Localization of Human ATP-Binding Cassette Transporter 1 (ABC1) in Normal and Atherosclerotic Tissues

Richard M. Lawn, David P. Wade, Tracey L. Couse, Josiah N. Wilcox

Abstract—The present study examines the expression of ATP-binding cassette transporter 1 (ABC1) mRNA in normal and atherosclerotic tissues by using in situ hybridization in an effort to better understand the function of this cholesterol transport protein. Samples of normal baboon tissues as well as human normal and atherosclerotic aortas were hybridized with 35S-labeled ABC1 sense and antisense riboprobes. Widespread expression of ABC1 was observed generally in tissues containing inflammatory cells and lymphocytes. Other noninflammatory cells that were also sites of ABC1 synthesis included the ductal cells of the kidney medulla, Leydig cells in the testis, and glial cells in the baboon cerebellum. Although normal veins and arteries did not express ABC1 mRNA, it was found to be upregulated in the setting of atherosclerosis, where widespread expression was found in macrophages within atherosclerotic lesions. These results are consistent with the proposed role of ABC1 in cholesterol transport in inflammatory cells. The specific upregulation of ABC1 mRNA in the setting of atherosclerosis probably reflects the response of leukocytes to cholesterol loading. However, the presence of ABC1 in ductal cells of the kidney medulla and in the small intestine suggest a more general role for this protein in cholesterol transport in other cell types. (Arterioscler Thromb Vasc Biol. 2001;21:378-385.)

Key Words: HDL ■ cholesterol ■ atherosclerosis ■ ATP-binding cassette transporter 1 (ABC1) ■ ATB-binding cassette transporter A1 (ABCA1)

Low plasma HDL concentration is a major risk factor for cardiovascular disease, yet the mechanisms that control the flux of cholesterol through HDL transport in plasma remain poorly understood. The biochemical understanding of this pathway was enhanced by the identification of ATP-binding cassette transporter 1 (ABC1, also known as ABCA1), an ATP-dependent sterol transporter, as the genetic defect in Tangier disease, a monogenic disorder marked by near-zero levels of circulating HDL.1–4 ABC1 is a member of the family of ATP-binding cassette proteins that transport ligands across the plasma membrane. This family includes the cystic fibrosis transporter (CFTR), several multidrug resistance P glycoproteins, and the ocular protein ABC-R.5 Intracellular roles are also likely for some of the ABC proteins. Ever since its identification as the defective gene in Tangier disease, most investigations have focused on the role of ABC1 in apolipoprotein-mediated efflux of cholesterol and phospholipids from macrophages. To date, cell culture studies using inhibition and overexpression suggest that ABC1 may play a rate-limiting step in that process.4,6 Similarly, the intermediate levels of HDL in humans and knockout mice heterozygous for ABC1 gene deficiency suggest that ABC1 is a vital determinant of plasma HDL concentration.7–9 Because a hallmark of Tangier disease is massive cholesterol deposition in tissue macrophages, the initial focus on this cell type seems justified.10 However, ABC1 message has been detected in various tissues, including liver, lung, adrenals, small intestine, and brain.11

Because tissue macrophages may not represent the source of ABC1 in all organs, we used in situ hybridization to localize its expression in human and baboon tissues. Although its presence in macrophages in arterial lesions supports an expected role in the process of atherosclerosis, detection of ABC1 mRNA in liver hepatocytes, villi of the small intestine, tubule cells in the kidney, and cells of the central nervous system serves notice that ABC1 plays a number of roles in lipid transport and homeostasis in addition to the early lipidation of nascent HDL particles in the vascular wall.

Methods

Tissue Preparation

Protocols for obtaining tissue samples were approved by the Emory University Hospital Human Investigation Committee and were in accordance with the principles of the Declaration of Helsinki. Human aorta samples representative of different stages of atherosclerosis were obtained from 9 transplant donors. Human internal mammary arteries (n=2) were used as nonatherosclerotic controls and were obtained from patients undergoing coronary artery bypass surgery. The stages of atherosclerotic development were based on the degree of intimal lesion formation, the presence of inflammatory...
cells, and regions of necrosis as previously described. Normal vessel segments (n=3) had almost no intimal development and no inflammatory cells, as defined by CD68 immunohistochemistry. Atherosclerotic aortas (n=6) had either minor intimal development with scattered macrophage staining just under the luminal surface or were advanced atherosclerotic lesions with a thickened intima, numerous CD68-positive macrophages in the intima and media, and regions of necrosis and cholesterol deposits. The normal tissue distribution of ABC1 mRNA was determined by using tissues obtained from male baboons, which were collected in the course of other studies. All primates were obtained under protocols approved by the Emory University Institutional Animal Care and Use Committee.

Tissue samples were fixed overnight in 4% (wt/vol) paraformaldehyde in 0.1 mol/L sodium phosphate (pH 7.4) at 4°C and then processed by using standard paraffin techniques. Other tissue samples were collected, immediately immersed in 4% (wt/vol) paraformaldehyde in 0.1 mol/L sodium phosphate (pH 7.4) at 4°C for 3 to 4 hours, cryoprotected in 15% (wt/vol) sucrose/isotonic PBS overnight at 4°C, and processed for frozen sections as previously described. Equivalent in situ hybridization results were obtained with both methods.

### In Situ Hybridization

In situ hybridization was performed on paraffin sections with the use of human-specific 32P-labeled riboprobes as previously described. In situ hybridization results were photographed by polarized light epiluminescence microscopy (Leitz) so that the silver grains appeared white. The results were evaluated by 2 individuals and graded (−, +, ++, and ++++) on the basis of the number of cells expressing ABC1 mRNA in each tissue type.

### Probes

A plasmid template for the synthesis of sense and antisense riboprobes to ABC1 mRNA was constructed by the ligation of sequences from base pairs 350 to 1805 of the ABC1 cDNA (GenBank accession numbers AF285167) into vector pGEM 3Zf- (Promega). RNA probes constituting either strand of this sequence were synthesized by in vitro transcription from the T7 or SP6 RNA polymerase promoters flanking the insert after linearization of the plasmid by restriction digestion. Probe specificity was confirmed by hybridization of a 32P-labeled probe containing this insert sequence to Southern blots of human genomic DNA that had been digested with KpnI, BamHI, or EcoRI. Only the bands expected from restriction enzyme fragment prediction based on the human ABC1 gene sequence (GenBank accession numbers AF287262 and AF287263) were observed.

### Immunohistochemistry

Immunohistochemistry was used on serial sections to identify cells containing ABC1 mRNA. The following antibodies were used: CD68 for macrophages (Dako, 1:50 dilution), CD20 for B cells (Pharmagen, 1:125 dilution), CD3 for T cells (Dako, 1:25 dilution), and SM1 for smooth muscle cell α-actin (Sigma Chemical Co, 1:800 dilution). Sections were predigested with proteinase K (1 mg/mL, Sigma) or pronase E (1 mg/mL, Sigma), the primary antibodies were applied at the indicated dilutions, and the slides were stained by using ABC-AP (Vector Labs) as described. Serial sections treated with secondary antibodies only or with nonimmune IgG did not show any staining.

### Results

The cell types expressing ABC1 mRNA in a series of tissues taken from adult male baboons were determined by in situ hybridization and are summarized in Table 1. Generally, expression of ABC1 was found in tissues involved in cholesterol transport and utilization and in inflammatory cells, including macrophages, T cells, and B cells.

In the liver, ABC1 was expressed in some, but not all, hepatocytes (Figure 1). Strong consistent hybridization was seen in scattered CD68-positive macrophages and Kupffer cells. At least some of the hybridizing cells in the liver were likely to be B cells, as determined by serial section immunohistochemistry with the CD20 antibody (Figure I, available online at http://atvb.ahajournals.org). Strong hybridization was found in the region surrounding the portal vein and appeared to consist of connective tissue cells and CD68- and CD20-positive lymphocytes found in this region. The small intestine was found to have numerous ABC1-positive macrophages in the lamina propria of the intestinal villi. ABC1 mRNA was not detected in the epithelial cells lining the small intestine. In the spleen and lymph nodes, hybridization was found in regions consistent with the localization of macrophages and B cells. Some hybridization to T cells was likely in the marginal zone but could not be confirmed. In the testis, ABC1-positive cells were found in the Leydig cells surrounding the seminiferous tubules. In the kidney, strong hybridization that was localized to scattered tissue macrophages as well as cells lining the proximal and distal tubules in the cortex and medulla (Figure II, available online at http://atvb.ahajournals.org) was found. In a section of the baboon cerebellum, ABC1 mRNA was detected in glial cells in the white matter as well as in cells within the granular layer. Purkinje cells did not express ABC1 mRNA (Figure III, available online at http://atvb.ahajournals.org). In the lung, ABC1 was expressed by pulmonary microphages (Figure IV, available online at http://atvb.ahajournals.org).

Normal baboon aorta and carotid artery did not express ABC1 mRNA. Consistent with this result, no ABC1-expressing cells were found in samples of human internal

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**Table 1. Distribution of ABC1 mRNA in Normal Baboon Tissues by In Situ Hybridization**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>ABC1 Hybridization</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Carotid artery</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>+++</td>
<td>B cells in white pulp, scattered T cells and macrophages in red pulp and marginal zone</td>
</tr>
<tr>
<td>Lymph node</td>
<td>+++</td>
<td>B cells in germinal centers, scattered T cells and macrophages in paracortex</td>
</tr>
<tr>
<td>Thymus</td>
<td>+</td>
<td>Scattered cells in medulla</td>
</tr>
<tr>
<td>Liver</td>
<td>++</td>
<td>Kupfer cells, macrophages, B cells, portal vein, some but not all hepatocytes</td>
</tr>
<tr>
<td>Small intestine</td>
<td>++</td>
<td>Macrophages in lamina propria</td>
</tr>
<tr>
<td>Kidney</td>
<td>+++</td>
<td>Collecting tubules and ducts in medulla, proximal and distal tubules in cortex, scattered macrophages, perivascular connective tissue in cortex</td>
</tr>
<tr>
<td>Lung</td>
<td>++</td>
<td>Pulmonary macrophages</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>+++</td>
<td>Gial cells in white matter, granular layer neurons, Purkinje cells negative</td>
</tr>
<tr>
<td>Testis</td>
<td>++</td>
<td>Leydig cells</td>
</tr>
</tbody>
</table>

ABC1 hybridization is graded from − to +++ on the basis of the number of cells expressing ABC1 mRNA in each tissue type.
mammary artery or normal nonatherosclerotic aortas (Table 2). Atherosclerotic vessels demonstrated expression of ABC1 mRNA in scattered lipid-filled macrophages in early fatty streaks (Figure 2) as well as in the shoulder region of advanced lesions (see http://atvb.ahajournals.org). Nonatherosclerotic regions of these same vessel cross sections contained no ABC1-hybridizing cells. In at least 1 vessel, strong hybridization was found in an inflammatory zone filled with scattered macrophages without lipids (Figure 3). In the same tissue section, a portion of the vessel had a diffuse intimal thickening without inflammatory cells, which showed no ABC1 hybridization. In all cases, serial section staining of these vessels with CD68 suggested that ABC1 hybridization was to macrophages in the atherosclerotic vessels. No CD20-positive cells were found in the vascular tissues, so it is unlikely that B cells are the source of ABC1 mRNA; scattered CD3-positive T cells were present but differed morphologically from the ABC1-positive cells (Figures 2 and 3). However, it should be emphasized that not all CD68-positive macrophages hybridized with the ABC1 riboprobe. ABC1 mRNA was not detected in a number of non–lipid-associated CD68-positive cells found scattered in other parts of the vessel, especially in the adventitia.

**Discussion**

The genetic basis for understanding the function of ABC1 was its establishment as the defect in the monogenic disorder Tangier disease. These patients have near-zero levels of circulating HDL. Sequence analysis and metabolic and cell culture studies in the last decade have established that apolipoprotein components of HDL are normal in sequence and synthesis in these patients but that HDL is catabolized at an increased rate.15,16 Francis et al17 have shown that apolipoprotein-mediated cholesterol efflux is deficient in cells derived from patients with Tangier disease. Hence, the defect in Tangier disease represents an initial step in reverse cholesterol transport. The current understanding is that apolipoproteins or nascent lipid-poor HDL particles fail to acquire a "mature" lipid content and are relatively quickly cleared from the circulation. ABC1 is likely to function as a cell membrane transporter that facilitates the transfer of cholesterol and phospholipids to poorly lipidated apolipoproteins at an exofacial pore, which is ringed by 12 predicted trans-membrane domains.18 Although this may describe the role of ABC1 in resident macrophages coping with excessive cholesterol import, the potential functions of ABC1 in other
settings remain unclear. Involvement in activities as diverse as apoptotic cell engulfment and secretion of interleukin-1β has been proposed,19,20 with the former linked to the calcium-induced exposure of phosphatidylserine at the outer surface of the plasma membrane by a hypothesized “flippase” activity of ABC1.21

Patients with Tangier disease experience cholesterol deposition in many tissues, not only in the enlarged orange tonsils, noted as the hallmark of this disease, but in liver, spleen, intestinal mucosa, lymph nodes, cornea, and Schwann cells.10,22 Major clinical symptoms include not only HDL deficiency and increased incidence of cardiovascular disease but splenomegaly and neuropathy as well. The results in the present study support the role of ABC1 in the development of arterial lesions as well as a broader function in lipid distribution. Previous studies detected the presence of ABC1 message by blot hybridization to whole-organ RNA samples from heart, liver, spleen, kidney, lung, small intestine, and brain.11,23 In situ hybridization data have now confirmed and extended these observations to the level of individual cells within these tissues.

ABC1 RNA is readily detected in fatty macrophages in early atherosclerotic lesions (Figure 2). However, not all macrophages in the plaque specimens are positive. This raises the intriguing issue of the signals and responses that control its synthesis. Cell culture studies have demonstrated steroid-induced ABC1 transcription in parallel with enhancement of cholesterol efflux from such cells.24 This may be an adaptive response to maintain cholesterol homeostasis in cells containing scavenger receptors that allow unregulated uptake of oxidized or otherwise modified LDL. Cultured THP-1 macrophages show a steady increase of ABC1 RNA during 4 days of exposure to oxidized LDL.25 It remains to be determined whether some plaque macrophages fail to express ABC1 as a result of an insufficient signaling input or a lack of response due to signal overload or because they represent a unique subclass of macrophages. At this point, such questions remain unanswered, yet it is suggestive that we have detected less ABC1 RNA in macrophage-rich regions of plaques considered to be of an advanced nature. We speculate that these macrophages may represent foam cells incapable of ABC1-mediated cholesterol efflux.

A substantial portion of the ABC1 RNA detected in spleen, lung, liver, and small intestine can be attributed to resident macrophages. Splenomegaly with lipid-filled macrophages occurs in some patients with Tangier disease, supporting a role of ABC1 in sterol efflux from macrophages that must cope with a large flux of cholesterol derived from the uptake by the spleen of phagocytic cells and senescent cells.10 Expression of ABC1 in alveolar macrophages in the lung is consistent with a role in lipid homeostasis in these cells that are involved in the uptake and clearance of surfactant phospholipids and cholesterol, as well as diseased and damaged cells.

Much of the expression of ABC1 in liver resides in macrophages and Kupffer cells. However, cells lining the

### TABLE 2. Distribution of ABC1 mRNA in Normal and Atherosclerotic Human Vessels by In Situ Hybridization

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Patient Information</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMA</td>
<td>42-year-old female</td>
<td>—</td>
<td>...</td>
</tr>
<tr>
<td>IMA</td>
<td>61-year-old female</td>
<td>—</td>
<td>...</td>
</tr>
<tr>
<td>Normal aorta</td>
<td>49-year-old male</td>
<td>—</td>
<td>Normal artery with some diffuse intimal thickening but no intimal macrophages; numerous macrophages in the adventitia were negative for ABC1</td>
</tr>
<tr>
<td>Normal aorta</td>
<td>24-year-old female</td>
<td>—</td>
<td>Normal artery with some diffuse intimal thickening but no inflammatory cells</td>
</tr>
<tr>
<td>Normal aorta</td>
<td>16-year-old male</td>
<td>—</td>
<td>...</td>
</tr>
<tr>
<td>Normal aorta fatty streak</td>
<td>22-year-old male</td>
<td>++</td>
<td>Series of small raised lesions containing lipid-filled macrophages, which were uniformly positive for ABC1 mRNA; adjacent regions of normal vessel had no ABC1-positive cells</td>
</tr>
<tr>
<td>Normal aorta</td>
<td>75-year-old male</td>
<td>++</td>
<td>Inflammatory region present with numerous macrophages containing ABC1 mRNA; an adjacent region of diffuse intimal thickening without inflammatory cells was negative for ABC1</td>
</tr>
<tr>
<td>Atherosclerotic aorta</td>
<td>49-year-old female</td>
<td>++</td>
<td>Early type III lesion with ABC1-positive macrophage foam cells in the shoulder region of the plaque; ABC1 mRNA was not detected in macrophages in the necrotic core of the lesion</td>
</tr>
<tr>
<td>Atherosclerotic aorta</td>
<td>75-year-old female</td>
<td>++</td>
<td>Strong positive hybridization to numerous intimal macrophages</td>
</tr>
<tr>
<td>Atherosclerotic aorta</td>
<td>72-year-old male</td>
<td>—</td>
<td>ABC1 mRNA was not detected in this advanced type IV atherosclerotic lesion with large necrotic core containing numerous macrophages</td>
</tr>
<tr>
<td>Atherosclerotic aorta</td>
<td>75-year-old female</td>
<td>+/-</td>
<td>Advanced type IV lesion with ABC1-positive macrophages along the border of the necrotic core; the large necrotic core had many ABC1-negative macrophages</td>
</tr>
</tbody>
</table>

IMA indicates internal mammary artery.

*Note:* IMA indicates internal mammary artery.
Figure 2. ABC1 in situ hybridization to normal human aorta (A) and early aortic atherosclerotic lesion (B). ABC1 mRNA was localized to foamy macrophages, as indicated by serial section immunohistochemistry to CD68 antibody (C); little, if any, hybridization to T cells was seen, as indicated by CD3 staining (D).
portal vein, B cells, and some hepatocytes are also positive. It can be suggested that in hepatocytes the primary function of ABC1 is to mediate the formation of nascent HDL through the provision of cholesterol and phospholipid to lipid-free apolipoproteins rather than to promote the efflux of excess cholesterol as in macrophages. Such divergent functions may require ABC1 gene regulation to differ in these tissue types. Indeed, Costet et al.²⁶ have recently provided evidence that Hep G2 cells produce a variant of ABC1 mRNA that uses a distinct 5' exon, containing a different transcription and translation start site than that expressed in macrophages and cultured fibroblasts.²⁴,²⁶,²⁷ Because only the initial 22 amino acids of the protein are predicted to differ in the liver form of ABC1,²⁶ potential differences in transcriptional response of the gene may be a more important consequence of the alternatively spliced variants.

ABC1 expression in Leydig cells of the testes implies a role in cholesterol homeostasis in tissues that synthesize steroid hormones, as also noted by the detection of ABC1 message by blot hybridization to adrenal RNA.¹¹ Synthesis of ABC1 in the kidney suggests a role in sterol transport during filtration and reabsorption. Because apoA-I is known to be filtered in the kidney, renal clearance of poorly lipidated apoA-I–containing particles may play a key role in their scarcity in the plasma of patients with Tangier disease and potentially other individuals with low levels of HDL.²⁸,²⁹ The HDL-binding proteins megalin and cubilin are expressed in the kidney and have been proposed to mediate the uptake of apoA-I dissociated from the lipoprotein particle.³⁰ Our detection of ABC1 gene expression in tubules and collecting ducts in the kidney medulla suggest a renal function for ABC1 as well as these other proteins in secreting or recapturing cholesterol and not merely the dissociated protein components of HDL.

It had been suggested that the neuropathology associated with Tangier disease could be linked to the low concentra-

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**Figure 3.** ABC1 in situ hybridization to human aorta showing that noninflammatory diffuse intimal thickening was predominantly negative (A). The same cross section showed intense hybridization to an inflammatory zone with the use of antisense riboprobes (B) but not with sense probes (C). Positive cells were identified as macrophages by serial section immunohistochemistry to CD68 (D). CD3 (T cell, E) and SM1 (smooth muscle α-actin, F) staining is also shown.
tions of apoA-I and HDL, which could impair the unloading of cholesterol from macrophages and Schwann cells during myelination. The discovery of ABC1 synthesis in neuronal tissue suggests that this protein plays a direct role in the redistribution of cholesterol and phospholipid in the nervous system, as do other components of lipid transport pathways, such as apoE and members of the LDL receptor family.

The role of ABC1 in the intestine was not appreciated before the discovery that mice with a targeted disruption of this gene showed an increased absorption of cholesterol from the gut. A more recent report of drug-induced increase in ABC1 expression and reduction in cholesterol uptake in the mouse intestine supports this activity. This suggests that ABC1 acts as a unidirectional transporter to efflux some percentage of absorbed cholesterol back to the intestinal lumen. The cell type and the presumed acceptor apolipoprotein in this process remain to be determined. Although we detect a clear ABC1 RNA signal in intestinal villi, it appears to be uniformly distributed throughout the lamina propria, where it colocalizes with macrophages, not with epithelial cells. Thus, our data may not support a proposed role of ABC1 in cholesterol absorption by the intestine. Alternatively, they may suggest that the “barrier” to absorption may be occurring not in the epithelial layer but in macrophages within the lamina propria. This might be consistent with the report that cultured intestinal epithelioid CaCo2 cells did not show apoA-I-stimulated cholesterol efflux. However, until the full nature of the induction of this pathway in the intestine is elucidated, we cannot exclude the possibility that intestinal epithelial cells may not express ABC1 under certain conditions.

The discovery that ABC1 represents the defective gene in Tangier disease and plays a key role in the efflux of cholesterol and phospholipids from macrophages to nascent HDL particles was a key step in clarifying the initiating events in reverse cholesterol transport. Conditions that are known to increase cholesterol efflux from macrophages, including cholesterol loading, cAMP signaling, and treatment with ligands of nuclear hormone receptors, are now known to affect ABC1 transcription in parallel. Most cells regulate intracellular cholesterol by controlling its synthesis or its uptake by LDL receptor-mediated endocytosis. Macrophages that ingest cell debris and modified lipoproteins by scavenger receptors that are not downregulated are more dependent on the efflux pathway in which ABC1 may be a rate-limiting component. The studies of Chimini and colleagues add to this the involvement of ABC1 in membrane phospholipid distribution in the process of phagocytosis. However, the distribution of ABC1 gene expression suggests a function beyond that appropriate for phagocytic cells. It can be proposed that ABC1 serves not only to aid in the net efflux of lipids from nonpolarized cells, such as macrophages. It can be hypothesized that in polarized cells lining blood and lymphatic vessels or kidney tubules, ABC1 is involved in the physiological redistribution of lipids via transcytosis, delivering its cargo out of a luminal or basolateral surface. Further definition of all the functions of ABC1 will be gained by the development and use of additional tools, which include genetically manipulated mice, inhibiting antibodies, and selective drugs that alter the quantity or activity of this protein.


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