

Regulation of cholesterol homeostasis in macrophages and consequences for atherosclerotic lesion development

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Abstract

Foam cell formation due to excessive accumulation of cholesterol by macrophages is a pathological hallmark of atherosclerosis. Macrophages cannot limit the uptake of cholesterol and therefore depend on cholesterol efflux pathways for preventing their transformation into foam cells. Several ABC-transporters, including ABCA1 and ABCG1, facilitate the efflux of cholesterol from macrophages. These transporters, however, also affect membrane lipid asymmetry which may have important implications for cellular endocytotic pathways. We propose that in addition to the generally accepted role of these ABC-transporters in the prevention of foam cell formation by induction of cholesterol efflux from macrophages, they also influence the macrophage endocytotic uptake.

Introduction

Atherosclerotic cardiovascular disease is the major cause of morbidity and mortality worldwide (1). A pathological hallmark of atherosclerosis is the excessive accumulation of cholesterol by macrophages leading to their transformation into foam cells (2). Macrophages play an important role in the initiation of the early atherosclerotic lesions. In addition, during the further progression of the lesion, macrophages also contribute to the formation of the necrotic core and may affect the stability of the atherosclerotic lesion. Especially in the initiation of atherosclerosis, cholesterol homeostasis in macrophages is of prime importance, as dysregulation of the balance of cholesterol influx and cholesterol efflux will lead to excessive accumulation of cholesterol in the macrophage and their transformation into foam cells. This minireview highlights important aspects of macrophage cholesterol homeostasis.

Macrophage cholesterol accumulation

Cholesterol may enter macrophages via several different pathways. Macrophages express high levels of scavenger receptors, which bind and internalize oxidatively modified lipoproteins. Furthermore, macrophages also contain several other types of binding sites that are involved in the accumulation of unmodified lipoproteins and lipoprotein remnants, including the LDL receptor (LDLr), LDL receptor-related protein (LRP), VLDL receptor (VLDLr), and proteoglycans (see Fig1).

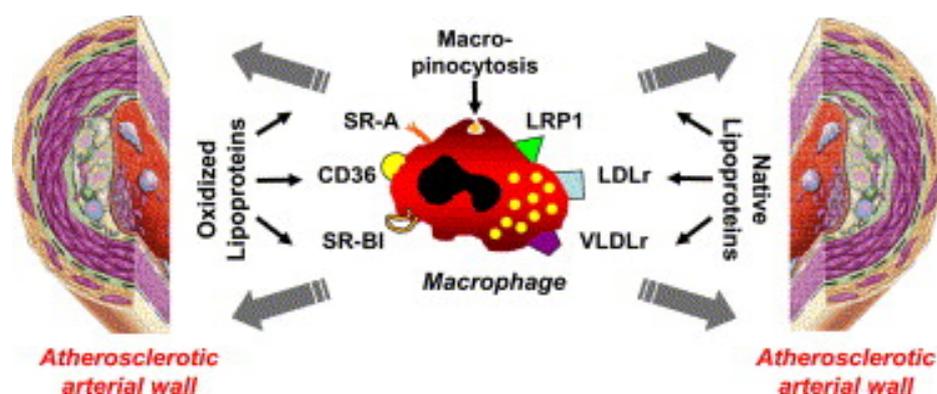


Figure 1. Macrophage cholesterol accumulation.

Cholesterol may enter macrophages via several different pathways and induce the transformation of macrophages into foam cells, the first step in atherosclerotic lesion development (2). Scavenger receptors, including scavenger receptor A (SR-A), scavenger receptor BI (SR-BI), and CD36 mediate the uptake of modified lipoproteins by macrophages. Native lipoproteins are taken up via the LDL receptor (LDLr), the VLDL receptor (VLDLr), and LDL receptor related protein 1 (LRP1). Furthermore, cholesterol may enter macrophages via fluid-phase macropinocytosis.

An important receptor implicated in the accumulation of oxidatively modified lipoproteins is scavenger receptor A (SR-A). SR-A is highly expressed in macrophage-derived foam cells in atherosclerotic plaques (3-5). It binds many polyanionic molecules, but the affinity of SR-A for modified lipoproteins varies. The uptake of acetylated LDL (AcLDL) and oxidized LDL (OxLDL) by SR-A deficient macrophages was 30% and 70% of that in wildtype macrophages, respectively (6, 7), while AcLDL degradation in SR-A deficient macrophages was 17% of control (8). Total body SR-A deficiency resulted in 50% and 20% reduction in atherosclerotic lesion size in apoE knockout (apoE^{-/-}) and LDL receptor knockout mice (LDLR^{-/-}), respectively (6, 9). Using the technique of bone marrow transplantation, Linton et al. (10) generated C57Bl/6 mice and LDLR^{-/-} mice that were selectively deficient for SR-A in macrophages. In both mouse models a 60% reduction in lesion area was observed in absence of macrophage SR-A, indicating an important pro-atherogenic role of SR-A expression by macrophages. Macrophage SR-A overexpression in LDLR^{-/-} mice and apoE^{-/-} mice, however, did not significantly affect atherosclerotic lesion development (11, 12).

SR-A belongs to a growing list of scavenger receptor family members (for review (13)). In addition to SR-A, also CD36 was shown to bind and internalize minimally modified forms of LDL (14). Peritoneal macrophages of CD36 deficient mice exhibit a 60–80% decrease in OxLDL binding (15). While SR-A appeared to be more important for AcLDL and fully oxidized LDL binding and degradation, CD36 was more active towards mildly oxidized LDL (16). In macrophages from SR-A/CD36 double knockout mice the degradation of AcLDL and OxLDL was inhibited for 75–90% while the cholesteryl esters from modified lipoproteins failed to accumulate and no foam cell forming was possible. These data establish that SR-A and CD36 are responsible for the preponderance of modified LDL uptake in macrophages and that other scavenger receptors do not compensate for their absence. Zhao et al. (17) showed recently that the lipid accumulation in macrophages induced by native LDL from apoE^{-/-} mice is also blocked for 80% by the absence of SR-A and CD36. ApoE^{-/-} mice reconstituted with CD36-deficient bone marrow displayed a 88% reduction in lesion area after 12 weeks Western-type diet feeding (18), establishing the essential role of macrophage CD36 in lesion formation. On the other hand, Moore et al. recently reported that deletion of SR-A or CD36 does not ameliorate atherosclerosis in apoE^{-/-} mice (19). The reason for this absence of an effect is presently unclear. It might either indicate that modified lipoproteins are less important in lesion formation as currently thought or that additional (pathological) stimulants, i.e. bacterial pathogens, are needed to evoke the crucial role of these scavenger receptors.

Scavenger receptor BI (SR-BI), an HDL receptor which mediates the selective uptake of cholesterol esters from HDL by the liver (20, 21) is also expressed by lipid-laden macrophages in human and murine atherosclerotic lesions (22-24). It binds native and modified lipoproteins, anionic phospholipids, and apoptotic cells (25). In addition to its role in the selective uptake of HDL cholesteryl esters, SR-BI stimulates the bi-directional flux of free cholesterol between cells and HDL and the rate of cholesterol efflux from various cell types correlates with the expression of SR-BI (26-28). Bone marrow transplantation studies have shown that SR-BI on macrophages reduces the development of advanced atherosclerotic lesions in LDLr^{-/-} (29, 30) and apoE^{-/-} mice (31). In contrast, the development of small fatty streak lesions in LDLr^{-/-} mice is facilitated by macrophage SR-BI (30). It thus appears that, depending on the stage of lesion development, SR-BI in macrophages is either proatherogenic or antiatherogenic, indicating a dual role for SR-BI in the pathogenesis of atherosclerosis. This unique dual role is probably a direct effect of the finding that SR-BI is a multi-functional, multi-ligand receptor that facilitates the binding of a wide array of native and modified lipoproteins and mediates the bi-directional flux of cholesterol between HDL and cells. Its function in the binding of atherogenic lipoproteins, like native VLDL and oxidized LDL is expected to induce foam cell formation, while efflux of intracellular cholesterol to HDL will prevent foam cell formation and thus atherosclerotic lesion development.

In addition to SR-BI, also members of the LDL receptor family, including the LDL receptor, LRP1, and the VLDL receptor have been implicated in macrophage foam cell formation. The LDL receptor is a major pathway for the uptake of VLDL by macrophages (32,33). However, for long the role of the macrophage LDL receptor in foam cell formation and atherosclerotic lesion development was thought to be limited, as it is rapidly downregulated upon cellular cholesterol accumulation(34). However, by performing bone marrow transplantation studies, we(35) and others (36) have provided in vivo evidence that the macrophage LDL receptor facilitates diet-induced atherosclerosis. In addition, to the LDL receptor, also the VLDL receptor is abundantly expressed by macrophage-derived foam cells in atherosclerotic lesions (37-39). In contrast to the LDL receptor, the expression of the VLDL receptor is not responsive to cholesterol loading (40). Furthermore, reconstitution of macrophage VLDL receptor expression in VLDL receptor knockout mice largely increased atherosclerotic lesion development, indicating that the macrophage VLDLr is a pro-atherogenic factor(41). LRP1 is a multi-ligand and multi-functional receptor involved in a variety of physiological processes, including the uptake of apoE-containing lipoproteins (42). Macrophage-specific LRP1

knockout mice, however, display a reduced susceptibility to atherosclerotic lesion development, indicating that macrophage LRP1 is protective and that the pro-atherogenic function of LRP1 in the accumulation of lipids by macrophages is limited in vivo (43). The majority of the processes described above for macrophage cholesterol accumulation involve rapid receptor-mediated coated-pit endocytosis. In addition, modified LDL is taken up by macrophages in part by the slower process of macropinocytosis (44). At high concentrations (0.5–2 mg/ml), native LDL can induce macrophage foam cell formation in PMA-activated (45, 46) and M-CSF-differentiated (47) human monocyte-derived macrophages by fluid-phase macropinocytosis. Macropinocytosis, first described by Lewis in 1931 (48), is the actin-dependent formation of large vesicles, allowing the internalization of large quantities of fluid-phase solute (for review (49)). It is a major endocytotic pathway in epithelial cells, fibroblasts, neutrophils, and macrophages that occurs constitutively, but is highly increased by growth factors, such as epidermal growth factor (EGF) (50), macrophage colony stimulating factor (M-CSF) (51, 52), and phorbol esters (53, 54). Macropinosomes are dynamic structures formed by the closure of lamellipodia at ruffling membranes and range in size from 0.2 to 5 μ m in diameter (49). In unstimulated macrophages, membrane ruffles are relatively small and seldom form macropinosomes (54). After stimulation by PMA or M-CSF, the ruffles transform into longer and broader lamellipodia that regularly form macropinosomes by resealing with the cell surface, enclosing extracellular medium (52, 54). Macropinosomes start as early endosomes derived from the plasma and rapidly mature into late endosomes and finally merge into a stable, resident lysosomal compartment (55). Although most studies on macropinocytosis are performed on cells in vitro, in vivo spleen, lymph nodes, and liver are organs active in fluid-phase endocytosis (56). Furthermore, fluid-phase uptake in Kupffer cells, resident macrophages of the liver, takes place via macropinocytosis in vivo (57). Thus, it is also plausible that this constitutive uptake of fluid-phase contributes to macrophage foam cell formation in the arterial wall.

ABC-transporters: key molecules for macrophage cholesterol efflux

Macrophages are incapable of limiting the uptake of lipids via the wide variety of uptake mechanisms described above and therefore, largely depend on cholesterol efflux pathways to maintain cellular lipid homeostasis. A key transporter involved in the efflux of cholesterol and phospholipids from macrophages is ATP-binding cassette transporter A1 (ABCA1)(58) (see Fig 2). ABCA1 is a 2261-amino acid, 240-kDa protein belonging to a large family of

conserved transmembrane proteins that use ATP as an energy source to transport a wide variety of substrates across cellular membranes(59). It is a full transporter, consisting of two 6-helix transmembrane domains that serve as a pathway for the translocation of substrates across membranes and two nucleotide-binding domains that bind ATP and provide the energy for transport. ABCA1 mediates the transport of cholesterol and phospholipids to lipid-free apolipoproteins such as apoAI (60). Macrophage ABCA1 expression is highly upregulated by oxysterol-dependent transactivation of the ABCA1 promoter by the liver X receptor (LXR) (61, 62). Furthermore, ABCA1 is highly expressed in atherosclerotic lesions, where it co-localizes with cholesterol-loaded macrophages (63). Bone marrow transplantation experiments showed that disruption of ABCA1 in macrophages results in a marked increase in atherosclerotic lesion development (64, 65). Thus, ABCA1-dependent cholesterol efflux is a crucial factor in the prevention of excessive cholesterol accumulation in macrophages of the arterial wall and their transformation into foam cells. By transplantation of ABCA1 overexpressing bone marrow into LDLr^{-/-} mice, we have recently provided the first evidence that ABCA1 expression by macrophages plays a critical role in the protection against the progression of atherosclerosis (66).

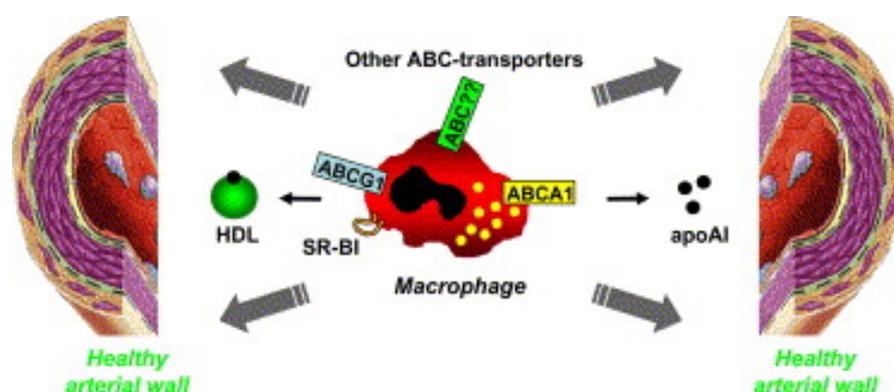


Fig. 2. Macrophage cholesterol efflux. Macrophages cannot limit the uptake of cholesterol and therefore depend on cholesterol efflux pathways for the prevention of excessive cholesterol accumulation and atherosclerotic lesion development (2). ABCA1 facilitates the efflux of cholesterol from macrophages to lipid-poor apoAI, while ABCG1 and SR-BI mediate the efflux of cholesterol to mature HDL. In addition, other transporters of the ABC-transporter superfamily might mediate cholesterol efflux from macrophages.

In addition to ABCA1, macrophages also express ABCG1, which is induced during cholesterol uptake in macrophages (67, 68) and is activated via LXR (69, 70). ABCG1 is a half transporter with a single 6-helix transmembrane domain and a single nucleotide domain that needs to form a homodimer or a heterodimer with another ABC-transporter to be functional. In contrast to ABCA1, ABCG1 facilitates cellular cholesterol and phospholipid

efflux from macrophages to mature HDL, but not to lipid-free apolipoproteins (71-73). ABCA1-mediated lipid efflux, however, transforms lipid-free apoAI into an efficient substrate for ABCG1-dependent efflux, suggesting that ABCA1 and ABCG1 might synergize to mediate cholesterol efflux to apoAI (75). Macrophages isolated from ABCG1 knockout mice display a reduction in cholesterol efflux capacity upon treatment with LXR activators (72, 75, 76). Furthermore, targeted disruption of ABCG1 in mice results in massive lipid accumulation in macrophages within lungs and multiple other tissues upon high-fat, high-cholesterol diet feeding, while overexpression of ABCG1 protects tissues from dietary induced lipid accumulation (72, 75). ABCG1 thus plays a critical role in preventing cellular lipid accumulation. Since ABCG1 is expressed by macrophage-derived foam cells in the human atherosclerotic plaque (77), it is anticipated that macrophage ABCG1 will also play an important role in atherosclerotic lesion development. To assess the role of macrophage ABCG1 in atherosclerosis, we recently generated LDLr^{-/-} mice that are selectively deficient in macrophage ABCG1 by using bone marrow transfer (78). After 12 weeks of feeding a high-cholesterol diet containing 0.25% cholesterol and 15% fat lungs of the LDLr^{-/-} mice, reconstituted with ABCG1 knockout bone marrow showed a striking accumulation of lipids in macrophages localized to the subpleural region. Furthermore, both after 6 weeks and 12 weeks of high-cholesterol diet feeding macrophage ABCG1 deficiency resulted in a moderate 33–36% increase in lesion formation. Under the same conditions, macrophage ABCA1 deficiency, however, did lead to a 2-fold increase in lesion development in LDLr^{-/-} mice after both 6 (unpublished results) and 12 weeks on the high-cholesterol diet (64), establishing that at both time points the potential effect of ABCG1 deficiency on atherosclerotic lesion formation is certainly less prominent as compared to ABCA1.

Both ABCA1 and ABCG1 belong to a large family of evolutionary conserved transmembrane proteins that use the energy of ATP hydrolysis to translocate a wide variety of substrates across cellular membranes. To date, 51 members of the family of ABC-transporters have been identified, which, based on structural similarities, have been subdivided into seven families, designated ABC A-G (59, 79, 80). A vast majority of these ABC-transporter genes are expressed by macrophages and show cholesterol influx or efflux dependent gene regulation (67, 81). It is therefore conceivable that a significant portion of the cholesterol-responsive ABC-transporters may be involved in macrophage lipid homeostasis and play pivotal roles in foam cell formation and atherogenesis.

The closest relatives of ABCA1 that are expressed by macrophages and display cholesterol-responsive regulation are ABCA2 (82), ABCA6 (83), ABCA7 (84-87), ABCA9 (88), and

ABCA10 (89). ABCA7 has recently been shown to mediate phospholipid efflux to apoAI (86, 87, 90), while also a role in cholesterol efflux has been indicated (87). Like ABCA1, ABCA7 is sensitive to LXR activation (81), and the expression is upregulated by cholesterol loading and downregulated upon cholesterol efflux to HDL (84). However, disruption of ABCA7 expression in macrophages did not affect phospholipid or cholesterol efflux to ApoAI (86). Moreover, in ABCA1-knockout macrophages, there was no detectable apoAI-stimulated phospholipid efflux, inconsistent with a residual role of ABCA7.

Also members of the ABCB-subfamily of ABC-transporters have been implicated in macrophage lipid homeostasis. ABCB1, a ubiquitous transporter which confers multidrug resistance (91) is upregulated upon differentiation from monocytes to macrophages and is highly responsive to activation by LXR agonists (81). Furthermore, increased ABCB1 mRNA was found in atherosclerotic specimens, suggesting a role for ABCB1 in atherosclerotic lesion development in vivo (92). The exact role of ABCB1 in macrophage lipid homeostasis, however is still unclear. Some studies suggest that ABCB1 mediates the esterification of plasma membrane cholesterol (93, 94), while more recently using a specific inhibitor of ABCB1 evidence was provided that the esterification of cholesterol is not correlated with ABCB1 activity (95). Recently, Le Goff et al. also showed that cholesterol efflux is increased in ABCB1 stably transfected drug-selected LLC-MDR1 cells, but not in the ABCB1-inducible HeLaMDR-Tet and 77.1 MDR-Tet cells (96). Another ABC-transporter that is expressed by macrophages is ABCB4, which plays an important role in the secretion of phospholipids into the bile (97). Interestingly, in contrast to ABCA1, expression of ABCB4 is downregulated by cholesterol loading and upregulated by cholesterol efflux (67). In addition to ABCG1 (70) and (67), also ABCG4 (98) of the ABCG subfamily of ABC-transporters shows cholesterol-responsive regulation in macrophages and promotes cholesterol efflux from cells to HDL (74). ABCG4 has been suggested to be the heterodimeric partner for ABCG1 (99). However, no effect of macrophage ABCG4 deficiency was observed on cholesterol efflux from macrophages (77). Finally, other LXR-regulated ABC-transporters expressed by macrophages include ABCB9, ABCB11, ABCC2, ABCC5, ABCD1, ABCD1, ABCD4, and ABCG2 (81). Thus although some members of the ABC-transporter superfamily might be more attractive candidates than others, currently still little is known about the exact function of most of the cholesterol-responsive ABC-transporters in macrophages in vivo and their potential relevance for the process of foam cell formation and atherosclerotic lesion development.

ABC-transporters in regulation of plasma membrane lipid asymmetry; implications for macrophage foam cell formation

Lipids in plasma membranes of eukaryotic cells are asymmetrically distributed between the inner and outer membrane leaflet (100). The choline-containing phospholipids, phosphatidylcholine (PC) and sphingomyelin (SM) are primarily located on the external leaflet, while the amine-containing glycerophospholipids, phosphatidylserine (PS) and phosphatidylethanolamine (PE) are concentrated in the internal leaflet. In addition, the minor phospholipids phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylinositol-4-monophosphate (PIP), and phosphatidylinositol-4,5-biphosphate (PIP₂) are enriched at the cytoplasmic leaflet. The generation and maintenance of membrane lipid asymmetry is mediated by the interplay of different transporters (for review (101, 102)). Two classes of ATP-dependent transporters of lipids can be distinguished: (1) the aminophospholipid translocase or “flippase”, which transports PS and PE from the outer leaflet to the inner leaflet and (2) “floppases” that transport lipids in the opposite direction from inside to outside. In circumstances of cell activation, cell injury, or programmed cell death (apoptosis) extensive remodeling of membrane phospholipids asymmetry occurs resulting in rapid egress of PS and PE to the cell surface. This is mediated by non-selective “scramblases” (103) (see Fig 3).

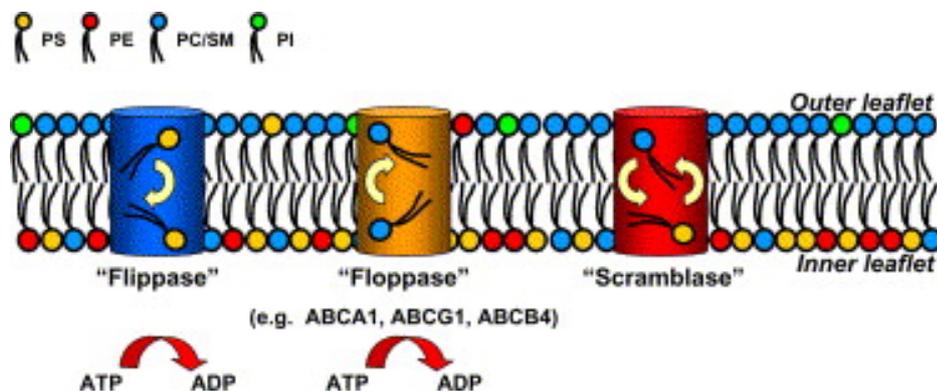


Fig. 3. Regulation of membrane asymmetry by transbilayer movement of phospholipids. Lipids in plasma membranes of eukaryotic cells are asymmetrically distributed between the inner and outer membrane leaflet. Phosphatidylcholine (PC) and sphingomyelin (SM) are primarily located on the external leaflet, while phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) are concentrated in the internal leaflet. The generation and maintenance of membrane lipid asymmetry is mediated by the interplay of different transporters, including the aminophospholipid translocase or “flippase”, which transports PS and PE from the outer leaflet to the inner leaflet and “floppases” that transport lipids in the opposite direction from inside to outside. Several members of the ABC-transporter superfamily, including ABCA1, ABCG1, and ABCB4 are recognized as “floppases”. “Scramblases”, which are activated by e.g. cell injury or programmed cell death, lead to loss of the membrane asymmetry. Adapted from Graham, T.R. (132).

Interestingly, several members of the ABC-transporter superfamily are recognized as “floppases”. ABCC1, or multi-drug resistance-associated protein MRP1, has been implicated in the active transport of PC and SM to the outer leaflet of the membrane. Inhibition of ABCC1 results in decreased amounts of BSA-extractable NBD-labelled PC and SM analogs in erythrocyte membranes (104). Furthermore, erythrocytes from ABCC1 knockout mice show enhanced accumulation of NBD-labelled PS analog (105). Interestingly, inhibition of ABCC1 for prolonged periods of time also results in significantly smaller amounts of PC and SM present in the outer leaflet of erythrocyte membranes, suggesting that ABCC1 might also be involved in the maintenance of the outward orientation of endogenous choline-containing phospholipids (104). ABCB1, a ubiquitous transporter which confers multidrug resistance, actively transports hydrophobic molecules from the inner to the outer leaflet of the plasma membrane (91). Recently, it was demonstrated that it functions as a broad-specificity “floppase” for NBD-labeled phospholipid analogs, including PC, PE, and SM (106, 107) and simple glycosphingolipids (108). ABCB4 functions as a selective “floppase” for PC (106). Mice deficient for ABCB4 develop severe damage to both the hepatocytes and the bile ducts of the liver as a result of impaired phospholipid secretion into the bile (97). Furthermore, using fibroblasts from transgenic mice expressing human ABCB4 it was confirmed that ABCB4 promotes the transfer of PC from the inner to the outer leaflet of the plasma membrane (109). Erythrocytes from mice with a homozygous disruption of ABCB1 or ABCB4 displayed reduced PC cell surface translocation, supporting a role for these transporters in natural PC translocation (110). Although both ABCB4 and ABCB1 can translocate PC, ABCB1 is unable to compensate for the absence of ABCB4 in transporting PC into the bile of ABCB4 knockout mice (97).

Several studies have shown that the levels of PS in the outer leaflet of cells are directly related to the expression level of ABCA1, providing evidence that ABCA1 functions as a PS “floppase” and pumps PS from the inner leaflet to the outer leaflet of the cellular membrane (111-113). Indeed, compared to ABCA1 wild-type mice, erythrocytes from ABCA1 knockout mice expose reduced amounts of PS after stimulation with a Ca²⁺-ionophore (111). In addition, ABCA1 mediates the efflux of PC (114). Recently, Kobayashi et al. provided evidence that in addition to PC, ABCA1 also transports SM, but that it has a preference for PC (115).

ABCG1, which facilitates cholesterol efflux to mature HDL, also transports PC (67, 74) and SM (115). In contrast to ABCA1, ABCG1 preferentially mediates the secretion of SM over PC (115). Interestingly, disruption of the ABCG1 gene in mice not only results in the tissue

accumulation of cholesterol, but also of phospholipids, while overexpression of ABCG1 results in decreased tissue phospholipid levels (73). Mutations in ABCG5 and ABCG8 cause sitosterolemia which is characterized by elevated plasma levels of phytosterols due to increased intestinal absorption and impaired biliary secretion of sterols (116). In contrast to ABC-transporters with outward directed “floppase” activity, the retina-specific ABC-transporter ABCA4 is an inward directed “flippase” that transports retinal PE derivatives (117). In the presence of an acceptor molecule, the ‘flopping’ of lipids from the inner to the outer leaflet of the membrane can result in a net flux of lipids, such as phospholipids and cholesterol, across the lipid bilayer and into the luminal space. This process of cholesterol efflux will prevent the transformation of macrophages into foam cells. On the other hand, alterations in the lipid balance across the bilayer of the plasma membrane plays a critical role in membrane budding and endocytosis (118, 119). This is a combined effect of the increase in the number of lipids in one leaflet as well as the molecular shape of the phospholipids. The headgroup and the lipid backbone of PC and PS have similar cross-sectional areas and are thus cylindrical, while PE with a small headgroup is cone-shaped (120). Transbilayer transport of lipids thus leads to a difference in the surface area between both membrane monolayers and induces bending of the membrane. In agreement, incorporation of additional amounts of PC and SM in the outer leaflet of the plasma membrane results in reduced endocytosis (121). Furthermore, transport of PS from the external to the internal leaflet of the plasma membrane by the “flippase” aminophospholipid translocase enhances endocytosis (122, 123), while disruption of this transport results in defective endocytosis (124). Interestingly, absence of ABCA1 in Tangier fibroblasts has also been associated with enhanced endocytosis, probably as a result of reduction of the surface area difference between the two membrane leaflets due to the transport of PS to the outer leaflet (125). Conversely, Alder-Baerens et al. demonstrated that overexpression of ABCA1-GFP results in reduced receptor-mediated endocytosis of fluorescent transferrin and reduced fluid-phase endocytosis (126). Increased endocytosis in absence of ABCA1 can be normalized through the addition of synthetic PS to the outer leaflet (125). Thus, ABC-transporters with “floppase” activity can reduce receptor-mediated and fluid-phase endocytosis by alteration the asymmetry of the plasma membrane lipid bilayer. Both receptor-mediated endocytosis and fluid-phase macropinocytosis are important processes involved in the induction of macrophage foam cell formation. Thus, in addition to the generally accepted role of the ABC-transporters ABCA1 and ABCG1 in prevention of foam cell formation by induction of cholesterol efflux from macrophages, these transporters are also expected to inhibit the uptake of lipids by

macrophages. In agreement, it was shown that upregulation of macrophage ABCA1 and ABCG1 by LXR activation, not only reduces macrophage foam cell formation by inducing macrophage cholesterol efflux but also by inhibiting fluid-phase macropinocytosis of LDL (127). In addition, recently we have demonstrated that specific disruption of ABCB4 in macrophages promoted macrophage foam cell formation and atherosclerotic lesion development (128). The increased foam cell formation in absence of ABCB4 was not the effect of macrophage ABCB4-deficiency on cholesterol and PC efflux, but rather of increased accumulation of modified LDL (129).

Perspectives

Current therapeutic strategies to prevent atherosclerosis are primarily based on the use of statins, inhibitors of the novo cholesterol synthesis that decrease serum LDL cholesterol levels thereby inhibiting the uptake of native and oxidatively modified LDL by macrophages in the arterial wall (130, 131). Despite the proven effectiveness of statins and their widespread use, the incidence of cardiovascular disease still remains high, indicating that there is an important need for new alternative therapies. With the discovery of the role of ABCA1 in macrophage cholesterol efflux, we have entered a new era in which the superfamily of evolutionary conserved ABC-transporters is linked to the pathogenesis of atherosclerosis. Although since the discovery of ABCA1 already several additional ABC-transporters have been implicated in macrophage cholesterol efflux, the quantitative role of these transporters in macrophage cholesterol homeostasis is still unknown. For instance, SR-BI and ABCG1 contribute for only 20% and 22%, respectively, to the transport of cholesterol from macrophages to HDL indicating that still unidentified gene products are responsible for the cholesterol efflux to HDL. The identification of additional relevant transporters for cholesterol efflux to HDL forms a short term scientific challenge. On a longer term the modulation of ABC-transporters might lead to new therapeutic approaches which will lead to regression of (advanced) atherosclerotic lesions with potential beneficial effects for treatment of cardiovascular disease.

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