl-LDL receptor. Such ligands include acetoacetylated LDL (20), maleylated LDL (4), succinylated LDL (4), and malondialdehyde-treated LDL (18,

periods of time (4). These paradoxical findings led to a search for altered forms of LDL that could be internalized by macrophages at rapid rates.

Receptor for Acetyl-LDL

The first plasma lipoprotein demonstrated to enter macrophages by receptor-mediated endocytosis was human LDL that had been reacted with acetic anhydride in vitro to form acetyl-LDL (4). These studies were conducted with monolayers of resident mouse peritoneal macrophages isolated by the classic techniques developed by Cohn and co-workers (reviewed in 17). Unlike most other cell types, normal tissue macrophages from the mouse and other species express few if any receptors for native LDL (4–6). When incubated with ^{125}I-labeled LDL in vitro, mouse peritoneal macrophages internalize only minimal amounts of the lipoprotein and do not increase cellular cholesterol content (4, 8).[2] In contrast, LDL that has been modified by chemical acetylation is taken up with extremely high efficiency by macrophages, resulting in massive cholesterol accumulation within the cells (4, 8).

BIOCHEMICAL PROPERTIES OF THE ACETYL-LDL RECEPTOR Studies with ^{125}I-labeled acetyl-LDL showed that the rapid uptake by mouse macrophages is mediated by an initial binding of the lipoprotein to a limited number of high affinity binding sites (20,000–40,000 sites/cell) that recognize acetyl-LDL but not native LDL (4, 18). Binding leads to rapid internalization of acetyl-LDL by endocytosis and delivery to lysosomes. Within 60 min, virtually all of the cell-bound ^{125}I-acetyl-LDL is hydrolyzed and the label is excreted from the cell in the form of ^{125}I-monoiodotyrosine (4). The receptor for acetyl-LDL is just beginning to be characterized biochemically. It is not yet clear whether it is a single molecular entity or is comprised of several different molecular species, each of which is capable of binding acetyl-LDL and mediating its rapid internalization by the cell. All of the surface binding sites for ^{125}I-acetyl-LDL are destroyed when the cells are treated briefly with low concentrations of trypsin or pronase (4), suggesting that all of the receptors are composed of protein. Half-maximal binding of ^{125}I-acetyl-LDL is achieved at an acetyl-LDL concentration of 5 μg pro-

[2]Although small amounts of ^{125}I-LDL are taken up and degraded by mouse peritoneal macrophages, this uptake does not appear to be mediated by the classic LDL receptor in that it is competitively inhibited nonspecifically by lipoproteins, such as acetyl-LDL [see Figure 2 f in (5)] and typical HDL (24), which do not bind to the LDL receptor. The nature of this nonspecific uptake process for ^{125}I-LDL by tissue macrophages is not clear; it may be related to the ability of lipoproteins to bind nonspecifically to a site on cell membranes that recognizes multiple lipoproteins, i.e. LDL, HDL, methyl-LDL, and acetyl-LDL (93, 105).

The identity of the negatively charged residues on acetyl-LDL that mediate binding to the acetyl-LDL receptor is not known. LDL is a complex particle that contains negatively charged lipids as well as amino acids and carbohydrates. To simplify analysis of ligand-receptor interactions, experiments were performed with less complex polyanionic ligands that bind to the acetyl-LDL receptor and thus compete for the binding, uptake, and degradation of ^{125}I-acetyl-LDL. In general, acetylation of other proteins (such as albumin, gamma globulin, α-1-antitrypsin, transferrin, ferritin, ovalbumin, histones, ovomucoid, α-1-acid glycoprotein, and HDL) does not convert them into ligands for the acetyl-LDL receptor (37). However, maleylated albumin binds with high affinity (4). In contrast to acetylation, reaction with the dicarboxylic acid maleate not only removes positive charges on lysine residues but also adds additional negatively charged residues in the form of carboxyl groups. These experiments suggest that most native proteins, such as albumin, do not contain a sufficient number or arrangement of negatively charged residues to bind to the acetyl-LDL receptor, even when all of the positive charges on the available lysine residues have been obliterated. However, the addition of new negative charges in the form of maleate converts the molecule into a binding moiety. The unique aspect of LDL is that it contains sufficient negatively charged residues so that elimination of the positive lysine residues induces binding to the acetyl-LDL receptor without a requirement for additional negative charges. HDL behaves like albumin in that it requires maleylation in order to be recognized by the acetyl-LDL receptor (37).

The acetyl-LDL receptor also recognizes compounds in which the negative charges reside on noncarboxyl moieties (4, 18), such as sulfate (e.g. polyvinyl sulfate, dextran sulfate, and fucoidin) or phosphate (e.g. polyinosinic acid and polyxanthinylic acid). All binding polyanions have a high molecular weight. Low molecular weight polyanions (e.g. ATP and GTP) do not bind, as judged by their inability to compete for the uptake of ^{125}I-acetyl-LDL (37).

Table 1 lists a large number of compounds that were tested for binding to the acetyl-LDL receptor. Testing was performed by measuring the ability of each molecule to compete with ^{125}I-acetyl-LDL for uptake and degradation by the mouse peritoneal macrophage receptor. Multiple negative charges are necessary but not sufficient for receptor binding. Certain contrasts are striking. For example, certain polypurines (such as polyinosinic acid, polyguanylic acid, and polyxanthinylic acid) compete effectively for the binding of ^{125}I-acetyl-LDL, while another polypurine (polyadenylic acid) does not compete. Adenylic acid differs from the first three purines in that it has an amino group in place of a keto group at carbon 6. However, polyguanylic acid, which has an amino group at carbon 2, is recognized by