ABCA1-Deficient Mice
Insights Into the Role of Monocyte Lipid Efflux in HDL Formation
and Inflammation

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Abstract—Studies with ATP-binding cassette transporter (ABCA1)–deficient mice have been critical in demonstrating the relation between ABCA1 expression, cellular lipid efflux, and HDL metabolism. The phenotype of the ABCA1-deficient mouse parallels the phenotype observed in human Tangier disease, including substantial reductions in both apolipoprotein B and apolipoprotein AI with confounding affects on atherosclerosis. (Arterioscler Thromb Vasc Biol. 2003;23:972-980.)

Key Words: Tangier disease ■ ATP-binding cassette transporter A1 ■ cholesterol efflux ■ HDL ■ macrophages ■ atherosclerosis

Two distinct strains of ATP-binding cassette transporter (ABCA1)–deficient mice have been generated by using homologous recombination. McNeish et al1 used a targeting construct to replace exons 17 to 22 that encode the first nucleotide-binding fold of ABCA1. Target cell lines from DBA/1J embryonic stem cells were microinjected into C57BL/6 blastocysts, and the resulting chimeric mice were back-crossed to DBA/1J mice. Christiansen-Weber et al2 used an 8.5-kb mouse DNA fragment containing exons 18 to 22 of the ABCA1 gene to prepare the knockout (KO) construct, in which there was a disruption of the 3 exons encoding the N-terminal region of the protein. Gene targeting was induced in E14 embryonic stem cells and established on a pure C57BL/6 background. Additional strains of ABCA1-deficient mice have been generated by crossing ABCA1-deficient mice on a DBA/1J background with both apolipoprotein E–deficient mice and LDL receptor (LDLR)–deficient mice on C57BL/6 backgrounds.3 Differences in the extent and severity of lipid accumulation in various tissues of these animals have been reported, suggesting that the genetic background of the mice greatly influences the phenotype.1-4 However, the absence of HDL in the ABCA1-deficient homozygous mice is a phenotype common to all strains.

Reproduction
Homozygous female ABCA1-deficient mice are difficult to breed, have a marked reduction in the number of pregnancies, and produce small litter sizes.1 Because the uptake of HDL is the primary pathway by which steroidogenic tissues in mice acquire cholesterol,5,6 it is likely that the reductions in litter size and number of pregnancies may be attributed to altered steroidogenesis associated with a reduction in both estrogen and progesterone levels in pregnant females and subsequent malformation of the placenta.2 In support of this mechanism,
cholesteryl ester levels are decreased in the adrenals and ovarian tissues of ABCA1-deficient mice, and lipid staining of the steroidogenic zone of the adrenal cortex is also reduced. The reduced cholesteryl ester storage in the steroidogenic tissues of the ABCA1-deficient mouse may indicate a phenotype similar to that of the apoAI and scavenger receptor, class B, type-1 (SR-B1) KO models. Our observations, as well as those of Christiansen-Weber et al., contrast reports from Orso et al., who described increased lipid staining in the adrenals from KO mice.

Homozygous ABCA1-deficient pups produced by mating of ABCA1-deficient males and females were found to be less likely to survive to weaning at 3 weeks of age. Therefore, deviations from mendelian inheritance were observed at weaning in both the C57BL/6 strain generated by Christiansen-Weber et al. and in the DBA/1J strain generated by McNeish et al., consistent with a perinatal lethality in ABCA1-deficient pups. Necropsy of these pups indicated that their lungs were congested with blood, accompanied by extensive bronchopulmonary dysplasia, consistent with severe pulmonary distress. However, ABCA1-deficient mice that survived to weaning appeared to develop normally and matured into apparently healthy adults. As shown in Figure 1, both the survival rate and body weight gains after weaning of the homozygous ABCA1-deficient mice paralleled those of control mice. In contrast, Orso et al. reported a decreased survival rate and reduced body weight in these mice, possibly related to deficiencies in vitamin absorption and platelet aggregation, as well as severe pathologies of the small intestine, which were not observed in mice from McNeish et al. or Christiansen-Weber et al. Because the strain of mouse is identical to that reported by McNeish et al., the reason for the observed differences is unclear and remains to be determined.

In addition to background strain differences, dietary differences have been implicated as possible explanations for a number of variations in phenotypes observed among laboratories. Orso et al. reported differences in a number of pathological findings when mice were fed the Altromin 1324 diet compared with mice fed a Formullab 5008 diet rich in fat and vitamins (Christiansen-Weber et al.). We have fed ABCA1-KO mice on a DBA1J background both a standard Purina Rodent Chow 5001 and a Western-type diet high in fat and cholesterol and observed no differences in survival or overall phenotype. Feeding the Western-type diet to hypercholesterolemic homozygous ABCA1-/-apoE-/- and ABCA1-/-/LDLR-/- deficient mice exacerbated the pruritic ulcerative dermal lesions observed in these animals, making it difficult to maintain these mice for extended periods of time.

**Role in HDL Biosynthesis**

From its primary sequence, ABCA1 appears to be a pore-forming protein consisting of 6 + 6 transmembrane domains connected by a hydrophobic segment. X-ray crystallographic analysis of MsbA, a bacterial homologue of a multidrug-resistance transporter from *Escherichia coli*, suggests that the transporter has a central "chamber," easily accessible by substrates from the inner leaflet of the membrane while excluding substrates from the outer leaflet. How these structural segments form the postulated transmembrane "pore" of ABCA1 and determine substrate specificity remains to be determined. Natural mutations in ABCA1 support the hypothesis that this transporter promotes unidirectional efflux of cholesterol and phospholipids from cell membranes to apoproteins; however, its substrate specificity has not been defined. It has been proposed that ABCA1 facilitates the efflux of phospholipids, mostly phosphatidylcholine, toward nascent apolipoproteins. These apolipoproteins can then accept cholesterol, forming a lipid-poor particle and thereby triggering the maturation and formation of HDL. Whether phospholipids and cholesterol are transported simultaneously or sequentially by the same or independent mechanisms remains unclear. Several apolipoproteins have been shown to mediate phospholipid and cholesterol efflux by the ABCA1 pathway. ApoAI, the most abundant and most-studied apoprotein on HDL, shows the greatest ability to efflux cholesterol from macrophages by the ABCA1 pathway. All exchangeable apolipoproteins, including AI, AIV, CI, CII, CIII, and E, promote ABCA1-dependent efflux. It has been postulated that the minimum critical domain required for interaction of apoprotein-containing acceptor particles with ABCA1 and subsequent lipid efflux is the presence of amphipathic helices. In contrast, no stimulation of efflux is observed when cells are incubated with apoprotein-free acceptors, such as 2-OH-β-cyclodextrin, small unilamellar vesicles, or bile acid micelles, further indicating that an apoprotein-specific acceptor is required. The physiological relevance and relative contribution of various apoproteins to cholesterol efflux and HDL synthesis remain unclear. How-
ever, it is likely that the overall level of lipid efflux to specific apoprotein-containing particles depends on the relative concentration of apoproteins in the extracellular fluid rather than in the plasma, and this is determined by the rate of synthesis, catabolism, and cycles of dissociation and reassociation with lipoproteins in the plasma compartment.

Studies in ABCA1-deficient mice have demonstrated a critical role of ABCA1 in the biogenesis of HDL. In these models, the absence of ABCA1 resulted in almost undetectable levels of HDL, implying that the interaction of lipid-poor apolipoproteins with the plasma membrane of cells is the first and most critical step in the biogenesis of HDL.1-21,22 This association also suggests that the synthesis of HDL requires at least 2 well-defined steps. In the first step, apoAI, in addition to other apoproteins, is lipidated through the actions of ABCA1. This process most likely occurs in the interstitial space and leads to the formation of lipid-poor, nascent HDL particles, also known as pre-β-HDLs. These nascent HDLs are transformed into large-size HDL particles with α-mobility by lecithin:cholesterol acyltransferase. Subsequent maturation and formation of larger HDL species are dependent on the interaction and continuous remodeling induced by various plasma proteins and surface receptors, resulting in the structural heterogeneity of circulating HDLs. Compositional analysis of plasma lipoproteins suggests that the absence of ABCA1 leads to profound changes in the phospholipid composition of HDL that, in turn, alters its metabolic stability and maturation, leading to the virtual absence of HDL in the plasma of deficient animals. Despite ubiquitous expression of ABCA1 in these animals,23 the accumulation of cholesterol observed in Tangier disease or in ABCA1-deficient mice appears to be restricted primarily to the macrophage,24-26 raising the intriguing question of whether the efflux of lipids from macrophages significantly contributes to the systemic levels of HDL. Studies of mice expressing ABCA1 in macrophages or with selected inactivation in macrophages demonstrated that monocyte/macrophage ABCA1 contributes to HDL formation; however, the contribution is minimal.27 Very recently, several laboratories reported the generation of mice overexpressing ABCA128-30 and demonstrated a general related dose responsiveness of HDL cholesterol, supporting the hypothesis that ABCA1 plays a critical role in the generation of HDL. Mice overexpressing ABCA1 in hepatocytes and macrophages also show significant increases in plasma HDL cholesterol. Because macrophages have a minimal contribution to HDL levels,27 these findings suggest that hepatocytes contribute significantly to the biogenesis of the majority of HDL present in the plasma compartment, suggesting that the liver is a major source of circulating HDL. Additional studies of mice with either targeted inactivation or overexpression of ABCA1 in the liver are required to confirm these findings and determine the relative contribution of various cell types and tissues to HDL.

**Foam Cell Accumulation**

Inactivation of ABCA1 in mice produces a phenotype similar to that observed in humans with Tangier disease, in which there is lipid accumulation that occurs predominantly in tissues with high cell turnover and large resident populations of macrophages. The phenotype of these mice is both strain and age dependent. Christiansen-Weber et al2 identified macrophages in the testes, thymus, liver, and placenta as the primary cell types affected in an ABCA1-deficient C57bBL/6 strain. The main pathological finding in the ABCA1-deficient DBA/1J strain reported by McNeish et al1 was limited to lipid accumulation in the lung, consistent with accumulations of foamy intra-alveolar macrophages and type II pneumocytes. Ultrastructurally, the alveolar macrophages contained lipid and cell debris, whereas the type II pneumocytes had multiple intracytoplasmic vacuoles containing lamellar material and amorphous lipid that was considered consistent with “aberrant unsecreted lamellar bodies.” In comparison, when ABCA1-deficient mice on a DBA/1J background were crossed with either hypercholesterolemic apoE- or LDLR-deficient mice, tissue accumulation of lipid-laden macrophages became widespread.3 The skin and the uterus were most severely affected, but foam cells were also consistently present in lung, lymph nodes, stomach, kidney, and liver and were also occasionally observed in the thymus, pancreas, mesentery, prostate, ovary, large and small intestine, urinary bladder, lymphatic vessels, spleen, and periodontal tissue. Based on the large numbers of organs infiltrated by these foam cells over time, it is likely that these foamy macrophages could be found in any organ in which an inflammatory reaction is present.

In ABCA1-deficient mice crossed to apoE-deficient mice, the severity and distribution of the cellular infiltrates were correlated with the age of the animals, possibly accounting for some of the differences in phenotype reported among
laboratories. Microscopically, these lesions were correlated with the accumulations of mononucleated and rare multinucleated foam cells containing occasional intracytoplasmic acicular clefts (cholesterol clefts). Histologically, the foam cells were positively stained for lipid by oil red O and resembled macrophages, possessing round and occasionally a spindle-shaped cytoplasm with 1 or more nuclei, 7 to 10 μm in diameter. Ultrastructurally, these cells contained a myriad of emptied intracytoplasmic vacuoles (Figure 2). Occasionally, the vacuoles contained a partially electron-dense material consistent with lipid. It is likely that the lipids from the other vacuoles were lost during processing of the tissue for electron microscopy. In addition to these vacuoles, there was also an increase in round, electron-dense material in the cytoplasm of the cells, consistent with secondary lysosomes. Analysis by immunohistochemistry demonstrated positive staining of these foam cells for 2 macrophage markers, F4/80 and lysozyme. Like macrophages, the foam cells infiltrated multiple tissues. Moreover, in tissues with large resident populations of macrophages, such as the liver, these foam cells were found lining the surface of the sinusoidal spaces consistent with Kupffer cells. Of particular interest, however, is the accumulation of these cells in focal areas in tissues not generally associated with monocyte infiltration. For example, in the kidney, the foam cells were mostly located in the interstitium from the upper third of the medulla. In the stomach, the foam cells were consistently located in the lamina propria of the squamous mucosa at the squamous-glandular gastric junction.

The reason why macrophages lacking ABCA1 target and accumulate in these specific areas of tissues is unclear. van Eck et al reported increased macrophage infiltration in a number of tissues, including the vessel wall and peritoneal cavity, in LDLR-deficient mice repopulated with macrophages from ABCA1-deficient mice. Although slight increases in the number of progenitor cells have been observed, ABCA1 expression does not seem to be a result of increased hematopoiesis or circulating monocyte numbers (Table 1). However, in the hypercholesterolemic strains, circulating monocytes, as well as neutrophils and lymphocytes, are increased. The increased circulating leukocyte numbers may be the result of inflammation present at different sites and organs (skin) and not a primary response resulting from ABCA1 deficiency. The hypercholes-

terolemic ABCA1-deficient mouse may prove to be a valuable tool to unravel the sequence of signals responsible and required for recruiting monocytes to specific and focal regions of tissues, a process that is the hallmark of atherosclerosis and other inflammatory diseases.

Because the most extensive foam cell accumulation occurred in hypercholesterolemic ABCA1-deficient mice, it stands to reason that the hypercholesterolemia exacerbated the foam cell accumulation. For example, extensive foam cell accumulation, xanthomatosis, and deposition of unesterified cholesterol occur in other murine KO models, such as acyl coenzyme A:cholesterol O-acyltransferase–deficient mice that exhibit severe hypercholesterolemia as a result of either apoE or LDLR deficiency. However, it is interesting to note that feeding ABCA1-deficient mice on a DBA/1J background a high-fat diet for 20 weeks could not reproduce the phenotype observed in the double-KO mouse described above, and more extensive foam cell accumulation was observed in the very young ABCA1-deficient mice on a C57BL/6 background compared with ABCA1-deficient mice on a DBA/1J background. Thus, other genetic factors present in the ABCA1-deficient mice on a C57BL/6 background, in addition to hypercholesterolemia, may be necessary to capitate the phenotype observed in Tangier patients, and these models may provide additional insight as to why some Tangier patients develop atherosclerosis while others develop splenomegaly.

Development of Atherosclerosis

Despite massive cholesteryl ester accumulation in their peripheral tissues and a nearly complete absence of HDL and apoAI, the incidence of clinically relevant vascular disease observed in homozygous Tangier patients is increased only 4-fold. This apparent paradox is reinforced by recent studies from our laboratory demonstrating that atherosclerotic lesions were not found in ABCA1-deficient mice on a DBA/1J background fed either a chow or an atherogenic diet for several months. Moreover, the absence of ABCA1 did not increase the development, progression, or composition of atherosclerosis in ABCA1-deficient mice crossed to either hypercholesterolemic apoE- or LDLR-deficient mice. Interestingly, several years ago, Poernama et al also found no apparent increase in atherosclerosis in the Wisconsin Hypo
Alpha Mutant (WHAM) chicken, a model characterized by low plasma HDL and apoA1 levels due to a naturally occurring mutation in ABCA1.

Although a complete absence of ABCA1 is not always associated with increases in atherosclerosis, the accumulation of cholesteryl ester–rich foam cells in other tissues is a common finding in ABCA1 deficiency in humans and ABCA1-deficient mice. Thus, because monocyte infiltration and the accumulation of cholesteryl esters in arterial cells are the hallmarks of atherosclerosis, it was surprising that massive foam cell formation occurred in certain tissues, such as the skin and uterus, while there was no effect on atherosclerosis.

Oram proposed that the source of cholesterol resulting in the accumulation of cholesteryl esters in macrophages might differ between the vessel wall and other peripheral tissues. Macrophages infiltrate most tissues to dispose of apoptotic and necrotic cells, suggesting that a major source of cholesterol for peripheral tissue macrophages is likely to be derived from phagocytized cell membranes. Conversely, lipoproteins may be the major source of cholesterol in arterial macrophages, and therefore, a reduction in atherogenic lipoproteins may have a greater affect on lesional macrophages than on macrophages in other tissues. In ABCA1-deficient mice, as in homozygous Tangier patients, plasma apoB-containing lipoprotein levels are 40% of normal. Approximately 50% reductions in plasma apoB-containing lipoproteins are also seen in WHAM chickens. Thus, it has been suggested that any proatherogenic effect that might be anticipated to result from an ABCA1 deficiency in Tangier patients and in the ABCA1-deficient mouse would be compensated for by a less atherogenic lipid profile. Recent findings by 2 separate laboratories using bone marrow from donor ABCA1-deficient mice transplanted into either apoE- or LDLR-deficient mice support this suggestion, demonstrating that without changes in the levels of apoB-containing lipoproteins, ABCA1 deficiencies will markedly increase atherosclerosis.

Studies on bone marrow transplanted from ABCA1-deficient mice into normal mice have demonstrated that ABCA1-mediated efflux from macrophages was markedly decreased while plasma lipoprotein levels remained unchanged. Similar results were obtained when bone marrow was transplanted from ABCA1-deficient mice into either the apoE- or the LDLR-deficient mouse support this suggestion, demonstrating that without changes in the levels of apoB-containing lipoproteins, ABCA1 deficiencies will markedly increase atherosclerosis.

Cholesterol Absorption
The findings by Repa et al., showing that the association between retinoic X receptor (RXR) and liver X receptor (LXR) agonists results in dramatic increases in ABCA1 expression and reductions in cholesterol absorption, have sparked a debate about whether ABCA1 plays a role in cholesterol absorption. Several conflicting reports have led to this controversy, including disparate results obtained in ABCA1-deficient mice, differences reported in the cell-specific location and subcellular distribution of ABCA1, and the finding that other ABC transporters are also regulated by LXR and RXR ligands.

A role for ABCA1 in the intestines was not considered until the discovery that mice with targeted disruption of ABCA1 showed increases in cholesterol absorption from the.

lesterol ester. It may be that the inability of monocytes to regulate their intracellular cholesterol levels results in a particular subtype of "proinflammatory monocyte" available for recruitment into the vessel wall. Distinct macrophage subtypes have been identified, which respond differently to proinflammatory stimuli and express different integrins and chemotactic receptors. For example, Han et al. very eloquently demonstrated that the effect of LDL on the MCP-1 receptor (CCR2) expression was caused by increases in cellular levels of cholesterol, possibly as the result of sterol-regulatory element-like sequences in the 5'untranslated region of the CCR2 gene. Functional abnormalities such as altered lipid metabolism and altered monocyte function have been observed in animal models and in hypercholesterolemic patients. Peritoneal macrophages isolated from ABCA1-deficient mice with an elevated cholesteryl ester content showed increased responsiveness to chemotactic factors such as monocyte chemoattractant protein-1 (authors' unpublished observations). This response has been seen in ABCA1-deficient mice crossed with either control or hypercholesterolemic mice; however, mice bred on the normal cholesterol-emic background remained resistant to atherosclerosis, despite an accumulation of cholesteryl ester–loaded, macrophage-like cells in several tissues.

The site of the observed foam cell accumulation noted previously has differed between laboratories, which may relate to the background strains of the mice. This suggests that in addition to the primary mechanisms that are altered in response to ABCA1 deficiencies, other genetic components determine the combinatorial array of receptors and chemotactic gradients required for leukocytes to navigate from plasma to specific tissues, such as the arterial wall. Pagien and others have identified a number of mouse strain differences in their susceptibility to atherosclerosis. It is therefore possible that some of these genetic differences can either exacerbate or attenuate the effects of ABCA1 deletion. Regardless of the mechanism, it is clear that deletion of leukocyte ABCA1 in the absence of changes in plasma lipids has a pronounced effect on atherosclerosis. The ABCA1-deficient mouse has therefore provided a valid explanation for the lower-than-expected incidence of atherosclerosis in Tangier patients, in whom there is also a clear decrease in levels of both HDL and LDL.
The potential role of ABCA1 in the transport of cholesterol from the enterocyte to the lumen of the small intestines would require that ABCA1 protein be present in the apical membrane of the enterocyte. ABCA1 protein is present in the enterocyte and is most abundant in the villus lining the epithelium and persists throughout the lifetime of the epithelial cell.51,52 Enterocytes from ABCA1-deficient mice were also found to have ultrastructural abnormalities, consisting of hyperplasia and dilation of the Golgi apparatus associated with reduced numbers of mitochondria. Nuefeld et al53 suggested that ABCA1 cycles between the plasma membrane and endosome. Recent observations, however, have challenged the hypothesis that ABCA1 regulates cholesterol absorption by acting at the apical membrane. Murphy et al54 demonstrated that LXR/RXR activation enhances basolateral but not apical efflux of cholesterol in CaCo2 cells. Finally, Lawn et al51 found that ABCA1 mRNA levels colocalized with macrophages in the intestinal villi, suggesting that ABCA1 in the macrophages, not epithelial cells, would act as a barrier to cholesterol absorption. Studies designed to fully establish the conditions under which ABCA1 expression is modulated and effective antibodies to ABCA1 will be required to establish the subcellular localization of ABCA1 and the role of intestinal ABCA1.

Differences in cholesterol absorption have been noted in ABCA1-deficient mice, and the absolute effects of ABCA1 deficiency on cholesterol absorptions remain unsettled. For example, McNeil et al1 used the fecal ratio of sestanol and cholesterol as a measure of cholesterol absorption to generate an increase in cholesterol absorption in ABCA1-deficient mice compared with wild-type DBA/1J mice, a result consistent with the findings of Repa et al.49 Groen et al55 reported no change in fecal neutral-sterol output, biliary secretion of bile salts, or cholesterol levels in the same DBA/1J strain of ABCA1-deficient mice. In contrast, Drobnik et al4 reported that fecal sterol output was increased owing to decreased cholesterol absorption in the C57BL/6 strain of ABCA1-deficient mice compared with the wild-type strain. Because ABCA1 is thought to be a phospholipid transporter, these researchers suggested that the decrease in cholesterol absorption and increase in fecal neutral-sterol output were the result of decreased biliary phospholipid secretion. This phenotype is similar to that observed in mice deficient in Mdr2,56,57 a multidrug resistance p-glycoprotein that belongs to the ABC transporter family.58 Although background strains have measurable differences in cholesterol absorption rates and methodologies have been used to explain some of these differences, these data would suggest that if ABCA1 expression affects cholesterol absorption, then it might play a minimal role.

The increasing evidence that ABCA1 is a phospholipid transporter has further questioned the hypothesis that ABCA1 is involved in cholesterol efflux from the apical membrane of the enterocyte. Moreover, recent findings have provided a more plausible explanation for the LXR-mediated decrease in cholesterol absorption. The ABCG5/8 heterodimer has been shown to be responsible for decreased plant sterol absorption and effects on cholesterol absorption. In addition, the transporters ABCG5 and ABCG8,59–61 as well as several other ABC transporters of unknown function, such as ABCA2, ABCA6, and ABCA7, are also induced by cholesterol feeding.62–66 Plosch et al67 found that inactivation of LXR with a synthetic LXR ligand resulted in increased biliary and fecal excretion of cholesterol equally in wild-type and ABCA1-deficient mice. Thus, the underlying mechanism for the changes in cholesterol absorption due to LXR activation appear to be independent of ABCA1. At present, the function of intestinal ABCA1 is still an important pharmacological issue and remains to be resolved. Based on studies in ABCA1-deficient mice, if ABCA1 does facilitate or inhibit cholesterol absorption, it appears to be limited to a small percentage of the total fraction absorbed.

**ApoB Levels and ABCA1 Deficiency**

Since the linkage between ABCA1 and Tangier disease was made,68–70 the evidence that ABCA1 plays an essential role in cellular lipid efflux and HDL metabolism is quite convincing. Although studies have begun to decipher the mechanism by which ABCA1 deficiency causes a reduction in HDL, the effect of ABCA1 expression and/or cholesterol efflux on the levels of apoB-containing lipoproteins remains uncertain. Differences in apoB production rates, apoB clearance rates, and hepatic lipid output have not fully explained the reduction in apoB-containing lipoproteins in Tangier patients,24,71 in the WHAM38,40 chicken, or in hypercholesterolemic ABCA1-deficient mice.3 At present, there is no evidence that reduced hepatic output resulting from reduced HDL-mediated return of cholesterol from the periphery to the liver accounts for these changes. Groen et al55 used a state-of-the-art Mass Isotopomer distribution analysis (MIDA) to demonstrate that there was no difference in total body cholesterol fluxes among homozygous and/or heterozygous ABCA1-deficient mice and wild-type mice. These surprising data suggest that the supply of cholesterol to the liver is not affected in the absence of HDL in ABCA1-deficient mice. Results of measurements of endogenous hepatic cholesterol production, reflected by changes in the expression of genes controlling the key steps in cholesterol biosynthesis, are inconclusive and differ among laboratories.1,4,55 Groen et al55 also reported that genes encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase, the LDLR, or sterile response element binding protein (SREBP2) were unchanged, whereas McNeil et al1 and Drobnik et al4 reported decreases in 3-hydroxy-3-methylglutaryl coenzyme A reductase levels. Clearly, gene expression does not exclude changes in enzyme activities. We recently observed that in ABCA1-deficient mice crossed to apoE-deficient mice, there was no difference in the incorpo-
ration of $[^{14}]$Cacetate in liver cholesterol and no difference in VLDL output measured by using Triton WR1339 methodologies and $[^{32}]$P clearance of LDL cholesterol (authors’ unpublished observations). Similarly, Vaisman et al. demonstrated that after injection of Triton WR1339 and $[^{35}]$Smethionine, increases in plasma apoB levels in human ABCA1-transgenic mice were not due to increased hepatic apoB secretion. Two major pathways for apoB lipoprotein clearance, the LDLR and apoE receptor, can also be excluded because reductions in apoB are found in ABCA1-deficient mice crossed to either apoE- or LDLR-deficient mice. This information leads to 2 questions: (1) Is ABCA1 lipid-mediated efflux required for the lipidation of apoB-containing lipoproteins to prevent altered metabolism in a hyperlipidemic setting, or (2) is the observed reduction in apoB lipoprotein clearance by an alternative pathway independent of LDL, such as the LDLR-related protein? Clearly, additional work is needed to fully explain the mechanism by which ABCA1 affects apoB-containing lipoprotein levels, as they may have major implications in the design of pharmaceuticals intending to upregulate ABCA1 as a means of increasing HDL levels and reducing atherosclerosis.

**Macrophage ABCA1 Expression and Future Directions**

To determine the influence of macrophage ABCA1 on plasma lipid levels and atherosclerosis, several laboratories have carried out bone marrow transplantation studies between wild-type and ABCA1-deficient mice, with each mouse serving as donor or recipient. Results from these studies demonstrated that foam cell accumulation and atherosclerosis are significantly influenced by the expression of macrophage ABCA1. Thus, it appears that ABCA1-deficient monocytes are being directed to, recruited by, and retained in tissues typical of an inflammatory response. This suggests that there is a dual function for ABCA1 in both lipid metabolism and inflammation. However, the ABCA1-deficient mouse may be providing us with additional evidence that the processes of HDL metabolism and inflammation are closely linked.

**References**


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