Atherosclerosis and Lipoproteins

Increased Atherosclerosis in Hyperlipidemic Mice With Inactivation of ABCA1 in Macrophages

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Abstract—The ATP-binding cassette transporter A1 (ABCA1) encodes a membrane protein that promotes cholesterol and phospholipid efflux from cells. Mutations in ABCA1 lead to HDL deficiency and tissue accumulation of macrophages in patients with homozygous Tangier disease. In this study, we examined whether the complete absence of ABCA1 or selected inactivation in macrophages is accompanied by an increase in atherosclerotic lesion progression in hypercholesterolemic apolipoprotein E–deficient (apoE–/–) mice and LDLR receptor–deficient (LDLR0/–) mice. The absence of ABCA1 led to reduced plasma cholesterol levels in both the apoE–/– and LDLR0/– mice, along with severe skin xanthomatosis characterized by marked foamy macrophages and cholesterol ester accumulation. However, the complete absence of ABCA1 did not affect the development, progression, or composition of atherosclerotic lesions in either the LDLR0/– or the apoE–/– mice fed a chow or atherogenic diet. In contrast, bone marrow transplantation studies demonstrated that the selective inactivation of ABCA1 in macrophages markedly increased atherosclerosis and foam cell accumulation in apoE–/–. Taken together, these findings demonstrate that the complete absence of ABCA1 has a major impact on plasma lipoprotein homeostasis, and the proposed antiatherogenic effect resulting from ABCA1 deficiency is compensated by a less atherogenic profile. ABCA1 deficiency in macrophages, however, demonstrates the antiatherogenic properties of ABCA1 independent of plasma lipids and HDL levels. (Arterioscler Thromb Vasc Biol. 2002;22:630-637.)

Key Words: ABCA1 • atherosclerosis • xanthomatosis • HDL

The ATP-binding cassette (ABC) transporter superfamily contains membrane proteins that use ATP as a source of energy to translocate a variety of substrates across extra- and intra-cellular membranes.1 ABCs are defined by the presence of nucleotide-binding domains containing two conserved peptide motifs known as walker A and walker B, with a unique signature between the two walker motifs. Genetic variation in these genes is the cause of, or contributor to, a wide variety of human disorders with Mendelian and complex inheritance, including cystic fibrosis, neurological disease, retinal degeneration, cholesterol, and bile transport defects, anemia, and drug response.2

ABCA1, a member of the ABCA subfamily, has near-ubiquitous expression in different mammalian tissues and cell types.3,4 This transporter is particularly abundant in macrophages, where its expression is tightly controlled by intracellular cholesterol levels.5 In addition, its activity is increased by protein kinases and is modulated at the transcriptional level by several metabolites including cAMP,6 cis-retinoic acid,6 peroxisomal proliferator-activated receptor,7,8 interferon γ,9 and oxysterol ligands for LXR.10,11 Several functions have been attributed to ABCA1 since its discovery. It has been implicated in the engulfment of apoptotic cells and11 secretion of leaderless proteins such as interleukin-1β13 and as an electroneutral anion exchanger.14 More recently, ABCA1 has been shown to be mutated in patients with Tangier disease.15–17 In vivo models with either natural mutations18 or targeted inactivation of ABCA119 have confirmed these findings and demonstrated the pivotal role of ABCA1 in the trafficking of lipids, biogenesis of HDL, and overall cholesterol homeostasis as demonstrated by the deposition of cholesterol in tissue macrophages.

Since the discovery of the inverse relationship between HDL levels and coronary artery disease, modification of HDL metabolism and ABCA1 expression in particular have been considered potential targets for therapeutic interventions to prevent atherosclerosis. Tangier disease patients have an increased risk of coronary artery disease20,21 but not as dramatic as one would expect in individuals with an almost complete absence of HDL. It is possible that Tangier patients are partially protected from the development of atherosclerosis because of low levels of circulating LDL.22

The aim of this study was to examine the pathophysiological consequences of ABCA1 deletion in two mouse models of atherosclerosis in the setting of severe hypercholesterolemia. The ABCA1-deficient mice on a DBA1/J background...
were crossbred to apolipoprotein E–deficient (apoE<sup>−/−</sup>) mice and LDLR receptor–deficient (LDLr<sup>−/−</sup>) mice. The findings from these studies revealed that the complete absence of ABCA1 led to plasma lipid reductions and severe foam cell accumulation in the skin and uterus without affecting the development of atherosclerosis. In contrast, specific inactivation of ABCA1 in monocytes markedly increased the development of atherosclerosis in apoE<sup>−/−</sup> mice without altering plasma cholesterol homeostasis.

**Methods**

**Animals and Diets**

ApoE<sup>−/−</sup> mice, backcrossed to C57BL6 for 10 generations, and ABCA1<sup>+/−</sup> mice in a DBA1/J inbred background, were mated, and the double heterozygous offspring were intercrossed. The resulting apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> offspring were mated to produce apoE<sup>−/−</sup> and apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice. LDLr<sup>−/−</sup> mice backcrossed to C57BL6 for 9 generations were mated to ABCA1<sup>−/−</sup> mice, and double heterozygous were mated to produce LDLr<sup>−/−</sup> and LDLr<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice. Genotypes were determined by using polymerase chain reaction analysis. Mice were maintained on a 12-hour light/dark cycle and fed a rodent chow diet (Purine Prolab RMH 3000 rodent diet) or Western-type diet (HFHC) containing 0.15% cholesterol and 20% a rodent chow diet.

**Tissue Preparation and Lipid Staining**

Mice were anesthetized with ketamine:xylazine:PBS (1:1:2) and perfusion-fixed with 4% paraformaldehyde. Mice were euthanized by exsanguination under anesthesia. After removal and fixation in 10% neutral-buffered formalin, tissues were infiltrated with 30% gum sucrose for 24 hours at 4°C, embedded in Tissue-Tek OCT compound (Sakura Finetek), cross-sectioned (10 μm) at −20°C, stained for lipids with Oil red O (Polyscientific), and counterstained with Gill III hematoxylin (Sigma). Additional samples of these tissues were post-perfusion fixed in formalin and embedded in paraffin for immunohistochemical analysis. Cholesterol and cholesteryl ester content of skin was determined in 1-cm² samples taken from the back of the neck. Skin samples from the apoE<sup>−/−</sup> and ABCA1<sup>−/−</sup> mice were mostly macroscopically involved, as shown in Figure 1A, compared with skin samples from littermates. Samples were 10-minute incubation with Dako streptavidin-horseradish peroxidase, extracted by using chloroform:mehanol (2:1) as described by Folch et al. Cholesterol and cholesteryl esters were quantified on a gas chromatograph (Hewlett-Packard) and expressed as μg/mg of tissue.

**Immunohistochemistry**

Rat IgG1 (Serotec) anti-F4/80 and rabbit IgG (Dako) anti-lysozyme antibodies were used as macrophage markers while a mouse IgG2a anti–smooth muscle actin (Dako) antibody was used as a specific marker for actin in formalin-fixed, paraffin-embedded sections. For F4/80, sections were blocked with 10% normal rabbit serum, incubated with the anti-F4/80 antibody followed by incubation with a peroxidase-labeled rabbit anti-rat IgG antibody (Vector). Sections were then incubated with the Elite ABC kit (Vector) for 30 minutes, stained with the Dako Liquid DAB for 5 minutes, and counterstained with hematoxylin. For smooth muscle actin, sections were deparaffinized, rehydrated, and steamed for 20 minutes in Citra pH 6 buffer (Biogenex). Mouse IgG2a anti–smooth muscle actin (Dako) was mixed with the biotinylated anti-mouse IgG2a antibody and mouse serum per the ARK kit instructions (Dako). Sections were incubated with the mixture for 1 hour at room temperature followed by a 45-minute incubation with Dako streptavidin-horseradish peroxidase, stained with DAB, and counterstained with hematoxylin. For lysozymes, sections were incubated with proteinase K (Dako), blocked with 10% normal goat serum, and incubated with a rabbit IgG (Dako) anti-lysozyme antibody diluted 1:200 in PBS mixed with 1% normal goat serum, followed by incubation with peroxidase-labeled goat anti-rabbit IgG antibody (Vector) diluted 1:150 in PBS mixed with 1% normal goat serum at room temperature. The sections were visualized with the Vector Elite ABC kit DAB substrate stained with Dako Liquid DAB and counterstained with hematoxylin. Tissue sections incubated without primary antibody and the appropriate IgG isotypes were used as negative control.

**Aortic Tree Analysis**

Mice were anesthetized with ketamine:xylazine:PBS (1:1:2) and perfusion-fixed with 4% paraformaldehyde. The entire aortic tree was removed, cleaned of adventitia, and laid out on a piece of polystyrene, and a digital image was obtained with a digital camera (Sony). The percent aortic surface covered by lesions was determined by using an en face preparation as previously described.

**Atherosclerotic Lesion Analysis**

To determine cross-sectional lesion area, hearts were perfusion-fixed in 4% paraformaldehyde, infiltrated with 30% gum sucrose for 24 hours at 4°C, and embedded in OCT compound. Tissues were sectioned (10 μm, −18°C) as previously described, stained with Oil red O, and counterstained with hematoxylin. Results are expressed as the average lesion size per section or as the percent of the total cross sectional vessel wall stained with Oil red O as previously described. For each animal, the average lesion area of 12 to 16 sections was determined, and data are expressed as lesion size or mean percent lesion area ± SD.

**Bone Marrow Transplantation**

Recipient female apoE<sup>−/−</sup> mice were divided into two groups of mice at 12 weeks of age. Bone marrow was harvested from male apoE<sup>−/−</sup>
mice and apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> littermates and transplanted into lethally irradiated recipient mice as previously described. Twelve weeks after transplantation, animals were sacrificed, and tissues were collected. Repopulation with donor bone marrow was confirmed by the presence of the ABCA1<sup>−/−</sup> allele and chromosome quantitation in the DNA harvested from bone marrow and peritoneal macrophages by using polymerase chain reaction analysis as previously described.

**Plasma Lipids and Lipoprotein Analysis**

Plasma samples were isolated from blood collected either retro-orbitally or by terminal bleeds. Cholesterol, triglyceride, and phospholipid levels were determined by using enzymatic colorimetric assays (Wako Biochemicals). Apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB) were determined by using ELISA as previously described. Lipoproteins were isolated from pooled plasma from six apoE<sup>−/−</sup>, apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup>, LDLr<sup>−/−</sup>, and LDLr<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice fed either a chow or a HFHC diet by fast protein liquid chromatography (FPLC) as previously described.

**Statistical Analysis**

Results are expressed as mean ± SD. Significant differences between means were determined by using the Student’s two-tailed t test.

**Results**

**Morphological Analysis**

A complete necropsy was performed to determine morphological changes related to the absence of ABCA1 in apoE<sup>−/−</sup> mice. The main necropsy finding in hypercholesterolemic mice possessing the null allele for ABCA1 was the accumulation of foam cells with identical characteristics in several tissues, most notably the skin and uterus, without changes in atherosclerotic lesion size. Accumulation of macrophage-derived foam cells was also observed, although to a lesser extent, in the lamina propria of the squamous mucosa at the squamous-glandular gastric junction, lung, liver, the upper one third of the kidney medulla, adrenal, and cortico-medullary junction of lymph nodes (Figure 1). In addition, pancreas, mesentery, ovary, large and small intestine, urinary bladder, lymphatic vessels, spleen, and periodontal tissue showed occasional presence of foam cells which progressed with age (data not shown). Several tissues, including blood vessels and brain, did not show accumulation of macrophages, suggesting that the absence of ABCA1 in a setting of hyperlipidemia leads to a selective and targeted trafficking and/or retention of macrophages.

In apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice, cutaneous abnormalities became macroscopically visible at an early age and were characterized by the presence of ulcerations and excoriations leading to pruritus (Figure 2b). Microscopically, extensive intercytoplasmic staining with Oil red O was consistently observed in apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice (Figure 2d) when compared with apoE<sup>−/−</sup> control littermates (Figure 2c). The lipid accumulation and the severity of the cell infiltrates correlated with age and were independent of sex. A similar accumulation of foamy cells in the skin and uterus was observed in the LDLr<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice fed a HFHC diet (data not shown).

To quantify the accumulation of lipids in skin, 1-cm<sup>2</sup> tissue samples were taken from the back of the necks of age- and sex-matched apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> and apoE<sup>−/−</sup> mice. After homogenization, tissue lipids were extracted and cholesterol and cholesteryl esters determined by gas chromatography. Free cholesterol was increased by 2.5-fold in apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice compared with apoE<sup>−/−</sup> control littermates (217.8 ± 80 μg/mg tissue vs 88.7 ± 45 μg/mg tissue, n = 6, P < 0.01). Cholesteryl esters were elevated by 7.5-fold in apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice when compared with apoE<sup>−/−</sup> control mice (121.6 ± 48 μg/mg tissue vs 16.3 ± 6.4 μg/mg, n = 6, P < 0.0001).

To identify the cell type of the foam cells, paraffin sections of skin, uterus, stomach, and lymph nodes were stained with smooth muscle actin and two macrophage markers, F4/80 and lysozyme. A representative paraffin section from the uterus from an apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> mouse is shown in Figure 3. The lipid-filled foam cells (Figure 3a) did not stain for smooth muscle actin (Figure 3b) but were positive for both macrophage markers (Figure 3c and d, respectively). The F4/80 staining was strong and mostly located at the periphery of the cells while the lysozyme staining was weaker and intracytoplasmic. These findings were identical in all tissues in which
foam cell accumulation was observed and indicate the presence of macrophage-derived foam cells.

Development and Progression of Atherosclerotic Lesions in Mice Lacking ABCA1

Because the original strain of ABCA1−/− mice on a normal cholesterolemic DBA1J background do not develop atherosclerosis, ABCA1−/− mice were mated to two atherosclerosis-susceptible strains of mouse: apoE−/− and LDLr−/− mice. Offspring from the same mating fed either a chow or a HFHC diet were used to determine the extent of atherosclerosis by cross sections at the aortic valve, the brachiocephalic region, or the entire aorta from aortic arch to the renal branch after en face preparations (Figure 4). In general, lesions in the apoE−/− strains on the mixed DBA/BL6 background were smaller and less complex than lesions previously reported for apoE−/− mice on a pure C57/BL6 background.24 Lesions from 16-week-old apoE−/−/ABCA1−/− and the apoE−/− mice consisted mainly of fatty streaks ranging from superficial foam cells to layers of foam cells within the lesions. Lesions in both groups were mainly confined to the proximal aorta and aortic arch. Unexpectedly, no greater foam cell accumulation was observed in apoE−/−/ABCA1−/− mice when compared with control apoE−/− littermates. Consistent with this finding, the atherosclerotic lesion area was not different between genotypes in 20-week-old mice fed a chow diet (Figure 1e and 1f and Table 1). An additional experiment was carried out to explore lesion development at a younger age.

As with animals studied at 20 weeks of age, there was no difference in the extent of atherosclerosis in 12-week-old mice lacking ABCA1 compared with their control littermates in the aortic valve sinus or throughout the entire aortic tree (Table 1). In addition, a dietary challenge consisting of feeding mice for 8 weeks with a HFHC diet did not significantly affect the development of atherosclerosis (Table 1) but there was a trend toward increased lesion area in the en face preparations (Table 1). In all animals, paraffin sections of the brachiocephalic region were stained with Movat's Pentachrome and evaluated for compositional differences. At all ages studied, lesions in this region in both the apoE−/−/ABCA1−/− and their littermate controls (apoE−/−) were small, consisting mainly of fatty streaks, and no difference in lesion composition was observed between genotypes (data not shown). In contrast to the simple lesion found in the brachiocephalic region of the apoE−/− strain, much more distinct

Figure 3. Macrophage-rich foam cell accumulation in apoE−/−/ABCA1−/− mice. Representative uterine section stained with Oil red O from a 20-week-old apoE−/−/ABCA1−/− mouse fed a chow diet (a), anti-actin (b), anti-F4/80 antibody (c), or lysosomes (d) as indicated in Methods. The arrows indicate foam cells. Bars=50 μm.

Figure 4. Plasma lipoprotein cholesterol distribution in mice of different genotypes fed a chow or a HFHC diet. Pooled plasma samples from apoE−/− (closed circles), apoE−/−/ABCA1−/− (open circles), LDLr−/− (closed squares) and LDLr−/−/ABCA1−/− (open squares) mice were separated by gel filtration as described in Methods. Cholesterol content of each fraction was determined enzymatically and plotted as a function of FPLC fractions.
atherosclerotic lesions were produced in the LDLr<sup>−/−</sup> strains fed a HFHC diet. The complex lesions consisting of multi-layered foam cells with areas of calcification necrosis and cholesterol clefts were found in both the LDLr<sup>−/−</sup> and the LDLr<sup>−/−</sup>/ABCA1<sup>−/−</sup> fed a HFHC diet. Lesion size in the aortic valve and throughout the entire aortic tree was also identical between age-matched littermates of LDLr<sup>−/−</sup> mice with and without the null alleles for ABCA1, fed either a chow or HFHC diet for 8 and 16 weeks (Table 1). Taken together, these findings suggest no measurable consequences of the complete absence of ABCA1 in the progression and extent of atherosclerosis development.

Plasma Lipids and Lipoproteins Analysis

In the absence of ABCA1, apoE<sup>−/−</sup> mice have a marked reduction on plasma apoAI levels. On a chow diet, plasma levels of apoAI were reduced to 87% of controls in apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice (Table 2). Identical results were observed when mice were fed a HFHC diet (Table 2), consistent with previous studies indicating a near complete absence of HDL in mice lacking ABCA1 even in the setting of hypercholesterolemia. Plasma triglyceride and phospholipid concentrations were not significantly different between genotypes. The disparity in cholesterol levels is more prominent in mice fed a HFHC diet on the LDLr<sup>−/−</sup> background (Table 2). As observed in the apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice, LDLr<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice had significantly lower cholesterol levels than control mice when fed the HFHC diet. The relatively lower plasma cholesterol levels in apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice and in the LDLr<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice could be largely accounted for by reductions in the VLDL and LDL fractions in mice fed either a chow or a HFHC diet as demonstrated by FPLC (Figure 4).

### TABLE 1. Percent Atherosclerotic Lesion Area in Aortic Sinus, Valve and En Face Preparations of Mice of Different Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age, wk</th>
<th>n</th>
<th>Diet</th>
<th>Sinus</th>
<th>Valve</th>
<th>En Face</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>12</td>
<td>7</td>
<td>Chow</td>
<td>32.7±7.8</td>
<td>26.3±6.0</td>
<td>5.3±1.9</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/ABCA1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>5</td>
<td></td>
<td>30.7±6.5</td>
<td>32.1±4.4</td>
<td>4.1±2.7</td>
<td></td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>20</td>
<td>6</td>
<td>Chow</td>
<td>41.5±3.2</td>
<td>42.3±3.2</td>
<td>4.0±1.1</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/ABCA1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>6</td>
<td></td>
<td>44.3±1.8</td>
<td>46.4±3.9</td>
<td>4.6±3.1</td>
<td></td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>17</td>
<td>4</td>
<td>HFHC</td>
<td>36.7±4.4</td>
<td>40.2±10.9</td>
<td>11.7±4.2</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/ABCA1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>3</td>
<td></td>
<td>31.1±13.9</td>
<td>40.2±19.6</td>
<td>7.3±0.9</td>
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<tr>
<td>LDLr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>20</td>
<td>7</td>
<td>Chow</td>
<td>3.2±1.4</td>
<td>4.3±1.7</td>
<td>7.1±1.9</td>
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<tr>
<td>LDLr&lt;sup&gt;−/−&lt;/sup&gt;/ABCA1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>5</td>
<td></td>
<td>4.8±1.2</td>
<td>3.8±1.3</td>
<td>6.1±3.5</td>
<td></td>
</tr>
<tr>
<td>LDLr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>20</td>
<td>8</td>
<td>HFHC*</td>
<td>35.0±9.3</td>
<td>0.2±0.6</td>
<td>7.0±3.6</td>
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<tr>
<td>LDLr&lt;sup&gt;−/−&lt;/sup&gt;/ABCA1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>8</td>
<td></td>
<td>33.0±7.0</td>
<td>40.6±7.4</td>
<td>12.4±2.7</td>
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<tr>
<td>LDLr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>29</td>
<td>6</td>
<td>HFHC†</td>
<td>46.7±6.8</td>
<td>48.9±5.2</td>
<td>15.0±3.1</td>
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<tr>
<td>LDLr&lt;sup&gt;−/−&lt;/sup&gt;/ABCA1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>6</td>
<td></td>
<td>46.4±7.6</td>
<td>49.1±4.0</td>
<td>15.0±3.3</td>
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The extent of atherosclerosis was determined in 12- and 20-week-old apoE<sup>−/−</sup> and apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice fed a chow diet, 17-week-old apoE<sup>−/−</sup> and apoE<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice fed a Western-type diet (HFHC) for 8 weeks, 20-week-old LDLr<sup>−/−</sup> and LDLr<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice fed a chow diet, and 20- and 29-week-old LDLr<sup>−/−</sup> and LDLr<sup>−/−</sup>/ABCA1<sup>−/−</sup> mice fed a HFHC diet for 8* and 17† weeks, respectively. The mean percent lesion area in 10-μm sections of the aortic sinus and valve region was calculated after Oil red O staining, and the percent lesion area was determined morphometrically from whole aortas as described in Methods.

Values are reported as means±SD.

### TABLE 2. Lipid and Apolipoprotein Concentrations in Mice of Different Genotypes Fed a Chow or HFHC Diet

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
<th>ApoB</th>
<th>ApoA-I</th>
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<tbody>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Chow</td>
<td>601±135</td>
<td>245±27</td>
<td>309±85</td>
<td>0.34±0.08</td>
<td>0.29±0.14</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/ABCA1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Chow</td>
<td>313±7.1†</td>
<td>194±21</td>
<td>217±41</td>
<td>0.42±0.09</td>
<td>0.04±0.02‡</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>HFHC</td>
<td>1522±184</td>
<td>443±79</td>
<td>459±76</td>
<td>0.73±0.17</td>
<td>0.44±0.09</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/ABCA1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>HFHC</td>
<td>532±84‡</td>
<td>372±74</td>
<td>353±56</td>
<td>0.42±0.07†</td>
<td>0.03±0.01‡</td>
</tr>
<tr>
<td>LDLr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Chow</td>
<td>212±40</td>
<td>190±41</td>
<td>208±29</td>
<td>0.38±0.08</td>
<td>0.88±0.14</td>
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<tr>
<td>LDLr&lt;sup&gt;−/−&lt;/sup&gt;/ABCA1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Chow</td>
<td>109±22‡</td>
<td>154±34</td>
<td>115±34†</td>
<td>0.27±0.06*</td>
<td>0.04±0.02‡</td>
</tr>
<tr>
<td>LDLr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>HFHC</td>
<td>1262±206</td>
<td>507±100</td>
<td>375±18</td>
<td>0.88±0.17</td>
<td>1.01±0.13</td>
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<tr>
<td>LDLr&lt;sup&gt;−/−&lt;/sup&gt;/ABCA1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>HFHC</td>
<td>329±78‡</td>
<td>186±98†</td>
<td>191±40‡</td>
<td>0.40±0.11†</td>
<td>0.02±0.01‡</td>
</tr>
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</table>

Values shown are mean±SD from 7 to 15 mice. All lipid values are expressed in mg/dL of plasma. Mouse apoA-I and apoB are expressed as μg/mL of plasma.

*P<0.05, †P<0.005, and ‡P<0.001 compared with apoE<sup>−/−</sup> or LDLr<sup>−/−</sup>.
Macrophage Inactivation of ABCA1-Effects on Plasma Lipids and Atherosclerosis

To determine whether ABCA1 deficiency in macrophages contributed to the development of atherosclerosis a bone marrow transplantation study was performed in which bone marrow from apoE−/−/ABCA1−/− or apoE−/− control mice was transplanted into recipient mice. After transplantation, mice were fed a chow diet for 12 weeks. By then, apoE−/− mice which received bone marrow from apoE−/−/ABCA1−/− had no changes in plasma cholesterol, triglycerides or HDL when compared with control recipient mice (Figure 5). In contrast, histological analysis of aortic valve, sinus and entire aorta following en face preparations revealed a significant increase in atherosclerosis in these mice lacking ABCA1 in macrophages compared with controls (Figure 5). These findings demonstrate the antiatherogenic function of ABCA1 in macrophages independently of changes in plasma lipids.

Discussion

Analysis of ABCA1 in several in vitro and in vivo studies has provided compelling evidence that ABCA1 is intimately involved in the synthesis of HDL and lipoprotein homeostasis. Lack of function, as a consequence of natural mutations, leads to Tangier Disease, a rare autosomal recessive genetic disorder characterized by virtually no HDL, 40% reductions in LDL levels and abnormal deposition of cholesteryl esters in the reticuloendothelial system. Clinically, the majority of patients with severe HDL and apoAI deficiencies suffer from Tangier Disease as demonstrated by the decrease in plasma cholesterol and VLDL fractions from mice lacking ABCA1. Similar changes in plasma lipid profiles are observed in chickens,27 ABCA1 knockout mice19 and patients with Tangier Disease.23 Thus, we hypothesize that the proposed atherogenic effect resulting from ABCA1 deficiency was compensated for by a less atherogenic lipid profile, resulting in similar lesion areas between the groups. Consistent with this hypothesis, the selective inactivation of ABCA1 in macrophages significantly increased atherosclerosis development in the absence of changes in plasma lipid profiles. Since the contribution of macrophages to the overall plasma HDL levels is minimal25 these studies suggest that the antiatherogenic properties of ABCA1 are independent of plasma levels of HDL.

The mechanism by which ABCA1 affects plasma levels of apoB remains unclear. The lipid effects are seen in both the apoE−/− and the LDLr−/− strains, mouse models with severely compromised clearance of apoB containing lipoproteins. It is unlikely that the decrease in plasma cholesterol levels resulted from altered receptor clearance rates. Previous transcript imaging of ABCA1 deficient mice have shown that cholesterol biosynthetic enzymes are decreased compared with wild-type littermates,19 suggesting a decrease in cholesterol synthesis. In addition, studies in transgenic mice demonstrated that the increased levels of plasma apoB-containing lipoproteins observed in mice expressing human ABCA1 was not due to increased secretion of apoB.28 More recently, Joyce et al29 demonstrated that the overexpression of ABCA1 in apoE−/− mice led to increased atherosclerosis despite
minimal changes in plasma lipids. In contrast, overexpression of ABCA1 in C57BL/6 mice fed a HFHC diet significantly changed plasma lipid profiles. Clearly, additional studies are required to understand the mechanisms underlying these lipid modulating effects of ABCA1.

The most outstanding phenotype observed in this study is the massive foam cell accumulation in tissues other than atherosclerosis-prone vessels in mice lacking ABCA1. Marked macrophage and foam cell accumulation was observed in skin and uterus and to a lesser extent in stomach, lymph nodes, kidneys and lungs. Several genetic mouse models develop xanthomas and show a marked accumulation of cholesterol and cholesteryl esters characteristic of typical xanthomatosis observed in patients with genetic disorders of lipoprotein metabolism.30

The mechanism(s) leading to foam cell accumulation in certain tissues but not in others, particularly the vessel wall remains unclear. Severe hypercholesterolemia was necessary to exacerbate the xanthomatosis in the ABCA1-deficient mice, whereas normal cholesterolemic ABCA1-deficient mice do not form similar foam cell lesions.19 Because monocyte infiltration and the accumulation of cholesterol in lesional macrophages are the hallmark of atherosclerosis, it was surprising that there was massive foam cell formation in certain tissues without affecting atherosclerosis. One possible explanation is that tissues like skin and uterus, which undergo rapid cell division and proliferation, not only require an influx of lipoprotein-derived cholesterol as suggested by Accad et al.31 but also require the efficient removal of apoptotic bodies and cell membranes. Oram32 proposed that sterol accumulation in macrophages plays different functional roles in different tissues. In some tissues, macrophages infiltrate to clear apoptotic and necrotic cells, and thus, a major source of cholesterol is likely derived from cell membranes. Conversely, lipoproteins are considered the major source of cholesterol in arterial macrophages, and reduction in these atherogenic lipoproteins as observed in mice with complete inactivation of ABCA1 may explain why foam cell accumulation was greater in proliferating tissues than the arterial wall. This is consistent with the findings from the bone marrow transplantation study in which selective inactivation of ABCA1 in macrophages does not alter plasma cholesterol homeostasis and yet lead to increased foam cell accumulation at the vessel wall. Our finding of accumulation of foam cells in specific tissues may also suggest a targeted infiltration or retention of macrophages lacking functional ABCA1. The trafficking of monocytes to various tissues is known to be regulated by a complex multistep process involving specific cell-cell adhesion interactions between monocytes and endothelial cells, chemoattractant factors, and their receptors.33 The recruitment of monocytes to any given tissue appears to require a specific pattern of adhesion factors and chemokines.34 The ability of lipoprotein cholesterol to alter the expression of specific adhesion factors and chemokines receptors on circulating macrophages is well-documented in vivo and in vitro.35,36 It is therefore conceivable that changes in intracellular sterol levels resulting from the absence of ABCA1 causes a pleiotropic phenotype in which macrophages are selectively targeted to certain tissues and not others. This hypothesis was first suggested by Schmitz et al.37 to explain the differences in phenotypes observed among patients with Tangier disease. Our studies in hypercholesterolemic mice deficient in ABCA1 show massive tissue-specific accumulation of macrophages and may provide a valuable animal model to unravel the mechanisms and physiology of macrophage trafficking.

In conclusion, our findings demonstrate that the complete absence of ABCA1 has a major impact in plasma lipoprotein homeostasis, and the proposed antiatherogenic effect resulting from ABCA1 deficiency is likely compensated by a less atherogenic lipid profile. ABCA1 deficiency in macrophages, however, demonstrates the antiatherogenic properties of ABCA1 independent of plasma lipids and HDL levels. Our results also highlight ABCA1’s potential role in monocyte targeting and trafficking, and provide the basis for additional experiments to elucidate its function and relationship to the development of atherosclerosis.

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