Caveolins and macrophage lipid metabolism

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Abstract The identification of caveolin-1 more than a decade ago initiated active research into its role in the formation of caveolae, membrane trafficking, signal transduction pathways, and lipid homeostasis. Although caveolins are ubiquitously expressed, the majority of the available information comes from differentiated cells rich in caveolins, such as fibroblasts, adipocytes, and endothelial cells. During the development of atherosclerosis, macrophages play a pivotal role in the formation of the fatty streak lesions. They take up large amounts of lipids and accumulate in the subendothelial space, forming foam cells that fill up the lesion area. Since caveolins have been implicated in the regulation of cellular cholesterol metabolism in several cell types, it is of interest to examine their potential function in macrophages. In this review, we attempt to summarize current knowledge and views on the role of caveolins in cholesterol metabolism with emphasis on macrophages.—Gargalovic, P. and L. Dory. **Caveolins and macrophage lipid metabolism.** *J. Lipid Res.* **2003.** 44: **11–21.**

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Macrophages play a pivotal role in the development of atherosclerosis. During the initial stages of the disease, monocytes migrate into the subendothelial space of blood vessels and differentiate into macrophages. The uptake of excessive amounts of lipoprotein-derived lipids leads to their conversion into foam cells (1). The availability of oxidized lipids in the subendothelial space and the macrophage-specific expression of scavenger receptors, which lack feedback regulation (2, 3), mediate the high degree of lipid enrichment. Under physiological conditions, the net accumulation of lipids in the subendothelial space of blood vessels is a result of a complex set of events, including the overall local redox balance and the rate of cholesterol accumulation/uptake and its removal via the reverse cholesterol transport pathway (4, 5).

Caveolins and caveolin-1 specifically, have been implicated in the regulation of cellular cholesterol metabolism and lipid uptake, as well as efflux (6). The majority of the available information about caveolins comes primarily from studies on fibroblasts and endothelial cells, which are relatively rich in caveolae and express high amounts of caveolin-1 and caveolin-2. The evidence for caveolin expression in macrophages has been conflicting, at least in part due to the use of different macrophage-like cell lines in the various inquiries. Our laboratory has recently investigated caveolin expression in mouse primary macrophages as well as J774 cells in detail.

The potential role of caveolins in the intracellular transport of cholesterol and its physical association with the cholesterol-rich rafts makes it tempting to speculate that these proteins play an important role in cholesterol metabolism of macrophages. Macrophage cholesterol metabolism may be, in turn, a central determinant in maintaining cholesterol homeostasis in the arterial wall.

CAVEOLINS AND CAVEOLAE

Lipid rafts are tightly-packed, liquid-ordered plasma membrane microdomains enriched in cholesterol, sphingomyelin, and glycolipids. They are characterized by their insolubility in non-ionic detergents at 4°C and their light density on sucrose gradients. Their unique lipid composition may act to compartmentalize specific membrane proteins, including caveolins. Caveolae represent a subset of lipid rafts, characterized by high caveolin content and formation of 50–100 nm flask-shaped membrane invaginations (7).

Caveolin-1, a member of the caveolin family, was first identified as a tyrosine-phosphorylated protein in Rous sarcoma virus-transformed chick embryo fibroblasts (8, 9). To-date, three mammalian caveolin genes have been identified (10). Of the three isoforms, caveolin-1 and -2 are expressed ubiq-

uitously, while caveolin-3 expression is restricted to striated muscle cells (11, 12).

The primary sequence of caveolin-1 has several interesting features. Caveolin-1 is inserted into the membrane via a 33 amino acid hydrophobic region with N- and C-termini facing the cytoplasm, thus forming a hairpin-like structure (13–15). The hydrophobic hairpin structure penetrates but does not traverse the lipid bilayer (15, 16) and is flanked by membrane attachment domains, which further anchor caveolin to the membrane (17, 18). These domains also mediate caveolin oligomerization and are thought to be responsible for its lack of solubility in non-ionic detergents (17, 19, 20). Caveolin-1 and -3 are acylated on cysteine residues flanking the transmembrane domain toward the C-terminus, which appears to be important in the binding of cholesterol and its transport to caveolae (21). The 20 amino acid scaffolding domain flanks the transmembrane domain towards the N-terminus. This domain mediates the interaction of caveolin-1 with several caveolae-associated proteins. Based on the above-mentioned characteristics of caveolin-1, it is not surprising that many studies demonstrate its involvement in a variety of cell functions, possibly acting as a coordinating bridge between signaling molecules and membrane domains of specific lipid composition.

Localization of caveolins in cells is not restricted to cell surface caveolae; the available evidence suggests that caveolin-1 can behave as a trafficking molecule, cycling between the Golgi apparatus and plasma membrane or enter the secretory pathway (22–25).

Caveolin-1 has been suggested to have three main functions. It is essential for the assembly of caveolae and directly influences the composition and function of these organelles (26–28). Several studies demonstrate that homooligomers of caveolin-1 or heterooligomers of caveolin-1 and caveolin-2 (11, 29) decorate the cytoplasmic surface of the caveolar coat. The recently generated caveolin-1 knockout mice, characterized by a complete loss of caveolae, confirm the importance of caveolin-1 for the formation of morphologically distinct caveolae (30–32). Caveolin-1 is also involved in the maintenance of cellular cholesterol homeostasis and lipid transport, (23–25, 33) and appears to act as a general inhibitor of enzymes and various signaling molecules (e.g., eNOS, Gα, H-Ras, MEK, ERK, Src, and Fyn) (34–36).

Much less is known about the function of caveolin-2. Caveolin-2 has less than a 60% amino acid similarity to and only a 38% identity with caveolin-1 and is the least studied isoform of the three caveolins (37). Unlike other caveolins, caveolin-2 does not form high molecular weight homooligomers, and this may contribute to its inability to drive the formation of caveolae (38, 39). It has been suggested that it is an accessory protein for the caveolin-1-mediated formation of caveolae (11, 36). In the absence or low levels of caveolin-1, caveolin-2 accumulates in the Golgi compartment (39–41). Recent data suggest that caveolin-2 has also a lipid droplet targeting sequence and can be found on the membrane surface of lipid droplets (42).

Based on the amino acid sequence homology, caveolin-3 has an 85% similarity to and a 65% identity with caveolin-1.

It is thought to substitute for caveolin-1 in striated muscle cells with respect to its role in the formation of caveolae (43). Mutation in the caveolin-3 gene and its deficiency of expression can lead to a rare, autosomally dominant form of limb-girdle muscular dystrophy, while over-expression of caveolin-3 in transgenic animals causes a Duchenne-like muscular dystrophy phenotype (44, 45). A review of the muscle-specific roles of caveolin-3 is available (46).

CAVEOLIN EXPRESSION AND LOCALIZATION IN VARIOUS MACROPHAGE CELL TYPES

There has been significant controversy regarding the expression of caveolins in macrophages. The absence of caveolin expression in macrophages was initially reported without specific elaboration on the detection methods used (33). In contrast, caveolin-1 presence in mouse bone marrow macrophages was demonstrated by immunofluorescence microscopic studies (47). Caveolin-1 expression in these cells was not confirmed by other approaches, but immunoblotting and RT-PCR was later used to demonstrate caveolin-1 expression in mouse peritoneal macrophages (48). The expression of caveolin-2 in macrophages was not investigated. Reports from studies on rat peritoneal macrophages suggest that resident macrophages express caveolin-1, and its expression is increased upon elicitation by Freund's adjuvant (49). The increase in caveolin-1 expression is associated with increased formation of morphologically distinguishable caveolar invaginations, as visualized by electron microscopy.

Caveolin-2 expression was detected by immunogold labeling and immunofluorescence microscopy, but immunoblotting experiments failed to identify caveolin-2 of correct molecular mass. The authors suggest that, based on these data, rat peritoneal macrophages express a modified or novel caveolin-2-related protein (of 29 kDa), which is recognized by antibodies against caveolin-1 and caveolin-2 and is specific to macrophages (50).

Recent data from our laboratory unequivocally established caveolin-1 and caveolin-2 protein expression in mouse primary macrophages by Western blotting and immunofluorescence, and mRNA expression by RT-PCR and nuclease protection assay (41).

The identification of caveolin expression in macrophage-like cell lines has also been controversial. The THP-1, J774, and Raw macrophage cell lines have been widely used in the past as models to study macrophage lipid metabolism (51–54). Caveolin-1 expression was detected in THP-1 cells by immunoblotting and RT-PCR and was shown to be increased 50-fold upon differentiation into macrophages (52, 55). Interestingly, while caveolin-1 expression was detected in J774 cells by RT-PCR and in Raw264.7 cells by RT-PCR and immunoblotting by some (48), caveolin-1 and caveolin-2 expression could not be detected by immunoblotting in J774 and Raw cells by others (55). We also failed to detect caveolin-1 expression in J774 cells but detected caveolin-2 expression using a variety of approaches, including Western blots, nuclease protection assay, and immunofluorescence (41). The primers designed to detect caveolin expression

in our studies were designed to span two exons and thus eliminate genomic DNA as a potential source of false-positive caveolin-1 expression in J774 cells. The available data on caveolin expression in macrophages and macrophage cell lines are summarized in **Table 1**.

We have undertaken the characterization of caveolin expression in three different macrophage cell types: thioglycollate-elicited mouse peritoneal macrophages, resident mouse peritoneal macrophages, and J774 cells (41). Several independent approaches were taken to identify and characterize caveolins in these cells and obtain a relative measure of caveolin expression when compared to fibroblasts.

Relative to 3T3 fibroblasts, resident and thioglycollate-elicited macrophages express caveolin-1 at levels 45% and 15% of that found in 3T3 cells, respectively. Caveolin-2 expression in resident as well as thioglycollate-elicited macrophages, on the other hand, is quantitatively similar to the extent of expression in fibroblasts (80 and 90% of the levels in fibroblasts, respectively). These data correspond well with the levels of respective mRNAs, as measured by ribonuclease protection assays (41). Surprisingly, despite similar mRNA levels, caveolin-2 expression in J774 cells is only 10% of that observed in fibroblasts, possibly due to a more rapid degradation in the absence of caveolin-1.

In contrast to fibroblasts, there is little overlap in caveolin-1 and -2 subcellular localization in macrophages (41). As shown in **Fig. 1**, caveolin-1 is expressed primarily on the surface of mouse primary macrophages, while caveolin-2 is found almost exclusively in the Golgi apparatus (41), an observation characteristic of cells expressing low levels of caveolin-1 and caveolae (39, 40). Over-expression of caveolin-1 in non-macrophage cells, which normally lack caveolin-1, is associated with the appearance of caveolin-2 at the cell surface and the formation of caveolae (39, 40). Based on these reports, one would expect that increased expression of caveolin-1 results in an increased movement of caveolin-2 from the Golgi compartment to the plasma membrane. Data from our laboratory are consistent with this notion. In the complete absence of caveolin-1 expression, as exemplified in J774 cells, caveolin-2 is not detectable at the cell surface. Significant caveolin-1 expression, as seen in resident mouse peritoneal mac-

rophages (45% of that in fibroblasts), on the other hand, is accompanied by an appearance of caveolin-2 at the cell surface. Thioglycollate-elicited mouse peritoneal macrophages, characterized by low caveolin-1 expression (one third of that of resident macrophages), contain detectable but very small amounts of caveolin-2 on the cell surface (41). Indeed, the requirement of threshold caveolin-1 expression levels for the formation of caveolae in caveolin-1-transfected lymphocytes has also been observed (56). In the absence of caveolin-1 expression, caveolin-2 is present in the Golgi compartment of K562 human erythroleukemic cells and, as expected, in the Triton X-100-soluble fraction (40). Upon transfection with caveolin-1, caveolin-2 exits the Golgi compartment and becomes resistant to Triton X-100 extraction, indicative of its incorporation into the lipid raft membranes (40). As shown in **Fig. 2**, in thioglycollate-elicited mouse peritoneal macrophages, the majority of caveolin-1 is in the Triton X-100-insoluble membranes (lipid rafts), while caveolin-2 is found almost exclusively in the Triton X-100-soluble fraction, which is identified as the Golgi compartment by marker enzyme immunofluorescence studies (41). The lack of morphologically distinguishable flask shaped surface caveolae in mouse primary macrophages (Gargalovic and Dory, unpublished observations) is thus likely the result of "subthreshold" expression of caveolin-1 and/or the failure to recruit caveolin-2 for the formation of these organelles.

It is now apparent that the pattern of caveolin expression in primary macrophages does not parallel that of caveolin expression in various macrophage-like cell lines (41, 48, 55). The choice of the appropriate cell to study various aspects of lipid metabolism may therefore be of significance, as the extent of caveolin expression may have a profound effect on various aspects of cholesterol/phospholipid metabolism.

LIPIDS AND REGULATION OF CAVEOLIN EXPRESSION

Non-macrophage cells

The ability of caveolin-1 to bind cholesterol and fatty acids (57, 58) is suggestive of its involvement in the trafficking

TABLE 1. Expression of caveolin isoforms in macrophages

Macrophage	Caveolin-1	Method	Caveolin-2	Method	References
RES-MPM	+	RT-PCR, RPA, WB, IC	+	RT-PCR, RPA, WB, IC	41
Tg-MPM	+	RT-PCR, RPA, WB, IC	+	RT-PCR, RPA, WB, IC	41, 48
Res-RPM	+	RT-PCR, WB, IC, IE	+	IC, IE	49, 50
Fr-RPM	+	RT-PCR, WB, IC, IE	+	IC, IE	49, 50
MBM	+	IC	?		47
J774A.1	−	RT-PCR, RPA, WB, IC	+	RT-PCR, RPA, WB, IC	41, 55, 79
THP-1	+	RT-PCR, WB	?		52, 55
Raw 264.7[a]	+	RT-PCR, WB	?		48

Res-MPM, resident mouse peritoneal macrophages; Tg-MPM, thioglycollate-elicited mouse peritoneal macrophages; Res-RPM, resident rat peritoneal macrophages; Fr-RPM, Freund's adjuvant-elicited rat peritoneal macrophages; MBM, mouse bone marrow-derived macrophages; RT-PCR, reverse transcription-polymerase chain reaction; RPA, ribonuclease protection assay; WB, Western blotting; IC, immunocytochemistry; IE, immunoelectron microscopy.

[a] Matveev and colleagues (55, 79) reported lack of caveolin-1 in Raw cells but did not specify which of the available Raw cell lines they used.
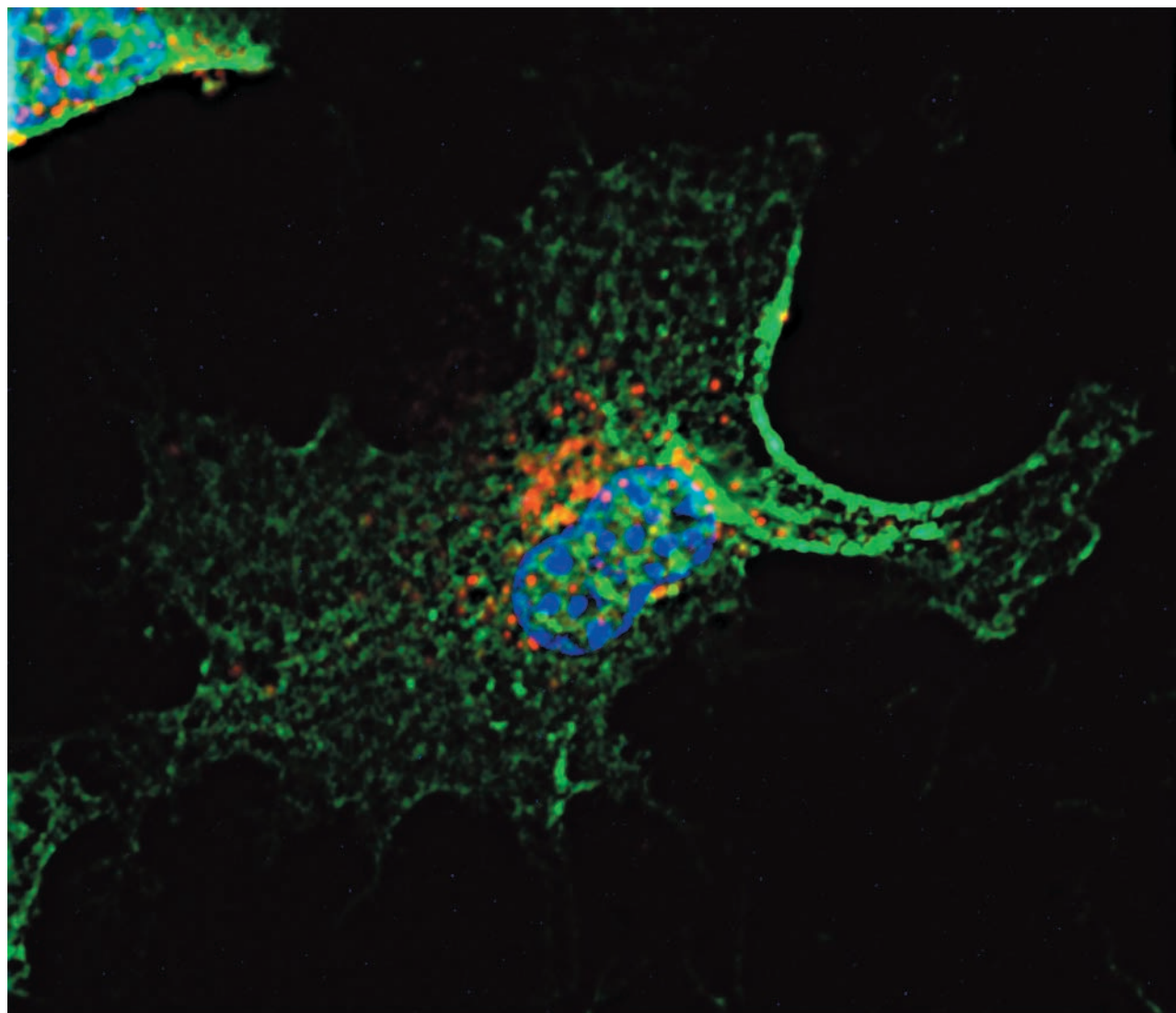
Fig. 1. Immunolocalization of caveolin-1 and -2 in mouse peritoneal macrophages. Resident mouse peritoneal macrophages were doubly immunostained with specific antibodies against caveolin-1 (green) and caveolin-2 (red), and visualized by incubation with distinctly tagged fluorescent secondary antibodies. Nuclear DNA (blue) was visualized by using 4′,6-diamidino-2-phenylindole-DNA dye.

of lipids between the various intracellular compartments and the cell membrane. In support of this notion, several studies demonstrate that in fibroblasts caveolin-1 can cycle between the ER, Golgi apparatus, and plasma membrane (24, 59). This behavior is reminiscent of that of the oxysterol-binding protein, which rapidly moves to the Golgi apparatus after binding 25-hydroxycholesterol (60). Caveolin-1 expression in fibroblasts may be especially important in the delivery of newly synthesized cholesterol to the membrane caveolae. Indeed, transfection of caveolin-1 into cells devoid of caveolin induces the formation of caveolae (23, 32), accompanied by significantly increased rates of cholesterol transport to the cell surface (24). It has been proposed that at least some of the newly synthesized cholesterol is transported to the cell surface, by-passing the Golgi apparatus, in a soluble cytosolic complex containing caveolin-1 and heat-shock proteins (21, 61). While caveolin-1 may be involved in the delivery of cholesterol to specific areas of plasma membrane, the extent of

its binding to the membrane appears to depend on the local cholesterol concentration (62).

In fibroblasts and MDCK cells the extent of caveolin-1 expression appears to correlate positively with the intracellular cholesterol content (63, 64). Cholesterol-dependent regulation of caveolin-1 mRNA levels in human skin fibroblasts has been proposed to be mediated by the sterol responsive element binding protein (SREBP) in a manner opposite to that of other known genes regulated by sterols (65). In support of this observation, the expression of an active form of SREBP-1 in HaCaT cells results in a 50% down-regulation of caveolin-1 expression (66). The promoter of the caveolin-1 gene contains DNA sequences similar to the SRE sequences found in other genes regulated by SREBP (65, 67). Somewhat paradoxically, cholesterol loading increases, while incubation with oxysterols decreases caveolin-1 mRNA levels in primary human fibroblasts (63). Incubation of MDCK cells with 25-hydroxycholesterol was also reported to lead to decreased caveo-
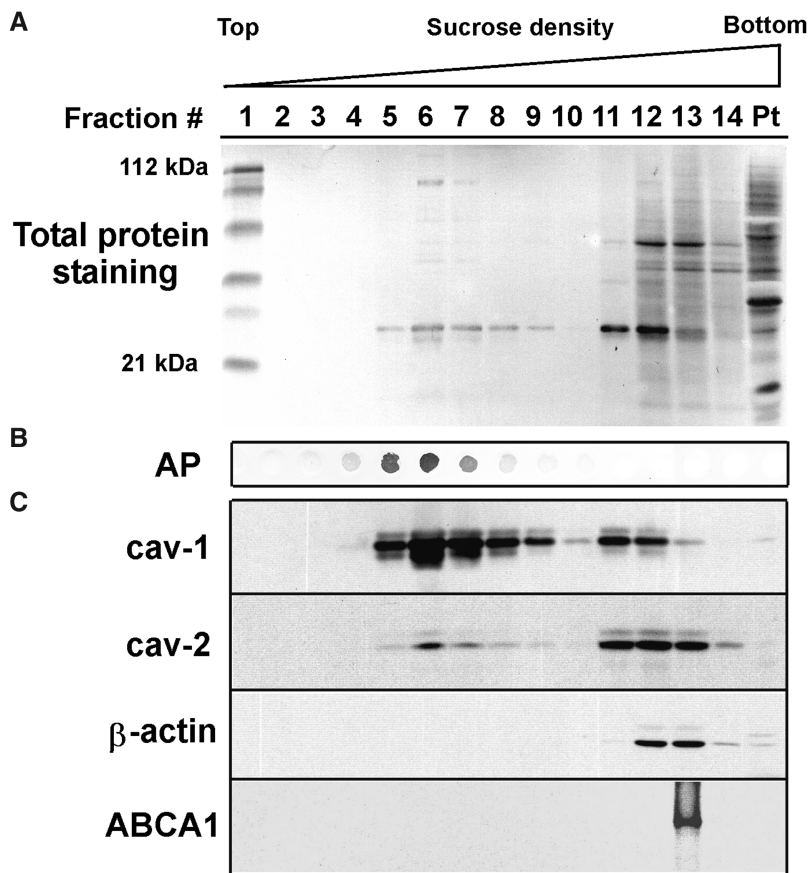
Fig. 2. Caveolin partitioning to lipid raft/caveolae membrane domains in mouse primary macrophages. Lipid raft/caveolae membranes were isolated as described by Machleidt et al. (20). Cells were solubilized in 1% Triton X-100 and lipid raft/caveolae membrane fraction separated by centrifugation in a (10–30%) linear sucrose gradient. After centrifugation for 21 h in SW41 rotor fractions (1–14) were collected from the top and together with pelleted material (Pt) analyzed by SDS-PAGE and immunoblotting. Total protein loading after transfer to PVDF membrane was visualized by Ponceau S staining (A). Portion from each fraction was analyzed for alkaline phosphatase (AP) activity (lipid raft/caveolae marker) using an alkaline phosphatase substrate kit (Bio-Rad) (B). Caveolin-1, caveolin-2, β-actin, and ABCA1 expression was analyzed by immuno-blotting (C). Rabbit anti-ABCA1 antibody was a gift from Michael L. Fitzgerald and Mason W. Freeman (Massachusetts General Hospital and Harvard Medical School).

lin-1 mRNA levels (64). The apparently opposing effects of cholesterol and oxysterols on the caveolin-1 mRNA levels in these cells are difficult to interpret in the context of our present understanding of sterol-mediated regulation of gene expression. If the caveolin-1 gene is regulated in a manner opposite to that of other known SRE-containing genes, then oxysterols would be expected to have a stimulatory effect on caveolin-1 mRNA expression, since oxysterols are well-known inhibitors of SREBP cleavage and SRE-mediated gene transcription (68, 69).

Macrophages

In contrast to observations made in fibroblasts and MDCK cells, 25-hydroxycholesterol (or cholesterol) has no effect on caveolin-1 mRNA levels in primary mouse macrophages (Gargalovic and Dory, unpublished observations). While several-fold increases in cellular cholesterol (and unesterified cholesterol) mass have no effect on caveolin-1 mRNA, they tend to increase the cellular pool size of caveolin, possibly by protecting it from degradation (41). We could not detect gross differences in cellular caveolin isoform distribution under conditions of cholesterol loading or HDL-mediated efflux (41). Future studies to examine the rates of caveolin-1 transcription and translation, as well as mRNA and protein stability, as affected by cellular cholesterol mass and distribution, are essential.

In the THP-1 monocytic cell line, caveolin-1 expression is induced upon phorbol ester-mediated differentiation into macrophages. Differentiation of THP-1 cells is also associated with an increase in total cellular cholesterol mass as well as the cholesterol associated with isolated caveolae/lipid rafts (52, 55). The increase in cellular cholesterol upon differentiation of these cells appears to be independent of caveolin-1 upregulation, since the inhibition of caveolin-1 expression by anti-sense technology fails to have an effect on the free and esterified cholesterol mass (52).

The apparent differences in the regulation of caveolin expression in macrophage-like cells, fibroblasts, and other cells, as described above, may well be the result of the differences in cell phenotype and the different regulatory

mechanisms operating in these cells. The relatively low (or none in J774 cells) level of caveolin-1 expression, the lack of caveolin isoform co-localization, as well as the lack of formation of caveolae on the one hand and active cholesterol metabolism on the other hand, are likely to contribute to the differences in regulation of caveolin expression in macrophages.

CAVEOLINS AND LIPID TRANSPORT IN MACROPHAGES

We have described the evidence suggesting that caveolin-1 plays a role in the transport of cholesterol to plasma membrane rafts, where it remains tightly packed in a liquid ordered state. The rafts may be an important source of cholesterol for the surrounding membrane through lateral diffusion. The ability of caveolin to bind cholesterol and the high concentration of cholesterol in caveolae or rafts suggest that caveolin and caveolin-rich domains of plasma membranes are involved in the maintenance of cellular cholesterol homeostasis as well as in its exchange with the extracellular environment (23). Newly synthesized cholesterol is transported from the ER to caveolae before diffusing into the bulk of the membrane (23), and caveolin-1 appears to play a role in this process. Caveolins may play a similar role in macrophages.

Cholesterol uptake

Caveolae are also believed to be involved in the uptake of cholesterol from HDL by the selective cholesteryl-ester uptake pathway (70). CD36 and SR-BI, two members of the class B scavenger receptors, are localized in caveolae or lipid rafts (71–73). Both CD36 and SR-BI have been shown to bind HDL and selectively transfer core cholesteryl esters to the cells (74, 75). CD36 binds HDL with a relatively high affinity; it is however much less efficient in the selective cholesteryl ester uptake than the SR-BI (74). SR-BI may also be at least partially responsible for cholesterol efflux, as it has been shown to promote a bi-directional exchange of free cholesterol between cells and acceptors, a process independent of selective cholesteryl ester uptake (76–78). Transfection of caveolin-1 into J774 and RAW macrophages inhibits HDL-mediated cholesteryl ester uptake but has no effect on HDL-mediated cholesterol efflux (79). On the other hand, increased expression of caveolin-1 as a result of differentiation of THP-1 cells is associated with increased HDL cholesteryl ester uptake by CD36 (55, 79) and increased cholesterol efflux (52). CD36 expression is also known to increase upon differentiation of human monocytes to macrophages (80). RAW cells on the other hand do not express detectable levels of CD36 and caveolin-1 but express SR-BI (79). Transfection of these cells with caveolin-1 leads to reduction of selective cholesteryl ester uptake from HDL (79).

The mechanism of regulation of the selective cholesterol uptake by caveolin-1, if any, is not known at the present time. Caveolin-binding sequence motifs have not been found in the SR-BI sequence and chemical cross-linking and co-immunoprecipitation of these two proteins also failed (79). The evidence of a direct interaction between caveolin-1 and SR-BI is therefore lacking.

While the selective uptake of HDL-derived cholesterol via lipid raft-associated CD36 or SR-BI is likely to be of limited physiological significance in macrophages, there is virtually no information about the relationship between caveolin expression and activity of the various receptors for oxidized/modified LDL that play an important role in the pathophysiology of atherosclerosis.

Cholesterol efflux

Cholesterol efflux from most peripheral cells is likely of limited physiological importance since their cholesterol uptake and synthesis are tightly regulated. On the other hand, the unregulated nature of cholesterol uptake by macrophages makes these cells particularly dependent on cholesterol efflux mechanisms through appropriate acceptors to maintain their cholesterol homeostasis (81, 82). Macrophages and lipid loaded foam cells can rapidly efflux cholesterol to media containing HDL or apoA-I (81–84). The physiological relevance of cholesterol efflux mediated by HDL or its subfractions is also supported by several epidemiological studies (85–87). We attempt to summarize the available data on the potential role of rafts and caveolin on cholesterol efflux from these cells below.

Cholesterol in plasma membranes can be present in two distinct lipid environments. In the lipid raft membrane domains, cholesterol is tightly packed primarily with sphingolipids in a liquid-ordered state, characterized by increased melting temperatures and high resistance to solubility in non-ionic detergents (88–90). On the other hand, cholesterol in non-raft membranes is primarily surrounded by phospholipids, and at relatively lower concentrations in a liquid-disordered state, and readily extractable by detergents (91, 92). Importantly, lateral diffusion of lipids assures a steady-state equilibrium between these two domains. Their tightly packed liquid-ordered state, highly resistant to solubilization by non-ionic detergents, makes rafts an unfavorable direct source of cholesterol for efflux (89, 92, 93).

While some studies demonstrate that caveolae and caveolin-1 expression is associated with enhancement of cholesterol efflux (52, 94, 95), others indicate the opposite (96). Indeed, a significant body of evidence suggests that cholesterol located in lipid rafts/caveolae is more resistant to HDL- and cyclodextrin-mediated efflux (91, 92). In fact, recent observations in human fibroblasts demonstrate that the ABCA1 transporter does not associate with lipid rafts and that cholesterol released to apoA-I is primarily of non-raft origin (93).

As shown in **Fig. 3**, cholesterol can be removed from macrophages by at least four distinct mechanisms. We propose that membrane shedding is the only mechanism of cholesterol efflux/loss that directly involves lipid rafts. Lipid raft cholesterol, resistant to extraction by acceptor-mediated mechanisms, can be released to the medium, along with caveolin-1, by membrane shedding (97). Proliferating and tumor cells are particularly active in membrane shedding (98); extensive shedding of the plasmalemmal vesi-
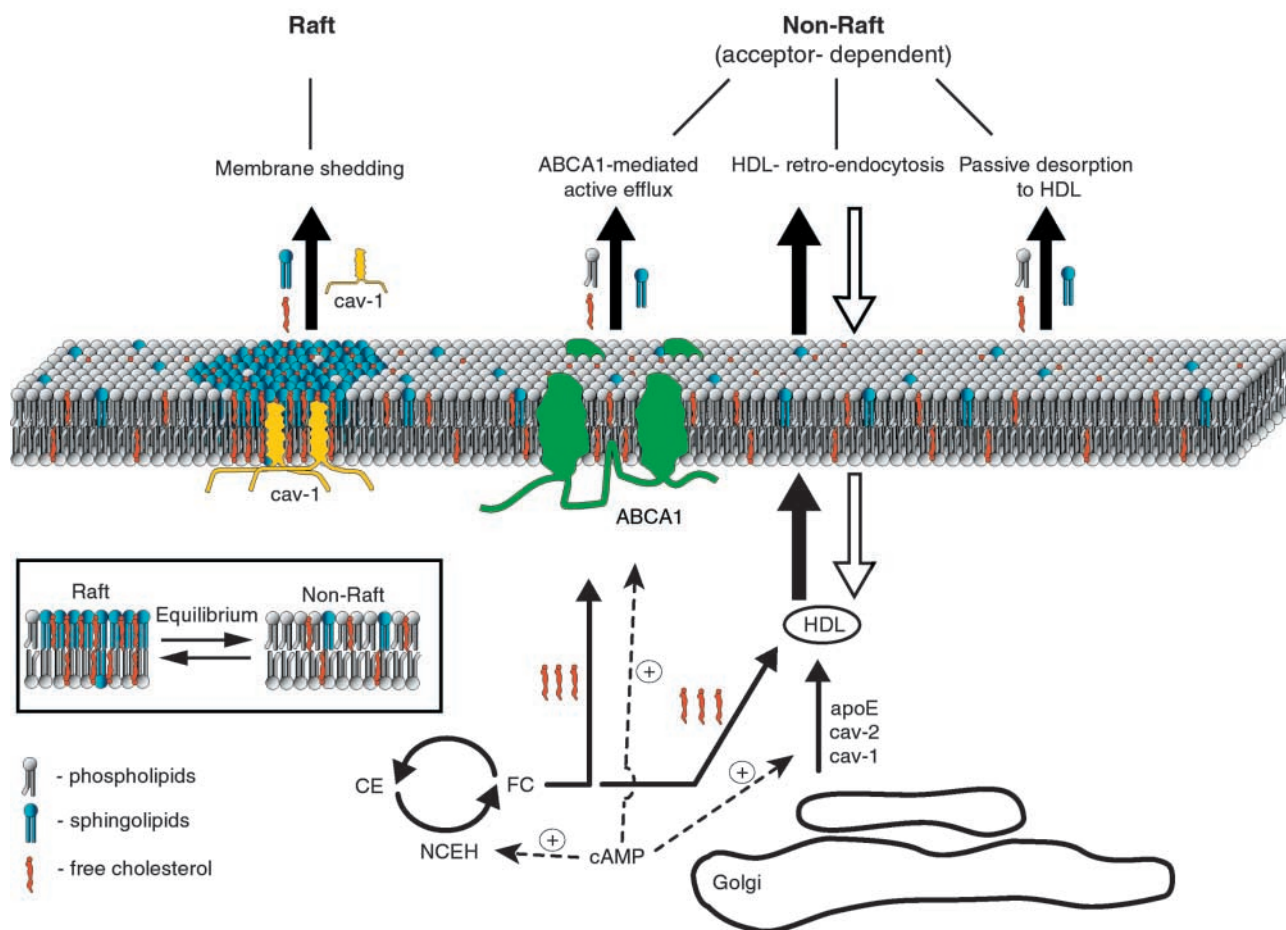
Cholesterol efflux in macrophages

Fig. 3. Role of caveolin and lipid raft domains in cholesterol efflux from macrophages. Plasma membrane cholesterol in the lipid rafts is surrounded by sphingolipids in a tight, liquid-ordered state and is highly resistant to extraction by nonionic detergents. Lipid raft cholesterol as well as other lipids and associated proteins (e.g., caveolin-1) may be released to the medium by membrane shedding in an acceptor-independent manner. Albeit at higher concentrations, the highly insoluble cholesterol in the lipid rafts is an unfavorable source for acceptor-mediated efflux, which takes place primarily in the non-raft, liquid disordered membranes. In passive diffusion, cholesterol is desorbed from the cell surface to an acceptor along the concentration gradient in an energy-independent manner. The second, energy- and acceptor-dependent mechanism is mediated by the ABCA1 transporter, and involves primarily the transport of cholesterol from intracellular pools to lipid-poor apo-AI. A third mechanism of cholesterol efflux, operating in macrophages, is mediated via HDL retro-endocytosis by interacting with intracellular lipid-rich pools. Retro-endocytosis is also a likely mechanism by which HDL in macrophages can acquire apoE and Golgi-associated non-raft cav-2 and cav-1. Increased cAMP production plays a major coordinating role in the removal of intracellular cholesterol by stimulating the NCEH, ABCA1 transcription, and apoE secretion.

cles from the surface of macrophage foam cells in atherosclerotic lesions has also been documented, suggesting that this mechanism can contribute to cholesterol efflux from macrophages (99). The physiological relevance of this pathway is unknown at the present time and awaits future studies.

The major mechanisms of cholesterol efflux in macrophages appear to be raft (and thus caveolin-1) independent. Active ABCA1-mediated efflux to lipid poor apoA-I (preβ-HDL) or passive desorption along the concentration gradient to HDL are the major, acceptor-dependent pathways of cholesterol efflux from macrophages. In contrast to caveolin-1, the expression of the ABCA1 transporter is highly regulated by cholesterol loading/efflux

(100) and does not partition to lipid raft membranes in macrophages (Fig. 2). The absence of raft localization of the ABCA1 transporter and the resistance of raft-associated cholesterol to extraction suggest that the acceptor-dependent mechanisms of cholesterol efflux in macrophages do not involve lipid rafts and caveolin-1. Caveolin-1 may, however, play an indirect role by transporting cholesterol to membrane rafts from which it is subsequently redistributed, by lateral diffusion, to non-raft, more efflux-friendly portions of the plasma membrane.

Another potential macrophage-specific mechanism of cholesterol removal may involve retroendocytosis of HDL. Retroendocytosis of HDL by macrophages has been previously documented (101–104). Our own data, using gold-

labeled apoE-free HDL, also demonstrate HDL retroendocytosis (data not shown). The HDL-containing endosomes can be found in a time dependent manner in a close proximity to or fused with apoE-containing secretory vesicles (Dory and Rudick, unpublished observations); this is followed by re-secretion of the gold-labeled-, apoE-enriched HDL. Parallel experiments with gold-labeled LDL (larger gold particles) demonstrate a different itinerary with LDL ending up in the lysosomal compartment. Data from our laboratory demonstrate apoE, caveolin-1, and -2 secretion by cholesterol-loaded mouse peritoneal macrophages in the presence of HDL (41, 105). Moreover, we have also demonstrated that secreted caveolins have a density similar to that of HDL, suggesting either their presence on HDL or their secretion in distinct particles with a similar density (Gargalovic and Dory, unpublished observations). Caveolins may therefore be acquired in a fashion similar to apoE. Secretion of caveolin may play an active role in lipid removal from cholesterol-rich macrophages by this pathway or be simply a by-product of phospholipid and cholesterol transfer to HDL. Based on the relative ratio of secreted caveolin-1/caveolin-2 (close to unity), we propose that both originate from the Golgi compartment and are brought out by HDL undergoing retroendocytosis. Overall, this process is likely to account for only a small fraction of the total cholesterol efflux and may not be physiologically significant.

There is evidence that signaling pathways involving cAMP may play a major coordinating role in cholesterol efflux via the non-raft, acceptor-dependent pathways in macrophages. ABCA1 transcription and cholesterol efflux to apoA-I is increased by cAMP (51, 106). Formation of cAMP also leads to a stimulation of neutral cholesteryl ester hydrolase (NCEH), thus increasing the concentration of free cholesterol available for efflux (107). Secretion of apoE from peritoneal macrophages is also stimulated in the cAMP-dependent manner (Dory, unpublished observations), supporting the role of cAMP as a central regulator of the active cholesterol transport pathways.

Clearly, further studies are needed to elucidate the mechanisms of cholesterol efflux in macrophages and the potential role of caveolins in this process. Recent generation of caveolin-1 knockout mice (30, 31) is likely to shed more light on this interesting subject.

CONCLUSIONS AND FUTURE PERSPECTIVES

The rapid growth in caveolin-related research in recent years provides us with many potentially important insights into the regulation of cholesterol metabolism. Some of these studies provide compelling evidence that the regulation of the signal transduction pathways is intimately linked to cellular lipid homeostasis, thus interlocking these two events into a complex bio-regulatory system (108). Caveolae and lipid rafts are good examples of domains with such regulatory functions (7). Because of the wide variety of functions ascribed to caveolins, it is possible that caveolins may coordinate signal transduction, lipid homeostasis, and subcellular trafficking of molecules. Evidence to date supports the role

of caveolins in the maintenance of such regulatory domains, probably unique to each cell type.

Although the precise function of caveolins in macrophage lipid/cholesterol metabolism is currently unknown, the evidence presented is suggestive of their involvement in cholesterol transport to the membrane; the evidence of its involvement in cholesterol efflux mechanisms is less compelling. The specific involvement of caveolins in the maintenance of cholesterol homeostasis and macrophage-foam cell formation must be further examined. The availability caveolin-1 knockout mice provides an additional powerful tool for future studies.

Caveolins may also be involved in the modulation of macrophage immune responses (48) (e.g., to oxLDL), and in the clearance of apoptotic cells in the lesion area (109). These outstanding issues are likely to draw a lot of attention in the future, and will extend our knowledge of the various mechanisms involved in atherosclerotic disease progression, providing additional opportunities for effective treatment.

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