Induction of acyl-coenzyme A:cholesterol acyltransferase-1 by 1,25-dihydroxyvitamin D_3 or 9-*cis*-retinoic acid in undifferentiated THP-1 cells

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Abstract **We have previously shown that acyl-coenzyme A: cholesterol acyltransferase-1 (ACAT-1) protein content increases significantly during the human monocyte-macrophage differentiation process. To gain further insight, we used undifferentiated human monocytic THP-1 cells as a model system with which to examine whether ACAT-1 mRNA and protein content can be increased by treating cells with 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] or with 9-*cis*-retinoic acid (9-*cis*-RA), two agents known to upregulate the expression of various genes during the monocyte-macrophage differentiation process. Immunoblot analysis with anti-human ACAT-1 antibodies revealed that ACAT-1 protein was increased by 2.6-fold, using 1,25-$(OH)_2D_3$ at a physiological concentration (100 pM). ACAT-1 protein was also increased when using 9-*cis*-RA, but only at relatively high concentrations (0.1–1 μM). Northern blot analysis revealed that among the four ACAT-1 mRNA transcripts (2.8, 3.6, 4.2, and 7.0 kb) examined, only the 2.8- and 3.6-kb transcripts were selectively increased. On the basis of enzyme assays in vitro, ACAT activity was increased 3.0-fold by using 100 nM 1,25-$(OH)_2D_3$, and 1.8-fold by using 1 μM 9-*cis*-RA. Together, our results suggest that 1,25-$(OH)_3$ participates in ACAT-1 gene expression during the monocyte-macrophage differentiation process.**—Maung, K. K., A. Miyazaki, H. Nomiyama, C. C. Y. Chang, T-Y. Chang, and S. Horiuchi. **Induction of acyl-coenzyme A:cholesterol acyltransferase-1 by 1,25-dihydroxyvitamin D_3 or 9-*cis*-retinoic acid in undifferentiated THP-1 cells.** ***J. Lipid Res.*** **2001.** 42: **181–187.**

Supplementary key words ACAT • ACAT-1 • monocyte-macrophage differentiation

Acyl-coenzyme A:cholesterol acyltransferase (ACAT) is an intracellular enzyme that catalyzes the formation of cholesteryl esters from cholesterol and long-chain fatty acyl-coenzyme A (1). ACAT plays essential roles in various physiological processes including cellular cholesterol homeostasis, hepatic lipoprotein assembly, and dietary cholesterol absorption in intestine (1). Under pathological conditions, ACAT plays a crucial role in the accumulation of cholesterol esters in macrophage-derived foam cells (2). The first ACAT-1 cDNA was cloned in 1993 (3). Genomic organization of human *ACAT-1* was then determined (4). The full-length ACAT-1 cDNAs in other mammalian species have subsequently been cloned (5–7). The characteristics of the ACAT-1 gene knockout mouse (8) strongly suggested the presence of another ACAT gene (*ACAT-2*), which was eventually cloned as a homolog of *ACAT-1* (9–11). ACAT-1 is ubiquitously expressed in various tissues and cells including adrenal glands, kidney (3, 5, 7), and macrophages (12), whereas ACAT-2 is expressed only in intestine and liver (9–11). Immunodepletion experiments using ACAT-1-specific antibodies (DM10) suggested that the major ACAT isozyme in adult human liver is ACAT-1 (13). To support this interpretation, immunohistochemical staining of adult human liver with DM10 indicated that human hepatocytes amply express ACAT-1 (12).

We demonstrated by immunohistochemical methods that ACAT-1 is highly expressed in human atherosclerotic lesions, particularly in macrophage-derived foam cells (14). We (14) and others (15) also found that the ACAT-1 mRNAs and protein content increase significantly when blood-borne human monocytes spontaneously differentiate into macrophages. The cellular factors involved in causing increases in ACAT-1 protein and mRNAs during the monocytic differentiation process have not been identified. In an attempt to identify such factors, we used THP-1 cells as a model for human monocytes (16–19) and tested various agents for their possible effects on ACAT-1 expression. We found that 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] and

Abbreviations: ACAT, acyl-coenzyme A:cholesterol acyltransferase; FCS, fetal calf serum; HODE, hydroxyoctadecadienoic acid; 25-OH D_3, 25-hydroxyvitamin D_3; 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; PC, phosphatidylcholine; 9-*cis*-RA, 9-*cis*-retinoic acid; PMA, phorbol 12-myristate 13-acetate; RARE, retinoic acid response element; RXR, retinoid X receptor; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; VDR, vitamin D receptor; VDRE, vitamin D response element.

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9-*cis*-retinoic acid (9-*cis*-RA) increased ACAT-1 mRNA as well as ACAT-1 protein and ACAT activity in undifferentiated THP-1 cells.

MATERIALS AND METHODS

Materials

THP-1 cells, a human monocytic leukemia cell line, were purchased from the American Type Culture Collection (Manassas, VA). RPMI 1640, fetal calf serum (FCS), penicillin G and streptomycin, and a TRIzol total RNA extraction kit were purchased from GIBCO-BRL (Glasgow, UK). Vitamin D_3, 25-hydroxyvitamin D_3 (25-OH D_3), 1,25-$(OH)_2D_3$, 9-*cis*-RA, and phorbol 12-myristate 13-acetate (PMA) were from Sigma (St. Louis, MO). 9- and 13-hydroxyoctadecadienoic acids (HODE) were from Cayman Chemical (Ann Arbor, MI). [^{14}C]oleoyl-CoA (1.85 MBq/ml), Hybond N+ nylon membrane, and an enhanced chemiluminescence (ECL) Western blotting detection reagent kit were obtained from Amersham (Buckinghamshire, UK). Goat anti-rabbit IgG antibody conjugated with horseradish peroxidase was obtained from Zymed (South San Francisco, CA). A digoxigenin (DIG) Northern starter kit for mRNA detection was purchased from Boehringer (Mannheim, Germany). DNA ligation kit was purchased from Promega (Madison, WI) and pBluescript II SK+ vector was purchased from Stratagene (La Jolla, CA). Other chemicals were the best grade available from commercial sources.

Cell culture

THP-1 cells were grown in RPMI 1640 supplemented with 10% FCS, streptomycin (100 μg/ml), and penicillin (100 U/ml) (medium A). For the ACAT assay and Northern blotting, THP-1 cells (1×10^7) in 10 ml of medium A were seeded to each 10-cm culture dish and incubated with the indicated concentrations of 1,25-$(OH)_2D_3$ or 9-*cis*-RA dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the medium was 0.1% (v/v). For immunoblot analysis, THP-1 cells (3×10^6) in 3 ml of medium A were seeded to each 6-cm dish and treated with the indicated concentrations of 1,25-$(OH)_2D_3$ or 9-*cis*-RA together with controls.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis

Cells were washed twice with ice-cold phosphate-buffered saline (PBS) and solubilized with 100–150 μl of 10% sodium dodecyl sulfate (SDS). Protein concentrations were determined by the method of Lowry et al. (20). In a standard experiment, 25 μg of protein was applied to each lane and separated by 10% SDS-polyacrylamide gel electrophoresis (PAGE) according to the method of Laemmli, Beguin, and Gujer-Kellenberger (21). The proteins were transferred to a nitrocellulose transfer membrane and incubated for 1 h with a 0.25-μg/ml concentration of affinity-purified rabbit polyclonal antibody to human ACAT-1 (DM10) (22). The membrane was washed and further incubated for 1 h with goat anti-rabbit IgG antibody conjugated with horseradish peroxidase as a second antibody. The immunoreactive bands were detected by using the ECL detection system.

Assay for ACAT activity

The reconstituted ACAT assay was performed according to the method of Chang et al. (23), with minor modifications. Briefly, cells were washed twice with PBS and treated with 1 mM Tris and 1 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.4) for hypotonic shock, followed by homogenization with 100–150 μl of 50 mM Tris and 1 mM EDTA (pH 7.8) (buffer A). To the cell homogenates, 2 M KCl and 10% 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate (CHAPS) in buffer A were added to obtain the final concentrations of 1 M and 2%, respectively. Protein concentrations of the homogenates were adjusted to 4 mg/ml with buffer A containing 1 M KCl, 2% CHAPS, and 0.2 mM phenylmethylsulfonyl fluoride.

Cell homogenates (20 μl) were mixed with 100 μl of sodium taurocholate-cholesterol-phosphatidylcholine (PC) mixed micelles (0.2 cholesterol-PC molar ratio) (23) and incubated for 20 min on ice. The reaction was initiated by adding 20 μl of [^{14}C]oleoyl-CoA mixture, which contained 25 μM [^{14}C]oleoyl-CoA (20 dpm/pmol), fatty acid-free bovine serum albumin (12.5 mg/ml), and 100 mM Tris (pH 7.8) and incubating the mixture for 10 min at 37°C. The enzyme reaction was terminated by adding 3 ml of chloroform–methanol 2:1 (v/v). Lipids were extracted and the radioactive cholesteryl [^{14}C]oleate was determined by thin-layer chromatography (24).

Northern blot analysis

The first-strand cDNA primed with oligo(dT) was synthesized from 4 μg of total RNA obtained from THP-1 cells. The sequences of the forward primer and the reverse primer for polymerase chain reaction (PCR) amplification of human ACAT-1 cDNA were GCCTCAGACAATACAATGG (nucleotides 1382 to 1400) and AAACACGTAACGACAAGTCC (nucleotides 3027 to 3046), respectively (3). The PCR product was digested with *Hinc*II to obtain a 727-bp fragment (nucleotides 1551 to 2278). The fragment was ligated into pBluescriptII SK+ at the *Hinc*II restriction site and amplified in *Escherichia coli*. DIG-labeled antisense RNA probes were generated in the presence of DIG-labeled UTP according to the manufacturer's instructions (DIG Northern starter kit from Boehringer) (25).

Total RNA (12 μg/lane) was denatured in formamide-formaldehyde and subjected to electrophoresis on a 1.2% agarose gel containing formaldehyde. The RNAs were transferred to a Hybond N+ nylon membrane by capillary transfer with 20× SSC and cross-linked by UV (FS1500; Funakosi, Tokyo) at 12 J/cm^2. The membrane was prehybridized for 30 min at 65°C in 40 ml of hybridization buffer (Boehringer) followed by incubation for 4 h at 65°C with heat-denatured RNA probe (80 ng/ml) in 40 ml of prewarmed hybridization buffer. Stringency washing was done twice for 5 min at room temperature with 2× SSC and 0.1% SDS and then twice for 30 min at 68°C with 0.1× SSC and 0.1% SDS. The membrane was then blocked for 30 min at room temperature with 100 ml of blocking solution (Boehringer). The membrane was further incubated for 30 min at room temperature with anti-DIG antibody conjugated with alkaline phosphatase (1:10,000 dilution) in 20 ml of the blocking solution, followed by washing twice with 100 ml of 0.1% maleic acid and 0.3% Tween 20. Eventually, ACAT-1 mRNA was detected by soaking the membrane for 5 min in CDP-Star (Boehringer) chemiluminescent substrate, followed by exposure to an X-ray film for 1–5 min.

Other methods

Data were evaluated by Student's *t*-test. The difference was judged to be significant at $P < 0.05$. The intensities of the bands of immunoblot analysis and Northern blot analysis were quantified by densitometric scanning of the films.

RESULTS

Effects of 1,25-$(OH)_2D_3$ and 9-*cis*-RA on ACAT-1 protein expression in THP-1 cells

Figure 1 shows dose-dependent effects of 1,25-$(OH)_2D_3$ or 9-*cis*-RA on ACAT-1 protein expression in undifferenti-

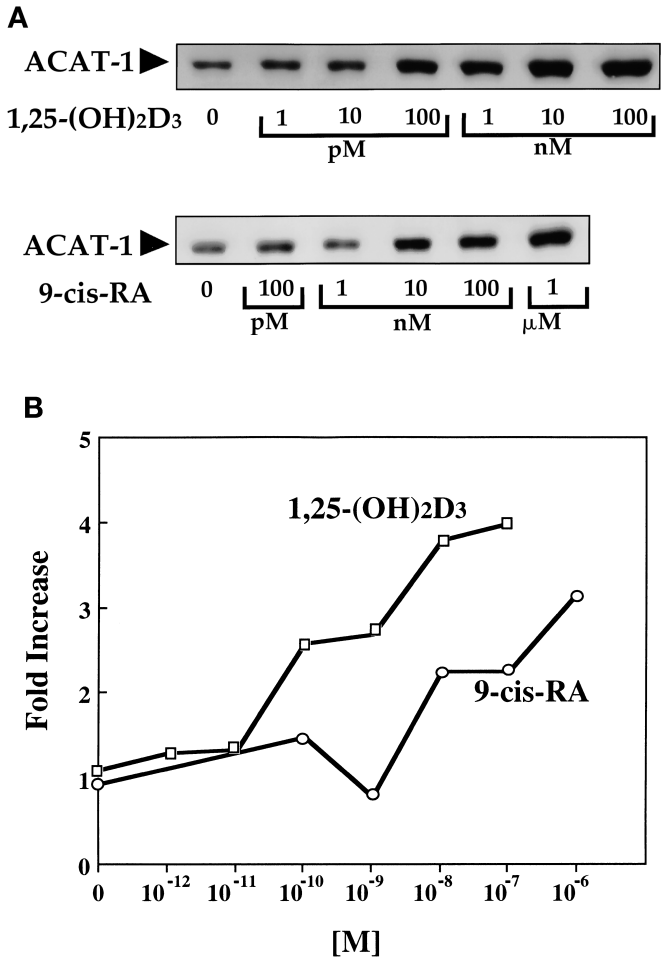

Fig. 1. Dose-dependent effects of 1,25-$(OH)_2D_3$ and 9-*cis*-RA on ACAT-1 protein content in THP-1 cells. A: THP-1 cells (3×10^6) in 3 ml of medium A were incubated for 48 h with the indicated concentrations of 1,25-$(OH)_2D_3$ or 9-*cis*-RA. Cells were solubilized and subjected to immunoblot analysis with anti-human ACAT-1 antibody. B: Densitometric scanning of A.

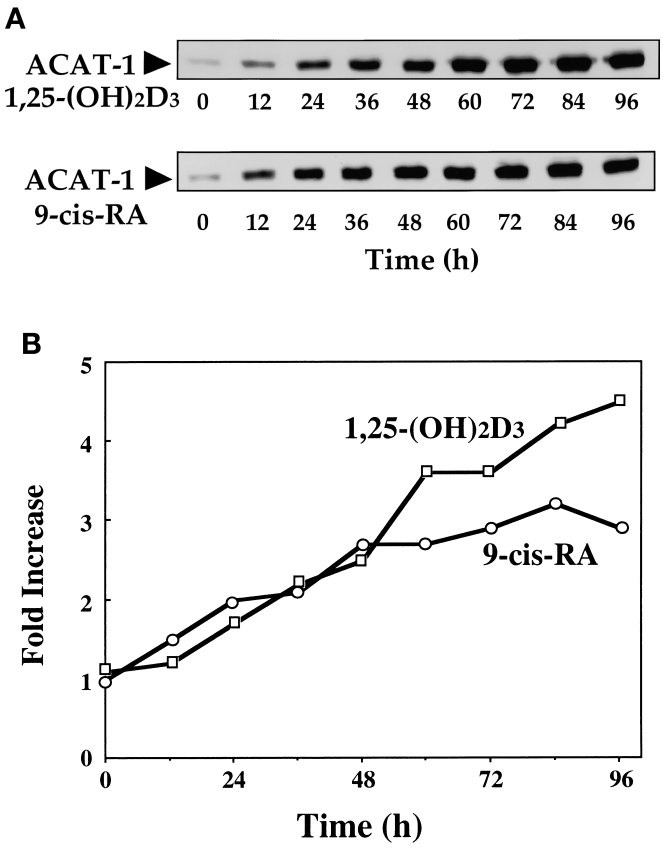

Fig. 2. Time course study of the effect of 1,25-$(OH)_2D_3$ and 9-*cis*-RA on ACAT-1 protein content in THP-1 cells. A: THP-1 cells (3×10^6) in 3 ml of medium A were treated for the indicated time periods with 100 nM 1,25-$(OH)_2D_3$ or 1 μM 9-*cis*-RA. Cells were solubilized and subjected to immunoblot analysis with anti-human ACAT-1 antibody. B: Densitometric scanning of A.

ated THP-1 cells. This result showed that the lowest concentration of 1,25-$(OH)_2D_3$ that displayed a significant effect (100 pM) was within the physiological concentrations reported in the literature (62–155 pM) (26). ACAT-1 was also increased by 9-*cis*-RA, but only when used at much higher concentrations (Fig. 1A). When compared at the same concentrations, 1,25-$(OH)_2D_3$ is much more effective than 9-*cis*-RA (Fig. 1B). At all concentrations of 1,25-$(OH)_2D_3$ and 9-*cis*-RA, THP-1 cells were still floating, without showing the typical phenotype of macrophages. Time course studies (**Fig. 2A**) were conducted with the most effective concentrations of 1,25-$(OH)_2D_3$ (100 nM) and 9-*cis*-RA (1 μM). The results showed that ACAT-1 protein continued to increase with 1,25-$(OH)_2D_3$ during the experiment whereas with 9-*cis*-RA, the increase reached a plateau level at 48 h (Fig. 2A).

Vitamin D_3 is derived from 7-dehydrocholesterol, the immediate precursor in the cholesterol biosynthetic pathway. In vivo, vitamin D_3 is converted to 25-OH D_3 in the liver, and then converted to biologically active 1,25-$(OH)_2D_3$ in the kidney. To examine the specificity of 1,25-$(OH)_2D_3$ in our system, we tested various compounds that are structurally related to 1,25-$(OH)_2D_3$ (**Fig. 3**). Cholesterol, a substrate of ACAT-1, did not show any effect on ACAT-1 protein expression. We tested the potential effect of 1,25-$(OH)_2D_3$ precursors at a single concentration (100 nM), and found that the ACAT-1 protein content was slightly increased

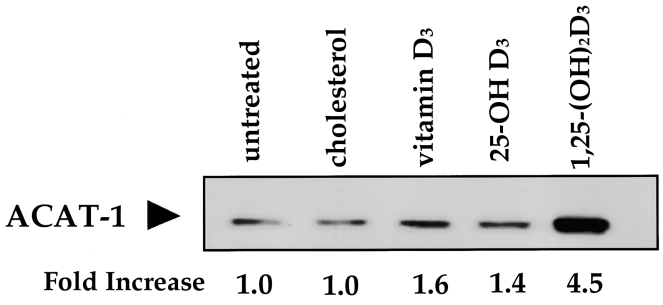

Fig. 3. Effects of vitamin D_3-related compounds on ACAT-1 protein expression in THP-1 cells. THP-1 cells (3×10^6) in 3 ml of medium A were incubated for 48 h with 100 nM vitamin D_3, 25-OH D_3, 1,25-$(OH)_2D_3$, or cholesterol. Cells were solubilized and subjected to immunoblot analysis with anti-human ACAT-1 antibody. Data were analyzed by densitometric scanning.

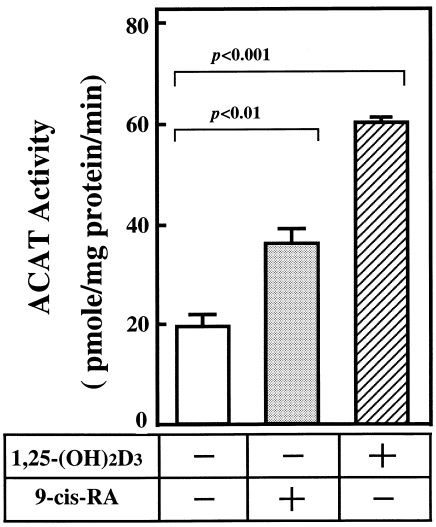

Fig. 4. ACAT activity in THP-1 cells after treatment with 1,25-$(OH)_2D_3$ or 9-*cis*-RA. THP-1 cells (1×10^7) in 10 ml of medium A were incubated for 48 h with 100 nM 1,25-$(OH)_2D_3$ or 1 μM 9-*cis*-RA. Cells were harvested by hypotonic shock, followed by determination of ACAT activities. Data are representative of three separate experiments with triplicate assays. Bars show the standard deviation.

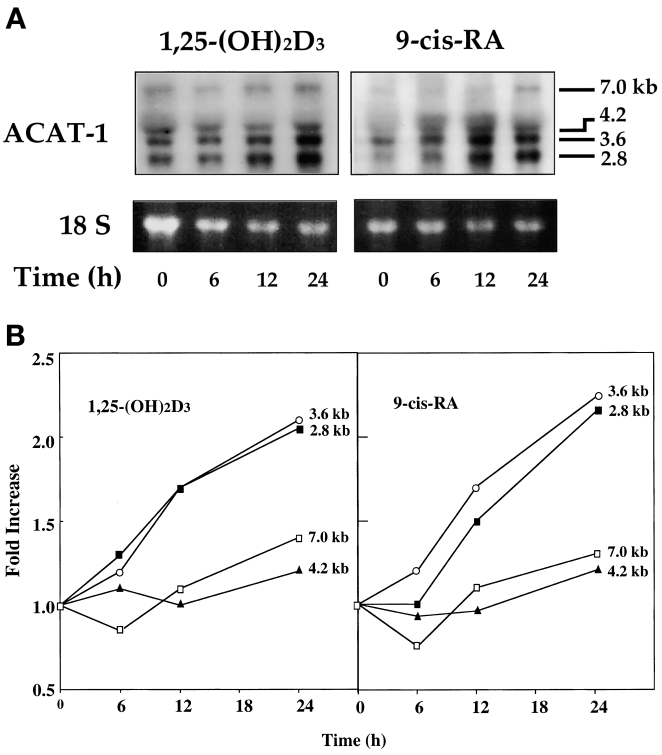

Fig. 5. Northern blot analysis of THP-1 cells treated with 1,25-$(OH)_2D_3$ or 9-*cis*-RA. A: THP-1 cells were incubated for the indicated time periods with 100 nM 1,25-$(OH)_2D_3$ or 1 μM 9-*cis*-RA. Total RNA was extracted and ACAT-1 mRNAs were detected by Northern blot analysis. B: Densitometric scanning of A. The intensities of 18S bands were used as internal controls to normalize the intensities of ACAT-1 mRNA subspecies.

when using vitamin D_3 or 25-OH D_3 (by 1.4- to 1.6-fold), while it was markedly increased when using 1,25-$(OH)_2D_3$ (by 4.5-fold) (Fig. 3). PMA is able to induce differentiation of THP-1 cells into macrophage-like cells. We tested the effect of PMA on ACAT-1 expression and found that its effect was negligible (data not shown). HODEs, which are oxidation products of linoleic acid, are generated during low density lipoprotein (LDL) oxidation and behave as ligands for the nuclear receptor, peroxisomal proliferator activated receptor γ, and stimulate monocyte-macrophage differentiation (16, 17). We tested the possible effects of 9- and 13-HODE on ACAT-1 expression, and found that neither 9- nor 13-HODE at 10–15 μM had any detectable effect on ACAT-1 protein content (results not shown). We performed additional experiments and showed that when THP-1 cells were differentiated to macrophage-like cells with PMA, there was no longer any appreciable effect on ACAT-1 by either 1,25-$(OH)_2D_3$ or 9-*cis*-RA (data not shown), indicating that the ACAT-1 induction in THP-1 cells by these agents is restricted to the undifferentiated stage. We also tested several other human cell types, including HepG2 cells and human embryonic kidney 293 cells, and found that 1,25-$(OH)_2D_3$ and 9-*cis*-RA did not increase ACAT-1 protein content in these cells (data not shown). Together, our results show that the effect of 1,25-$(OH)_2D_3$ and 9-*cis*-RA on ACAT-1 protein content is restricted to the undifferentiated monocytic cell lineage.

ACAT enzyme activity

ACAT enzyme activity was determined in THP-1 cells after treatment for 48 h with 1,25-$(OH)_2D_3$ or with 9-*cis*-RA. The activity in untreated THP-1 cells was about 20 pmol/mg/min. On treatment with 1 μM 9-*cis*-RA, the activity was increased by 1.8-fold. Treatment of THP-1 cell with 100 nM 1,25-$(OH)_2D_3$ led to a 3.0-fold increase in ACAT activity (**Fig. 4**). This result demonstrates that ACAT-1 protein in THP-1 cells treated with 1,25-$(OH)_2D_3$ or 9-*cis*-RA is enzymatically active.

Northern blot analysis

THP-1 cells were treated for the indicated time periods with 100 nM 1,25-$(OH)_2D_3$ or with 1 μM 9-*cis*-RA. Northern blot analysis showed that four mRNA transcripts (7.0, 4.2, 3.6, and 2.8 kb) are present (**Fig. 5A**). This result is consistent with previous studies using other types of human cells such as HepG2 cells (24), human monocyte-macrophages (15), and melanoma cells (4). Among the four transcripts, the 2.8- and 3.6-kb transcripts were increased steadily from 12 h on, after cells were treated with either 1,25-$(OH)_2D_3$ or with 9-*cis*-RA. The other two transcripts (4.2 and 7.0 kb) remained essentially unchanged during the course of the experiment (Fig. 5A and B). The differential increase in ACAT-1 mRNAs is consistent with earlier results obtained during the spontaneous macrophage differentiation process, using blood-borne human monocytes as the starting material (15).

Synergistic effect of 1,25-$(OH)_2D_3$ and 9-*cis*-RA on ACAT-1 induction in THP-1 cells

To examine the possible synergistic effect of 1,25-$(OH)_2D_3$ and 9-*cis*-RA on ACAT-1 protein increase, THP-1

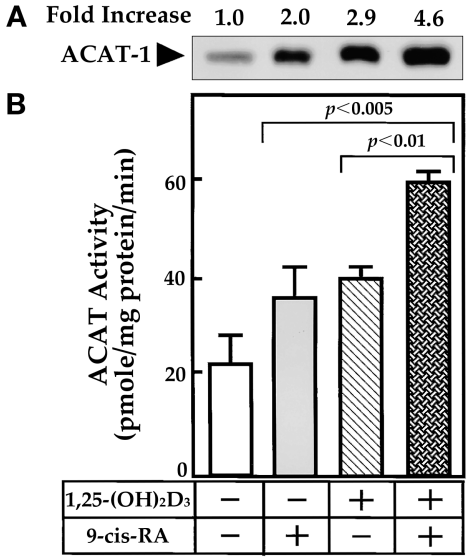

Fig. 6. Combined effect of 1,25-$(OH)_2D_3$ and 9-*cis*-RA on ACAT-1 protein content (A) and ACAT activity (B) in THP-1 cells. A: THP-1 cells (3×10^6) in 3 ml of medium A were incubated for 48 h with 1 nM 1,25-$(OH)_2D_3$ and/or 10 nM 9-*cis*-RA. Cells were solubilized and subjected to immunoblot analysis with anti-human ACAT-1 antibody. B: THP-1 cells (1×10^7) in 10 ml of medium A were incubated for 48 h with 1 nM 1,25-$(OH)_2D_3$ and/or 10 nM 9-*cis*-RA. Cells were harvested by hypotonic shock, followed by determination of ACAT activities. Data are representative of three separate experiments with triplicate assays. Bars show the standard deviation.

cells were treated with relatively low concentrations of 1,25-$(OH)_2D_3$ (1 nM) and 9-*cis*-RA (10 nM). As shown in **Fig. 6A**, ACAT-1 protein was increased by 2.9-fold with 1,25-$(OH)_2D_3$ and by 2.0-fold with 9-*cis*-RA. A combination of these two agents led to a further increase (4.6-fold), indicating a synergistic effect of 1,25-$(OH)_2D_3$ and 9-*cis*-RA. Consistent with this result, a parallel experiment showed that ACAT activity was increased by 1.8-fold with 1,25-$(OH)_2D_3$, 1.6-fold with 9-*cis*-RA, and 2.7-fold with both agents (Fig. 6B). We tested higher concentrations of 1,25-$(OH)_2D_3$ (100 nM) and 9-*cis*-RA (1 μM), and found that the synergism is lost.

DISCUSSION

Previous studies showed that ACAT-1 mRNAs and protein content increased 5- to 10-fold during the human monocyte-macrophage differentiation process (15, 16). The cellular factors involved in causing the ACAT-1 mRNA and protein increase have not been identified. In the current study, we tested certain candidate agents, using the THP-1 cells as a model of human monocytes (16–19). In this report, we found that 1,25-$(OH)_2D_3$ or 9-*cis*-RA significantly increases the ACAT-1 protein content and ACAT enzyme activity; the increases correlate well with the increases in the ACAT-1 mRNAs, suggesting that the effect of these agents may be at the mRNA level.

It is known that 1,25-$(OH)_2D_3$ and 9-*cis*-RA induce expression of various genes during the monocyte-macrophage differentiation process (27–29). In particular, 1,25-$(OH)_2D_3$ has been reported to induce differentiation markers for monocyte-macrophages such as CD14 and CD11b in THP-1 cells (27, 29). Therefore, ACAT-1 can be regarded as one of the genes that is regulated by 1,25-$(OH)_2D_3$ and/or 9-*cis*-RA in differentiating monocytic cells. Among the various vitamin D derivatives tested (Fig. 3), our results show that 1,25-$(OH)_2D_3$ is the most effective derivative at the concentration tested (100 nM). On the other hand, because we tested the various vitamin D derivatives at only one concentration, the effects of vitamin D_3 or 25-OH D_3 at higher concentrations remain to be studied in the future.

Both 1,25-$(OH)_2D_3$ and 9-*cis*-RA are known to enter the cell interior and then bind to their respective nuclear receptors, that is, the vitamin D receptor (VDR) and the retinoid X receptor (RXR), which are ligand-activated transcriptional regulators (30, 31). The VDR binds to the vitamin D response element (VDRE) after forming heterodimers with the RXR (VDR/RXR), and then exerts its effect on the targeted genes. After binding of 9-*cis*-RA, the RXR behaves mainly as an auxiliary receptor for other nuclear receptors, also functioning independently as homodimers (RXR/RXR) that bind to the retinoic acid response element (RARE). ACAT-1 contains four discretely sized mRNA species (2.8, 3.6, 4.2, and 7 kb). We noted that only the levels of the two shorter mRNAs (2.8 and 3.6 kb) responded to 1,25-$(OH)_2D_3$ and 9-*cis*-RA; the levels of the longer ACAT-1 mRNAs (4.2 and 7.0 kb) remained essentially constant. Earlier work by Li et al. (4) showed that ACAT-1 contains two promoters; the P1 promoter is located at chromosome 1, while the P7 promoter is located at chromosome 7. The two short mRNAs are derived from the P1 promoter, while the 4.2-kb mRNA is believed to be the product of a novel mRNA recombination event, through *trans*-splicing of two mRNAs; one is produced from the P1 promoter while the other is produced from the P7 promoter. The origin of the 7.0-kb mRNA remains unclear. Our current results thus suggest that the ACAT-1 P1 promoter may contain specific *cis* element(s) that respond to 1,25-$(OH)_2D_3$ and 9-*cis*-RA. We examined the available nucleotide sequences of the ACAT-1 P1 and P7 promoters, but were not able to identify the sequences of the VDRE or the RARE. Therefore, the molecular mechanisms of ACAT-1 induction by 1,25-$(OH)_2D_3$ and 9-*cis*-RA remain unclear. It is possible that the VDRE or RARE-like sequence may be present in regions upstream of the available sequence. Our results do not exclude the possibility that the increase in ACAT-1 message may in part be due to increase(s) in ACAT-1 message stability, instead of to an increase in transcription. In the future, these two possibilities will be tested by performing nuclear run-on assays and mRNA stability assays.

On the basis of a review of the current literature, we propose the following scenario to describe various modes of ACAT-1 regulation in monocyte-macrophages within the arterial walls: during the monocyte-macrophage differentiation process, significant ACAT-1 gene expression

occurs, which may be triggered by certain serum components such as 1,25-$(OH)_2D_3$, as suggested by the current study. After differentiation, *ACAT-1* gene expression could be further induced by various factors present in situ, such as interferon γ (32) and steroid hormones (33). At the enzyme activity level, ACAT-1 is controlled at the substrate delivery level, through endocytic degradation of atherogenic lipoproteins such as oxidized LDL and remnant lipoproteins (2). ACAT enzyme activity is upregulated by the substrate cholesterol allosterically (1, 23). In addition, the redistribution of ACAT-1 protein from the tubular endoplasmic reticulum to certain small-sized vesicles during foam cell formation, as demonstrated by Sakashita et al. (12), may be a device for enhancing the accessibility of free cholesterol to ACAT-1 for esterification inside the macrophages.

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