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MACROPHAGE FOAM CELLS AND ATHEROSCLEROSIS

Howard S. Kruth

Section of Experimental Atherosclerosis, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

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1. ABSTRACT

Focal buildup of cholesterol in arteries is the process that produces atherosclerotic plaques, the cause of most coronary artery disease and strokes. Monocyte-derived macrophages are central cells that accumulate this cholesterol in atherosclerotic lesions, a manifestation of the scavenging function of the macrophage. Different types of cholesterol-containing lipid particles found in atherosclerotic lesions may enter macrophages by a variety of endocytic pathways. The fate of cholesterol that enters macrophages determines whether macrophages help or hinder cholesterol removal from the vessel wall. Macrophages may function to carry cholesterol out of lesions, or to process the cholesterol for excretion in association with small protein-phospholipid complexes. Alternatively, macrophages that do not efficiently function to remove cholesterol from lesions may ultimately undergo cell death. Some cytokines, hormones, and pharmacologic agents show potential to modulate these processes and may be useful in directing macrophage function in atherosclerotic lesions towards beneficial rather than harmful effects.

2. INTRODUCTION

Early pathological studies of atherosclerotic lesions showed the presence of large cells with a foamy appearance. Because preparation of tissue sections for routine histology involves exposure of tissue to organic solvents, cellular lipid droplets were extracted, and this produced the foamy appearance. Although it was recognized that the foamy appearance represented extracted lipid droplets, the origin of the cells that contained the lipid droplets was in dispute for many years. Some investigators considered the cells to be derived from vascular smooth muscle cells, while other investigators believed that the cells were derived from macrophages of both monocyte and tissue origin. Ultrastructural characteristics (e.g., a basement membrane around smooth muscle cells), and later the development of monoclonal antibodies that labeled markers on these two cell types resolved the controversy by showing that both smooth muscle cells and macrophages in atherosclerotic lesions accumulate lipid and transform into foam cells (1-4). While this review will focus on macrophage-derived foam cells, it should be kept in mind that other types of cells in lesions besides macrophages

and smooth muscle cells can accumulate lipids within atherosclerotic lesions. Also, macrophage foam cells develop in other tissue sites besides the vascular wall as a manifestation of other diseases and metabolic disturbances.

Where do foam cells accumulate their lipid? Does this occur in the vessel wall or somewhere else with foam cells then transporting lipid into the vessel wall as proposed by early investigators (5). At the time, the idea was supported by the observation that circulating foam cells were found in the blood of cholesterol-fed animals that developed foam cell-filled atherosclerotic lesions. Also, foam cells were observed breaching the endothelium (reviewed in (6)). However, it was not initially known whether these foam cells were entering or leaving the vessel wall. Later studies indicated that foam cells accumulate their lipid within the vessel wall and some of them migrate across the endothelium and enter the blood (7-9).

3. NATURE OF LIPID IN FOAM CELLS

Two types of lipid oils, cholesteryl ester and triglyceride, can produce cellular lipid droplet accumulation and cause cells to have a foamy appearance in tissue sections prepared with extracting solvents. Lipid droplets in tissues or cells prepared without extracting solvents can be stained with lipid-soluble dyes such as oil red O. While lipid-soluble dyes do not differentiate triglyceride from cholesteryl ester, the presence of putative cholesteryl ester can be confirmed with chemical analysis or filipin staining to label cholesterol after enzymatic hydrolysis of cholesteryl ester (10, 11). Chemical studies of human atherosclerotic lesions show that the major lipid oil accumulating in lesions is cholesteryl ester and not triglyceride (12, 13). Some studies of foam cell formation using model systems have assumed cholesteryl ester accumulation based on observations of foaminess, staining with lipid-soluble dyes, or lipid droplets observed with phase microscopy. Such studies have overlooked the possibility that the accumulated lipid was triglyceride rather than cholesteryl ester (14).

4. SOURCES OF CHOLESTEROL THAT ACCUMULATES IN FOAM CELLS

What is the source of cholesterol that causes foam cell formation in atherosclerotic lesions? The fact that feeding cholesterol to animals elevates plasma cholesterol levels and simultaneously induces atherosclerosis (15) led to the widely accepted conclusion that plasma cholesterol carried in lipoproteins is the source of cholesterol that accumulates in atherosclerotic lesion foam cells. This idea was reinforced by studies showing a correlation of plasma cholesterol levels and low density lipoprotein (LDL), the major carrier of plasma cholesterol, with the development of coronary artery disease (16, 17), caused by cholesterol-buildup in atherosclerotic lesions.

Another point of view is that the source of cholesterol for macrophage foam cell formation is derived predominantly from cells of vessel-associated thrombi rather than from blood lipoproteins. In this hypothesis (reviewed in (18)), macrophages and vascular smooth muscle cells enter thrombi on the surface of arteries. These cells subsequently accumulate cholesterol derived from the platelets and red cells contained within the thrombi. Experimental evidence supports this hypothesis as platelet antigens can be localized within the substance of many coronary artery atherosclerotic lesions. Also, macrophages and vascular smooth muscle cells readily transform into foam cells *in vitro* when exposed to platelets, or cholesterol-rich lipid particles released from platelets (19-22). Atherogenic LDL promote platelet aggregation (23). Thus, LDL may contribute to atherosclerotic plaque development through promotion of thrombosis, as well as by depositing cholesterol in the vessel wall. It is likely that both plasma lipoproteins such as LDL and thrombi contribute to the cholesterol burden of atherosclerotic plaques and are sources of cholesterol ultimately accumulated within macrophage foam cells.

5. SIGNIFICANCE OF FOAM CELL FORMATION

5.1. Trapping lipid in lesions versus removing lipid from lesions

Why has there been such great interest in studying foam cell formation? The observation that foam cells accumulate in atherosclerotic lesions suggested the hypothesis that foam cell formation was the mechanism by which cholesterol accumulates in the vessel wall. The idea is that LDL enters and leaves the vessel wall. However, when macrophages are present in the vessel wall, they take up LDL, trapping its cholesterol within the macrophage and the vessel wall. In this model of vessel wall cholesterol accumulation, cholesterol would not accumulate if macrophages were not present to take up the LDL that enters the vessel wall. The foam cell hypothesis of atherosclerotic plaque development goes on to explain that formation of the advanced atherosclerotic plaque's dangerous lipid core is the result of cholesterol released from dying macrophage foam cells. This lipid core expands and contributes to rupture of the plaque. This can result in thrombosis and acute occlusion of the vessel causing heart attacks and strokes.

The foam cell hypothesis is the most commonly accepted hypothesis among investigators for explaining lipid accumulation in atherosclerosis. However, evidence does not support this hypothesis as an explanation for lipid accumulation in plaques. If the lipid core of atherosclerotic plaques is derived from released macrophage cholesteryl ester-containing lipid droplets, then the core lipid particles should resemble macrophage lipid droplets. However, this is not so. Fatty acid composition is different for cholesteryl esters from plaque regions containing foam cells compared with lipid core regions containing extracelluar spherical lipid particles that stain with lipid-soluble dyes (24-28). Intracellular lipid droplets of foam cells have cholesteryl esters with predominately oleate, while extracellular lipid particles have cholesteryl esters with predominately linoleate similar to the fatty acid profile of LDL cholesteryl esters. Ultrastructural studies of atherosclerotic plaques additionally showed that the extracellular lipid particles

Table 1. Types of macrophages used to study foam cell formation

Macrophage Type	Species	Primary or Cell Line	Comments
Blood monocyte-derived macrophages	human, pigeon, swine	primary	
Liver kupffer cells	human, mouse, rabbit, rat	primary	
Lung alveolar macrophages	human, bovine, rabbit, rat	primary	
Peritoneal macrophages	human, mouse, pigeon, rabbit, rat	primary	
U937	human	cell line	isolated from pleural effusion caused by diffuse histiocytic lymphoma
HL-60	human	cell line	isolated from promyelocytic leukemia
THP-1	human	cell line	isolated from acute monocytic leukemia, monocyte differentiation to macrophage induced by PMA
P388D1	mouse	cell line	isolated from methylcholanthrene-induced neoplasm
J774	mouse	cell line	isolated from tumor that arose in BALB/c mouse, do not synthesize apoE
RAW 264	mouse	cell line	isolated from ascites tumor induced by A-MuLV
IC-21	mouse	cell line	derived by SV40 transformation of normal C57BL/6 mouse peritoneal macrophages

were smaller (30-400 nm) than the lipid droplets (>400 nm in diameter) within foam cells (29). Confirmation of these chemical and particle size differences was made by analyzing cellular lipid droplets and extracellular spherical cholesteryl ester-rich lipid particles isolated from human atherosclerotic plaques (30-35).

Experimental atherosclerosis studies indicate that subendothelial lipid accumulation precedes entry of monocytes into the subendothelial space and foam cell formation (36-39). All of these findings support a different hypothesis about the role of foam cells in plaque development in which the foam cells do not cause LDL cholesterol to be trapped in atherosclerotic plaques. Rather, macrophages take up LDL-derived lipids that have accumulated in atherosclerotic plaques for another reason, possibly due to increased uptake of LDL into the vessel wall or extracellular matrix trapping of LDL that enters the vessel wall.

While the foam cell hypothesis of plaque development has provided a perhaps flawed rationale for most research on foam cells, nevertheless, macrophage accumulation of cholesterol has important implications for atherosclerotic plaque development. Rather than trapping cholesterol, macrophages may be functioning to help remove cholesterol from atherosclerotic plaques. As will be discussed below (Section 10), macrophages show mechanisms for eliminating the cholesterol they accumulate and these mechanisms potentially facilitate cholesterol removal from plaques. In addition, as mentioned above, foam cell macrophages can emigrate from atherosclerotic lesions (8), another potential mechanism for removal of cholesterol from plaques.

5.2. Alteration of macrophage function

Macrophage cholesterol accumulation can affect plaque development in other ways. Cholesterol-induced release of metalloproteinases may promote plaque rupture, and expression of tissue factor may promote thrombosis of ruptured lesions (40-42). Macrophage cholesterol-enrichment also causes enhanced expression of factors involved in cholesterol and lipoprotein metabolism such as LTP-1, apoE, sterol carrier protein-2, and enhancement of Lp(a) uptake (43-49). Further, cholesterol-enriched macrophages show increased production of monohydroxyeicosatetraenoic acid and interleukin 8, and increased expression of chemokine receptor CCR2 and urokinase-type plasminogen activator, factors that could contribute to the complex pathological process by which atherosclerotic plaques develop (50-53).

6.THE USE OF CULTURED MACROPHAGES TO STUDY FOAM CELL FORMATION

It was natural that macrophage foam cell formation would be studied most extensively with cultured macrophages. Most studies of cultured macrophages have relied on readily available macrophage cell lines and macrophages isolated from animals (Table 1). It is likely that differences in the metabolism of lipoproteins and cholesterol exist between these cultured macrophages and the macrophages within human atherosclerotic lesions. Many differences in metabolism of lipoproteins and cholesterol by cultured macrophages of different origins have been documented. For example, the types of receptors that recognize acetylated LDL (AcLDL) and oxidized LDL (OxLDL) and the fate of OxLDL cholesterol within macrophages (i.e., accumulation within lysosomes or lipid droplets) vary with macrophage origin (54-57).

Even similar type macrophages from different animal species show differences in cholesterol and lipoprotein metabolism. Mouse peritoneal macrophages show readily stimulated cholesterol efflux when exposed to high density lipoprotein (HDL), while rabbit and rat peritoneal macrophages (and also J774 and P388D1 mouse cell lines) show poor cholesterol efflux (58-60) (see (61) for other examples). Also, similar types of macrophages from different animal strains show differences in cholesterol metabolism. Peritoneal macrophages from White Carneau pigeons clear cholesteryl esters at a slower rate than do peritoneal macrophages from Show Racer pigeons (62). Peritoneal macrophages from atherosclerosis-prone C57B46J mice show greater beta-very low density lipoprotein (beta-VLDL)-induced cholesterol esterification and less cholesterol efflux compared with peritoneal macrophages from C3H/HeN atherosclerosis-resistant mice (63). Lastly, even presumably similar cell lines can be different. Two different J774 macrophage cell lines showed low and high cholesterol accumulation when incubated with LDL that was secondary to differences in cell line cholesterol esterification (64, 65). This was presumably due to either cell selection or mutation during culture of the two lines in different laboratories.

Other factors besides macrophage origin can influence macrophage and lipoprotein metabolism. An increased age of donor mice is associated with greater uptake of AcLDL by mouse peritoneal macrophages (66). Increased cell density decreases scavenger receptor activity and cholesterol accumulation induced in THP-1 cells by the scavenger receptor ligand AcLDL. In contrast, increased cell density increases scavenger receptor activity in human monocyte-derived macrophages (67) (68). The functional state of macrophages, specifically whether they are activated or not, would be expected to influence macrophage metabolism of lipoproteins and cholesterol. Activation with phorbol myristate acetate decreases AcLDL uptake by mouse peritoneal macrophages, and increases cholesteryl ester synthesis in IC-21 macrophages (69, 70). Activation of mouse peritoneal macrophages with bacterial products decreases synthesis and secretion of apoE (71), and endotoxin activation of human monocyte-macrophages suppresses expression of scavenger receptor activity (72).

Primary human monocyte-derived macrophages may be the best choice of macrophages for *in vitro* studies of foam cell formation. This is because most macrophages in plaques are derived from circulating monocytes that enter the vessel wall where they differentiate into macrophages (7-9). However, even with cultured human monocyte-derived macrophages, many questions remain about how these and other cultured macrophages relate to macrophages in plaques. For example, the macrophages in plaques are surrounded by a cellular matrix rather than as the monolayer of cells usually studied in tissue culture. Also, plaque macrophages are likely influenced by the cytokine environment produced by the many cell types found in lesions, and also by direct interaction with these other cells (73). Lastly, human monocyte-derived macrophages undergo progressive differentiation in culture during which their morphology and function change (74). The state of differentiation of macrophages in atherosclerotic plaques has not been determined.

Many functional changes that occur during macrophage differentiation in culture are known to affect lipoprotein and cholesterol metabolism. LDL receptor expression initially increases and then decreases. However, LDL receptor-related protein, scavenger receptor, apoE, ACAT, and ABC1 expression increase (67, 75-81). Chemical-induced differentiation of macrophage cell lines also changes expression of lipoprotein receptors. Interestingly, different receptor expression patterns can occur with different differentiation agents (82, 83). Undifferentiated HL-60 cells express the LDL receptor and lack the AcLDL scavenger receptor. However, HL-60 macrophages differentiated with tetramyristic phorbol acetate fail to show receptor-mediated degradation of LDL and AcLDL. On the other hand, HL-60 macrophages differentiated with 1,25-dihydroxyvitamin D3 show saturable receptors for LDL and AcLDL.

Heterogeneity of macrophage function should also be considered when studying cultured human monocyte-derived macrophages. For example, the time course of esterification of accumulated cholesterol by these macrophages is heterogeneous (84). Macrophage heterogeneity is presumably due to monocyte-macrophage subpopulations (85) (86, 87). More research is needed to learn about the state of macrophage differentiation in lesions so that studies of lipoprotein and cholesterol metabolism of cultured macrophages can better reflect the metabolism of macrophages in lesions.

7. *IN VITRO* FOAM CELL FORMATION

7.1. Plasma lipoproteins

A paradox, yet unresolved, is the finding that while LDL is believed to be the major source of cholesterol that accumulates in atherosclerotic plaques and in macrophage foam cells, producing greatly cholesterol-enriched macrophages (i.e., foam cells) has not been generally possible by incubating macrophages with LDL *in vitro* (see (65) for one exception). From this came the belief that LDL must be modified in some way that promotes its uptake by macrophages. Indeed, it was shown that imparting negative charge to LDL by acetylation causes the LDL to bind the so called macrophage scavenger receptor. The AcLDL is taken up through this receptor and metabolized within macrophages producing substantial cholesterol accumulation (88). Because this type of chemical modification is unlikely to occur *in vivo*, investigators sought to discover what other types of possibly more physiological modifications to LDL would induce LDL uptake by macrophages (Table 2). In conjunction with these studies, many new receptors (Table 3) have been identified that contribute to the scavenging cell function of the macrophage in clearing biologic and non-biologic debris from tissues.

Table 2. Modifications to LDL that promote macrophage uptake

Modification	Reference
Chemicals	
• Acetic anhydride	88
• Cyanate	295
• Glucose	296
• Glutaraldehyde	297
• Glycated phosphatidylethanolamine	298
• Hexanedione	299
• Hydroxynonenal	206
• Maleic anhydride	88
• Malondialdehyde	297
• Methylamine	300
• Methylglyoxal	301
• Pentanedione	299
• Trimethylpyrylium	299
Enzymes	
• Cholesterol esterase	128
• Cholesterol oxidase	302
• Alpha-chymotrypsin	168
• Neuraminidase	303
• Phospholipase A2	121
• Phospholipase C	119
• Phospholipase D	131
• Sphingomyelinase	118
• Trypsin	168

Oxidation of LDL is one modification that has attracted the attention of researchers and has led to the oxidation hypothesis of foam cell formation. This hypothesis suggests that oxidation of LDL alters LDL such that it is recognized and taken up by macrophage receptors converting the macrophage into a foam cell (89). Does oxidation of LDL explain how macrophages in atherosclerotic lesions accumulate massive amounts of cholesterol to become foam cells? *In vitro* studies do not support this idea because incubation of human monocyte-derived macrophages with even LDL strongly oxidized with artificial chemical systems produces no or little macrophage cholesterol accumulation (90, 91). Oxidation of LDL mediated by cells rather than with artificial chemical systems produces OxLDL with very low capacity for inducing cholesterol accumulation in macrophages (92). Also, OxLDL is metabolized poorly within lysosomes of most macrophage types because of partial inactivation of lysosomal enzymes that degrade LDL (93-98). This limits the capacity of OxLDL to induce cholesteryl ester lipid droplet formation, the hallmark of foam cell formation. Lastly, oxidation of LDL greatly increases its density (99), but LDL isolated from human atherosclerotic lesions shows a density more similar to plasma LDL than to OxLDL (100-102). While oxidation of LDL may not explain cholesterol accumulation within human monocyte-derived macrophages, nevertheless, oxidation of LDL has been shown to have many important biological effects that could influence atherosclerotic lesion development (103).

Another mechanism that promotes macrophage uptake of LDL involves its complexing with other macromolecules that bind macrophage receptors (104). Among the biologically occurring macromolecules are proteoglycans, heparin-fibronectin-collagen complexes, lipoprotein lipase, negatively-charged phospholipids, and antibodies that form immune complexes with LDL and are taken up by macrophage Fc receptors (105-116).

Aggregation of LDL also promotes LDL uptake by macrophages. Macrophages readily take up LDL aggregated by vortexing (117). Lipolysis of LDL's surface phospholipid by exposure to phospholipase A2, phospholipase C, or sphingomyelinase disturb the stable oil in water LDL emulsion and also cause LDL to aggregate (118-120). Aggregation of LDL by all these means leads to macrophage uptake of the aggregated LDL through both the LDL receptor and a non-LDL receptor mechanism that has not yet been characterized (118, 119, 121-124). Even uptake of OxLDL correlates best with its degree of aggregation induced by oxidation (125-127).

Other enzymatic treatments of LDL (e.g., cholesterol oxidase, cholesterol esterase, phospholipase D, and phospholipase A2) that hydrolyze specific lipid components of LDL also promote macrophage uptake of LDL without causing LDL to initially aggregate (128-131). However, LDL modified by various means (glycosylation, desialylation, oxidation, and malondialdehyde treatment) aggregate during incubation in culture medium, and this aggregation was necessary for uptake of these modified LDLs by cultured human aortic intimal smooth muscle cells (132). Therefore, LDL aggregation during culture could be an important factor in macrophage uptake of modified forms of LDL even when these modified LDLs might not be initially aggregated by the modification. Interestingly, lipoprotein (a) from normal subjects and LDL from patients with coronary atherosclerosis and diabetes also aggregate during culture and cause macrophage cholesterol accumulation (133).

While much emphasis has been placed on the role of LDL as an atherogenic lipoprotein, other plasma lipoproteins have also been considered sources of cholesterol accumulation in the vessel wall and macrophage foam cells. As discussed above, normal monomeric LDL does not produce much cholesterol accumulation in macrophages, even during prolonged (>4 days) incubations (134). VLDL induces moderate cholesterol accumulation in macrophages in some but not all studies (134-138). In contrast, beta-VLDL, occurring in the blood of cholesterol-fed animals and individuals with Type III hypercholesterolemia, consistently induces macrophages cholesterol accumulation (139-143). Beta-VLDL are composed of intestinal-derived cholesteryl ester-rich chylomicron remnants and liver-derived cholesteryl ester-rich lipoproteins (141).

Table 3. Major receptors implicated in macrophage cholesterol accumulation

Receptor	Lipoprotein Ligands	Reference
CD36	VLDL, LDL, HDL, AcLDL, OxLDL	(304-306)
CD68/macrosialin	AcLDL, OxLDL	(307)
CLA-1/SR-BI (CD36 and LIMPII analogous-1/scavenger receptor BI)	VLDL, LDL, HDL, AcLDL, OxLDL	(308-310)
Fc gamma RII-B2	OxLDL	(311, 312)
LDL (low density lipoprotein)	LDL, beta-VLDL	(146, 199)
LOX-1 (lectin-like oxidized LDL receptor-1)	OxLDL	(313, 314)
LRP (lipoprotein receptor-related protein)	beta-VLDL	(315)
LSR (lipolysis stimulated receptor)	chylomicron remnants, VLDL	(316)
SR-AI (scavenger receptor-AI)	AcLDL, OxLDL	(317, 318)
TGRLP (triglyceride-rich lipoprotein)	chylomicrons, HTG-VLDL, beta-VLDL	(319)
VLDL (very low density lipoprotein)	chylomicron remnants, VLDL, lipoprotein (a)	(320)

Much controversy has occurred concerning the identity of the binding sites that mediate beta-VLDL uptake by macrophages. Some studies identify the LDL receptor as one and possibly the sole beta-VLDL receptor (144-146). However, evidence for other beta-VLDL receptors including heparan sulphate proteoglycans has been obtained in other studies (142, 147-152). Beta-VLDL contains both apoB and apoE but it is apoE rather than apoB that mediates its binding to the LDL receptor (153, 154). It is possible that beta-VLDL's apoB does not have the necessary conformation to bind the LDL receptor, as has been shown for VLDL (155, 156).

Interestingly, beta-VLDL from apoE knockout mice retains its capacity to induce macrophage cholesteryl ester accumulation although it lacks apoE and is deficient in apoB 100. This suggests that uptake by the non-LDL receptor site does not depend on apoE or apoB 100 but may depend on apoB 48, which is rich in beta-VLDL (147, 157, 158). The triglyceride-rich lipoprotein receptor binds apoB 48, and apoB 48-containing lipoproteins that include chylomicrons, beta-VLDL from cholesterol-fed animals, and VLDL from hypertriglyceridemic humans (a lipoprotein fraction that includes chylomicron remnants) (159, 160). These are the very lipoproteins that can promote cholesterol accumulation in some types of cultured macrophages (161-166). Thus, the triglyceride-rich lipoprotein receptor may be identical with the non-LDL receptor site on macrophages reported in some studies to mediate macrophage uptake of beta-VLDL discussed above.

7.2. Non-lipoprotein forms of cholesterol and lipid particles isolated from lesions

While all the above mechanisms for uptake of lipoproteins are intended to explain macrophage foam cell formation, it should be noted that much (if not most) of the cholesterol in atherosclerotic lesions that macrophages would encounter does not resemble any blood lipoprotein (167). Rather, this lipid is contained in non-lipoprotein particles such as the extracellular spherical cholesteryl ester-rich lipid particles discussed in section 5. Similar cholesteryl ester-rich lipid particles can be generated through proteolytic-induced LDL fusion. These fused LDL particles produce substantial cholesterol accumulation when incubated with mouse peritoneal macrophages but do not produce cholesterol accumulation when incubated with normal human monocyte-derived macrophages (168) (unpublished observation).

Besides extracellular cholesteryl ester-rich droplets, cholesterol-rich phospholipid liposomes and cholesterol crystals occur in the extracellular spaces of lesions possibly derived from metabolism of plasma lipoproteins at the vessel surface or within the vessel wall (11, 169, 170). The liposomes may be derived from several sources including hydrolysis of LDL cholesteryl ester (171), cholesterol excreted by macrophages ((172, 173) and discussed in section 10), and lipolytic surface remnants of plasma lipoproteins (174). Incubation of macrophages with cholesterol-rich liposomes produced from *in vitro* hydrolysis of LDL cholesteryl ester causes substantial apoB-dependent cholesterol accumulation in macrophages (128), as does incubation of macrophages with cholesterol crystals or cholesterol-albumin coacervates produced *in vitro* (175-177).

When necrosis of advanced atherosclerotic lesions occurs, cholesterol-containing cellular membranes and lipid droplets released from vascular cells also became potential sources of cholesterol for macrophages to accumulate (178). J774 macrophage uptake of artificially

produced cholesteryl ester-rich lipid droplets has been achieved by inverting cultured macrophages over medium to allow contact between the floating lipid droplets and the macrophages (179). Some negatively charged phospholipid was incorporated into the cholesteryl ester-rich lipid droplets that most likely mediated lipid droplet binding and uptake through macrophage scavenger receptors (113). It is not known whether cholesteryl ester lipid droplets isolated from lesions would be taken up by macrophages.

ApoB-containing lipoproteins isolated from atherosclerotic lesions and purified by density gradient centrifugation and gel-filtration chromatography are taken up by cultured macrophages producing some cholesterol accumulation (101, 102, 180-186). In many of these studies the presence of non-plasma lipoprotein cholesterol-containing lipid particles (e.g., the cholesterol crystals, cholesterol-rich phospholipid liposomes, and small cholesteryl ester-rich lipid droplets) in the "purified" lipoprotein fractions was not assessed. It is possible that reported macrophage uptake of lipoproteins isolated from lesions reflects uptake of complex aggregates of these lipoproteins with non-lipoprotein particles and vessel wall proteoglycans, fibronectin, or immunoglobulins that are known to contaminate these lipoprotein fractions (102, 167, 180, 183, 186, 187). As discussed above, aggregation of LDL and complexing of LDL with these other constituents stimulates macrophage uptake of LDL.

8. ENDOCYTIC PATHWAYS BY WHICH FOAM CELLS MAY ACCUMULATE CHOLESTEROL

What are the endocytic pathways by which macrophages accumulate their cholesterol to become foam cells? Lipoprotein binding to the plasma membrane has been studied with different ligands (e.g., AcLDL, beta-VLDL, LDL, OxLDL) in different macrophage model systems. Depending on macrophage and ligand type, lipoproteins can bind the plasma membrane diffusely at 4°C and then cluster when cells are warmed to 37°C. Alternatively, lipoproteins can bind in a pre-clustered manner at 4-8°C(188-192). Generally, macrophage lipoprotein binding occurs in coated (clathrin-associated) pits on microvillous extensions, and on uncoated regions of plasma membrane ruffles (188, 193-197). Whether a lipoprotein type binds predominantly to coated or uncoated plasma membrane regions seems to depend on the specific lipoprotein-macrophage model system under examination, but binding by a given lipoprotein type to both plasma membrane sites in the same macrophage can occur (198). Both uncoated and coated pits function in the uptake and delivery of bound lipoproteins to lysosomes (190, 191, 193, 194, 199, 200, 201).

Other endocytic pathways have been described that may be unique to macrophages. Large beta-VLDL enter peripheral surface-connected tubules, so-called STEMs, before the beta-VLDL undergoes lysosomal degradation (202-204). Importantly, there are different fates for beta-VLDL cholesterol that enters macrophages through STEMs and LDL cholesterol that enters macrophages through a vesicular pathway having characteristics of coated pits. Beta-VLDL cholesterol delivered through STEMS leads to more efficient cholesterol esterification compared with LDL cholesterol delivered through coated pits.

In contrast to most other cells, macrophages also have the capacity to take up materials by phagocytosis. Macrophages can take up some types of aggregated LDL by phagocytosis that then leads to rapid lysosomal degradation of the accumulated aggregated LDL(205, 206). Chylomicron remnants and aggregated LDL have been reported to be taken up by phagocytosis because uptake was inhibited by cytochalasin D, and because electron microscopy suggested that lipoproteins accumulated in what appeared to be phagocytic vacuoles (108, 117, 119, 123, 164) (107, 207). However, as discussed below, this may not have been the case in all of these studies.

Patocytosis is a recently described pathway for human monocyte-macrophage uptake of aggregated lipoproteins, microcrystalline cholesterol, cholesterol-phospholipid liposomes, and other hydrophobic materials (122, 128, 175, 208). In this pathway, aggregated LDL induces surface invaginations that connect with a labyrinth of interconnected vacuolar compartments within the macrophage cytoplasm. What distinguishes patocytosis from phagocytosis is that in phagocytosis materials enter vacuoles formed from pinching off of macrophage plasma membrane. During patocytosis, this does not occur. Rather, the aggregated LDL accumulates within the cytoplasmic labyrinth that remains connected to the macrophage surface. While some accumulated aggregated LDL subsequently undergoes lysosomal degradation, most aggregated LDL remains in the surface-connected compartments of the labyrinth. The poor degradation of aggregated LDL taken up by patocytosis, differs from the rapid degradation of aggregated LDL taken up by phagocytosis (117, 206). Following patocytosis of aggregated LDL and exposure of macrophages to plasminogen, macrophage generated plasmin partially degrades LDL protein reversing LDL aggregation and allowing the dissaggregated LDL to efflux from the open surface-connected compartments back into the extracellular space (208a). Actin microfilaments function in lipoprotein uptake during patocytosis and phagocytosis but not during uptake of beta-VLDL into STEMs (122, 203). As a result, lipoprotein uptake mediated by patocytosis and phagocytosis is inhibited by cytochalasin D, an agent that interferes with actin polymerization.

Pinocytosis (uptake of fluid in small vesicles) and macropinocytosis (uptake of fluid in large vacuoles) also potentially function in uptake of lipoproteins by macrophages. Fibroblast pinocytotic uptake of LDL in bulk fluid is linearly related to LDL concentration, and the LDL taken up undergoes lysosomal degradation (209). Nevertheless, LDL cholesterol taken up through this endocytic pathway is not retained by the fibroblast but is excreted as free cholesterol. A similar pathway appears to function in macrophages but has not been extensively studied (139, 190). Uptake of lipoproteins bound to

plasma membrane areas that form macropinosomes is another mechanism by which lipoproteins may enter macrophages (193, 194, 210, 211).

Selective uptake of HDL and LDL cholesteryl ester independent of whole lipoprotein uptake has been shown for many cell types including macrophages. However, it has not been shown whether selective uptake of lipoprotein cholesteryl ester induces macrophage cholesterol accumulation (212, 213). Mouse peritoneal macrophage uptake of aggregated LDL into surface invaginations (possibly related to STEMs or surface-connected compartments) shows greater degradation of LDL cholesteryl ester compared with degradation of LDL apoB (124). This finding is characteristic of selective uptake of cholesteryl esters from lipoproteins, and suggests the possibility that macrophages selectively take up cholesteryl ester from aggregated LDL. In contrast, WHHL rabbit alveolar macrophage uptake of beta-VLDL by an unknown pathway shows selective apolipoprotein degradation without cholesteryl ester hydrolysis (214).

9. CHOLESTEROL TRAFFICKING WITHIN MACROPHAGES

As reviewed above, multiple pathways exist for lipoprotein and non-lipoprotein sources of cholesterol to enter macrophages. For cholesterol that reaches lysosomes, that portion esterified undergoes hydrolysis (177, 215). Perhaps depending on the pathway by which cholesterol reaches lysosomes, cholesterol is retained or excreted by the macrophage. Cholesterol that is retained moves to the plasma membrane (139) (216). Excess plasma membrane cholesterol then enters the cytoplasm where the cholesterol is esterified by acyl-CoA:cholesterol acytransferase (ACAT) located predominantly within the endoplasmic reticulum (217, 218). The esterified cholesterol is stored in cellular lipid droplets. Because the ACAT enzyme prefers oleate to linoleate during cellular esterification of cholesterol, and availability of oleate in the macrophage modulates how much cholesteryl ester is synthesized, the fatty acid profile of macrophage-derived cholesteryl esters is predominantly oleate (215, 219). This explains why lipid droplet cholesteryl ester in lesion foam cells shows a fatty acid profile different from LDL cholesteryl ester. Although macrophage-derived oleate is used to re-esterify cholesterol, linoleate released from hydrolysis of LDL cholesteryl ester is mainly incorporated into macrophage phospholipids (220).

Once macrophages esterify cholesterol, this cholesterol undergoes a continuous cycle of hydrolysis and re-esterification (221). Cholesterol released from hydrolysis of cholesteryl ester can traffic back to the plasma membrane where it can undergo efflux (discussed below) (222, 223). How cholesterol traffics through cells including macrophages is under intense investigation to learn whether this occurs in association with carrier proteins and whether cholesterol is transported by membrane vesicles or smaller macromolecular complexes.

One fate of cholesterol in macrophages that may preclude its availability for eventual cholesterol efflux is where cholesterol and other molecules become deposited in ceroid inclusions within macrophages. These inclusions contain insoluble oxidized and polymerized proteins and lipid presumably including cholesteryl ester. Ceroid inclusions can be produced *in vitro* when macrophages take up OxLDL or lipid particles containing lipids that are especially susceptible to oxidation (224-226).

10. CHOLESTEROL EFFLUX FROM MACROPHAGES

10.1. Plasma-derived HDL

Macrophages can excrete cholesterol they accumulate through many processes. What most of these processes have in common is the use of phospholipid-rich lipid particles that solubilize excess macrophage cholesterol and remove the cholesterol from the macrophage. HDLs are specialized lipoprotein cholesterol acceptors that circulate in the blood and can be found in atherosclerotic lesions. When incubated with macrophages they induce cholesterol efflux (215, 227) by stimulating translocation of cholesterol from intracellular membranes to the plasma membrane (223). Then, the HDL acquires excess plasma membrane cholesterol (228). This also occurs with lipid-free amphipathic apolipoproteins of HDL such as apoAI that associate with macrophage phospholipid and form nascent high density lipoprotein particles (229).

Alternatively, some studies show that HDL enters macrophages and acquires cholesterol through interaction with lipid droplets. Then, this cholesterol-enriched HDL is re-secreted by the macrophage (197, 230-232). A slower rate of hydrolysis of lipid droplet cholesteryl ester correlates with a slower rate of macrophage cholesterol efflux. This is consistent with cholesteryl ester hydrolysis functioning as a rate limiting step for efflux when cholesterol acceptors themselves are not rate limiting (62, 227, 233-236).

10.2. Macrophage excretion of cholesterol in discoidal particles

Macrophages also can produce their own HDL particles that mediate macrophage cholesterol efflux through an autocrine/paracrine mechanism. This occurs when macrophages secrete a particular amphipathic apolipoprotein, apoE, that associates with macrophage phospholipid (probably similar to the way that exogenous amphipathic proteins do) to form apoE-phospholipid discoidal complexes (49, 237). These apoE-phospholipid particles acquire cholesterol from the macrophage sufficiently to cause a decrease in cholesterol content of human monocyte-derived macrophages and 8-bromo-cyclic AMP-treated RAW264 mouse macrophages (173, 238, 239), but not untreated mouse peritoneal macrophages (240). Macrophage-specific expression of human apoE reduces atherosclerosis in hypercholesterolemic apoE-null mice (241), supporting a possible function of macrophage-produced apoE within lesions in promoting cholesterol efflux.

10.3. Macrophage excretion of cholesterol in liposomes

Macrophages also show an HDL-independent mechanism for removing excess cholesterol that occurs

through excretion of cholesterol-phospholipid liposomes (172, 242). Similar cholesterol-phospholipid liposomes surround macrophages in atherosclerotic lesions suggesting that this process occurs *in vivo* (11, 31). The extracellular liposomes range in size from 40-200 nm and should be too large to filter out of lesions. Possibly macrophage excretion of cholesterol-phospholipid liposomes is a process by which cholesterol can be temporarily stored in extracellular deposits before removal from tissue.

10.4.Macrophage excretion of 27-oxygenated cholesterol metabolites

Finally, a recently described mechanism for macrophage cholesterol excretion utilizes the enzyme sterol 27-hydroxylase to convert cholesterol to the more polar and soluble metabolites 27-hydroxycholesterol and 3-beta-hydroxy-cholesterolenoic acid (243). In the absence of cholesterol acceptors such as HDL, these compounds are produced and excreted from macrophages. Because this process is suppressed in the presence of HDL (244), it remains to be determined to what extent this pathway operates in cholesterol-enriched human monocyte-derived macrophages that produce their own HDL.

11. MODULATION OF FOAM CELL CHOLESTEROL METABOLISM

A discussion of all the pharmacologic agents used to modulate foam cell formation is beyond the scope of this review. However, the major agents and their effects will be discussed. These agents can function to limit cholesterol uptake, to alter cholesterol trafficking within the macrophage, and to enhance macrophage cholesterol efflux. It should be kept in mind that drug effects reported for one macrophage type and species may not apply to other macrophage types and species.

Some agents block the endocytic pathways through which the cholesterol-containing particles enter the macrophage. This would be the case for inhibition of actin-dependent and tubulin-dependent endocytic pathways by cytochalasin and nocodazole or colchicine, respectively. Still, other agents function to block cholesterol trafficking. Agents that neutralize the lysosomal proton gradient (ammonium chloride, chloroquine, and bafilomycin A1) block egress of cholesterol from the lysosome (215, 245). Because lysosomal acid cholesterol esterase ceases to function with disruption of low lysosome pH by these agents, lipoprotein-derived unhydrolyzed cholesteryl ester accumulates in lysosomes when macrophages are exposed to these agents. Ketoconazole causes unesterified cholesterol accumulation in macrophages possibly by blocking egress of cholesterol from lysosomes through an undefined mechanism (246, 247-249).

Agents such as S58-035 cause accumulation of unesterified cholesterol in macrophages by inhibiting ACAT. If the ACAT inhibitor is added prior to when cholesterol undergoes esterification, cholesterol is neither esterified nor stored in lipid droplets (221, 250). These agents cause accumulation of unesterified cholesterol even when added after cholesterol has been initially esterified by ACAT and stored in lipid droplets. This is because cholesteryl ester synthesized in cells continuously undergoes hydrolysis and re-esterification (221). Inhibition of ACAT facilitates cholesterol efflux in the presence of cholesterol acceptors, apparently by causing excess macrophage cholesterol to enter the plasma membrane where it is then available for removal by cholesterol acceptors (62, 222, 238, 250, 251).

Likewise, in some macrophage types, cholesterol efflux is stimulated by treatment of macrophages with cAMP that enhances the hydrolysis of stored cholesteryl esters and also stimulates transfer of this cholesterol to the plasma membrane (62, 222, 234, 239, 252). cAMP does not show stimulation of cholesteryl ester hydrolysis in all macrophage types including human macrophages. This may reflect the presence of a cAMP-independent lipase that hydrolyzes cholesteryl ester in these macrophages (253, 254).

Octimate, progesterone, and related steroids also interfere with cholesterol esterification (242, 255, 256). In contrast to agents that are strong inhibitors of ACAT enzymes (257), these agents may cause accumulation of unesterified cholesterol by interfering with cholesterol trafficking following hydrolysis of lipid droplet cholesteryl ester. With progesterone, it has been shown that this agent prevents movement to the plasma membrane of unesterified cholesterol derived from cholesteryl ester hydrolysis (222). The unesterified cholesterol becomes sequestered in phospholipid-rich lamellar membrane structures (256). This does not occur when macrophages accumulate unesterified cholesterol secondary to inhibition of ACAT enzyme with S58-035 (unpublished observation). Unesterified cholesterol sequestered in the lamellar structures can be mobilized and excreted when macrophages are incubated with HDL that enters macrophages and interacts with the lamellar structures (172, 222).

The calcium channel blockers, nifedipine and verapamil, inhibit cholesterol esterification in mouse and rabbit macrophages (242, 258-262). However, we have not observed inhibition of cholesterol esterification by nifedipine in human monocyte-derived macrophages (unpublished observation). The site of action of these agents has not been clearly established, but their action may be unrelated to their blocking of calcium entry (259).

The effects of biologic agents such as cytokines and other hormones besides progesterone on macrophage lipoprotein and cholesterol metabolism have been examined in many model macrophage systems and are listed in Table 4. Interestingly, bacterial products such as lipopolysaccharide increase LDL uptake and macrophage cholesterol accumulation (263-265). However, in these studies it is not clear whether LDL uptake occurs secondary to macrophage recognition and uptake of bacterial products that have formed complexes with LDL, or whether the bacterial product stimulates direct LDL uptake (266). For all effects, one should consider whether the cytokine or hormone is merely inducing differentiation of the macrophage that then shows a changed metabolism

because of a more differentiated state, or whether the agent's effect is more specifically affecting lipoprotein and cholesterol metabolism.

12.COMPARISON OF FOAM CELLS ISOLATED FROM ATHEROSCLEROTIC LESIONS WITH FOAM CELLS PRODUCED *IN VITRO*

While most studies of foam cell formation are carried out with cultured cells, formation of foam cells in atherosclerotic lesions is what is being modeled. What is known about lesion foam cells and how do foam cells produced *in vitro* compare to lesion foam cells? Study of lesion foam cells has been limited because of the difficulty of isolating them in sufficient quantities to carry out analysis and *in vitro* experiments with these cells.

Early studies of foam cells isolated from rabbit and monkey atherosclerotic lesions induced by cholesterol feeding showed two major foam cell types that probably represent smooth muscle cells and macrophages (267-270). Besides having cholesterol-containing lipid droplets, these cells showed cholesterol-enrichment of lysosomes (271, 272). During atherosclerotic lesion development lipid deposition initially occurs in lipid droplets (273, 274). Subsequently, more lipid is stored in lysosomes, suggesting that lysosomal storage of lipid results from prolonged and excessive storage of cholesterol in macrophages. *In vitro* studies show that lysosomal storage of lipid occurs when macrophages take up OxLDL or large amounts of synthetic cholesteryl ester lipid droplets (57, 179). With the latter, lysosomes accumulate unesterified cholesterol derived from lysosomal hydrolysis of lipid droplet cholesteryl ester. Excess accumulation of unesterified cholesterol in lysosomes can result in formation of intra-lysosomal cholesterol crystals (275, 276). Similar cholesteryl crystals are present in lysosomes of some foam cells in atherosclerotic lesions (271, 277).

Foam cells isolated from atherosclerotic lesions show much more cholesterol accumulation than that usually achieved with *in vitro* models of foam cell formation. Lesion foam cells have been reported to accumulate over 1000 nmoles/mg protein of cholesterol, greater than 50% of which is esterified (267, 269, 278-281). Many studies of foam cell formation *in vitro* do not report cholesterol mass accumulation but instead report indices of lipoprotein processing based on isotopically labeled cholesterol or protein. Examination of these data often reveals very low levels of potential cholesterol accumulation, far less than that which would characterize a foam cell. On the other hand, a pathway that delivers cholesterol very slowly to a macrophage could still prove significant. If such a pathway were not down regulated, and functioned over a long period, it could produce the massive cholesterol accumulation characteristic of lesion foam cells.

Macrophage foam cells isolated from rabbit and human atherosclerotic lesions take up beta-VLDL from cholesterol-fed rabbits and AcLDL (279, 282-284). Interestingly, for cells isolated from human lesions, LDL partially competed for beta-VLDL uptake by lesion smooth muscle cells but did not compete for beta-VLDL uptake by macrophage foam cells (283, 284). This suggested the presence of a non-LDL receptor mediating uptake of beta-VLDL by foam cell macrophages similar to the non-LDL receptor mediating uptake of beta-VLDL by non-foam cell macrophages discussed in Section 7.1. LDL uptake occurred with rabbit macrophage foam cells but was not seen with human macrophage foam cells. Rabbit macrophage foam cells also show binding and uptake of OxLDL (279, 285).

Although mouse peritoneal macrophages readily efflux their stored cholesterol to cholesterol acceptors present in plasma (227), foam cells isolated from rabbit atherosclerotic lesions do not show very great cholesterol efflux when cultured in the presence of cholesterol acceptors (236, 280, 281, 284). This may reflect differences in efflux potential of macrophages from different species or different sites, the vessel wall versus the peritoneal cavity.

Other characteristics of macrophage foam cells from rabbit atherosclerotic lesions are that they show high levels of acid lipase, nonspecific esterase, Fc receptors, C3 receptors, and secretion of apoE and metalloproteinases, but show low expression of lipoprotein lipase and loss of lysozyme activity (270) (42, 279, 280, 282, 284, 286, 287).

13. FOAM CELL DEATH

One fate of foam cells that could interfere with removal of cholesterol from atherosclerotic lesions is foam cell death. Death of foam cells would end macrophage function in reverse cholesterol transport and cause release of not only its lipid stores into the extracellular space, but also potentially damaging digestive enzymes.

Does massive cholesterol accumulation lead to foam cell death? Some macrophages accumulating cholesterol in the presence of an ACAT inhibitor show buildup of unesterified cholesterol in cellular membranes associated with macrophage death (288-290). However, macrophage accumulation of excessive unesterified cholesterol does not always cause macrophage death. The macrophage can respond to excess unesterified cholesterol by increasing phospholipid content that functions as a natural buffer for cholesterol (6, 288, 291-293). Whether toxicity occurs may also depend on where in the macrophage cholesterol accumulates. For example, when cholesterol entering lysosomes of mouse macrophages is prevented from trafficking out of lysosomes, toxicity of the cholesterol is reduced (276, 289, 290). Human monocyte-derived macrophages show no evidence of death even after accumulating large amounts of cholesterol crystals and without increasing their phospholipid content (175). This may be because these macrophages sequester the cholesterol crystals in surface-connected compartments if the crystals are small and in lysosomes if the crystals are large. Because oxysterols but not cholesterol are reported to be toxic to human monocyte-derived macrophages (294), macrophage conversion of cholesterol to oxysterols could be another factor determining the toxicity of excess cholesterol.

Table 4. Effect of hormones and cytokines on macrophage cholesterol and lipoprotein metabolism

Agent	Macrophage Type	Lipoprotein	Effect	Reference
activin-A	THP-1	AcLDL	9 cholesteryl ester accumulation 9 lipoprotein cell association and degradation	(321)
cortisol	human monocyte-macrophage	cholesterol-phospholipid liposomes	8 cholesteryl ester synthesis	(322)
dehydro-epiandrosterone	J774-1	AcLDL	9 cholesteryl ester accumulation 8 unesterified cholesterol accumulation	(323)
dexamethasone	mouse peritoneal macrophages J774	AcLDL	8 cholesteryl ester synthesis	(324)
dexamethasone	human monocyte-macrophage	cholesterol crystals, cholesterol-phospholipid liposomes	8 cholesteryl ester synthesis 8 ACAT activity 9 neutral cholesterol esterase activity	(325)
dexamethasone	human monocyte-macrophage	LDL/AcLDL	9 LDL receptor activity 8 AcLDL receptor activity	(326)
1,25-dihydroxyvitamin D3	human monocyte-macrophage	AcLDL	8 cholesteryl ester accumulation	(327)
17 beta-estradiol	THP-1	AcLDL	9 lipoprotein uptake	(328)
GM-CSF	human monocyte-macrophages	AcLDL	8 cholesteryl ester synthesis	(329)
interferon-gamma	J774	HTG-VLDL LDL	8 UC and EC accumulation 8 LDL binding and degradation	(330)
interferon-gamma	human monocyte-macrophages	AcLDL	9 lipoprotein binding and uptake due to decreased scavenger receptor expression	(78)
interferon-gamma	human monocyte-macrophages, mouse peritoneal macrophages	Lp(a)	9 Lp(a) receptor activity	(331)
interferon-gamma	mouse peritoneal macrophages	AcLDL	9 lipoprotein transport to lysosomes	(332)
interferron-gamma	mouse peritoneal macrophages	HDL_3	9 cholesterol efflux	(333)
interleukin-1	human monocyte-macrophages	AcLDL	8 cholesteryl ester synthesis	(329)
interleukin-1	J774	AcLDL	8 cholesteryl ester synthesis	(334)
M-CSF	mouse peritoneal macrophages	AcLDL	8 scavenger receptor synthesis	(335)
M-CSF	human monocyte-macrophage	AcLDL	8 lipoprotein binding 8 lipoprotein uptake and degradation 8 cholesteryl ester synthesis	(329)
M-CSF	human monocyte-macrophage	AcLDL	8 acid and neutral cholesteryl ester hydrolases	(336)
prednisolone	human monocyte-macrophage	AcLDL, OxLDL, cholesterol-phospholipid liposomes	8 cholesteryl ester synthesis	(322)

Table 4. contrinued

progesterone	human monocyte-macrophage	cholesterol-phospholipid liposomes	9 cholesteryl ester synthesis	(322)
progesterone	human monocyte-macrophages	AcLDL	9 cholesteryl ester accumulation in macrophages from female donors only	(337)
progesterone	mouse peritoneal macrophages	AcLDL	9 cholesteryl ester synthesis	(248)
prostaglandin E2	rat peritoneal macrophage	Beta-VLDL	9 cholesteryl ester synthesis	(338)

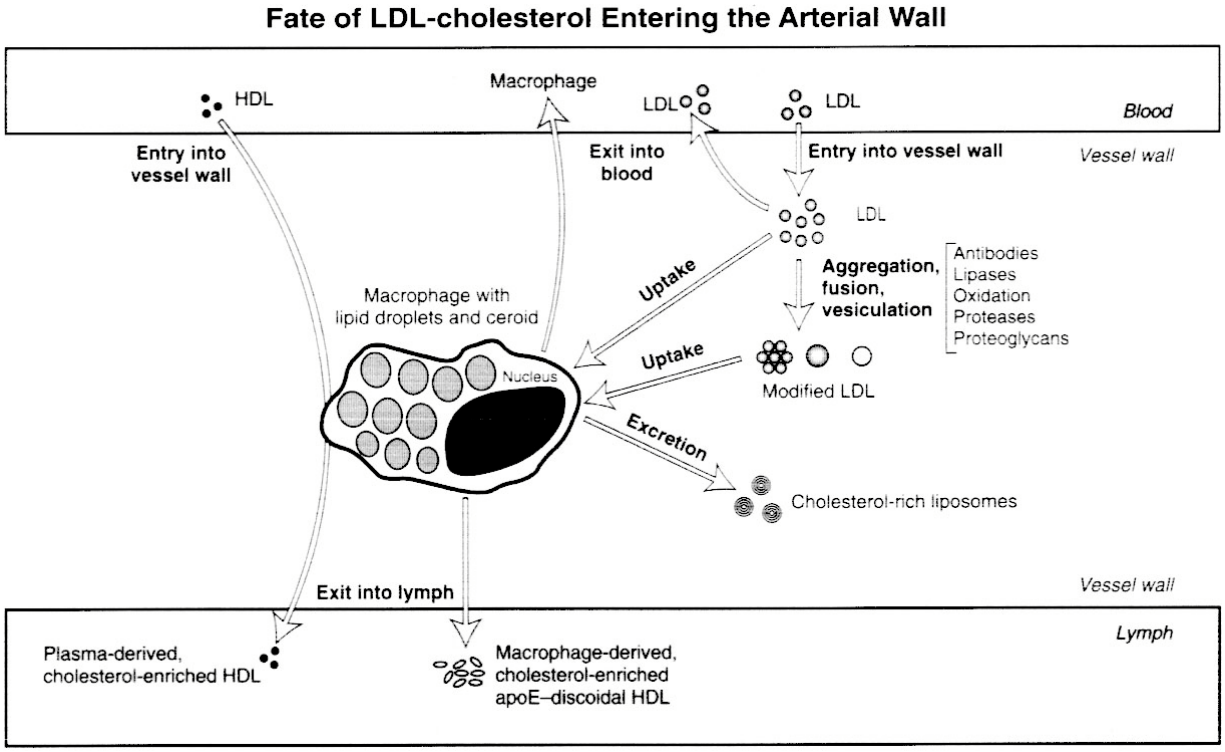

Figure 1. Macrophage foam cells and cholesterol trafficking in atherosclerotic lesions.

14. PERSPECTIVE

It is time to reassess macrophage function with respect to cholesterol deposition and removal from the vessel wall (Figure 1). Even with compelling evidence to the contrary, many investigators believe that macrophages cause cholesterol to accumulate in the vessel wall rather than that macrophages function to scavenge lipid that accumulates in the vessel wall for another reason. Some macrophage functions may help remove cholesterol from the vessel wall, while other macrophage functions related to inflammation may contribute to plaque rupture and thrombosis. Continued research of the many ways that macrophages function in atherosclerotic lesions should allow for development of strategies that seek to enhance the beneficial functions and to minimize the harmful functions.

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Abbreviations: Apolipoprotein B, apoB; Low Density Lipoprotein, LDL; Acetylated LDL, AcLDL; Beta-Very Low Density Lipoprotein, Beta-VLDL; oxidized LDL, OxLDL; High Density Lipoprotein, HDL; Very Low Density Lipoprotein, VLDL; Hypertriglyceridemic, HTG; acyl-CoA:Cholesterol Acytransferase, ACAT; Triglyceride-Rich Lipoprotein, TGRLP, Review

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Send correspondence to: Howard S. Kruth, M.D., Section of Experimental Atherosclerosis, National Heart, Lung, and Blood Institute, National Institutes of Health, Building 10, Room 5N-113, 10 Center Drive MSC 1422, Bethesda, MD 20892-1422, Tel: 301-496-4826, Fax: 301-402-4359, E-mail: kruthh@nhlbi.nih.gov