Expression of Scavenger Receptor BI Facilitates Sterol Movement between the Plasma Membrane and the Endoplasmic Reticulum in Macrophages[†]

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Received September 30, 2002; Revised Manuscript Received January 16, 2003

ABSTRACT: Scavenger receptor BI influences multiple aspects of cellular sterol metabolism. In this series of studies, we evaluated the effect of scavenger receptor BI expression on the distribution and movement of sterol between the plasma membrane and the endoplasmic reticulum in macrophages, by comparing control J774 cells to J774 cells in which SR-BI expression was constitutively increased 3-fold. J774 cells with increased expression of SR-BI (J774-SRBI cells) esterified plasma membrane cholesterol more rapidly as compared to control cells. The esterification of endogenously synthesized cholesterol was also more rapid in cells with increased SR-BI expression; this could be partially suppressed by removing cholesterol from the plasma membrane. The increased plasma membrane sterol esterification in J774-SRBI cells was not due to increased acyl-coA:cholesterol acyltransferase activity and was observed even though J774-SRBI cells manifested a smaller free cholesterol pool in the endoplasmic reticulum. Cholesterol ester hydrolysis was also more rapid in J774-SRBI cells. Increased expression of SR-BI also facilitated the clearance of cellular cholesterol ester to HDL_3. This latter observation, combined with the measurement of the smaller ER free cholesterol pool in J774-SRBI cells, suggests that the free cholesterol derived from the hydrolysis of cholesterol ester was rapidly transported back to the plasma membrane. It is concluded that expression of SR-BI in macrophages increases the rate of free cholesterol transport, and modulates free cholesterol distribution between the plasma membrane and the internal membrane compartments in macrophages.

Scavenger receptor BI is a recently described cell surface receptor that is expressed in multiple tissues and that has an important role in organismal and cellular lipid homeostasis (*1*−*3*). At the cellular level, it plays an important role in mediating the selective uptake of cholesterol ester from lipoproteins. It also appears capable of acting as a classic endocytic receptor for the uptake of extracellular particles, including advanced glycosylation end product-modified proteins and oxidized lipoproteins (*1*−*5*). In addition to selective uptake of cholesterol ester, or endocytic uptake of lipoprotein particle, SR-BI[1] can also stimulate free cholesterol flux in a bi-directional manner between cells and extracellular lipoprotein particles (*3*, *6*). In the presence of specific cholesterol acceptor particles, therefore, SR-BI can stimulate net cholesterol efflux from cells. Several lines of evidence suggest that the enhanced bi-directional flux of free cholesterol produced by SR-BI expression is not dependent on the binding of extracellular particles to SR-BI (*7*, *8*). This has led to the suggestion that SR-BI expression reorganizes plasma membrane lipid domains to facilitate cholesterol flux. This notion has received support from observations that expression of SR-BI alters the susceptibility of plasma membrane free cholesterol to oxidation by exogenously added cholesterol oxidase (*9*). In addition, the expression of SR-BI has been shown to alter cellular morphology, causing the formation of microvillar channels at the surface of cells (*10*). These observations suggest that SR-BI plays a fundamental role in plasma membrane organization. Therefore, it is reasonable to expect that SR-BI expression could also have a fundamental role in the distribution and metabolism of endogenous cellular sterol.

SR-BI has been found in atherosclerotic lesions and is expressed in macrophages (*11*). Factors that influence free cholesterol distribution and flux in macrophages are important in view of the important role played by these cells in the defense of vessel wall lipid homeostasis (*12*). In this series of studies, therefore, we evaluated the role of SR-BI in modulating the distribution and recycling of free cholesterol between the plasma membrane and the internal cellular membranes.

MATERIALS AND METHODS

Materials. [Oleoyl-1-^{14}C]coenzyme A, [1,2-3H(N)]cholesterol, ^{3}[H]acetate, and [1-^{14}C]oleic acid were purchased from Perkin-Elmer Life Sciences (Boston, MA). 25-hydroxycholesterol, βCD, and progesterone were purchased from

[†] This work was supported by Grants HL 39653 and HL 59489 (T.M.) and HL 28448 (Y.L.) from the National Institutes of Health.

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[1] Abbreviations: βCD, β cyclodextrin; SR-BI, scavenger receptor class B type I; ACAT, acyl-coA:cholesterol acyltransferase; ER, endoplasmic reticulum; TLC, thin-layer chromatography; A-LDL, acetylated LDL; CE, cholesterol ester.

10.1021/bi0269207 CCC: $25.00
Published on Web 03/13/2003

Sigma (St. Louis, MO). J774 and CHO cell lines were obtained from ATCC (Manassas, VA). HDL, LDL, and acetylated LDL were prepared as previously described (*13*).

Culture and Transfection of J774 Macrophages and CHO Cells. J774 macrophages were cultured in DMEM with 10% FCS. CHO cells were cultured in F-12 Hams medium supplemented with 10% FCS. J774-SRBI or J774-C cells were generated by stable transfection of J774 cells with a murine SR-BI cDNA (pcDNA3.1-mSRBI/Zeo$^+$) or a control plasmid (pcDNA3.1/Zeo$^+$), respectively, using a calcium phosphate transfection method as described in detail previously (*14*, *15*). CHO cells were stably transfected with the same plasmid pair using lipofectamine (Invitrogen, Carlsbad, CA). In pcDNA3.1-mSRBI/Zeo$^+$, the expression of the SR-BI cDNA is constitutively driven by the CMV promoter. Stably transfected cells were selected with 400 μg/mL of Zeocin, and the levels of SR-BI expression were quantified by Western blot analysis. Cells were used for experiments at 90% confluency.

Plasma Membrane Sterol Esterification. Plasma membrane was selectively labeled by incubating cells with 1−3 μCi/mL ^{3}[H]cholesterol in DMEM with 0.2% BSA for 30 min at 15 °C (*16*). After washing, 1 mL of pre-warmed DMEM with 0.2% BSA was added to the well and incubated at 37 °C, for the times indicated in the figure legends, to allow the internalization of the plasma membrane label. After incubation, cellular lipids were extracted with hexane and 2-propanol (3:2) and separated by thin-layer chromatography in heptane, ethyl ether, and acetic acid (90:30:1) (*16*). Steryl ester spots were isolated, and their radioactivity was determined by scintillation counting.

Measurement of Endogenous Sterol and Steryl Ester Synthesis Rate. Cells were seeded at 1.5×10^6/well in 6-well plates and cultured for 1 day or until 90% confluent. Additions were made as described in the figure legends. Cells were pulse labeled with 10 μCi/mL ^{3}[H]acetate in DMEM with 0.2% BSA for 2 h at 37 °C. After washing, cellular lipids were extracted with hexane and 2-propanol (3:2) and separated by TLC in heptane, ethyl ether, and acetic acid (90:30:1). Sterol and steryl ester spots were isolated, and their radioactivity was determined by scintillation counting. In some experiments, labeled sterol ester was hydrolyzed to free sterol plus fatty acid by saponification in ethanolic KOH for 1 h at 85 °C, prior to TLC.

Measurement of ACAT Activity in Cell Homogenates. J774-SRBI or J774-C macrophages were seeded in T75 flasks and cultured till 95% confluent. After washing with PBS, cells were homogenized with a Balch homogenizor at 4 °C, and the unbroken cells were removed by centrifugation at 500 rpm for 5 min. Cell homogenates were incubated with excess substrate (60 μM [^{14}C]oleoyl-CoA and 5 μg of cholesterol/Triton 10% WR-1339) at 37 °C over a period of 30 min (*16*). After incubation, lipids in the reaction mixtures were extracted with chloroform and methanol (2:1) and separated by TLC in hexane and ethyl acetate (90:10). Steryl ester and free cholesterol spots were isolated, and their radioactivity was determined by scintillation counting.

ER Sterol Pool Measurement. The size of the ER sterol pool was measured as described previously by Lange et al. (*17*, *18*). Briefly, J774-SRBI or J774-C macrophages were cultured in T75 flasks till 95% confluent. After washing the cells with cold PBS, cell homogenates were prepared, 60 μM [^{14}C]oleoyl-CoA, 1 mM dithiothreitol, and 1 mg/mL BSA were added, and the reaction mixtures were incubated at 37 °C for the times specified. Time course experiments were evaluated to ensure that the endogenous ER pool of free sterol was esterified to completion under our experimental conditions. After incubation, lipids in the reaction mixtures were extracted with chloroform/methanol (2:1) and separated by TLC in hexane/ethyl acetate (90:10). ^{3}[H]cholesteryl oleate was added as recovery standard. Steryl ester spots were isolated, and their radioactivity was determined by scintillation counting.

Measurement of Steryl Ester Hydrolysis Rate. J774-SRBI or J774-C cells were plated in 6-well plates and cultured till 95% confluent. After washing, cells were labeled with 3 μCi/mL ^{3}[H]cholesterol in DMEM with 0.2% BSA for 30 min at 15 °C followed by three washes with the pre-warmed (37 °C) medium. Cells were then incubated in DMEM with 0.2% BSA for 4 h at 37 °C to allow formation of plasma membrane-derived ^{3}[H]steryl ester. After incubation, cells were washed, and fresh DMEM with 0.2% BSA plus 5 μg/mL of the ACAT inhibitor, 58035, was added. At the times specified, cellular lipids were extracted with hexane and 2-propanol (3:2) and separated by TLC in heptane, ethyl ether, and acetic acid (90:30:1). Steryl ester spots were isolated, and their radioactivity was determined by scintillation counting.

Sterol Mass and Cell Protein Assays. Sterol and steryl ester mass were measured enzymatically using commercially available kits (Wako, Richmond, VA). Cellular protein was measured using the DC protein assay kit (Bio-Rad, Hercules, CA).

Statistical Analysis. Values shown in the figures are the mean ± SD from triplicate wells unless otherwise indicated. Figures are representative of 2−3 experiments with similar results. Statistical differences were analyzed for significance using ANOVA.

RESULTS

Stably transfected J774 cells that constitutively expressed SR-BI at a 2.6-fold higher level than control cells were selected for study. This 2.6-fold increased level of expression was chosen because it is within the range of induction of native macrophage SR-BI expression by its physiologic modulators (*11*). We first conducted a series of experiments to investigate if expression of SR-BI enhanced the movement of sterol from plasma membrane to internal membranes. The esterification of sterol occurs in the ER; therefore, we estimated the movement of sterol from the plasma membrane to the ER from the rate of esterification of plasma membrane free cholesterol. Plasma membrane sterol pools were selectively labeled with [^{3}H] cholesterol at 15 °C for 30 min (*16*) and then chased at 37 °C to allow the internalization of the sterol. Cellular free cholesterol counts were approximately 25−30% higher in J774-SRBI cells than in control cells (not shown). However, esterification rates of plasma membrane cholesterol were 3−5-fold higher in J774-SRBI cells as compared to J774-C cells (Figure 1). The addition of 25-hydroxycholesterol, previously shown to enhance the internalization of plasma membrane cholesterol to the ER (*17*), increased the rate of esterification in both cell types, and the difference between them was maintained (upper panel).

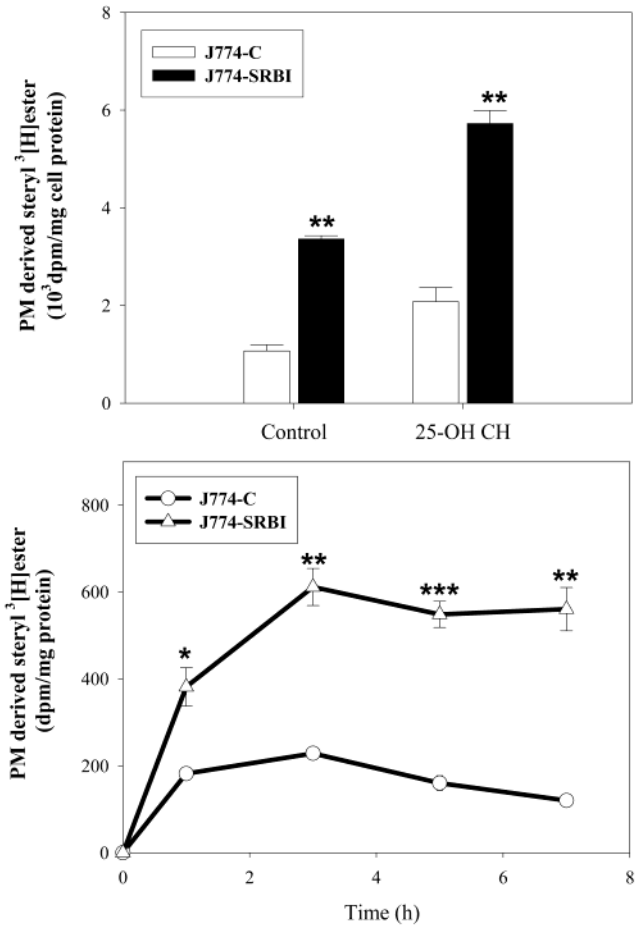

FIGURE 1: Esterification of plasma membrane sterol in J774-SRBI and J774-C macrophages. Macrophages were labeled with ^{3}[H]cholesterol in DMEM with 0.2% BSA for 30 min at 15 °C. After washing, cells were incubated in DMEM with 0.2% BSA in the presence or absence of 1 μM 25OH CH at 37 °C for 6 h (upper panel) or in 0.2% BSA for up to 6 h (lower panel). Cellular lipids were extracted, and steryl ester derived from the plasma membrane was analyzed by TLC as described in Materials and Methods. Values shown are the mean ± SD from triplicate wells. *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$ for J774-C vs J774-SRBI.

Increased expression of SR−BI was more effective in stimulating esterification of plasma membrane sterol than the addition of 25-hydroxycholesterol to control cells. Increased esterification of plasma membrane cholesterol could be detected at the earliest time point assayed, and by 3 h the rate of labeling of the cholesterol ester pool reached a plateau in both cell types. However, this equilibrium was reached at a higher steady-state level in J774-SRBI cells than in control cells.

Progesterone, which has no direct effect on ACAT activity, has been shown to inhibit the esterification of plasma membrane cholesterol in macrophages by inhibiting its movement to the ER (*16*, *18*). The effect of interrupting this movement on the level of esterification of plasma membrane cholesterol in J774-C and J774-SRBI cells was evaluated. As shown in Figure 2, the interruption of sterol movement from the plasma membrane to the ER by progesterone reduced esterification of plasma membrane sterol in both cell types and completely abolished any difference between them. The data in Figures 1 and 2 are consistent with the conclusion that expression of SR-BI in macrophages facilitates the movement of plasma membrane sterol to the ER. Additional

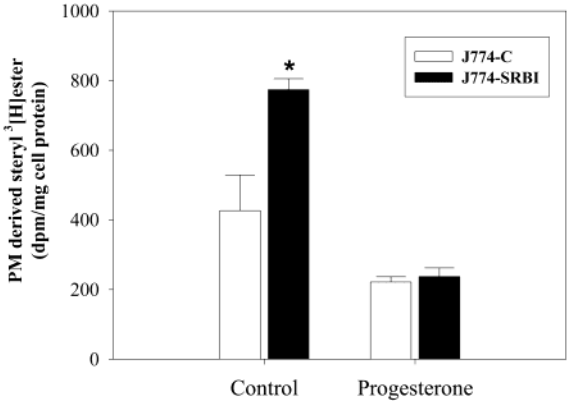

FIGURE 2: Progesterone eliminates the difference in plasma membrane sterol esterification between J774-C and J774-SRBI cells. Macrophages were labeled with ^{3}[H]cholesterol in DMEM with 0.2% BSA for 30 min at 15 °C. Cells were then warmed to 37 °C in 0.2% BSA in DMEM containing 10 μg/mL of progesterone or ethanol vehicle. After a 4-h incubation, cells were harvested for analysis as described in the legend to Figure 1. The values shown are the mean ± SD from triplicate wells. *$p < 0.05$ for J774-C vs J774-SRBI.

experiments were then conducted to rigorously exclude other potential mechanisms that could contribute to the results of the experiment presented in Figure 1.

A small expansion of total cell cholesterol has been previously reported in cells with increased SR-BI expression grown in 10% FBS (*9*). This expansion is likely due to SRBI-mediated uptake of cholesterol from complete medium. We measured free cholesterol and cholesterol ester mass in J774-C and J774-SRBI cells, and our results are consistent with these previous reports. Free cholesterol mass was 21.9 ± 1.4 and 25.8 ± 1.7 μg/mg cell protein in J774-C and J774-SRBI cells, respectively ($n = 4$). Cholesterol ester mass was 0.3 ± 0.9 and 3.6 ± 1.3 μg/mg cell protein in these cells, respectively. Therefore, the increase in plasma membrane cholesterol esterification we measured in Figure 1 could reflect an increase in overall cellular sterol. Because the expansion of total cell cholesterol mass modulates cholesterol esterification by expansion of the ER free cholesterol pool (*17*, *18*), we examined this potential mechanism by directly measuring the size of the ER cholesterol pool. This pool is also of special interest because it is the site for sterol esterification and the cellular site from which cholesterol synthesis rates and LDL receptor activity are modulated.

In previous studies, it was shown that the size of the ER sterol pool could be measured by an in vitro esterification assay in which the ER cholesterol pool is esterified to completion in cell homogenates (*17*, *19*). We utilized this same assay to estimate the effect of increased SR-BI expression on the size of the ER sterol pool in macrophages. As shown in Figure 3, expression of SR-BI decreased the size of the ER sterol pool. As an additional probe for the size of the ER cholesterol pool, we measured the rate of cholesterol synthesis in J774-SRBI and J774-C cells. The rate of cholesterol synthesis was higher in J774-SRBI cells (Figure 3, inset). This is contrary to what one would expect in the presence of an increase in cellular cholesterol content; however, the increased cholesterol synthetic rate is consistent with a smaller ER cholesterol pool.

We next examined whether the esterification of endogenously synthesized cholesterol was also enhanced in J774-

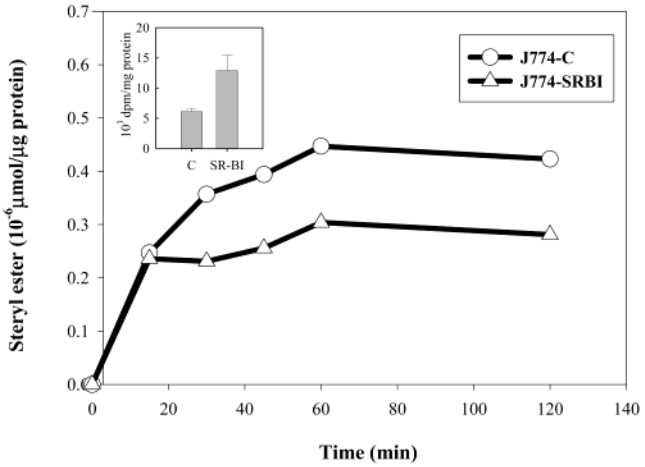

FIGURE 3: ER sterol pool size in J774-SRBI and J774-C macrophages. Macrophage homogenates were incubated with 60 μM [^{14}C]oleoyl-CoA at 37 °C for 15, 30, 45, 60, and 120 min. Esterified sterol was isolated and analyzed by TLC. Values shown are the mean from duplicate wells. Inset shows the cholesterol synthesis in J774-C and J774-SRBI. Cells were incubated with 0.2% BSA in DMEM for 24 h followed by 10 μCi/mL ^{3}[H]acetate for 2 h. Cellular cholesterol was separated and analyzed by TLC as described in Materials and Methods. Values shown are mean ± SD from triplicate wells.

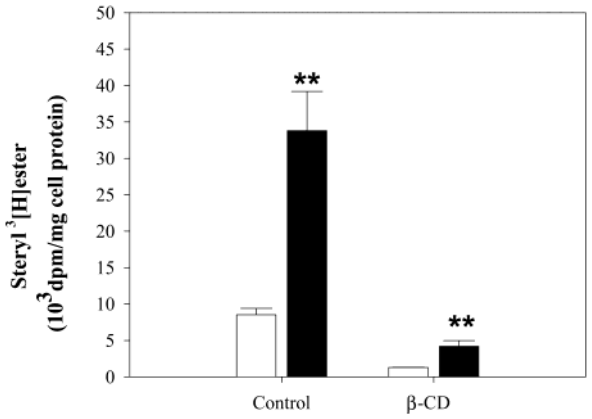

FIGURE 4: Steryl ester levels derived from the newly synthesized sterol. Macrophages were incubated for 2 h in 0.2% BSA with or without 2 mM methyl-β-CD. After incubation, cells were washed and labeled with 10 μCi/mL ^{3}[H]acetate in DMEM for 2 h. Cells were washed, cellular lipids were extracted, and radioactive label incorporation into steryl ester was analyzed by TLC as described in Materials and Methods. Values shown are the mean ± SD from triplicate wells of cells. **$p < 0.01$ for J774-C vs J774-SRBI. Open bars, J774-C cells; closed bars, J774-SRBI cells.

SRBI cells (Figure 4). Cells were incubated with labeled acetate for 2 h and then harvested for measurement of label in cholesterol ester. Again, a 3−4-fold increase in esterification was noted as a result of SR-BI expression. A similar increase was observed if labeled cholesterol ester was saponified prior to analysis to specifically detect label in sterol (3.9-fold increase, $p < 0.01$). To determine if esterification rates of newly synthesized cholesterol were modulated by plasma membrane sterol pool size, we briefly preincubated cells in 2 mM methyl-β-CD to reduce plasma membrane sterol, prior to the labeling period. The depletion of plasma membrane cholesterol pools during the preincubation dramatically decreased the incorporation of newly synthesized sterol into cholesterol ester in both cell types (Figure 4), suggesting that the newly synthesized sterol that

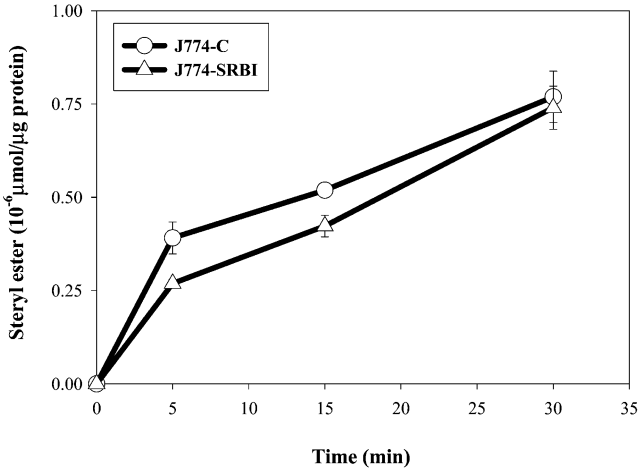

FIGURE 5: ACAT activity in J774-SRBI and J774-C macrophage homogenates. Macrophage homogenates were prepared as described in Materials and Methods and incubated with 5 μg of cholesterol/10%WR-1339 and 60 μM [^{14}C]oleoyl-CoA for up to 30 min at 37 °C. Esterified sterol was isolated and analyzed by TLC, and the radioactivity was determined. Values shown are the mean ± SD from triplicate wells.

served as substrate for esterification passes through the plasma membrane or through a pool that is in rapid equilibrium with the plasma membrane. However, even after the incubation in β-cyclodextrin, the cells expressing increased levels of SR-BI displayed a 2−3-fold increased level of esterification as compared to J774-C cells. These results indicate that SR-BI expression enhanced the esterification of endogenously synthesized cholesterol and exclude the possibility that the increased esterification of plasma membrane cholesterol observed in Figure 1 was due to a change in the labeling efficiency of plasma membrane sterol in J774-SRBI cells.

We next evaluated the effect of an increase in SR-BI expression on the esterification rate of plasma membrane sterol in CHO cells. A transfected CHO clone that expressed SR-BI at 3.6-fold higher than control CHO cells was selected for study. Similar to macrophages, expression of SR-BI expanded cellular sterol mass in CHO cells, primarily reflected by an increase in cholesterol ester mass (CE 1.3 ± 1.5 vs 4.8 ± 2.7 μg/mg in CHO-C vs CHO-SRBI cells, respectively). However, increased SR-BI expression did not enhance the esterification of plasma membrane sterol in CHO cells. This result suggested that the effect of SR-BI expression on esterification of plasma membrane sterol was cell-specific.

We designed the experiment in Figure 1 to probe for the internalization of plasma membrane free cholesterol and used esterification as a measure of the movement of plasma membrane cholesterol to the endoplasmic reticulum. However, the increased esterification of cell surface [^{3}H] cholesterol would also be observed if ACAT activity was increased in J774-SRBI cells as compared to controls. Figure 5 shows the results of an experiment directly measuring ACAT activity in macrophage homogenates. ACAT activity was similar in J774-C and J774-SRBI cells, and in some experiments ACAT activity even tended to be somewhat lower in J774-SRBI cells, as shown here. Increased ACAT activity, therefore, cannot explain the increased esterification of plasma membrane sterol as a result of SR-BI expression.

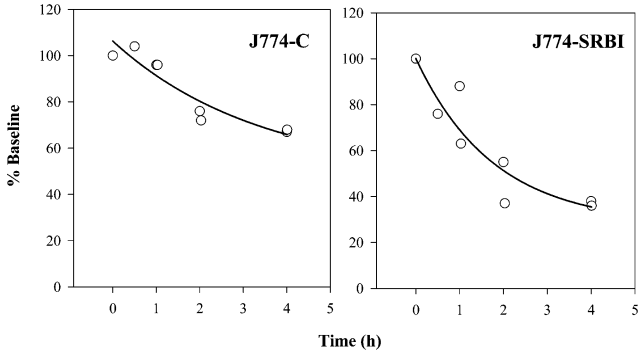

FIGURE 6: Steryl ester hydrolysis rate in J774-SRBI and J774-C macrophages. Macrophages were labeled with 3 μCi/mL ^{3}[H]cholesterol in DMEM with 0.2% BSA for 30 min at 15 °C. After washing the cells with pre-warmed medium, cells were incubated in DMEM and 0.2% BSA at 37 °C for 4 h, and then the fresh medium with 5 μg/mL of the ACAT inhibitor, 58035, was added (at 0 time) for the times indicated. After incubation, cells were washed, cellular lipids were extracted, and radioactive label in steryl ester was analyzed by TLC as described in Materials and Methods. The counts in CE present at each time point are expressed as a percentage of the baseline value, taken as the counts present at 0 time. The pooled data from two experiments for each cell line were fit by SigmaPlot (SPSS, Chicago, IL) to a first-order exponential expression. The half-times for the best fits were panel A (J774-C): 2.3 h ($R^2 = 0.88$) and panel B (J774-SRBI): 1.2 h ($R^2 = 0.85$).

Table 1: HDL_3 Mediated Efflux from Sterol-Loaded J774-C and J774-SRBI Cells

	Cellular sterol (μg/mg protein)		
	total	free	ester
	J774-C[a]		
before HDL_3	24.3 ± 2.2	17.6 ± 2.2	6.7 ± 3.3
after HDL_3	19.4 ± 3.0	16.6 ± 1.2	2.8 ± 1.1
	J774-SRBI[a]		
before HDL_3	49.1 ± 2.7	30.1 ± 2.3	19.0 ± 3.6
after HDL_3	25.1 ± 1.7	18.0 ± 1.4	7.1 ± 0.3

[a] J774-C and J774-SRBI cells were incubated with 50 μg/mL A-LDL for 48 h. At that time, some wells were harvested for measurement of total, free, and esterified cellular sterol (before HDL_3). Parallel wells were incubated for an additional 6 h with 400 μg/mL HDL_3 in 0.2% BSA before harvesting for the same measurements.

We next considered the effect of SR-BI expression on the rate of CE hydrolysis in macrophages. In Figure 6, plasma membrane cholesterol was labeled at 15 °C for 30 min, and the label was chased for 4 h at 37 °C to allow for the formation of plasma membrane derived [^{3}H] steryl ester. The cells were then treated with an inhibitor of ACAT, so as to allow the direct observation of the rate of hydrolysis of this steryl ester. As shown in Figure 6, hydrolysis was significantly more rapid in J774-SRBI as compared to J774-C cells (half-time for CE hydrolysis: 2.3 vs 1.2 h in J774-C vs J774-SRBI, respectively). Thus, the increased rates of esterification of plasma membrane cholesterol because of increased SR-BI expression are accompanied by increased hydrolysis of the cholesterol ester derived from plasma membrane sterol.

The availability of free cholesterol derived from the hydrolysis of cholesterol ester for movement to the plasma membrane was then evaluated by measuring its availability for efflux out of the cell (Table 1). Total cellular cholesterol and cholesterol ester were increased by preincubating cells with A-LDL at 50 μg/mL. Incubation of J774-SRBI cells with A-LDL led to increased cholesterol loading as compared to J774-C cells, likely related to SRBI-mediated uptake of A-LDL. Therefore, immediately after the 48-h preincubation in A-LDL (and before incubation in HDL_3) total cholesterol, free cholesterol, and cholesterol ester are all higher in J774-SRBI cells (24.8, 12.5, and 12.3 μg/mg higher in J774-SRBI cells vs J774-C cells, respectively). A subsequent 6-h incubation in HDL_3 reduced cholesterol levels more substantially in J774-SRBI cells than in J774-C cells. A substantial portion of the stored cholesterol ester in J774-SRBI cells is mobilized for efflux during a 6-h incubation in HDL_3 (12.3 μg/mg of 19.0 μg/mg cholesterol ester mobilized). In a separate experiment, we incubated J774-C and J774-SRBI macrophages with 100 μg/mL of A-LDL. After this incubation, total cell cholesterol was 49.8 ± 8.3 μg/mg in J774-C cells and 70.3 μg/mg in J774-SRBI cells. After incubation with HDL_3 for 6 h in this experiment, there was no fall in total cell cholesterol in J774-C cells (48.2 ± 7.8 μg/mg). However, incubation with HDL_3 reduced total cell cholesterol in J774-SRBI cells to 45.6 ± 5.2 μg/mg. Because HDL_3 mediates the efflux of free cholesterol (and not cholesterol ester) from cells, the results in Table 1 along with the results in Figures 1, 2, and 6 indicate that increased expression of SR-BI is associated with more rapid turnover of cholesterol ester, a smaller ER free cholesterol pool, and enhanced movement of free cholesterol between the plasma membrane and the ER.

DISCUSSION

Intracellular cholesterol transport likely occurs via several mechanisms, including vesicle budding and protein-mediated transport (*20*). The distribution of free cholesterol resulting from these transport mechanisms is highly compartmentalized, with greater than 80% of total cellular cholesterol residing in the plasma membrane (*20*). However, sterol within the plasma membrane continually recycles between the plasma membrane and the internal membrane compartments. These compartments include the ER, the endocytic recycling compartment, and the Golgi. The half-life for lipid recycling between the plasma membrane and the intracellular membrane compartments has been estimated to be 5−10 min (*20*−*23*). Transport between the plasma membrane and the ER is particularly important inasmuch as the signals that modulate cholesterol synthesis and uptake are initiated in this intracellular compartment. Moreover, cholesterol arriving in this compartment becomes a substrate for ACAT.

SR-BI has complex effects on cellular lipid homeostasis. Expression of this plasma membrane receptor facilitates the selective uptake of cholesterol ester from lipoproteins and holo-particle endocytic uptake of lipoproteins and magnifies the bi-directional flux of free cholesterol between cells and lipoproteins down a concentration gradient (*1*−*6*). Expression of this protein also has complex effects on plasma membrane lipid organization and structure. In Sf9 cells, expression of SR-BI induces the appearance of complex double membrane channels at the cell surface (*10*). These channels are specifically labeled with SR-BI antisera. In the adrenal gland, SR-BI expression is absolutely required for the expression of microvillar channels normally observed at the surface of adrenocortical cells (*24*). In COS7 cells, SR-BI expression induces enhanced susceptibility of plasma membrane cholesterol to oxidation by exogenous cholesterol oxidase (*9*).

In our studies, we evaluated the effect of SR-BI on the intracellular transport of sterol between the plasma membrane

and the ER in the absence of exogenous lipoproteins or cholesterol acceptors. Our experiments demonstrated that a physiologic increase in SR-BI expression enhances the esterification of plasma membrane cholesterol. We also showed that the esterification of endogenously synthesized sterol was also enhanced. The increased plasma membrane esterification, as a result of SR-BI expression, appeared to be cell-specific in that CHO cells with a similar increase in SR-BI expression did not show enhanced esterification of plasma membrane cholesterol. We showed that the increased esterification of plasma membrane cholesterol that accompanied the increased SR-BI expression was not accounted for by increased ACAT activity, nor was it accounted for by an overall expansion in cell sterol, as evidenced by the fact that the ER cholesterol pool was actually smaller in J774-SRBI cells. We suggest that the most likely explanation for the increased level of esterification of plasma membrane sterol is an increased rate of transport of free cholesterol to the ER from the plasma membrane. This increased rate of transport of free cholesterol from the plasma membrane to the ER could result from an expansion of the plasma membrane sterol pool, even in the presence of a smaller ER sterol pool, if SR-BI expression alters the steady-state distribution of free cholesterol between plasma membrane and ER. Altered sterol transport and distribution as a result of SR-BI expression helps to rationalize the increase in endogenous cholesterol synthesis even with an increase in total cell sterol and the increased esterification rate of sterol even with a contracted ER free sterol pool. The increased transport to the ER could also result from a reorganization of free cholesterol into micro-domains within the plasma membrane that are in more rapid equilibrium with the ER. Such a redistribution within domains of the plasma membrane would be consistent with observations of others concerning the effect of SR-BI expression on the susceptibility of plasma membrane free cholesterol to exogenous cholesterol oxidase, as well as with observations suggesting a structural reorganization of the plasma membrane after SR-BI expression. Our results are also consistent with the possibility that SR-BI may selectively recycle sterol between the plasma membrane and the ER.

In J774-SRBI cells at steady-state there is a small, but significant, increase in cholesterol ester mass, and cholesterol ester hydrolysis rates are increased. Despite this, the ER free cholesterol pool is smaller, and the cholesterol synthesis rate is higher in these cells. This suggests that the free cholesterol derived from the intracellular hydrolysis of cholesterol ester is not returned to the ER and most likely moves back to the plasma membrane in J774-SRBI cells. Transport back to the plasma membrane is also suggested by the effective mobilization of stored cholesterol ester by exogenous cholesterol acceptors in cells expressing increased SR-BI levels. We did not investigate the mechanism for the increase in cholesterol ester hydrolase activity in J774-SRBI cells. In macrophages, activation of cholesterol ester hydrolase activity, and subsequent increased cholesterol ester hydrolysis, has also been observed after treatment of macrophages with insulin or leptin (*25*). Cholesterol ester hydrolase activity in macrophages may result from the activity of more than one enzyme. For example, hormone-sensitive lipase can give rise to cholesterol ester hydrolase activity, but it has been observed that cholesterol ester hydrolase activity is not reduced in macrophages obtained from hormone-sensitive lipase knockout mice (*26*). Consistent with this, a separate neutral cholesterol ester hydrolase has been recently cloned from a human macrophage library (*27*). Thus, the molecular identity, characteristics, and regulation of neutral cholesterol ester hydrolase activity in macrophages will require further study.

There are multiple potential mechanisms by which SR-BI expression can alter sterol transport between the plasma membrane and the ER, as discussed above, that will also require further study. It is of interest that we observed this effect in macrophages but not in CHO cells, even though relative increases in SR-BI expression as compared to their respective controls were similar. There may be a unique relationship between the ER and the plasma membrane in macrophage cells, related to their high phagocytic activity. For example, recently it was reported that the ER membrane can fuse with the macrophage plasmalemma and serve as a source for phagosome formation in macrophages (*28*). Thus, there may be unique aspects of membrane lipid transfer between ER and plasma membrane in macrophages that can be influenced by the level of SR-BI expression. Our results show that increased SR-BI expression in macrophages mediates important changes in intracellular sterol transport and distribution. SR-BI expression in macrophages may, therefore, modulate atherogenesis not only by influencing the flux of sterol between cells and lipoproteins but also by modulating intracellular cholesterol trafficking.

ACKNOWLEDGMENT

The authors thank Stephanie Thompson for assistance with manuscript preparation.

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BI0269207