Co-expression of scavenger receptor-BI and caveolin-1 is associated with enhanced selective cholesteryl ester uptake in THP-1 macrophages

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Abstract **Scavenger receptor (SR)-BI mediates the selective uptake of high density lipoprotein (HDL) cholesteryl esters and the efflux of free cholesterol. In Chinese hamster ovary (CHO) cells, SR-BI is predominantly associated with caveolae which we have recently demonstrated are the initial loci for membrane transfer of HDL cholesteryl esters. Because cholesterol accumulation in macrophages is a critical event in atherogenesis, we investigated the expression of SR-BI and caveolin-1 in several macrophage cell lines. Human THP-1 monocytes were examined before and after differentiation to macrophages by treatment with 200 nM phorbol ester for 72 h. Undifferentiated THP-1 cells expressed caveolin-1 weakly whereas differentiation up-regulated caveolin-1 expression greater than 50-fold. In contrast, both undifferentiated and differentiated THP-1 cells expressed similar levels of SR-BI. Differentiation of THP-1 cells increased the percent of membrane cholesterol associated with caveolae from 12% ± 1.9% to 38% ± 3.1%. The increase in caveolin-1 expression was associated with a 2- to 3-fold increase in selective cholesterol ether uptake from HDL. Two mouse macrophage cell lines, J774 and RAW, expressed levels of SR-BI similar to differentiated THP-1 cells but did not express detectable levels of caveolin-1. In comparison to differentiated THP-1 cells, RAW and J774 cells internalized 9- to 10-fold less cholesteryl ester. We conclude that differentiated THP-1 cells express both caveolin-1 and SR-BI and that their co-expression is associated with enhanced selective cholesteryl ester uptake.**—Matveev, S., D. R. van der Westhuyzen, and E. J. Smart. **Co-expression of scavenger receptor-BI and caveolin-1 is associated with enhanced selective cholesteryl ester uptake in THP-1 macrophages.** ***J. Lipid Res.*** **1999.** 40: **1647–1654.**

Supplementary key words caveolae • SR-BI • CD36 • HDL • foam cells

Plasma concentrations of high density lipoprotein (HDL) cholesterol are inversely correlated to the risk of developing atherosclerosis and coronary artery disease (1, 2). HDL is thought to protect against lesion development through its role in the process of reverse cholesterol transport (3, 4). In this process, cholesterol is removed from peripheral cells and delivered to the liver as cholesteryl ester. The delivery of cholesteryl ester to cells from HDL occurs by a process called selective lipid uptake (5, 6). In selective lipid uptake, the cholesteryl ester associated with HDL is transferred to cells without concomitant uptake and degradation of HDL proteins (5, 6). In contrast, clearance of low density lipoprotein (LDL) involves whole particle uptake by receptor-mediated endocytosis (7). Recently, an HDL receptor called scavenger receptor class B, type I (SR-BI), has been shown to mediate the selective uptake of HDL cholesteryl ester into cells (8–10). SR-BI also mediates the efflux of cholesterol from cells to HDL (11, 12).

SR-BI receptors are concentrated in cholesterol/sphingomyelin-rich microdomains in the plasma membrane called caveolae (13, 14) where they are thought to function in cellular cholesterol homeostasis. Fielding and Fielding (15) demonstrated that caveolae mediate the efflux of free cholesterol derived from either de novo synthesis or LDL. We recently demonstrated that caveolae are the initial acceptors of SR-BI-mediated selective cholesteryl ester uptake from HDL (13). In addition, we have demonstrated that newly synthesized cholesterol translocates from the ER to caveolae in a protein chaperone complex that includes caveolin (16). Caveolin is a 22 kDa cholesterol binding protein that is necessary for caveolae formation (17, 18) and the presence of caveolin is indicative of caveolae (19).

Because caveolae and SR-BI play a role in cholesteryl ester uptake and cholesterol efflux they should be present in cells involved in reverse cholesterol transport. Indeed, caveolae (20) and SR-BI (21) are found in liver. Macrophages would also be expected to contain caveolae and SR-BI, however, the presence of caveolae and SR-BI in macrophages is controversial. Murao et al. (22) has reported that SR-BI expression is down-regulated in differentiated

Abbreviations: HDL, high-density lipoprotein; CHO, Chinese hamster ovary; SR-BI, scavenger receptor, class B, type I; LDL, low-density lipoprotein; PMA, phorbol-12-myristate-13-acetate; CE, cholesteryl-oleoyl ether; LPDS, human lipoprotein-deficient serum.

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THP-cells and Fielding and Fielding (23) reported that macrophages do not contain caveolae. However, other reports demonstrate that elicited macrophages express caveolin (24) and contain morphologically identifiable caveolae (25).

In this report we have addressed three questions. *1*) Do macrophages express SR-BI? *2*) Do macrophages express caveolin and contain caveolae? *3*) Are SR-BI and caveolin expression associated with enhanced selective cholesteryl ester uptake in macrophages? We have demonstrated that THP-1, RAW, and J774 cells express similar amounts of SR-BI. Furthermore, we demonstrated that differentiation of THP-1 cells up-regulates caveolin-1 expression which is associated with enhanced selective cholesteryl ester uptake. In contrast, J774 and RAW macrophages do not express detectable levels of caveolin-1 and selectively internalize 9- to 10-fold less cholesteryl ester than differentiated THP-1 cells. These data suggest that caveolae and SR-BI play a role in macrophage cholesterol homeostasis.

MATERIAL AND METHODS

Materials

RPMI medium 1640, DMEM high glucose medium, fetal calf serum, l-glutamine, trypsin-EDTA, penicillin-streptomycin, and OptiPrep were purchased from Life Technologies Inc. (Grand Island, NY). Percoll, PVDF membrane, and Tween 20 were purchased from Sigma (St. Louis, MO). Bradford reagent was purchased from Bio-Rad (Hercules, CA). The analytical silica gel thin-layer chromatography plates, heptane, petroleum ether, ethyl ether, acetic acid, and 2-propanol were from Fisher. The lipid standards cholesterol, sphingomyelin, and phosphatidylcholine were from Supelco (Bellefonte, PA). Rabbit IgG directed against caveolin-1 and mouse monoclonal IgG directed against clathrin were obtained from Transduction Laboratories (Lexington, KY). Mouse IgG directed against the human transferrin receptor was supplied by Zymed Laboratories (San Francisco, CA). Rabbit IgG directed against cyclophilin A was supplied by Affinity BioReagents (Neshanic Station, NJ). Rabbit IgG directed against the extracellular domain of SR-BI (RED-1) was produced at the University of Kentucky. CD36 IgG (OKM5) was kindly provided by Columbia Diagnostics, Inc. Horseradish peroxidase-conjugated IgGs were supplied by Cappel (West Chester, PA). SuperSignal® chemiluminescent substrate was purchased from Pierce (Rockford, IL). [1α, 2α (n)-^{3}H]cholesteryl-oleoyl ether (47 Ci/mmol) and [^{3}H]acetate (5.21 Ci/mmol) were supplied by Amersham (Arlington Heights, IL). ^{125}I-labeled Na (1 mCi/ml) was purchased from NEN (Boston, MA).

Buffers

Sample buffer (5×) consisted of 0.31 m Tris, pH 6.8, 2.5% (w/v) SDS, 50% (v/v) glycerol, and 0.125% (w/v) bromophenol blue. TBS consisted of 20 mm Tris, pH 7.6, and 137 mm NaCl. Blocking buffer consisted of TBS plus 0.5% Tween 20 and 5% dry milk. Wash buffer consisted of TBS plus 0.5% Tween 20 and 0.2% dry milk.

Cell culture

THP-1, RAW, and J774 cells were grown in 100-mm dishes or 12-well plates. THP-1 cells were grown in RPMI 1640 medium and RAW and J774 cells were grown in DMEM high glucose medium. Each medium also contained 10% fetal calf serum, 100 units/ml penicillin/streptomycin, and 2 mm l-glutamine. The medium was changed every 48 h. THP-1 cells were grown in the presence of 200 nm phorbol-12-myristate-13-acetate (PMA) for 12 h (to promote attachment to the cell culture dishes) or for 72 h (for cell differentiation). Cellular lipid pools were radiolabeled by incubating the cells in the presence of [^{3}H]acetate (30 mCi/dish) for 18 h at 37°C in serum-free medium.

Northern blots

Total RNA was isolated with Tri-Reagent according to the manufacturer's instructions. The cDNAs for caveolin-1, human SR-BI, and S30 were labeled with [^{32}P]dCTP (3000 Ci/mmol) by random prime labeling. Northern blot hybridization analysis was performed as described (26). After the hybridization, the membranes were washed in 0.2×SSC, 0.1% SDS for 1 h at 60°C. Radioactive signal was visualized with a phosphoimager.

Enzyme assays

Galactosyl transferase and NADPH cytochrome C reductase were assayed using methods adapted from Graham and Higgins (27).

Isolation of caveolae

Caveolae were isolated as described previously (16, 28). This method generates a highly purified fraction of caveolae that is enriched in caveolin and free of non-caveola markers such as transferrin receptor and clathrin (28). The yield of caveolae was determine by immunoblot analysis of caveolin in the linear range of detection. The estimated yield of caveolae in the present study was 56% ± 7%.

Radiolabeled lipid determinations

Thin-layer chromatography and liquid scintillation counting were used to quantify the relative amounts of radiolabeled cholesterol, sphingomyelin, and phosphatidylcholine in plasma membranes and caveola membranes. Each sample was adjusted to a volume of 3 ml with distilled water. A mixture of chloroform–methanol 1:2 (9 ml) was added and vortexed for 30 sec. Chloroform (3 ml) was added and the sample was vortexed for 30 sec. Finally, 3 ml of distilled water was added and vortexed for an additional 30 sec. The organic and aqueous phases were separated in a Beckman Clinical Centrifuge at 2500 rpm, 15 min, room temp. The organic phase was dried under nitrogen and then suspended in 50 μl of chloroform–methanol 1:1. Commercially available cholesterol, sphingomyelin, and phosphatidylcholine standards (5 μg) were dissolved in chloroform and the standards and samples were resolved by thin-layer chromatography (sterol analysis: petroleum ether–ethyl ether–acetic acid 80:20:1 or phospholipid analysis: chloroform–methanol–acetic acid–water 60:50:1:4). Lipids were visualized by charring with sulfuric acid–ethanol and heating at 180°C for 10 min. The appropriate spots were scraped and the amount of radiation was quantified by liquid scintillation counting.

Lipoprotein isolation and radiolabeling

HDL (d 1.063–1.21 g/ml) was isolated from fresh human plasma by density gradient ultracentrifugation as described previously (29). The HDL_3 subfraction (d 1.13–1.18 g/ml) was isolated from other HDL subfractions using a density gradient fractionator (ISCO). [1α, 2α-^{3}H]cholesteryl-oleoyl ether was incorporated into HDL_3 as described previously (30). The specific radioactivity of [^{3}H]CE-HDL ranged from 32 to 35 DPM/ng cholesterol. HDL_3 apolipoproteins were iodinated with iodine monochloride (31) to a specific radioactivity of 400 to 600 CPM/ng protein.

Uptake and ligand binding assays

The selective uptake of cholesterol ethers from HDL into cells was determined using non-hydrolyzable [^{3}H]cholesteryl-oleoyl ether (CE) (32). Cells were rinsed twice with PBS (37°C). Me-

dium containing 5% human lipoprotein-deficient serum (LPDS) and 10 μg/ml [^{3}H]CE-HDL or ^{125}I-labeled HDL was added to the cells for the indicated intervals. After incubation, uptake was terminated by aspirating the medium and washing cell monolayers four times with Tris–saline (4°C). The cells were dissolved in 1 m NaOH and radioactivity was determined in aliquots by liquid scintillation counting. To compare cell associated ^{125}I-labeled HDL with [^{3}H]CE-HDL, [^{3}H]CE uptake was expressed as apparent HDL protein uptake, assuming that [^{3}H]CE uptake resulted from whole HDL particle uptake as described by Knecht and Pittman (32).

SDS-PAGE and immunoblotting

Cellular fractions were dissolved in 0.015% (w/v) deoxycholate, concentrated by precipitation with 7% (w/v) trichloroacetic acid and washed in acetone (28). Pellets were suspended in 1× sample buffer plus 1.2% (v/v) β-mercaptoethanol and heated to 95°C for 5 min immediately prior to loading. Proteins were separated on a 12.5% polyacrylamide gel at 50 mA (constant current) and subsequently transferred to PVDF membrane at 50 V (constant voltage) for 2 h. Membranes were blocked with blocking buffer for 60 min at 22°C. Primary antibodies were diluted (SR-BI, 1/5,000; caveolin-1, 1/10,000; clathrin, 1/1,000; transferrin receptor, 1/500) in blocking buffer and incubated with blocked membranes for 60 min at 22°C. Membranes were washed four times for 10 min in wash buffer. Horseradish peroxidase-conjugated IgGs directed against the appropriate host IgG were diluted (1/30,000) and incubated with membranes as described for primary antibodies. Membranes were washed four times for 10 min in wash buffer and visualized using chemiluminescence.

Quantification

The up-regulation of SR-BI and caveolin-1 proteins were quantified by immunoblot analysis. Linear standard curves were prepared for each protein. At least two measurements within the linear range of the standard curve were obtained in each experiment. The reported values are the average of at least four experiments.

RESULTS

Expression of SR-BI and caveolin-1

We examined three macrophage cell lines, RAW, J774, and THP-1 (200 nm PMA for 72 h), for the expression of SR-BI and caveolin-1. Both SR-BI protein (**Fig. 1A**) and mRNA (Fig. 1B) were expressed abundantly and to the same magnitude in all three macrophage lines and in undifferentiated THP-1 cells (Fig. 1). Caveolin-1 was not expressed at detectable levels in RAW and J774 cells and was expressed at low levels in undifferentiated THP-1 cells (Fig. 1). The SR-BI and caveolin-1 IgGs used in these studies recognize and bind both human and mouse antigens equally well (data not shown). Differentiation of THP-1 cells up-regulated both caveolin-1 mRNA and protein greater than 50-fold (Fig. 1). Caveolin-1 protein was maximally up-regulated by 60 h of treatment with 200 nm of PMA (**Fig. 2**). Treatment of RAW and J774 cells with 200 nm of PMA for 72 h did not alter the expression of SR-BI or caveolin-1 (data not shown). Caveolin-2 was not detected in any of the cell lines under any of the conditions used in this study (data not shown).

Isolation of caveolae

We defined the presence of biochemical caveola microdomains in undifferentiated and differentiated THP-1 cells by an established non-detergent isolation procedure (16, 28). Briefly, the cells were lysed and post-nuclear supernatants were generated. The post-nuclear supernatants were separated into cytosol, plasma membrane, and intracellular membrane (ER, Golgi, endosomes, etc.) fractions by centrifugation in Percoll. The plasma membranes were then sonicated and fractionated by density gradient centrifugation to isolate caveolae. Similar to other cell types studied (18, 33), the caveola fractions from undifferentiated and differentiated cells contained less than 0.6% of the protein found in the starting post-nuclear supernatant fraction (**Table 1**).

The most probable contaminates of the plasma membrane and caveola fractions are the endoplasmic reticulum (ER) and Golgi. Therefore, we measured the amount of NADPH cytochrome reductase (ER) and galactosyl transferase (Golgi) activity in each of the subcellular fractions (**Table 2**). The majority of the activities were associated with the intracellular membrane fractions. The plasma membrane fractions contained less than 2% of the total post-nuclear supernatant activities and the caveola fractions did not contain any detectable NADPH cytochrome reductase or galactosyl transferase activity.

To further characterize the caveola fractions, we resolved an equal amount of protein from each subcellular fraction by SDS-PAGE and immunoblotted for various caveola and non-caveola proteins (**Fig. 3**). Caveolin-1 and SR-BI were enriched in the caveola fractions isolated from both undifferentiated and differentiated THP-1 cells. However, the non-caveola proteins, clathrin and transferrin receptor, were completely excluded from the caveola fractions. Cell equivalent loads and immunoblot analysis of SR-BI in the linear range of detection demonstrated that 55–70% of the total cellular SR-BI was associated with the caveola fractions in both undifferentiated and differentiated THP-1 cells (data not shown). The yield of caveolae was determined by immunoblot analysis of caveolin in the linear range of detection (data not shown). The estimated yield of caveolae in both undifferentiated and differentiated cells was 56% ± 7%.

Caveolae are characteristically enriched in both cholesterol and sphingomyelin (19, 34, 35) although caveolae can be enriched only in cholesterol (36) or sphingomyelin (16, 34). Therefore, we determined the relative enrichment of cholesterol, sphingomyelin, and phosphatidylcholine in caveola membranes and plasma membranes isolated from undifferentiated and differentiated THP-1 cells. The cells were labeled for 18 h with [^{3}H]acetate prior to isolating plasma membranes and caveola membranes. Each subcellular fraction was extracted with chloroform/methanol and the lipids were separated by thin-layer chromatography. The relative amount of each lipid analyzed was determined by liquid scintillation counting. Plasma membranes isolated from undifferentiated and differentiated cells were similarly radiolabeled, thus, differentiation did not significantly alter the synthesis or degradation of cholesterol, sphingomyelin, and phosphatidylcholine

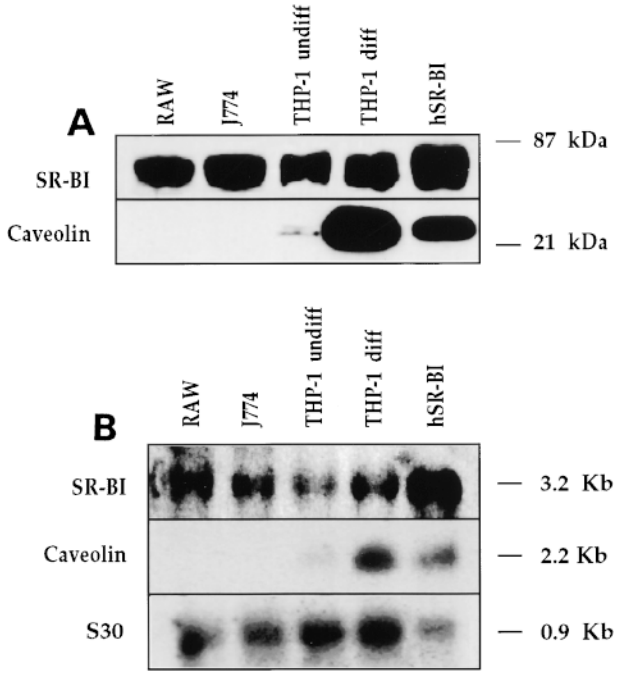

Fig. 1. Expression of caveolin-1 and SR-BI in RAW, J774, undifferentiated THP-1, and differentiated THP-1 cells. Cells were cultured and processed as described in Methods. A) Immunoblot analysis of post-nuclear supernatants from RAW, J774, undifferentiated and differentiated THP-1 cells for SR-BI and caveolin-1. An equal amount of protein (50 μg) from each cell line and 1 μg of lysate from CHO cells expressing SR-BI (hSR-BI) was resolved by SDS-PAGE and immunoblotted with IgGs for SR-BI and caveolin-1. Signal for SR-BI was evident at 82 kDa and signal for caveolin-1 was detected at 22 kDa. The immunoblots were developed by the method of chemiluminescence (20 sec exposures). Longer exposures (2 h) did not detect caveolin-1 in RAW or J774 cells (data not shown). In addition, SR-BII and caveolin-2 were not expressed in these cell lines (data not shown). B) Ten μg of total RNA from each cell line was separated by agarose gel electrophoresis and transferred to nylon membrane. The membranes were hybridized with random prime labeled fragments of full-length SR-BI cDNA (SR-BI), caveolin cDNA (Caveolin), and ribosomal S30 cDNA (S30). The S30 serves as an internal control for the amount of RNA present in each lane. Undifferentiated cells are represented by undiff and differentiated cells by diff. Representative data from one of three independent experiments is shown.

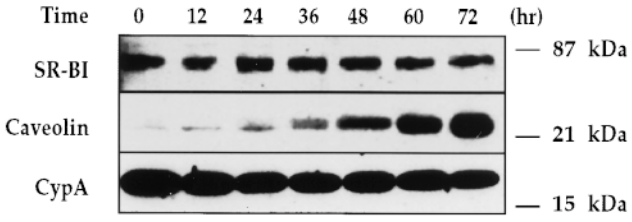

Fig. 2. Time course of caveolin-1 up-regulation in differentiating THP-1 cells. THP-1 monocytes were treated with 200 nm of PMA for the indicated intervals and then post-nuclear supernatants were prepared. An equal amount of protein (50 μg) from each sample was resolved by SDS-PAGE and immunoblotted with IgGs for SR-BI (SR-BI), caveolin-1 (Caveolin), and cyclophilin A (CypA). The cyclophilin A immunoblot serves as a control for the amount of protein loaded in each lane. The immunoblots were developed by the method of chemiluminescence (20 sec exposures). Representative data from one of three independent experiments.

TABLE 1. Protein profile of subcellular fractionations isolated from undifferentiated and differentiated THP-1 cells

Subcellular Fractions	THP-1 Undiff	THP-1 Diff
	mg	*mg*
PNS	5.50 ± 0.99	9.80 ± 1.79
CYTO	1.89 ± 0.79	4.00 ± 1.07
IM	0.53 ± 0.21	0.86 ± 0.39
PM	1.11 ± 0.36	2.16 ± 0.72
CM	0.03 ± 0.01	0.06 ± 0.03

Approximately 10 million undifferentiated and differentiated THP-1 cells were fractionated to isolate caveolae. The total amount of protein in each subcellular fraction is shown. Subcellular fractions are designated as PNS, post-nuclear supernatant; CYTO, cytosol; IM, total intracellular membranes; PM, total plasma membrane; CM, caveola membrane. Undifferentiated cells are represented by undiff and differentiated cells by diff. Presented data are the mean ± SEM from three independent experiments, n = 3.

(**Fig. 4**). Caveolae isolated from both sets of cells were highly enriched in sphingomyelin (Fig. 4B) but not enriched in phosphatidylcholine (Fig. 4C). Cholesterol was not enriched in caveolae isolated from undifferentiated THP-1 cells whereas differentiation increased the amount of cholesterol associated with caveolae by 4- to 6-fold (Fig. 4A).

Selective cholesterol ether uptake

We recently demonstrated that caveolae are the initial acceptor membranes for HDL-derived cholesteryl ester (13). Therefore, we determined the ability of caveolin-deficient macrophages, RAW and J774, and caveolin-containing macrophages, differentiated THP-1 cells, to selectively internalize [^{3}H]cholesterol ether. Undifferentiated THP-1, differentiated THP-1, RAW, and J774 cells associated with similar amounts of ^{125}I-labeled HDL (**Fig. 5A, B**) which is consistent with the levels of SR-BI expression shown in Fig. 1. However, RAW and J774 cells selectively internalized only modest amounts of HDL-derived [^{3}H]cholesterol ether (Fig. 5C). In contrast, 2-

TABLE 2. Galactosyl transferase and NADPH cytochrome C reductase activities in subcellular fractionations isolated from undifferentiated and differentiated THP-1 cells

Subcellular Fractions	THP-1 Undiff		THP-1 Diff	
	GT	NADPH CR	GT	NADPH CR
	%	%	%	%
PNS	100	100	100	100
CYTO	9.80 ± 1.20	4.70 ± 0.71	11.1 ± 2.20	6.7 ± 0.99
IM	89.1 ± 6.20	93.5 ± 7.60	87.2 ± 4.01	91.7 ± 6.42
PM	1.10 ± 0.20	1.80 ± 0.32	1.70 ± 0.33	1.60 ± 0.11
CM	ND	ND	ND	ND

Galactosyl transferase (GT) and NADPH cytochrome C reductase (NADPH CR) activities were measured in each fraction and normalized with respect to the values obtained for the post-nuclear supernatant fractions. Subcellular fractions are designated as PNS, post-nuclear supernatant; CYTO, cytosol; IM, total intracellular membranes; PM, total plasma membrane; CM, caveola membrane. Undifferentiated cells are represented by undiff and differentiated cells by diff. Presented data are the mean ± SEM from three independent experiments, n = 3; ND, not detected.

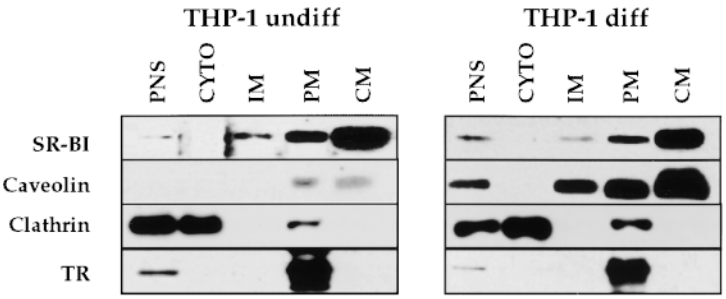

Fig. 3. Characterization of caveolae isolated from undifferentiated and differentiated THP-1 cells. Caveolae were isolated as described in Methods. Thirty micrograms of protein from each subcellular fraction was resolved by SDS-PAGE (7.5–20% gradient) and transferred to a PVDF membrane. The membrane was then immunoblotted with IgGs for SR-BI (SR-BI), caveolin-1 (Caveolin), clathrin (Clathrin), and transferrin receptor (TR). The immunoblots were developed by the method of chemiluminescence (20 sec exposures for Caveolin and SR-BI; 60 sec exposures for Clathrin and TR). Representative data from one of three independent experiments. PNS, post nuclear supernatant; CYTO, cytosol; IM, intracellular membranes; PM, total plasma membranes; CM, caveola membranes. Undifferentiated cells are represented by undiff and differentiated cells by diff.

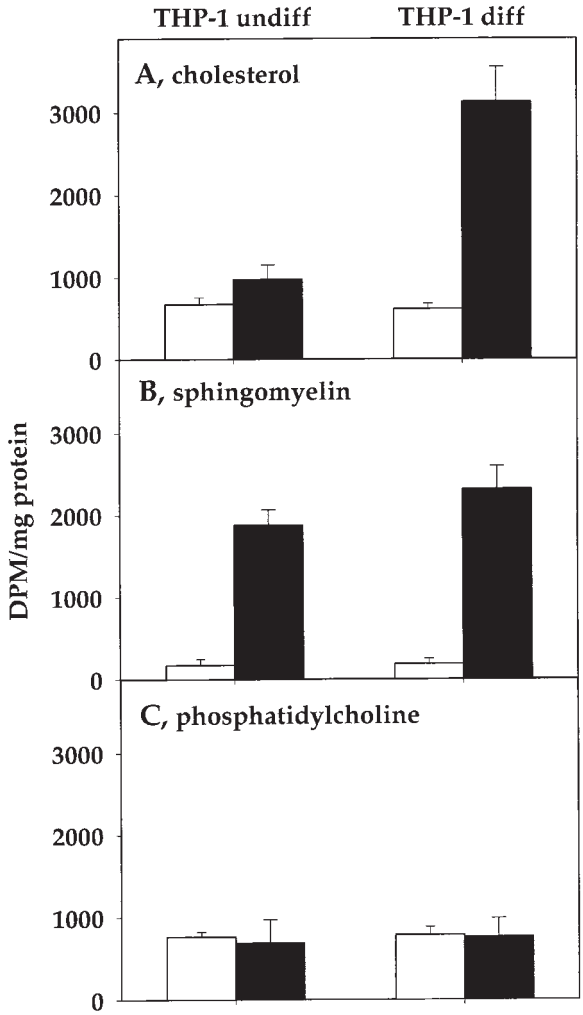

Fig. 4. Differentiation of THP-1 cells increases the amount of cholesterol associated with caveolae. Undifferentiated and differentiated THP-1 cells were labeled with [^{3}H]acetate for 18 h at 37°C. The cells were then washed and processed to isolate caveolae. Purified plasma membranes and caveola membranes were extracted with chloroform/methanol and the lipids were resolved by thin-layer chromatography. Commercially available cholesterol, sphingomyelin, and phosphatidylcholine were used as standards. The relative amount of (A) cholesterol (B) sphingomyelin, and (C) phosphatidylcholine in plasma membranes and caveola membranes was determined by scraping the appropriate spots and quantifying by liquid scintillation counting. Undifferentiated cells are represented by undiff and differentiated cells by diff. Open bars represent plasma membranes and closed bars represent caveola membranes; mean ± SEM, n = 6.

to 3-fold more [^{3}H]cholesterol ether was selectively internalized by undifferentiated THP-1 cells (Fig. 5D, undiff) and 5- to 7-fold more [^{3}H]cholesterol ether was selectively internalized by differentiated THP-1 cells (Fig. 5D, diff). Furthermore, the addition of 200 nm of PMA to RAW and J774 cells for 72 h did not affect ^{125}I-labeled HDL association or the selective uptake of [^{3}H]cholesterol ether (data not shown). Control experiments with ^{125}I-labeled HDL demonstrated that less than 2% of the lipoprotein was degraded in any of the experiments.

DISCUSSION

Macrophages express SR-BI

Circulating monocytes are recruited to developing atheromatous lesions where they differentiate into macrophages, accumulate lipid, and become foam cells (37, 38). The recent identification of SR-BI as a functional HDL receptor opens the possibility that this receptor is involved in foam cell formation. For instance, SR-BI could potentially inhibit foam cell formation by effluxing free cholesterol or SR-BI could facilitate foam cell formation by selectively internalizing cholesteryl esters. We report here that SR-BI is expressed abundantly and to a similar extent in three different macrophage cell lines, RAW, J774, and differentiated THP-1 cells. Furthermore, differentiation of THP-1 cells did not significantly affect the expression level of SR-BI (Fig. 1). SR-BII, an alternatively spliced form of SR-BI, was not detected in any of the cell lines used in these studies (data not shown).

THP-1 macrophages contain caveolae

We recently demonstrated that SR-BI is concentrated in caveolae (13, 14) and that caveolae are the initial sites of selective cholesteryl ester uptake into CHO cells (13). Therefore, we predicted that macrophages would also contain caveolae. In this report we describe the biochemical isolation of a microdomain with the characteristics of caveolae. First, the isolated microdomain only contained 0.6% of the post-nuclear supernatant and was not contaminated with ER or Golgi membranes. Second, the microdomain was highly enriched in caveolin. Caveolin is a 22 kDa protein that is thought to be part of the cytoplasmic coat of caveolae (39, 40) and is an accepted marker for ca-

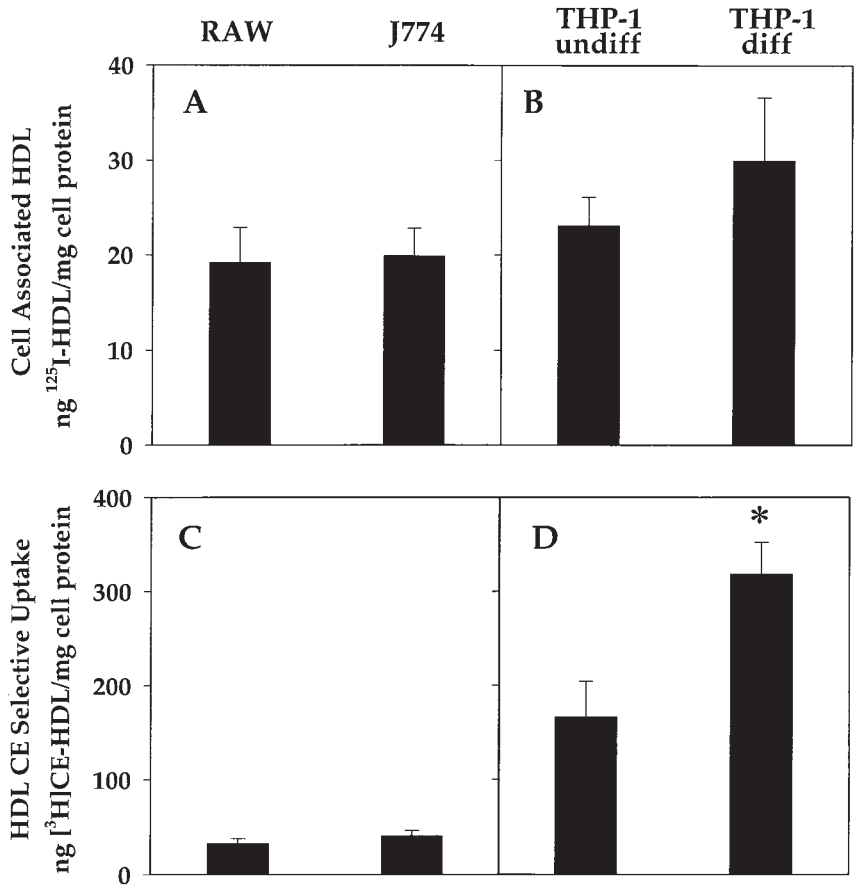

Fig. 5. Cell association of ^{125}I-labeled HDL and selective [^{3}H]cholesterol ether uptake. Undifferentiated and differentiated THP-1, RAW, and J774 cells were grown as described in Methods. The cells were then washed and incubated for 5 h with 10 μg/ml of either ^{125}I-labeled HDL or [^{3}H]CE-HDL in the absence or presence of 200 μg/ml of unlabeled HDL. The specific ^{125}I-labeled HDL binding (A and B) and [^{3}H]CE-HDL uptake (C and D) was calculated by subtracting the values obtained in the presence of 20-fold excess unlabeled HDL from the total values. For [^{3}H]CE-HDL uptake, undifferentiated THP-1 cells accumulated significantly more [^{3}H]CE than RAW and J774 cells ($P < 0.001$) and differentiated THP-1 cells accumulated significantly more [^{3}H]CE than undifferentiated THP-1 cells ($P < 0.001$) as determined by Tukey's HSD. Less than 2% of the ^{125}I-labeled HDL was degraded. Undifferentiated cells are represented by undiff and differentiated cells by diff. Representative data from one of three independent experiments; mean ± SD, n = 3.

veolae. Third, the microdomain was enriched in cholesterol and sphingomyelin and not enriched in phosphatidylcholine (Fig. 4). Caveolae isolated from other cell types are also enriched in cholesterol and sphingomyelin (41). Based on the above findings we conclude that differentiated THP-1 cells contain caveolae. In contrast, Fielding and Fielding (23) reported that macrophages do not contain caveolae. However, others have demonstrated that elicited macrophages express caveolin (24) and contain morphologically identifiable caveolae (25). Our present findings demonstrate that RAW and J774 cells do not express detectable levels of caveolin-1 but that undifferentiated THP-1 cells express small amounts of caveolin-1 (Fig. 1). Furthermore, caveolin-1 was up-regulated greater than 50-fold upon differentiation of THP-1 cells (Figs. 1 and 2). Our data provide a possible explanation for the discrepancy in the literature concerning macrophage caveolae, namely that caveolae are present in some macrophage cell lines but not others. This finding is not unexpected because caveolin and caveolae are often down-regulated when cells are grown in culture (42). The mechanism of caveolin and caveolae down-regulation is not known.

SR-BI and caveolin-1 expression is associated with enhanced selective cholesterol ether uptake

We examined the ability of three macrophage cell lines to selectively internalize cholesterol ether. Even though THP-1 cells expressed SR-BI to a similar extent as RAW and J774 cells, undifferentiated and differentiated THP-1 cells selectively internalized 2- to 3-fold and 5- to 7-fold more cholesterol ether, respectively. Upon differentiation, caveolin-1 was dramatically up-regulated which has been shown to induce the formation of caveolae (17, 18, 43). Because many caveolin molecules are required to form a single caveola, a large increase in caveolin expression would be expected to result in a modest increase in the number of caveolae. Our findings suggest that while a low rate of selective cholesterol ether uptake occurs in the

absence of caveolae (RAW and J774 cells), the presence of caveolae facilitates this process (THP-1 cells). Alternatively, CD36, another class B scavenger receptor that can be up-regulated in macrophages (44) and is associated with caveolae (45, 46), could have been responsible for the increase in selective cholesterol ether uptake. However, Gu et al. (47) recently demonstrated that CD36 binds HDL but does not facilitate selective cholesteryl ester uptake. We confirmed these observations by using the CD36 blocking antibody, OKM5, in our studies (data not shown). The addition of OKM5 to the uptake assays did not inhibit the selective uptake of [^{3}H]cholesterol ether demonstrating that selective uptake is most likely mediated by SR-BI.

In summary, we have shown the presence of caveolae and SR-BI in undifferentiated and differentiated THP-1 cells and that THP-1 cells selectively internalize cholesterol ethers. In THP-1 cells, the up-regulation of caveolin-1 is accompanied by an increase in selective cholesterol ether uptake in the absence of an increase in SR-BI expression. Further studies are needed to determine the role of caveolae and SR-BI in regulating foam cell formation.

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