Expression of ACAT-1 Protein in Human Atherosclerotic Lesions and Cultured Human Monocytes-Macrophages

Akira Miyazaki, Naomi Sakashita, Oneil Lee, Kiyoshi Takahashi, Seikoh Horiuchi, Hideki Hakamata, Peter M. Morganelli, Catherine C.Y. Chang, Ta-Yuan Chang

Abstract—The acyl coenzyme A:cholesterol acyltransferase (ACAT) gene was first cloned in 1993 (Chang et al., J Biol Chem. 1993;268:20747–20755; designated ACAT-1). Using affinity-purified antibodies raised against the N-terminal portion of human ACAT-1 protein, we performed immunohistochemical localization studies and showed that the ACAT-1 protein was highly expressed in atherosclerotic lesions of the human aorta. We also performed cell-specific localization studies using double immunostaining and showed that ACAT-1 was predominantly expressed in macrophages but not in smooth muscle cells. We then used a cell culture system in vitro to monitor the ACAT-1 expression in differentiating monocytes-macrophages. The ACAT-1 protein content increased by up to 10-fold when monocytes spontaneously differentiated into macrophages. This increase occurred within the first 2 days of culturing the monocytes and reached a plateau level within 4 days of culturing, indicating that the increase in ACAT-1 protein content is an early event during the monocyte differentiation process. The ACAT-1 protein expressed in the differentiating monocytes-macrophages was shown to be active by enzyme assay in vitro. The high levels of ACAT-1 present in macrophages maintained in culture can explain the high ACAT-1 contents found in atherosclerotic lesions. Our results thus support the idea that ACAT-1 plays an important role in differentiating monocytes and in forming macrophage foam cells during the development of human atherosclerosis. (Arterioscler Thromb Vasc Biol. 1998;18:1568-1574.)

Key Words: ACAT ■ atherosclerosis ■ immunohistochemistry ■ differentiation ■ monocytes

Acyl coenzyme A:cholesterol acyltransferase (ACAT) is an intracellular enzyme that catalyzes formation of cholesteryl esters (CEs) from cholesterol and fatty acyl coenzyme A (for a recent review, see Reference 1). ACAT activity is present in various tissues, including the liver, intestine, adrenal gland, and aorta. This enzyme is believed to play significant roles in intracellular cholesterol storage, lipoprotein assembly, steroid hormone production, and dietary cholesterol absorption. In addition, under pathological conditions, formation and accumulation of CEs as lipid droplets by ACAT within macrophages constitute a characteristic feature of early lesions of atherosclerotic plaques (for a recent review, see Reference 2).

The ACAT cDNA was first cloned and functionally expressed in 1993 by Chang and colleagues; the full-length cDNA was isolated from a cDNA library of THP-1 cells, a human monocytic leukemia cell line. This gene is now designated ACAT-1. Subsequently, its homologues in the mouse, hamster, rabbit, yeast, and rat have been cloned. The human ACAT-1 cDNA contains 1650-bp nucleotides encoding 550 amino acids. Recently, the ACAT-1 gene knockout mouse has been generated. The homozygous knockout mice showed markedly reduced amounts of CEs in the adrenal glands (3% of wild type). On cholesterol loading with acetylated LDL, peritoneal macrophages from homozygous knockout mice showed negligible accumulation of CE (6% of wild type) and significant accumulation of free cholesterol. These observations indicate that ACAT enzyme activities in the adrenal glands and in peritoneal macrophages are virtually disrupted in the knockout mouse. In contrast, liver ACAT activity was not reduced in these mice, suggesting the possibility that the structure of liver ACAT may be different from that in the adrenal glands and peritoneal macrophages. A second ACAT gene has recently been cloned and functionally expressed by R. Farese and colleagues (from mouse) and by L. Rudel and colleagues in collaboration with S. Sturley and colleagues (in monkeys); these results were announced at the 70th Scientific Sessions of the American Heart Association. This gene has been designated ACAT-2 (personal communications with R. Farese and L. Rudel, 1997). By Northern blot analyses, the major sites of tissue expression of the ACAT-2 gene in the mouse and monkey are in the liver and intestines, but not in macrophages. There is no amino acid sequence similarity in the first 100 amino acids between the ACAT-1 and ACAT-2 proteins; in the remaining amino acids (>400), there is significant...

We recently engineered and produced a glutathione S-transferase (GST) fusion protein that contains the N-terminal portion of human ACAT-1 (amino acids 1 to 131). Using this fusion protein as the antigen, we obtained higher-titer specific polyclonal antibodies (DM10). The antibodies were affinity-purified by column chromatography before use. The ACAT-1 protein in various human cells could be detected as a single 50-kDa protein band by Western blotting with DM10.13 We also showed that cholesterol loading does not affect the ACAT-1 protein levels in human fibroblasts or in human HepG2 cells.13 Additional experiments showed that cholesterol loading did not affect the ACAT-1 mRNA levels in HepG2 cells or in rabbit liver cells.13,15 However, using an in vitro system, Cheng et al18 showed that cholesterol itself served as an activator of ACAT-1, besides serving as a substrate, suggesting the possibility that ACAT may be an allosteric enzyme and that cholesterol may activate the enzyme by causing configurational change(s) of the protein. Other studies showed that additional mode(s) of ACAT regulation also exist. Wang et al17 showed that ACAT-1 enzyme by causing configurational change(s) of the protein. 

Other studies showed that additional mode(s) of ACAT regulation also exist. Wang et al17 showed that ACAT-1 mRNAs increased significantly (by 3- to 5-fold) during human monocyte differentiation to macrophages in vitro. Cheng et al18 reported that administration of dexamethasone to cultured human macrophages caused an increase in the ACAT-1 mRNA level. In animal experiments, cholesterol feeding increased ACAT-1 mRNAs by 2- to 3-fold in mouse and rabbit aortas, respectively, suggesting that the ACAT-1 message level may be regulated in a tissue-specific manner. Earlier studies showed that the rabbit aorta is easily susceptible to diet-induced atherosclerosis.19–21 Together, these results suggest the possibility that ACAT-1 may be abundantly expressed in atherosclerotic lesions.

In the current study, we examined ACAT-1 protein expression in human atherosclerotic lesions by immunohistochemical methods. We also monitored the change in ACAT-1 protein content and in ACAT enzyme activity during spontaneous differentiation of cultured human monocytes to macrophages in vitro. Our results support the idea that ACAT-1 plays an important role in differentiating monocytes and in the formation of macrophage-derived foam cells during the development of atherosclerosis.

Methods

Tissue Preparation

Tissue samples for immunohistochemistry analysis were obtained from atherosclerotic lesions of the aorta from 10 autopsy cases (8 men and 2 women; 41 to 75 years old) within 4 hours postmortem. After macroscopic inspection of the intimal surface, several tissue specimens were removed from the thoracic or abdominal aorta of these cases. For histological examination, complicated atherosclerotic lesions that included ulceration or thrombosis were excluded; diffuse intimal thickening, fatty streak lesions, and atherosclerotic plaques were selected as tissue samples for histochemical and immunohistochemical staining. Tissue specimens were fixed in an ice-cold 2% periodate-lysine-paraformaldehyde fixative for 6 hours and washed with PBS (pH 7.2) containing a graded series of sucrose (10%, 15%, and 20%). To inhibit ice crystal formation, the specimens were immersed in PBS containing 20% sucrose and 10% glycercin for 30 minutes and then embedded in OCT compound (Miles). These embedded materials were frozen and cut sequentially on a cryostat (Microm) into 5-μm-thick sections.

Histochemistry

The cryostat sections were stained with the lipid stain oil red O at 37°C for 30 minutes, counterstained with hematoxylin, and mounted with Malinol (Mutoh Chemical Co).

Antibodies

Rabbit polyclonal antibodies against the GST fusion protein of human ACAT-1 (amino acids 1 to 131) were prepared and purified as described.13 The ACAT-specific IgG fractions (designated DM10) were affinity-purified by column chromatography and were used as the ligand in affinity column chromatography.13 The EBM11 antibodies that specifically recognize human macrophages22 were purchased from Becton-Dickinson, and the HHF35 antibodies that specifically recognize human SMCs23 were purchased from Dako.

Immunohistochemistry

The sections were stained by using the indirect immunoperoxidase method as described previously.24 In brief, after the sections were rinsed with ice-cold PBS for 5 minutes, the method of Isobe et al20 was performed to block endogenous peroxidase activity. After they were washed, the sections were incubated for 20 minutes with 5% donkey serum and then reacted with anti–ACAT-1 antibodies DM10 (diluted 1:200 from a 0.22 mg/mL stock) as the primary antibody for 1 hour at room temperature. The sections were next rinsed 5 times with PBS and incubated with peroxidase-labeled anti-rabbit immunoglobulin F(ab′)2 (Amersham) diluted 1:100 from the stock as the secondary antibody. After the sections were washed again, peroxidase activity was visualized with 3,3'-diaminobenzidine as the substrate (Dojin Chemical Co), and the sections were counterstained with hematoxylin and then mounted with Malinol (Mutoh Chemical Co). Control immunohistochemical staining was done with nonimmune rabbit IgG instead of DM10 showed only background staining. The results of these negative controls were the same as the negative controls previously observed and published.26,27

Double Immunohistochemical Staining

To determine the cell types that express ACAT at high levels in the atherosclerotic lesions, we performed double immunohistochemical staining with DM10 and EBM11 or HHF35 by using previously described procedures.24 In brief, in the first step the sections were stained with DM10 and the peroxidase-conjugated secondary antibodies; 3,3'-diaminobenzidine was used to visualize the peroxidase activity (brown). Next, the sections were rinsed twice with 0.1 mol/L glycine/HCl buffer (pH 2.2) for 15 minutes to remove the antibody–pigment complex. In the second step, the same sections were incubated with either EBM11 or HHF35 at 4°C overnight. After they were washed, the sections were treated with rabbit anti-mouse immunoglobulin (Dako) at room temperature for 1 hour, rinsed with Tris-buffered saline (pH 7.6), and treated with alkaline phosphatase/anti–alkaline phosphatase complex (Dako) for 1 hour. To visualize the alkaline phosphatase activity (blue), the sections were incubated with a solution containing 0.2 mmol/L naphthol AS-MX phosphate, 1 mmol/L fast blue BB salt, and 1 mmol/L levamisole (Sigma Chemical Co) in 50 mmol/L Tris-HCl buffer (pH 8.7) at room temperature for 10 minutes and while avoiding exposure to light.

Cell Cultures

Human monocytes were obtained from leukapheresis packs of normal healthy donors as described previously.25 Purified human monocytes were resuspended at 105 cells/mL in serum-free Dulbecco’s modified Eagle’s Medium (DMEM) containing 25 mmol/L HEPES and 25 μg/mL gentamicin (medium A). Ten milliliters of cell suspension (107 cells) was seeded onto a 10-cm tissue culture dish (Corning) and incubated in a humidified incubator at 37°C and 10% CO2 for 1 to 2 hours to allow cell adherence. Afterward, the nonadherent cells were removed by aspiration, and the remaining
adherent monocytes were incubated in 10 mL per dish of medium A containing 10% pooled human serum. Cells at this stage were designated the zero-time culture. They were further cultured for up to 7 days without changing the growth medium.

Primary cultures of human aortic endothelial cells (ECs) and human aortic SMCs were purchased from Clonetics (San Diego, Calif). Cells were seeded into 10-cm dishes and cultured in 10 mL MCDB 131 medium (Sigma) containing 10% FBS, 10 μg/mL human epidermal growth factor, and 1.0 μg/mL hydrocortisone as described. When the cell density reached confluence, cells were harvested for analyses by Western blotting as described below.

**Immunoblotting**

With the use of various cultured human cells, our previous studies showed that the ACAT-1 protein solubilized in SDS solution tends to form aggregate(s) of higher molecular weights during SDS polyacrylamide gel electrophoresis Western blot analysis; the extent of aggregation tends to increase if the protein samples remain in SDS for >1 day, either at room temperature or in the cold. We also found that the extent of ACAT-1 protein aggregation was partially preventable if a high concentration of DTT (25 to 100 mmol/L) was included in the SDS solution (result not shown). To prevent the aggregation from occurring, a method was devised to harvest the cells for immunoblot analysis. At indicated times, monolayers of cells were washed several times with PBS and stored at 80°C as frozen and dried monolayers for up to 7 days. At the end of cell culture, the frozen cell monolayers were thawed and extracted with 0.1 mL 10% SDS per dish. Cells were scraped and sheared by using syringes with 25-gauge needles. Protein concentrations of cellular extracts were determined by the method of Lowry et al. Samples were separated by 10% SDS polyacrylamide gel electrophoresis and subjected to immunoblotting as described previously. The primary antibodies (DM10) were used at a final concentration of 0.5 μg/mL.

**ACAT Enzyme Activity Determined by the Reconstituted-Vesicle Assay**

This assay was performed essentially as described previously. It measures the ACAT activity independent of the endogenous lipid composition associated with the cellular extract. In brief, human monocytes-macrophages were harvested and homogenized by the hypotonic shock-scraping method. The protein concentration of the broken homogenate was kept at 2 to 4 mg/mL. To solubilize the enzyme, a deoxycholate (DOC)-phosphatidylcholine (PC) stock solution in buffer A was added to the cell homogenate to obtain a final concentration of 1% DOC. The DOC-solubilized cell extracts were reconstituted into PC vesicles that contained a cholesterol to PC molar ratio of 0.3 and were then used as the enzyme source. [1H]Oleoyl coenzyme A was added to the assay mixture to initiate the enzyme reaction. The assay was performed at 37°C for 20 minutes.

**Results**

To elucidate the histological distribution of ACAT-1 protein in human atherosclerotic aortas, immunohistochemical staining with anti–ACAT-1 polyclonal antibody (DM10) was performed. We examined 10 different cases that were affected by atherosclerotic lesions at various stages, including diffuse intimal thickening lesions, fatty streak lesions, and atheromatous plaques. Fatty streak lesions are characterized by many blood-derived macrophages with foam cell transformation and a limited number of elongated SMCs filled with intracytoplasmic lipid droplets; the lesions consist of foam cells, proliferating SMCs, macrophages, other mononuclear inflammatory cells, and extracellular matrix. Atheromatous plaques contain a fibrous cap and necrotic center; they are the more advanced lesion; they consist of cell debris, cholesterol crystals, degenerated foam cells, and calcium deposits. In the lesions with diffuse intimal thickening, both the ECs and the infiltrating mononuclear cells were stained weakly with DM10 (brown) (Figure 1a). Markedly enhanced ACAT staining was observed in the fatty streak lesions, particularly in the infiltrated macrophages with foamy transformation (Figure 1b). In contrast, there seemed to be no significant difference in immunoreactivity between the ECs in the diffuse intimal thickening lesions (Figure 1a) and those in the fatty streak lesions (Figure 1b).

Macrophages and SMCs are the major cellular components of atherosclerotic lesions. To determine the cell type(s) that exhibited high ACAT-1 expression in the lesions, we performed histochemical staining and double immunostaining experiments. In these experiments, we used cell type–specific antibodies, with the monoclonal antibody EBM11 for staining monocytes-macrophages, and the monoclonal antibody HHF35 for staining SMCs. The histochemical staining with oil red O showed that the atherosclerotic plaques contained massive deposits of lipid droplets, particularly in the atherosclerotic plaque “shoulder” (the central portion of Figure 2a), whereas the necrotic center (the acellular area on the right side of Figure 2a) contained a much smaller number of lipid droplets. Figure 2b and 2c shows the double immunostaining with DM10 and EBM11 of the serial sections of Figure 2a. The necrotic center was only marginally stained with DM10; however, the atherosclerotic plaque shoulder, which con-
tained numerous lipid droplets (Figure 2a), was markedly stained with DM10 (Figure 2b). Moreover, most of the DM10-positive cells (brown) were also positive for EBM11 (blue) (Figure 2b and 2c), indicating that monocytes-macrophages are the major cellular component of the ACAT-1–expressing cells in atherosclerotic lesions. A similar result was obtained from samples that contained fatty streak lesions (data not shown). We also used DM10 and the anti-SMC–specific antibody HHF35 to perform double immunostaining of fatty streak lesions (Figure 3a) and atherosclerotic plaques (Figure 3b). Although these lesions contained both macrophage-derived foam cells and SMC-derived foam cells (a representative of the latter cell type is indicated by an arrowhead in Figure 3a), most of the high-ACAT-1–expressing cells (brown) were not stained with HHF35 (blue) (Figure 3a and 3b), indicating that intimal SMCs are not the major cell type expressing ACAT-1 protein in atherosclerotic lesions. The results described in Figures 2 and 3 were consistently seen in samples prepared from at least 3 different cases. A total of 10 different cases were used in the current study. The clinicopathological information of these 10 cases and the ACAT-1 immunoreactivity at various stages of human aortic atherosclerosis are summarized in the Table.

As a first step in exploring the mechanism of ACAT-1 expression in macrophages in atherosclerotic lesions, we used a cell culture system to monitor the ACAT-1 protein contents and ACAT activities of human monocytes that spontaneously differentiate into macrophages in vitro. Western blotting with DM10 was used to monitor the ACAT-1 protein content. In Western blot analysis, the anti–ACAT-1 DM10 antibodies specifically recognized a single protein band from cell extracts of monocytes and macrophages, with an apparent molecular weight of ~50 kDa. No other protein signal(s) was detectable (results not shown). Additional results revealed that the ACAT protein content increased rapidly on day 1 of culturing the monocytes; this increase reached a peak value of 5- to 10-fold above the zero-time value on day 4. This finding was consistently seen in 3
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Clinicopathological Information and ACAT-1 Immunoreactivity of 10 Cases in the Current Immunohistochemical Study

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, y</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Samples</th>
<th>ACAT-1 Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>F</td>
<td>ATL + AML</td>
<td>FS</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>M</td>
<td>AML + sepsis</td>
<td>FS</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>M</td>
<td>Lung cancer</td>
<td>AP</td>
<td>+++</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>F</td>
<td>Multiple myeloma</td>
<td>AP</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>71</td>
<td>M</td>
<td>Lung cancer</td>
<td>AP</td>
<td>+++</td>
</tr>
<tr>
<td>6</td>
<td>54</td>
<td>M</td>
<td>Esophageal cancer</td>
<td>DIT</td>
<td>±</td>
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<tr>
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<td>M</td>
<td>Cholangiocarcinoma</td>
<td>AP</td>
<td>+++</td>
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<tr>
<td>8</td>
<td>41</td>
<td>M</td>
<td>Pancreatic cancer</td>
<td>DIT</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>71</td>
<td>M</td>
<td>Lung cancer</td>
<td>AP</td>
<td>+++</td>
</tr>
<tr>
<td>10</td>
<td>67</td>
<td>M</td>
<td>MDS</td>
<td>FS</td>
<td>+</td>
</tr>
</tbody>
</table>

ATL indicates adult T-cell leukemia; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; FS, fatty streak; AP, atheromatous plaque; DIT, diffuse intimal thickening; ±, fairly positive; +, constantly positive; ++, strongly positive; and ++++, extremely positive.

Figure 4. Immunoblotting of ACAT-1 protein from cultured human monocytes-macrophages, SMCs, and ECs. Human monocytes (10^7 cells) were seeded onto 10-cm dishes and cultured in 10 mL of DMEM containing 10% pooled human serum. Cells were harvested at the indicated times and submitted to ACAT activity determination in triplicate by using the reconstituted-vesicle assay. This result indicates that the increase in ACAT-1 protein content is an early event of monocyte differentiation. We also found that the ACAT protein content of human monocytes (day 0) was comparable to that of cultured ECs and cultured SMCs (Figure 4), whereas the monocyte-derived macrophages (day 7) expressed much higher levels of ACAT-1 protein.

We next used the reconstituted-vesicle assay to monitor the ACAT activities in vitro in differentiating monocytes-macrophages. ACAT is a membrane-bound enzyme in the endoplasmic reticulum. In broken-cell homogenates, the enzyme activity is affected by the cellular lipid associated with the enzyme. The reconstituted-vesicle assay measures the ACAT activity in a manner independent of cellular lipid composition, thus circumventing the uncertainty in endogenous lipid composition. Using this assay, we found that the monocytes at time zero expressed basal ACAT activity (11 pmol·min^−1·mg^−1), a value comparable to those found in various human tissue culture cells such as fibroblasts, HepG2 cells, and Caco-2 cells. During monocyte differentiation, we found that the ACAT activity rapidly increased, reaching values 10 times as high as the zero-time value by day 4. Figure 5 shows a typical result. This finding was consistently seen in 2 separate experiments. This experiment demonstrates that the high ACAT protein content expressed in human monocyte–derived macrophages is enzymatically active in vitro.

Discussion

Earlier studies showed that when various animal species were fed an atherogenic diet, the ACAT enzyme activities in aortas isolated from these animals were significantly increased (eg, see Reference 35). Owing to the lack of ACAT molecular probes and of refined ACAT enzyme assay conditions, it could not be determined from these studies whether the increase in ACAT enzyme activity was due to an increase in ACAT protein content, to an increase in cholesterol available to ACAT as the substrate, or to a change in some other factor(s) that caused the increase in ACAT enzyme activity. More recently, it was shown that feeding rabbits an atherogenic diet caused a 2- to 3-fold increase in ACAT-1 mRNAs isolated from these aortas. With the use of immunostaining, the current results amply demonstrated the presence of the ACAT-1 protein in human aortas affected with atherosclerosis. The control experiments showed that in mild lesions with diffuse intimal thickening, the ECs and infiltrating monocytes stained only weakly with the ACAT-1 antibodies. The intimal layers of the human atherosclerotic lesions mainly consist of macrophages and SMCs. Using double immunostaining, we showed that macrophages, but not SMCs, were the principal cell type that expressed high levels of ACAT-1 protein.
As shown in Figure 3, SMC-derived foam cells do not abundantly express ACAT-1 protein as macrophage-derived foam cells. What might be the mechanism of foam cell formation in SMCs? It is possible that a lower level of ACAT-1 expression may be enough for SMCs to accumulate CE, although such a process may require a longer time to develop. Alternatively, a more plausible mechanism may be that SMCs have an ACAT-independent mechanism of foam cell formation. In fact, it has been shown that CE from lysed J774 macrophage–derived foam cells are adsorbed by SMCs, which results in accumulation of lipid droplets in the cytoplasm of SMCs in vitro. Third, SMCs may express an ACAT isoform other than ACAT-1. Very recently, another ACAT gene (ACAT-2) has been cloned; in the mouse and monkey, Northern blot analyses showed that ACAT-2 message expression seemed to be restricted to the liver and intestines. In the future, the availability of specific ACAT-2 antibodies will be needed to test the possible expression of ACAT-2 protein in the atherosclerotic lesions.

To begin exploring the mechanism(s) of ACAT expression in atherosclerotic lesions, we monitored the ACAT-1 protein contents and ACAT enzyme activities in human monocytes spontaneously differentiating to macrophages in vitro. Using this system, we showed that the ACAT-1 protein content rapidly increased during the early stage of differentiation. This result implies that the upregulation of ACAT-1 is an important phenotypic characteristic of differentiating monocytes. We also showed that human macrophages express ACAT-1 protein at levels much higher (by 10- to 20-fold) than many other human cell types that we have examined thus far, including cultured aortic SMCs, cultured ECs (reported in this study), fibroblast cells, liver hepatoma HepG2 cells, as well as intestinal Caco-2 cells (reported in our earlier study). ACAT-1 expressed in the differentiating monocytes-macrophages was enzymatically active in vitro, as tested by the reconstituted-vesicle assay. In Figures 4 and 5, the fold increases in ACAT-1 protein (5.9-fold; Figure 4) and ACAT activities (>10-fold; Figure 5) appear to be somewhat different. This could largely be due to the limitations of using Western blotting for quantifying the protein content. In this particular experiment, we needed a long exposure time to obtain significant signals for the ACAT-1 protein expressed in human aortic ECs and SMCs; both samples were present in the same membrane. The long exposure time caused the intensity of the ACAT-1 signal expressed in monocyte-derived macrophages to plateau. In other experiments with less exposure time, we had observed a fold increase of >10 in ACAT-1 protein content by Western blotting during the human monocyte–macrophage differentiation process.

The finding that high levels of ACAT-1 protein are present in differentiating monocytes maintained in culture explains at least in part the high levels of ACAT-1 protein found in the macrophages of human atherosclerotic lesions. ACAT catalyzes the conversion of cellular cholesterol into CEs. In atherogenesis, the accumulation of CEs as cytosolic lipid droplets within the macrophages is a critical event of foam cell transformation. Our results presented in this article support the idea that ACAT-1 plays an important role in the formation of macrophage-derived foam cells during the development of atherosclerosis.

The mechanism(s) involved in the upregulation of ACAT-1 protein content during the monocyte-macrophage differentiation process is not clear at present. Using Northern blot analysis, Wang et al previously demonstrated that the ACAT-1 message levels in differentiating monocytes were significantly upregulated; specifically, the ACAT transcripts increased by 3- to 5-fold within 6 days of culturing the monocytes. In principle, the increase in ACAT-1 protein content may involve an increase in the ACAT-1 gene transcription rate, a decrease in the ACAT-1 message turnover rate, or both; in addition, an increase in translational efficiency of the ACAT-1 messages may also be involved. Future studies are needed to address this issue.

The high levels of ACAT enzyme activities present in macrophages imply that these cells have a large capacity to produce CEs from incoming cholesterol. This implication is consistent with the proposed role of macrophages in clearing up denatured lipoprotein complexes in the blood. During atherogenesis, a major source for the incoming cholesterol in macrophages is believed to be modified LDL, probably in oxidized form (for reviews, see References 37 and 38). At least 3 types of membrane protein receptor capable of taking up oxidized LDL are present on the cell surfaces of macrophages: the macrophage scavenger receptor (for a review, see Reference 39), CD36, and CD68 (macrosialin). The macrophage scavenger receptor (MSR) gene produces 2 kinds of protein isoform, MSR type I and MSR type II. The expression of the MSR type I protein increases significantly during differentiation of human monocytes. The expression of CD36, a different receptor for oxidized LDL, is also upregulated during differentiation of human monocytes to macrophages. These studies, along with our current results, point to the theme that during differentiation of monocytes to macrophages, the cellular capacity to accumulate CEs is intensified by increased expressions of the ACAT enzyme and the receptors for oxidized LDL. It is thus tempting to speculate that agents that regulate the scavenger receptor(s) may also be involved in regulating ACAT during monocyte differentiation or atherosclerotic lesion development. This possibility is currently being investigated in our laboratories.

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