THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 273, No. 3, Issue of January 16, pp. 1562–1567, 1998

Printed in U.S.A.

Acquisition of Secretion of Transforming Growth Factor-β1 Leads to Autonomous Suppression of Scavenger Receptor Activity in a Monocyte-Macrophage Cell Line, THP-1*

(Received for publication, June 2, 1997, and in revised form, October 30, 1997)

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Macrophage cells derived from the human monocytic leukemia cell line, THP-1, accumulate esterified cholesterol when cultivated in the presence of acetylated low density lipoprotein (Ac-LDL) through scavenger receptors (ScR). In the present study, we isolated a subtype of THP-1 cells that failed to accumulate esterified cholesterol when cultivated in the presence of Ac-LDL. The cells had negligible amounts of cell association and degradation of Ac-LDL compared with the parent THP-1 cells. The subtype THP-1 cells did not express ScR mRNA as well as that of lipoprotein lipase. In contrast, the expression of apolipoprotein E mRNA was greater than that found in parent THP-1 cells. The culture medium of subtype THP-1 cells treated with 12-*O*-tetradecanoylphorbol-13-acetate inhibited the uptake of Ac-LDL and the expression of ScR in parent THP-1 cells. After a 48-h incubation in the culture medium containing 12-*O*-tetradecanoylphorbol-13-acetate, the culture medium of differentiated subtype THP-1 cells contained 6.9 ng/ml transforming growth factor (TGF)-β1, while that of parent THP-1 cells secreted below detection level, which was less than 3 ng/ml. This inhibitory effect of the conditioned medium on the expression of ScR in parent THP-1 cells was abolished by pretreatment of the culture medium with anti-TGF-β1 antibodies. Parent THP-1 cells expressed as much TGF-β1 mRNA as sTHP-1 cells after stimulation of differentiation. Although the precursor forms of TGF-β1 that were synthesized in both parent and subtype THP-1 cells were of similar size and were expressed at similar levels, latent TGF-β1-binding protein, which is necessary for the secretion of TGF-β1, could only be co-immunoprecipitated with anti-TGF-β1 antibody from subtype THP-1 cells. This suggests that subtype THP-1 cells secrete TGF-β1 into the medium by forming a functional complex with the latent TGF-β1-binding protein. We conclude that subtype THP-1 cells could not take up Ac-LDL because ScR was inhibited (leading to a loss of function) caused by the secreted TGF-β1.

Foam cells carrying lipid droplets are often found in the early stage of atheromatous lesions (1, 2). Some of the foam cells are reported to originate from blood monocytes (3, 4). When monocytes differentiate into macrophages, they change shape, reduce the number of the microvilli on the cell surface, and generate podocytes. At the terminal stage of the differentiation, low density lipoprotein (LDL)[1] receptors are down-regulated, and acetylated LDL (Ac-LDL) receptors (scavenger receptors; ScR) are induced to express (5, 6). In addition, macrophages begin to synthesize and secrete apolipoprotein E (apoE) and lipoprotein lipase (LPL) (7, 8), which results in a dramatic change in their lipid metabolism. Macrophages accumulate lipids including cholesterol and become foam cells (9). Since the macrophages in atheromatous lesions express ScR, it is expected that ScR plays an important role in the development of atherosclerotic lesions (10, 11). The mechanism of the regulation of ScR activity, however, has not been fully understood.

Human monocyte-like cell line, THP-1, established by Tsuchiya *et al.* (12), differentiates into macrophage-like cells by the treatment with 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) (13). As ScR, LPL, LRP, and apoE start to express, and LDL receptor is down-regulated during the terminal differentiation of the cells (14–18), THP-1 cells are often used as a model for monocyte-macrophages.

The induced expression of apoE, LPL, and ScR in macrophages is regulated by a number of factors. The expression of ApoE is stimulated by cholesterol loading (19) and transforming growth factor-β1 (TGF-β1) (20), while LPL expression is inhibited by oxysterols (21), TGF-β (22), and lipopolysaccharides (23). Bottalico *et al.* (24) reported that TGF-β1 inhibits ScR activity in THP-1 cells. TGF-β informs a family that inhibits the growth of most epithelial cells, and it has potent effects on the accumulation of extracellular matrix components (25). TGF-β is known to induce the expression of natriuretic peptide type C (CNP) in vascular endothelial cells (26), and the secretion of CNP inhibits the proliferation of smooth muscle cells (27). Thus, TGF-β may play an important role in vascular remodeling.

In the present study, we have isolated a subtype of THP-1 cells (sTHP-1) that could not accumulate esterified cholesterol by Ac-LDL loading. The cells did not express ScR activity even

* This work was supported by grants from the Science and Technology Agency (Encourage System of Center of Excellence) and Ministry of Education, Science, Sports and Culture Grant 07457228. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: LDL, low density lipoprotein; Ac-LDL, acetylated low density lipoprotein; sTHP-1, subtype of THP-1; LPDS, lipoprotein-deficient serum; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; TGF-β, transforming growth factor β; LTBP, latent TGF-β1-binding protein; ScR, scavenger receptor(s); ApoE, apolipoprotein E; LPL, lipoprotein lipase; GAPDH, glyceroaldehyde-3-phosphate dehydrogenase; DMEM, Dulbecco's modified Eagle's medium; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; sT-CM, sTHP-1-conditioned medium; DiI-Ac-LDL, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-Ac-LDL.

after they were induced to differentiate. In the differentiated sTHP-1 cells, transcripts of ScR and LPL were below the detection level, and that of apoE was significantly increased compared with that of parent THP-1 cells. The conditioned medium of the differentiated sTHP-1 cells contained sufficient amounts of active TGF-β1 to suppress the expression of ScR in parent THP-1 cells. sTHP-1 cells synthesized a 190-kDa latent TGF-β1-binding protein (LTBP), which was not detectable in parent cells. We conclude that differentiated sTHP-1 cells have acquired an ability to secrete large amounts of active form of TGF-β1, which may due to its ability to express and synthesize LTBP. sTHP-1 cells may prove to be a useful model for understanding the secretion and activation mechanisms of TGF-β1 and their roles in vascular systems.

EXPERIMENTAL PROCEDURES

Materials—THP-1 cells were kindly provided by Dr. S. Tsuchiya (Department of Pediatrics, Tohoku University, School of Medicine). LDL ($1.019 < d < 1.063$ g/ml) and lipoprotein-deficient serum (LPDS, $d > 1.215$) were prepared from normal human plasma by sequential ultracentrifugation as described (28). Ac-LDL was prepared by chemical modification of the native LDL as described by Basu (29). Ac-LDL was iodinated with $Na^{125}I$ (NEN Life Science Products) by the iodine monochloride method (30). The specific activity of ^{125}I-Ac-LDL was more than 100 cpm/ng protein. Modified LDL, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-Ac-LDL (DiI-Ac-LDL) was purchased from Biomedical Technologies Inc. The cDNAs of human ScR, LPL, and apoE3 were kindly provided by Dr. Kodama (Tokyo University) (31), Japanese Cancer Research Resources Bank (JCRB, Tokyo, Japan) (32) and Mitsubishi Kagaku (Tokyo, Japan) (33), respectively. A DNA fragment of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was purchased from CLONTECH. THP-1 cells were maintained in RPMI 1640 supplemented with 10% fetal calf serum (Hyclone AB, Lund, Sweden) in a humidified incubator equilibrated with 5% CO_2, at 37 °C. The differentiation of the cells was induced by cultivating the cells in the differentiation medium, which is Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum or 10% (v/v) LPDS and 50 ng/ml of TPA (Nakarai Tesque, Kyoto, Japan).

Oil Red-O Staining—The differentiated cells that were incubated with 10 μg/ml Ac-LDL treatment were fixed in 4% formaldehyde. Then the cells were rinsed with propyleneglycol, and the lipids were stained with 10% oil red-O in propyleneglycol followed by staining of the nuclei with Mayer's hematoxylin (34).

Measurement of Lipids—THP-1 cells were treated with TPA for 4 days, causing differentiation into macrophages, and were subsequently incubated with or without 25–500 μg/ml Ac-LDL. The cells were washed and lysed in 1 ml of 1 N NaOH. Lipids were extracted three times with chloroform and methanol (2:1). Total cholesterol and unesterified cholesterol were determined by the enzymatic method (35). The esterified cholesterol content was calculated by subtracting the amount of unesterified cholesterol from that of total cholesterol. The protein concentration was determined by the method of Lowry *et al.* (36).

Determination of Cell-associated and Degraded Ac-LDL—The binding activity of ScR was measured after incubating the cells with ^{125}I-Ac-LDL or DiI-Ac-LDL at 37 °C. For the measurement of cell-associated and degraded ^{125}I-Ac-LDL, the cells were incubated with 10 μg/ml ^{125}I-Ac-LDL for 5 h at 37 °C. At the end of the incubation, trichloroacetic acid-soluble radioactivity in the medium was determined. This represented the degradation product of ^{125}I-labeled Ac-LDL. The cells were washed and lysed in 1 ml of 1 N NaOH, and the radioactivities were determined (37). To determine the uptake of DiI-Ac-LDL, the cells were incubated in the presence of 5 μg/ml DiI-Ac-LDL for 4 h at 37 °C, and they were washed, dissolved, and analyzed for fluorescent intensity by spectrofluorometer (Hitachi, Tokyo) with excitation at 520 nm and emission at 570 nm (38, 39). The specific activities for the associated and degraded Ac-LDL were calculated by subtracting the values in the presence of a 100-fold excess amount of unlabeled lipoproteins.

Preparation of Conditioned Medium—After the cells were cultivated in the presence of TPA for 24–48 h, the culture medium was centrifuged at 1300 × *g* at 4 °C for 15 min. The supernatant fraction was filtered with a 0.22-μm pore size filter.

Determination of Cytokines in Conditioned Medium—The concentrations of TGF-β1, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ in the culture medium were determined using an enzyme-linked immunosorbent assay kit from R & D Systems, Japan Immunoreseach Laboratories Co., Ltd. (Gumma, Japan), and Zoning Industrial (Fleurus, Belgium), respectively (40–42). The concentration of TGF-β1 was measured after incubation in 0.1 N HCl.

Treatment of Conditioned Medium with Anti-TGF-β1 Antiserum—To the conditioned culture medium, 100 μl of anti-TGF-β1 antiserum or control nonimmune serum diluted with 300 μl of Dulbecco's phosphate-buffered saline was added with 100 μl of protein G-Sepharose 4FF (Pharmacia, Uppsala, Sweden). The mixtures were rotated at 4 °C overnight. The Sepharose beads, prebound to either anti-TGF-β1 antibodies (King Brewing Co. Ltd.) or control IgG were added to 2 ml of conditioned medium from sTHP-1 cells that had been treated with TPA for 48 h. The mixtures were incubated at 37 °C for 2 h, and the supernatant fractions were recovered by centrifugation at 420 × *g* for 10 min.

Northern Blot Analysis—Total RNA was isolated from THP-1 cells using the acid guanidium thiocyanate-phenol-chloroform method, described by Chomczynski and Sacchi (43). Total RNA (20 μg) was electrophoresed in 0.9% agarose gels containing 2.2 M formaldehyde and then transferred onto nylon membranes (Gene Screen, NEN Life Science Products). Then the membrane was hybridized at 42 °C overnight with the indicated probes, which were labeled with [^{32}P]dCTP (Amersham, Buckinghamshire, United Kingdom) by the multipriming method (44). The membranes were washed and exposed to a Fuji Imaging Plate (Fuji Photo Film, Tokyo, Japan). The radioactive bands were determined by a Fuji BAS 2000 image analyzer (Fuji Photo Film).

Immunoprecipitation of TGF-β1—TGF-β1 and its complex form were radiolabeled and immunoprecipitated as described previously by Miyazono *et al.* (45). Briefly, the two types of THP-1 cells, incubated in the medium containing TPA for 3 days, were incubated in cysteine- and methionine-free RPMI 1640 supplemented with 50 ng/ml TPA, 0.1% bovine serum albumin, L-[^{35}S]methionine (200 μCi/ml), and L-[^{35}S]cysteine (200 μCi/ml) at 37 °C for 2 h (pulse labeling). Then the culture medium was replaced with the RPMI 1640 supplemented with 50 ng/ml TPA, 0.1% bovine serum albumin, 75 mg/liter L-methionine, and 250 mg/liter L-cysteine and further incubated at 37 °C for 4 h (chase). The cells were then lysed and pretreated twice with control mouse IgG1κ and protein G-Sepharose. Then 1 ml of the supernatant fractions was immunoprecipitated with 2 μg of mouse anti-human TGF-β1 (BioSource International, Camarillo, CA) at 4 °C for 2 h. The antigen-antibody complex was recovered with 80 μl of protein G-Sepharose. The matrix was washed twice with detergent buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.0, 1% sodium deoxycholate, 1% Triton X-100) and once with high salt buffer (500 mM NaCl, 0.2% Triton X-100, 20 mM Tris-HCl, pH 7.0) and eluted with Laemmli's buffer. Samples were electrophoresed in a 8–16% polyacrylamide gradient gel. The gel was dried and exposed to Fuji Imaging Plate. The visualized data was analyzed using a BAS 2000 image analyzer.

RESULTS

Lipid Accumulation in Parent THP-1 and sTHP-1 Cells—THP-1 cells can be induced to differentiate into macrophage-like cells by TPA treatment. THP-1 cells, thus treated, efficiently accumulate esterified cholesterol into vacuoles when cultivated in the presence of Ac-LDL (14, 18). During the course of maintenance of THP-1 cells, we isolated by chance a subtype of THP-1 cells, which, when fully differentiated, failed to accumulate esterified cholesterol, even cultivated in the presence of Ac-LDL. As shown in Fig. 1, these sTHP-1 cells (*panel B*) differentiated into macrophage-like cells. Like parent THP-1 cells (*panel A*), they generated pseudopodia, but sTHP-1 cells could not accumulate lipids, even cultivated in the presence of Ac-LDL (10 μg/ml). Under identical conditions, parent THP-1 cells accumulated and formed oil red-O-positive vacuoles (Fig. 1*A*). These vacuoles were rarely found in sTHP-1 cells (Fig. 1*B*). The accumulation of esterified cholesterol in both cells was determined after incubation with 25–500 μg/ml Ac-LDL. In parent THP-1 cells, esterified cholesterol inside the cells significantly increased in a dose-dependent manner. In contrast, when sTHP-1 cells were treated with less than 100 μg/ml of Ac-LDL, almost no esterified cholesterol was accumulated (Fig. 2). Parent THP-1 cells accumulated about 2.5-fold more esterified cholesterol than sTHP-1 cells even in the presence of extraordinary high concentration of Ac-LDL (500 μg/ml) (Fig. 2). During the proliferative stage, the doubling time of sTHP-1

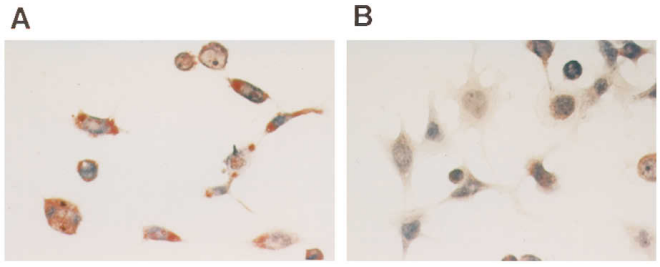

FIG. 1. **Micrographs of differentiated parent THP-1 cells (*A*) and sTHP-1 cells.** On day 0, 3×10^4 cells/mm^2 were seeded and cultivated on cover glass with 0.3 ml of DMEM containing 10% (v/v) fetal calf serum and 50 ng/ml TPA (differentiation medium). On day 3, the cells were washed with PBS, and the medium was replaced with fresh differentiation medium containing 10% LPDS instead of fetal calf serum and supplemented with 10 μg/ml Ac-LDL. On day 4, the cells were fixed and stained with Mayer's hematoxylin and oil red-O as described under "Experimental Procedures."

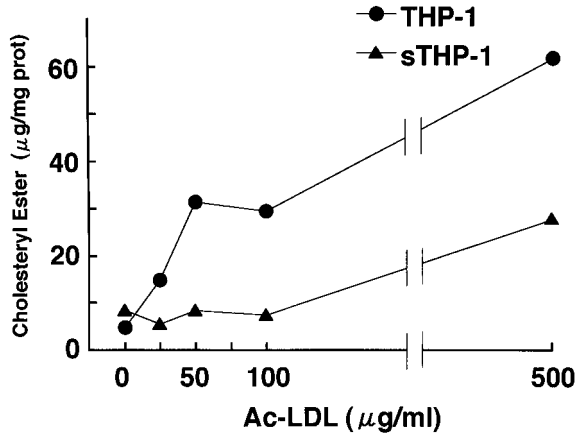

FIG. 2. **Accumulation of esterified cholesterol in parent THP-1 and sTHP-1 cells cultivated in the presence of Ac-LDL.** On day 0, 1.5×10^7 cells were seeded into 10-cm plastic dishes and cultivated in 6 ml of the differentiation medium containing 10% LPDS. On day 4, the medium was replaced with a fresh one supplemented with 0–500 μg/ml Ac-LDL. On day 6, the cells were harvested, and esterified cholesterol was determined. Each point is the mean of two samples.

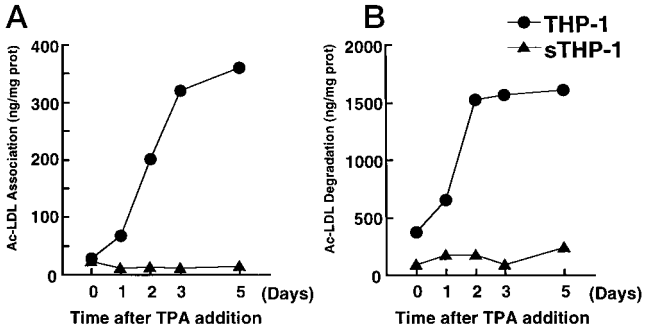

FIG. 3. **Time courses of the Ac-LDL binding (*A*) and degradation (*B*) activities of parent THP-1 and sTHP-1 cells after the addition of TPA.** On day 0, 5×10^6 cells were seeded into 6-cm plastic dishes and cultivated in 3 ml of the differentiation medium. After cultivation for the indicated time periods, the cells were assayed for their ability to bind and degrade ^{125}I-labeled Ac-LDL. Each point is the mean of three samples.

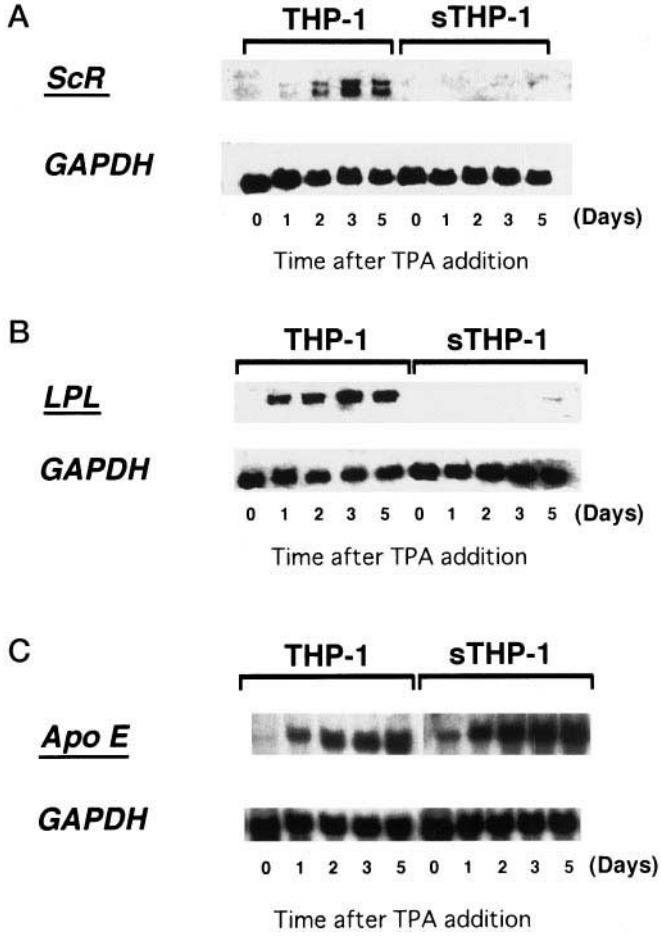

FIG. 4. **Northern blot analyses of ScR (*A*), LPL (*B*), and ApoE (*C*) in parent THP-1 and sTHP-1 cells.** On day 0, 1.5×10^7 cells were seeded into 10-cm plastic dishes and cultivated in the differentiation medium for the indicated time periods. Total RNA (20 μg) was electrophoresed in 0.9% agarose in the presence of 2.2 M formaldehyde, transferred onto nylon membrane, and hybridized with ^{32}P-labeled probes. Each membrane was rehybridized with human GAPDH probe as an internal standard.

cells was identical to that of parent THP-1 cells (data not shown).

Determination of ScR Activity—To determine whether the difference in accumulation of esterified cholesterol between the two cell types was directly due to the function of ScR, the binding and degradation activities of ^{125}I-labeled Ac-LDL were measured (Fig. 3, *A* and *B*). In parent THP-1 cells, the binding and degradation activities of labeled Ac-LDL increased after the addition of TPA. However, the cell-associated and degraded Ac-LDL were almost undetectable in sTHP-1 cells even after 5-day treatment with TPA. These data suggest that sTHP-1 cells had some defects in ScR activity.

Expression of ScR, LPL, and ApoE mRNAs—To examine if the defect of ScR activity in sTHP-1 cells was at the transcriptional level, the expression of ScR mRNA was determined. In differentiated parent THP-1 cells, the ScR mRNA was detected after one-day treatment with TPA, and increased thereafter (Fig. 4*A*). On the other hand, in sTHP-1 cells, ScR mRNA was not detected even after 5-day cultivation. The expression of mRNAs of LPL and apoE, which is reported to be induced after differentiation into macrophages, was also determined. While parent THP-1 cells increased the mRNA level of LPL by the TPA treatment, sTHP-1 cells showed little expression even after a 5-day incubation with TPA (Fig. 4*B*). After the TPA treatment, the induction of apoE mRNA was faster and greater in sTHP-1 cells than in parent THP-1 cells (Fig. 4*C*). The induction of apoE expression supports the theory that sTHP-1 cells were differentiated into macrophages by the TPA treatment.

Effect of sTHP-1-conditioned Medium (sT-CM) on the ScR Activity in Parent THP-1 Cells—In sTHP-1 cells, the induction of not only ScR mRNA but also LPL mRNA was suppressed; at the same time, however, induction of apoE was not suppressed

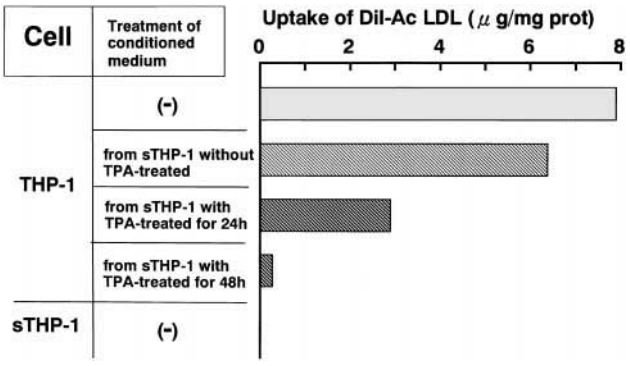

FIG. 5. **Effect of the culture medium of sTHP-1 cells (sT-CM) on the ScR activity in parent THP-1 cells.** sTHP-1 cells (1×10^7 cells), were seeded into 10-cm plastic dishes in the differentiated medium for 24 or 48 h, and the culture medium was recovered. Parent THP-1 cells (5×10^5 cells) were seeded into 3-cm plastic dishes, containing 0.5 ml of recovered conditioned medium, and cultivated for 72 h. The cells were then incubated with 5 μg/ml of DiI-Ac-LDL in fresh DMEM at 37 °C for 4 h. The cell-associated DiI-Ac-LDL was determined. Each experiment was the mean of duplicate determinations.

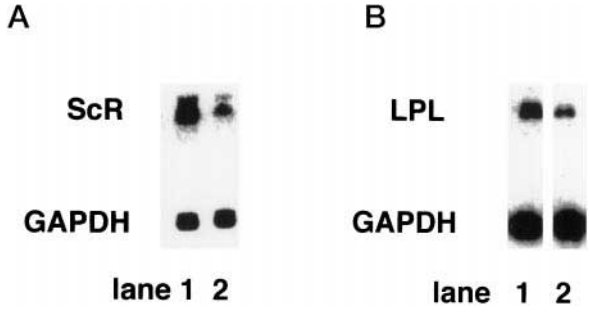

FIG. 6. **Effect of sT-CM on the expression of ScR and LPL mRNA in parent THP-1 cells.** Total RNA (20 μg/ml) was prepared from parent THP-1 cells cultivated for 48 h in the differentiation medium (*lane 1*) or sT-CM (*lane 2*) and was hybridized with ScR cDNA (*A*) or LPL cDNA (*B*). Each membrane sheet was rehybridized with human GAPDH probe as an internal standard.

by the TPA treatment. Thus, we expected that the phenotype of sTHP-1 cells that lack in ScR activity might be epigenetic. We first tested if the culture medium obtained from sTHP-1 cells treated with TPA (sT-CM) might have some effects on the expression of ScR activity in parent THP-1 cells. Expectedly, the sT-CM obtained after 24 and 48 h of cultivation in the presence of TPA inhibited the ScR activity in parent THP-1 cells 63 and 94%, respectively (Fig. 5). The sT-CM without TPA treatment showed almost no effect on ScR activity in parent THP-1 cells. This inhibitory effect was not directly on the binding of Ac-LDL to ScR, because the measurement of ScR binding activity was not affected with the sT-CM (data not shown). The sT-CM suppressed the transcript level of ScR mRNA in differentiated parent THP-1 cells to 31.8% of that of the control level. The suppression of the ScR activity in parent THP-1 cells was at least at the transcriptional level (Fig. 6*A*). Similarly, the sT-CM suppressed LPL mRNA expression in differentiated parent THP-1cells (Fig. 6*B*). These results suggest that the component(s) that was secreted into the medium from the differentiated sTHP-1 cells, was responsible for the observed suppression in parent THP-1 cells.

Measurement of Cytokines in the Conditioned Medium—To identify which component(s) in the sT-CM was responsible for the suppression of the ScR and LPL expression, the concentration of three cytokines, TGF-β1, TNF-α, and IFN-γ, which were reported to affect the expression of ScR and LPL, were determined (20, 22, 24, 46–49). While the culture media from undifferentiated sTHP-1 cells and differentiated and undifferentiated parent cells contained below the detection level of TGF-β1, those of differentiated sTHP-1 cells obtained after incubation with TPA for 24 and 48 h contained 3.9 and 6.7 ng/ml TGF-β1, respectively (Table I). Because there is no difference in concentration of TNF-α or IFN-γ in the culture media from parent THP-1 and sTHP-1, TGF-β1 was the candidate for the factor in the sT-CM that inhibits the expression of ScR and LPL mRNAs in parent THP-1 cells.

TABLE I
The concentration of TGF-β1, TNF-α, and IFN-γ in the conditioned medium obtained from the parent THP-1 cells and sTHP-1 with or without TPA treatment

The conditioned medium was obtained from the cells with TPA (50 ng/ml) treatment for 24–48 h (TPA(+)) or without TPA treatment for 48 h (TPA(−)). Each medium was subjected to measurement of the concentration of each cytokine using an enzyme-linked immunosorbent assay.

	sTHP-1, TPA(−)	sTHP-1, TPA(+) 24h	sTHP-1, TPA(+) 48h	THP-1, TPA(−)	THP-1, TPA(+) 48h
TGF-β1 (ng/ml)	<3	3.9	6.7	<3	<3
TNF-α (pg/ml)	<5	786	414	<5	626
IFN-γ (units/ml)	1.0	1.0	1.4	1.4	1.6

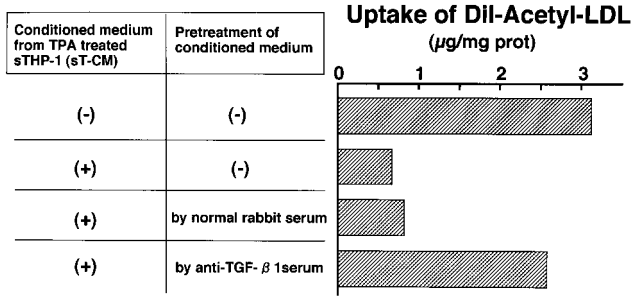

FIG. 7. **Abolition of the inhibitory effect of sT-CM on the ScR activity in parent THP-1 cells by pretreatment of the medium with anti-TGF-β1 antibodies.** Parent THP-1 cells (5×10^5 cells) were seeded into 3-cm plastic dishes and cultivated for 72 h in the differentiation medium, sT-CM, sT-CM treated with preimmune-immunoglobulin, or sT-CM pretreated with anti-TGF-β1 antibodies. After the cultivation the cells were incubated with 5 μg/ml DiI-Ac-LDL in DMEM at 37 °C for 4 h. The cell-associated DiI-Ac-LDL was determined. Each experiment was the mean of the duplicate determinations.

Neutralizing Effect of Anti-TGF-β1 Antibodies on the Inhibitory Effect of sT-CM on ScR Expression—The sT-CM was pretreated with anti-TGF-β1 antibody bound to protein G-Sepharose. The treated sT-CM, which contained TGF-β1 below the detection level, lost the inhibitory effect on that ScR activity in differentiated parent THP-1 cells, and the ScR activity was at a level similar to that of the cells treated without sT-CM (Fig. 7). The sT-CM treated with control antibodies suppressed ScR activity to the same extent as that without the antibody treatment. The data suggest that TGF-β1 significantly secreted from the differentiated sTHP-1 cells is a major factor that suppresses the expression of ScR activity in parent THP-1 cells. Thus, it was strongly suggested that the negligible level of ScR expression in sTHP-1 cells was due to the increased level of TGF-β1 produced by own cells.

Expression of TGF-β1 mRNA in sTHP-1 and the Parent THP-1 Cells—TGF-β1 mRNA expression was determined in parent THP-1 and sTHP-1 cells. Before the TPA treatment, neither type of cells expressed TGF-β1 mRNA. After TPA treatment, both types of cells started to express TGF-β1 mRNA (Fig. 8). The expression profiles of TGF-β1 mRNA in the two cell types were similar.

Synthesis of TGF-β1 in sTHP-1 and Parent THP-1 Cells—

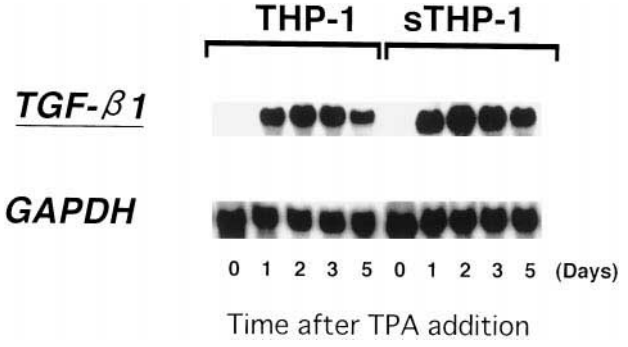

FIG. 8. **Northern blot analysis of TGF-β1 in parent THP-1 and sTHP-1 cells.** On day 0, 1.5×10^7 cells were seeded into 10-cm plastic dishes and cultivated in the differentiation medium for indicated time periods. Total RNA (20 μg) was electrophoresed in 0.9% agarose in the presence of 2.2 M formaldehyde, transferred onto nylon membrane, and hybridized with ^{32}P-labeled probes. The membrane was rehybridized with human GAPDH probe as an internal standard.

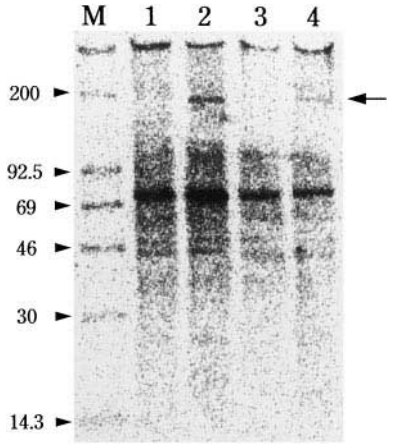

FIG. 9. **Immunoprecipitation of latent TGF-β1 in parent THP-1 and sTHP-1 cells.** On day 0, 3×10^6 cells were seeded into 6-cm plastic dishes and cultivated in differentiation medium. On day 3, parent THP-1 cells (*lanes 1* and *3*) and sTHP-1 cells (*lanes 2* and *4*) were pulse-labeled with L-[^{35}S]cysteine and L-[^{35}S]methionine for 2 h (*lanes 1* and *2*) and then chased with excess of unlabeled cysteine and methionine for 4 h (*lanes 3* and *4*). Cell lysates were collected and subjected to immunoprecipitation with anti-TGF-β1 antibody. Immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis. Molecular weight markers are indicated.

TGF-β1 is known to be synthesized as a precursor protein (about 48 kDa) that attaches to LTBP (about 195 kDa) to form the functional secretory complex (45). To know which of these steps is responsible for the overproduction of TGF-β1 in sTHP-1 cells, all cell proteins were radiolabeled, and then TGF-β1 was immunoprecipitated with specific antibody. As shown in Fig. 9, four radiolabeled bands, 190, 70, 48, and 45 kDa were observed in sTHP-1 cells (*lane 2*). The 190-kDa protein band, thought to be LTBP (45), was seen in sTHP-1 cells but not in parent THP-1 cells (compare *lanes 1* and *2*). The 48- and 45-kDa protein bands are thought to be the precursor form of TGF-β1 and were seen both in sTHP-1 and parent THP-1 cells. The 190-, 48-, and 45-kDa but not the 70-kDa bands decreased when the immunoprecipitation was performed in the presence of TGF-β1, suggesting that the 70-kDa protein precipitated nonspecifically. After a 4-h chase, 190-, 48-, and 45-kDa bands in sTHP-1 cells significantly decreased (*lane 4*). The 48- and 45-kDa bands in parent THP-1 cells decreased as well; however, the decrease level was small compared with those in sTHP-1 cells (*lane 3*). All of the results obtained indicate that the secretion of TGF-β1 was accelerated in sTHP-1 cells due to the increased formation of the functional secretory complex with LTBP.

DISCUSSION

ScR are able to bind and internalize modified lipoproteins, such as acetylated and oxidized LDL. Different from LDL receptor, the ScR activity is not regulated by the intracellular concentration of cholesterol. Therefore, the continuous uptake of modified LDL leads to great accumulation of cholesterol into the cells. It has been reported that nonmacrophage cells, transfected with the cDNA encoding ScR, accumulate cholesterol and become foam cells (50). ScR are expected to play an important role in the foam cell formation.

THP-1 cells possess many of the characteristics of monocyte-macrophages, especially in the lipid metabolism. They begin to express ScR and secrete greater amounts of apoE and LPL (14–18). For these reasons, THP-1 cells have been used as a model for foam cell formation (51–53). In the present study, we identified a subtype of THP-1, sTHP-1 cells, which demonstrated negligible amounts of ScR activity, even after the differentiation. Consequently, the cells failed to become foam cells by cultivation with Ac-LDL at a relatively low concentration. The lack of ScR activity in sTHP-1 cells was due to the suppression of the expression of ScR mRNA. The lack of ScR activity in sTHP-1 cells may not be due to dedifferentiation of the cells, because 1) the doubling time of sTHP-1 cells was similar to those of parent cells, and 2) apoE mRNA, which is a differentiation marker for macrophages, was induced to express in sTHP-1 cells with TPA treatment. A loss of regulation in the expression of these genes was indicated to be the cause for acquired new phenotypes of sTHP-1 cells. As expected, the sT-CM inhibited the ScR activity of parent THP-1 cells. The inhibitory effect of the conditioned medium on the induction of ScR activity depended on the cultivation time of the differentiated sTHP-1 cells. These results supported the hypothesis that factor(s) that caused the suppression of ScR mRNA in the parent THP-1 cells had been secreted from the differentiated sTHP-1 cells.

Macrophages have been reported to produce and secrete a large variety of cytokines, which are known to modulate the macrophage functions and cell marker expression (54). We considered the possibility that there was a factor that could change the expression of ScR and LPL in sTHP-1 cells. First, we measured the concentrations of cytokines in the culture medium of sTHP-1 cells. TGF-β1, TNF-α, and IFN-γ are known to suppress the expression of both ScR and LPL mRNAs (22, 24, 48, 55, 56, 58). The concentrations of TGF-β1 in culture media of sTHP-1 cells (sT-CM) and parent THP-1 cells were quite different. The sT-CM contained significantly higher concentrations of TGF-β1 compared with that of parent cells. TGF-β1 in the sT-CM increased in a time-dependent manner, which was parallel to the ability to inhibit the ScR activity (see Fig. 5). The treatment of sT-CM with anti-TGF-β1 antibodies abolished the inhibitory effect of the sT-CM on ScR activity of differentiated THP-1 cells. The results clearly indicate that the component that inhibited the ScR activity was TGF-β1, suggesting that sTHP-1 cells had acquired the ability to produce TGF-β1 in the presence of TPA.

TGF-β1 is reported to decrease the expression of ScR mRNA in THP-1 cells (24) and LPL mRNA in preadipocytes (22) and to increase apoE mRNA in macrophages (20). The present results agree with the findings in previous reports and show that the TGF-β1 secreted from sTHP-1 cells suppressed the transcript levels of ScR and LPL mRNAs and increased the level of ApoE mRNA. Due to the acquisition of an ability to secrete TGF-β1, sTHP-1 cells lost their ability to accumulate cholesterol.

Bottalico *et al.* (24) reported that 24 pM TGF-β1 has an ability to inhibit 70% of the uptake activity of Ac-LDL through

ScR in the differentiated THP-1 cells. The concentration of TGF-β1 required to acquire maximal suppression of ScR activity in THP-1 cells was 10 times higher in the present study than that reported by Bottalico *et al.* (24). In the present study, the concentration of TGF-β1 in the sT-CM after 24 and 48 h of treatment with TPA was 3.9 ng/ml, which is about 160 pM, and 6.7 ng/ml, which is about 270 pM, respectively. These concentrations of TGF-β1 inhibited 63 and 94% of the uptake activities of Ac-LDL by parent THP-1 cells, respectively (Fig. 5). TGF-β1 is known to be synthesized and secreted as latent complex from the producer cells. The latent TGF-β1 complex in human and rat platelets is composed of three different components: mature TGF-β1, the TGF-β1 latency-associated peptide, and the LTBP (57, 59, 60). The latent TGF-β1 is biologically inactive, and the activation of the latent complexes is an important step for the regulation of TGF-β1 activity. The N-terminal remnant of the TGF-β1 precursor is sufficient for TGF-β1 latency. TGF-β1 in this latent complex can be activated *in vitro* by treatment with acid, urea, or heat (59). It is reasonable to speculate that most of the TGF-β1 in the culture medium of sTHP-1 cells might be in the latent form. Since TGF-β1 concentration was measured by enzyme-linked immunosorbent assay in acidic conditions, both latent and active forms of TGF-β1 were included in the measurements. Therefore, a higher concentration of TGF-β1 was necessary for the maximal inhibition of the ScR activity in our assay system.

In our study, TGF-β1 was not detected in the culture medium of parent THP-1 cells, even after stimulation of TPA. The parent THP-1 cells showed almost as much TGF-β1 mRNA as did sTHP-1 cells (Fig. 8). In sTHP-1 cells, considerable amounts of LTBP were immunoprecipitated with anti-TGF-β1, suggesting that LTBP was forming a functional complex with TGF-β1 precursor only in sTHP-1 cells. This might explain the high concentration of TGF-β1 in the culture media of sTHP-1 cells, since LTBP is reported to play a crucial role in the secretion of TGF-β1 (45). An increased rate of formation of the TGF-β1-LTBP complex could be explained by either enhanced production of LTBP in sTHP-1 cells or a defect in complex formation in parent THP-1 cells. Since sTHP-1 cells are derived from THP-1 cells, it is reasonable to suppose that a defect(s), if it exists, should occur in sTHP-1 cells rather than in parent THP-1 cells. Therefore, a defect in sTHP-1 cells could possibly be the failure to suppress either the synthesis of LTBP or the formation of a functional secretion complex with LTBP. The latter possibility may be tested when a specific antibody against LTBP becomes available. sTHP-1 cells will be a useful model for investigating the regulation of secretion of TGF-β1.

Acknowledgments—We thank Dr. Kentaro Shimokado and Dr. Mikio Kinoshita for helpful discussions. We also thank Dr. Patrick Leahy for proofreading this article.

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