ApoE-dependent sterol efflux from macrophages is modulated by scavenger receptor class B type I expression

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Abstract **Macrophages express a number of proteins involved in sterol efflux pathways, including apolipoprotein E (apoE) and scavenger receptor class B type I (SR-BI). We have investigated a potential interaction between these two sterol efflux pathways in modulating overall macrophage sterol flux. We utilized an experimental system in which we increased expression of each of these proteins to a high physiologic range in order to perform our evaluation. We show that in apoE-expressing cells, a 4-fold increase in SR-BI expression leads to reduction of sterol and phospholipid efflux. SR-BI-mediated reduction in sterol efflux was only observed in cells that expressed endogenous apoE. In J774 cells that did not express apoE, a similar increase in SR-BI level led to increased sterol efflux. The divergent response of sterol efflux after increased SR-BI was maintained in the presence of a number of structurally diverse extracellular sterol acceptors. Increased SR-BI expression also enhanced sterol efflux to exogenously added apoE. Investigation of a potential mechanism for reduced efflux in apoE-expressing cells indicated that SR-BI expression reduced macrophage apoE by accelerating the degradation of newly synthesized apoE. This led to decreased secretion of apoE and reduced the fraction of apoE sequestered on the cell surface. Thus, enhanced SR-BI expression in macrophages can reduce the cellular level and secretion of apoE by accelerating degradation of the newly synthesized protein. This reduction of endogenous apoE is accompanied by reduced sterol efflux from macrophages.**—Huang, Z. H., and T. Mazzone. **ApoE-dependent sterol efflux from macrophages is modulated by scavenger receptor class B type I expression.** ***J. Lipid Res.*** **2002.** 43: **375–382**

Supplementary key words atherosclerosis • vascular wall

The importance of macrophages for preserving normal vessel wall homeostasis has been documented in many experimental systems. Macrophages are among the first cells to accumulate in the vessel wall after hyperlipemic injury, and are likely to play a key role in the regression or progression of nascent atherosclerotic lesions. Sterol metabolism in these cells has been specialized and permits large fluxes of sterol because of the expression of cell surface receptors (including members of the scavenger receptor A and scavenger receptor B family) that can internalize significant amounts of cholesterol from lipoproteins that are retained and modified in the vessel wall (1–4). Because the progressive and uncontrolled accumulation of sterol by macrophages may lead to cell death and the release of potentially harmful factors in the vessel wall (5), pathways for sterol efflux from macrophages are also important in maintaining normal vessel wall homeostasis. Macrophages are relatively unique among vessel wall cells in that they express significant levels of apolipoprotein E (apoE), a specialization that enhances sterol efflux from these cells (6–8).

One of the scavenger receptors expressed by vessel wall macrophages is scavenger receptor class B type I (SR-BI) (9). Expression of this receptor in multiple cell types has been shown to stimulate the bi-directional movement of sterol between cells and extracellular acceptors (10–12). Depending on the cell model and conditions of study, SR-BI can mediate net efflux of sterol from cells or net sterol uptake into cells. Interestingly, in macrophages, under some experimental conditions SR-BI may oppose sterol efflux mediated through ATP-binding cassette transporter A1 (ABCA1) by facilitating the reuptake of cellular lipids released to exogenously added apoA-I (13). In the current series of experiments, we have investigated if and how apoE and SR-BI expression in macrophages interact to influence sterol efflux from these cells. The genes coding each of these proteins in macrophages are subject to transcriptional regulation (over a range of several-fold) by a number of previously defined (9, 14–16) and, likely, as-yet-undefined factors. We, therefore, took an approach that would allow us to directly evaluate the effect of these two proteins and their interaction on lipid efflux without potentially confounding changes in the level of gene tran-

Abbreviations: βCD, 2 hydroxy-propyl β cyclodextrin; PC, phosphatidylcholine; SR-BI, scavenger receptor class B type I.

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scription for each. This was accomplished by utilizing cells that were transfected to constitutively express these proteins, alone or in combination, in the J774 macrophage cell line. This macrophage cell line was selected because it has been well characterized with respect to sterol metabolism. The J774 macrophage line does not express its native apoE gene and expresses low basal levels of SR-BI. We also selected specific transfected lines that expressed increased apoE and SR-BI levels that were well within a physiologically attainable range.

MATERIALS AND METHODS

Materials

The pcDNA 3.1 expression vector containing the neomycin or zeocin resistance construct was obtained from Invitrogen. Purified apoA-I (from human HDL) and apoE (from human VLDL) were obtained from Calbiochem. Phosphatidylcholine (PC) used for the generation of PC vesicles (6) was obtained from Avanti Polar Lipids. The rabbit anti-mouse SR-BI polyclonal antiserum was purchased from Novus Biologicals (Littleton, CO). The goat-antihuman apoE polyclonal antiserum was purchased from International Immunology (Murrieta, CA). All other materials were from previously identified sources (6, 17, 18).

Cell lines

J774 cells obtained from American Type Culture Collection were transfected to stably express a full-length human apoE-3 cDNA in pcDNA 3.1 neo, or a full-length murine SR-BI cDNA in pcDNA 3.1 zeo, as previously described in detail (18). Control cells were generated by transfecting with resistance vectors only. Clones were selected in the presence of G418 (400 μg/ml) and zeocin (500 μg/ml). An apoE-expressing clone that secreted 1 μg of apoE per mg cell protein over 24 h was chosen for study, as this level of secretion approximates the level produced by human monocyte-derived macrophages and mouse peritoneal macrophages. This clone was then used to stably express the SR-BI cDNA, and a clone that expressed SR-BI at a 4-fold increase over basal level was chosen for study. This level of expression was chosen as it is within level of induction reported for this receptor in macrophages (9). The engineered cell lines utilized for these studies are designated as follows: J774C are parental cells that express no apoE and basal SR-BI level; J774E cells express apoE and basal SR-BI; J774E-SR-BI cells were derived from J774E cells and express SR-BI levels at 4-fold higher than basal; J774C-SR-BI cells were derived from J774C cells and express SR-BI at a level 3.8-fold higher than basal. Expression of resistance vectors alone (without apoE or SR-BI) had no effect on sterol or phospholipid efflux. Expression of SR-BI, apoE, or both at the physiologic levels used in these studies did not alter cell viability. All cell lines appeared morphologically identical, and equivalent mass of cell protein was harvested from wells after plating equivalent numbers of cells from each cell line. As a further index of cell health, we measured the synthesis and secretion of total proteins from cell lines during a 45 min pulse label with [^{35}S]methionine. Values were not different between the cell lines. For example, in J774E cells, incorporation of label into total cell protein was 358,567 ± 23,747 dpm and into total secreted protein was 11,247 ± 550 dpm. The respective values in J774E-SR-BI cells were 332,890 ± 18,053 dpm and 11,121 ± 563 dpm.

Immunoblot analysis

For immunoblot analysis, cell medium or lysates were harvested in a buffer containing protease inhibitors as previously described in detail (6). An equivalent mass of cellular protein (15 μg of protein for cell lysate samples) or an equal volume of cell medium was loaded on to 10% SDS polyacrylamide gels and separated by electrophoresis, followed by transfer to nitrocellulose membranes. The membranes were probed with polyclonal antisera to SR-BI or to apoE for an overnight incubation. Detection was accomplished using ECL Western blotting reagents (Amersham) according to the manufacturer's instructions. Signals were quantified using an UMAX scanner and Zero Dscan Software (Scanalytics Inc., Fairfax, VA). The immunoblot results shown are representative of 3 or 4 similar experiments with similar results.

Lipid analyses

Cholesterol and phospholipid mass were measured as previously described (6). For the isotopic efflux assay, cells were labeled with ^{3}[H]cholesterol (1 μCi/ml) and/or ^{14}[C]choline (1 μCi/ml) for 48 h in DMEM plus 10% FCS, followed by equilibration as previously described in detail (6). The efflux assay was initiated by the addition of "control medium" (DMEM plus 0.1% BSA) alone or with the additions noted in the figure legends. Efflux is expressed as μg lipid/mg cell protein, calculated by dividing the lipid counts present in the medium by its cellular specific activity. Cholesterol and phospholipid mass measured at the start of the efflux incubation showed no significant differences between cell lines. For example, representative total sterol values after the 48 h incubation in 10% FCS were as follows: J774C, 22.2 ± 2.0; J774C-SR-BI, 24.3 ± 3.1; J774E, 24.1 ± 2.1; J774E-SR-BI, 26.9 ± 2.8 μg/mg cell protein.

Immunoprecipitation of medium and cellular apoE

ApoE in cell lysates and in media was isolated by immunoprecipitation with goat apoE antiserum as previously described in detail (17). Equal numbers of total trichloroacetic acid precipitable counts were taken for each time point in a pulse-chase experimental format to start the immunoprecipitation so changes in apoE are already corrected for changes in total protein synthesis and secretion. The samples were first cleared by incubating for 2 h at 4°C with 25 μl of non-immune goat serum, and then with 60 μl of r-protein G agarose beads for another 2 h. After centrifugation, the supernatant was incubated with 18 μl of apoE antisera and then with 60 μl of additional protein G agarose. After washing the protein G agarose beads with IP buffer (10 mM Na_2HPO_4, 15 mM NaCl, 10 mM methionine, 1% Triton X100), apoE was released by boiling in a dissociation buffer containing 5% SDS, 0.12 M Tris-HCl (pH = 6.8), and β-mercaptoethanol (40 μl/ml) for 15 min and then resolved by SDS-PAGE. The labeled apoE band was detected at its appropriate molecular weight using a Molecular Dynamics Phosphorimager. The signal in labeled apoE was quantitated using ImageQuant software. We have previously shown that this immunoisolation procedure allows for highly specific and quantitative recovery of biosynthetically labeled apoE (17, 19).

Biotinylation of cell surface apoE and SR-BI

Cell surface proteins were biotinylated using the water soluble biotinylation reagent ss-biotin, following a previously published procedure with minor modifications (17). Cells were rapidly cooled on ice for 5 min and washed with PBS at 4°C. The cells were then incubated with freshly prepared sulfo-NHS-ss-biotin/PBS solution (1 mg/ml) at 4°C for 45 min. After washing twice with PBS at 4°C, the cells were harvested for analysis. Cellular apoE was immunoprecipitated as described above with the following modifications. After washing the protein G agarose beads three times with IP buffer containing 10 mM iodoacetimide, 100 μl HEPES buffered saline containing 1% SDS, and 1 mM phenyl-

methylsulfonyl fluoride is added to the tubes and heated for 3 min at 90°C to release apoE-IgG complexes from the protein G agarose beads. Thereafter, 900 μl IP buffer containing 10 mM iodoacetimide is added to the tubes which are mixed and centrifuged to obtain the supernatant. Nine hundred μl of the supernatant is transferred to tubes that contain 100 μl of streptavidin agarose beads that have been washed twice with IP buffer and have been pelleted to the bottom. The samples are incubated for 1 h with rotation at 4°C. After centrifugation, the supernatant is collected for determination of unbiotinylated (intracellular fraction) apoE. The streptavidin agarose beads are then washed three times with IP buffer, and then a buffer containing 62.5 mM Tris-HCl (pH 6.8), 20% glycerol, 2% SDS, 5% β-mercaptoethanol, and 50 mM dithiothreitol was used to release biotinylated (cell surface fraction) apoE from the beads. The percentage of apoE on the cell surface was calculated by dividing cell surface apoE by intracellular plus cell surface apoE. Biotinylation of cell surface SR-BI was performed substituting an SR-BI polyclonal antiserum in the protocol described above.

Other methods

Cell protein was measured using a DC Protein assay kit (Bio-Rad, Hercules, CA). Cholesterol oxidase treatment of unfixed cells was performed as described (20). Statistical differences were analyzed for significance by ANOVA using SPSS software (SPSS Inc., Chicago, IL).

RESULTS

Expression of SR-BI reduces apoE-dependent efflux from macrophages

Utilizing the SR-BI cDNA in our transfection protocols increased the expression of an 82 kDa protein (identical in size to the native SR-BI protein) recognized by the SR-BI polyclonal antiserum. Biotinylation of cell surface proteins confirmed increased expression of SR-BI protein at the plasma membrane. Clones expressing SR-BI at high physiologic levels (3- to 4-fold above baseline) were selected for these studies. It has been reported that increased SR-BI expression produces a reorganization of plasma membrane free cholesterol that can be monitored by an increased accessibility to exogenous cholesterol oxidase (20). J774 cells expressing increased levels of SR-BI also showed increased accessibility of plasma membrane cholesterol to exogenous cholesterol oxidase (2-fold increase, $P < 0.01$). We have

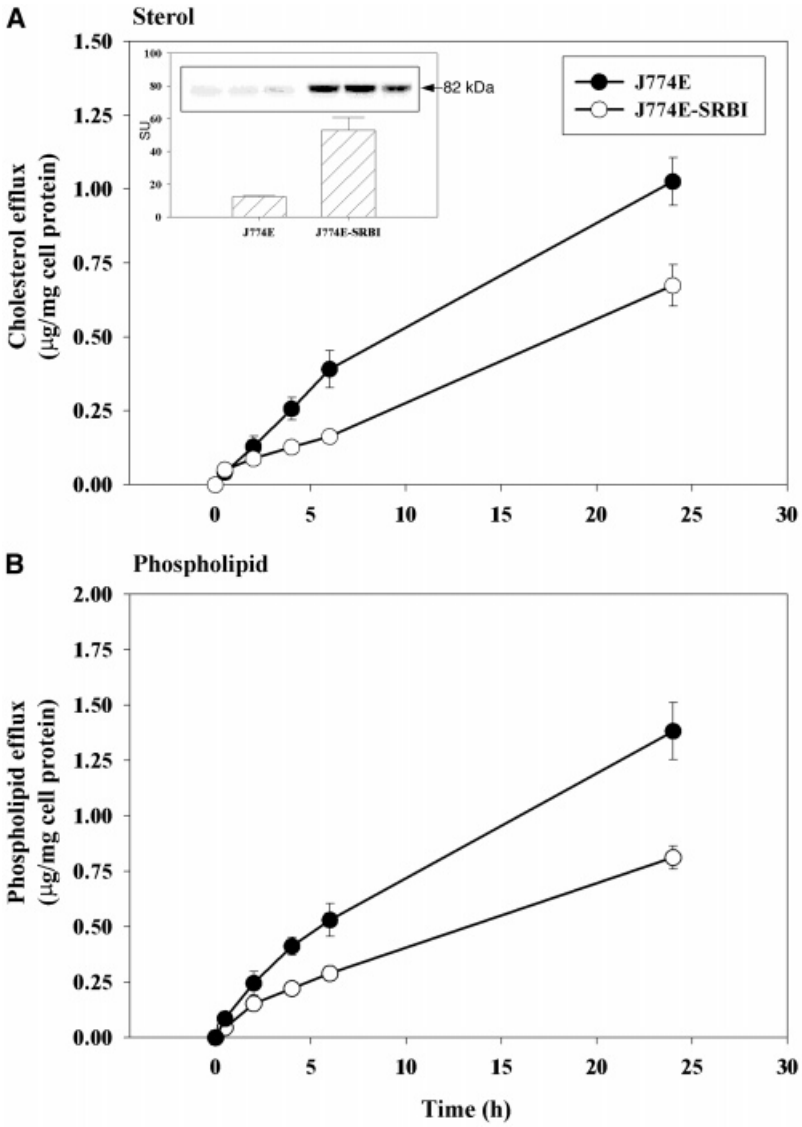

Fig. 1. Cholesterol and phospholipid efflux from J774E and J774E-SR-BI cells. Sterol and phospholipid efflux were measured over 24 h in 0.1% BSA/DMEM (control medium) as described in Materials and Methods, from J774E cells or J774E cells with increased expression of scavenger receptor class B type I (SR-BI) (J774E-SR-BI). Values shown are the mean ± SD from triplicate wells. Where SD bars are not visible, they are contained within the symbol. The differences in efflux between J774E and J774-SR-BI cells are significant at $P < 0.05$ for all time points past 2 h for sterol and phospholipid. The insert shows the results of an immunoblot analysis of SR-BI level in J774E and J774E-SR-BI cells. The mean ± SD from triplicate samples are shown, and the difference in SR-BI expression level is significant at $P < 0.05$. SU = scanning units.

TABLE 1. Mass of secreted sterol and phospholipid from J774E and J774E-SR-BI cells

	Medium Cholesterol	Medium Phospholipid
	μg/mg protein	
J774E	0.347 ± 0.053	0.333 ± 0.114
J774E-SR-BI	0.200 ± 0.039	0.079 ± 0.033

Cells in 75 cm^2 flasks were washed twice with DMEM and incubated in 0.1% BSA in DMEM. After 24 h, the media were collected for measurement of cholesterol and phospholipid mass. Values shown are the mean ± SD from triplicate flasks. J774E, apolipoprotein E (apoE) expressing cells; J774E-SR-BI, J774E cells with increased SR-BI expression. $P < 0.05$ for J774E versus J774E-SR-BI cells for both medium cholesterol and medium phospholipid mass.

previously shown that expression of physiologic levels of apoE leads to enhanced sterol and phospholipid efflux from J774 macrophages (6, 18). In the first series of experiments, we utilized an apoE-expressing clone (J774E) to express SR-BI at high physiologic levels (J774E-SR-BI) in order to examine the effect on sterol and phospholipid efflux. **Figure 1** shows the result of a representative experiment. Unexpectedly, we found that SR-BI expression reduced both sterol and phospholipid efflux from apoE-expressing cells (Fig. 1). The decrease due to SR-BI expression was evident as early as 4 h into the efflux time course analysis. We next confirmed this result by directly measuring the mass of sterol and phospholipid released from J774E and the J774E-SR-BI cells during a 24 h incubation in serum-free medium. The result of a representative experiment is shown in **Table 1**. The mass results shown in Table 1 confirm that SR-BI expression reduces both phospholipid and sterol release from apoE-expressing cells.

In order to determine if SR-BI expression was specifically reducing apoE-dependent efflux, we next evaluated the effect of increased SR-BI expression on sterol efflux from J774 cells that do not express apoE (J774C vs. J774C-SR-BI cells). This experiment was performed in the absence and presence of a group of structurally diverse

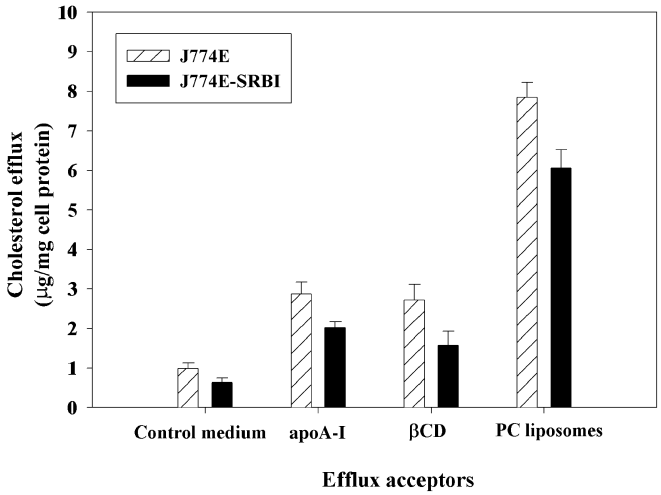

Fig. 3. Sterol efflux from J774E and J774E-SR-BI cells. The incubations and analyses described in the legend to Fig. 2 were performed using J774E cells and J774E cells with increased SR-BI (J774E-SR-BI). The differences between J774E and J774E-SR-BI cells are significant at $P < 0.05$ in control medium, apoA-I, and βCD; and at $P < 0.01$ in PC liposomes.

extracellular sterol acceptors, and is shown in **Fig. 2**. Expression of SR-BI in macrophages that do not express apoE increased sterol efflux under all incubation conditions examined. The increased efflux to a heterogeneous group of extracellular lipid-free acceptors is similar to what has been reported in other cell types as a result of increased SR-BI expression, and lends support to the notion that SR-BI expression facilitates the reorganization of cellular membranes to facilitate flux of cellular sterol (20–22). We next performed this same experiment in apoE-expressing cells (J774E vs. J774E-SR-BI cells). Consistent with the data in Fig. 1, expression of SR-BI in apoE-expressing cells reduced efflux in control medium. SR-BI expression also reduced sterol efflux from apoE-expressing cells in the presence of each of the sterol acceptors examined (**Fig. 3**).

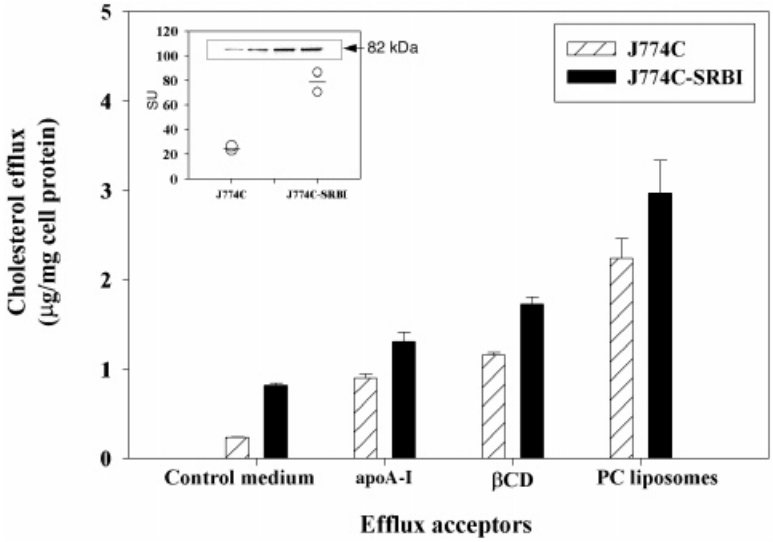

Fig. 2. Sterol efflux from J774C and J774C-SR-BI cells. Efflux of radiolabeled sterol during a 24 h incubation was measured from J774C cells (expressing no apoE and basal SR-BI level) and J774C cells with increased SR-BI expression (J774C-SR-BI). Incubations were carried out in control medium alone, or this medium plus lipid-free apolipoprotein A-I (apoA-I) (10 μg/ml); hydroxy-propyl β cyclodextrin (βCD) (2 mM); or PC liposomes (1 mg/ml). Values shown are the mean ± SD from triplicate wells. The differences between J774C and J774C-SR-BI cells are significant at $P < 0.001$ in control medium, $P < 0.01$ in βCD, and $P < 0.05$ in phosphatidylcholine (PC) liposomes and apoA-I. The insert shows the results of a representative immunoblot analysis of SR-BI level in J774C and J774C-SR-BI cells. Individual results from duplicate samples are shown (open circles) and the mean of the duplicates is shown by the horizontal line. SU = scanning units.

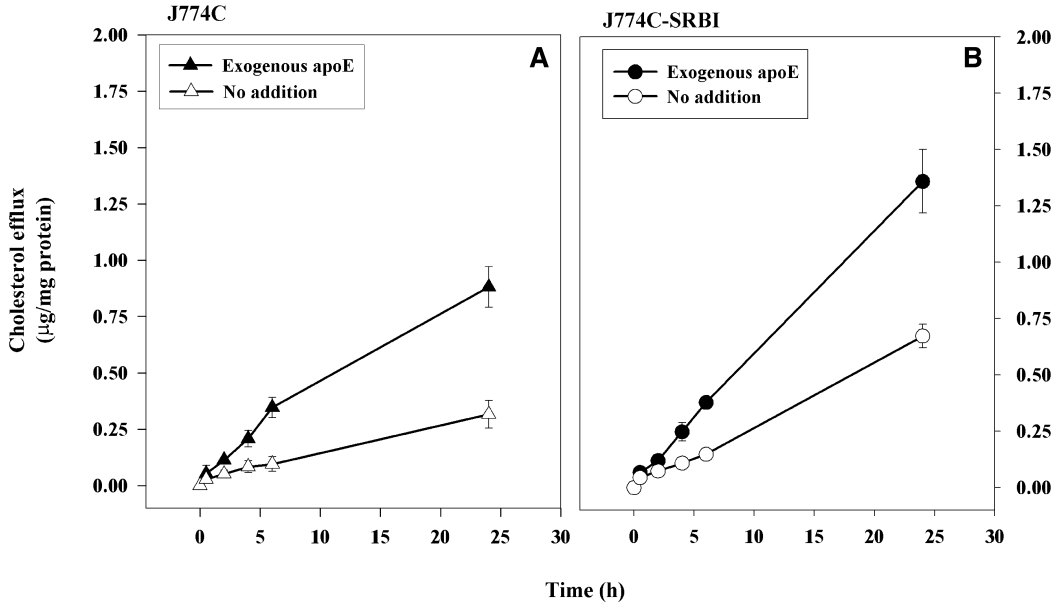

Fig. 4. Effect of exogenous lipid-free apoE on sterol efflux from J774C and J774C-SR-BI cells. Efflux of radiolabeled sterol was measured as described in Materials and Methods, in the presence or absence of 10 μg/ml lipid-free apoE (isolated from human VLDL). A: Efflux is shown for J774C cells. B: Efflux from J774C cells with increased expression of SR-BI is shown. The values shown are the mean ± SD from triplicate wells. Where SD bars are not visible, they are contained within the symbol.

Direct comparison of sterol efflux from J774C and J774E-SR-BI showed higher efflux from the latter (not shown). Therefore, even though SR-BI expression reduced apoE-dependent efflux, efflux remained higher compared to cells with basal levels of SR-BI that do not express apoE.

The results of the above experiments indicated that expression of SR-BI specifically reduced apoE-dependent sterol efflux from macrophages. To further investigate the mechanism for this, we examined whether the same reduction would be observed in the presence of exogenously added apoE. The results of a representative experiment to address this question are shown in **Fig. 4**. For this experiment, we utilized J774C cells and J774C-SR-BI as recipient cells. In Figure 4A, we added exogenous apoE (isolated from human VLDL) to J774C cells. This addition increased sterol efflux as we have previously reported in this cell model (6). The same addition, done in J774C-SR-BI cells, also increased sterol efflux (Fig. 4B). Therefore, increased expression of SR-BI did not attenuate the increased sterol efflux stimulated by the addition of exogenous lipid-free apoE. A similar result was observed when medium collected from apoE-expressing cells was used as the source of exogenous apoE (not shown). The results shown in Fig. 4 do not support a mechanism in which SR-BI expression reduces apoE-dependent efflux by interacting with extracellular apoE to enhance the reuptake of secreted cellular sterol. If this mechanism were operative, SR-BI expression would have also reduced the efflux resulting from the addition of exogenous apoE.

SR-BI reduces macrophage apoE level

In further considering potential mechanisms for SR-BI reduction of endogenous apoE-dependent efflux, we considered the possibility that cellular production of apoE could be altered by SR-BI expression level. **Figure 5** shows the result of an immunoblot analysis of apoE in the medium of apoE-expressing cells with and without increased expression of SR-BI. Increasing SR-BI expression reduced the amount of apoE present in the medium by almost 50% at each time point examined. In order to further probe the mechanism for this reduction, a pulse-chase

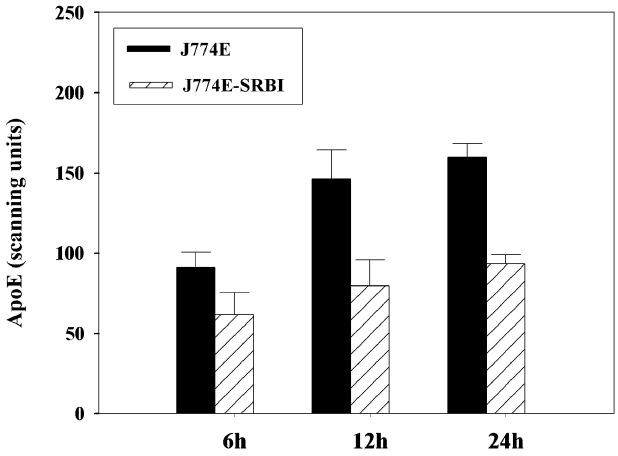

Fig. 5. Immunoblot analysis of secreted apoE level in J774E and J774E-SR-BI cells. Medium (0.1% BSA in DMEM) was collected from J774E (apoE-expressing) cells, or J774E cells with increased expression of SR-BI (J774E-SR-BI) at the times shown. Immunoblot analyses were performed for apoE as described in Materials and Methods. The values shown are the mean ± SD from triplicate wells. Differences are significant at $P < 0.05$ at 6 h, $P < 0.01$ at 12 h, and $P < 0.001$ at 24 h.

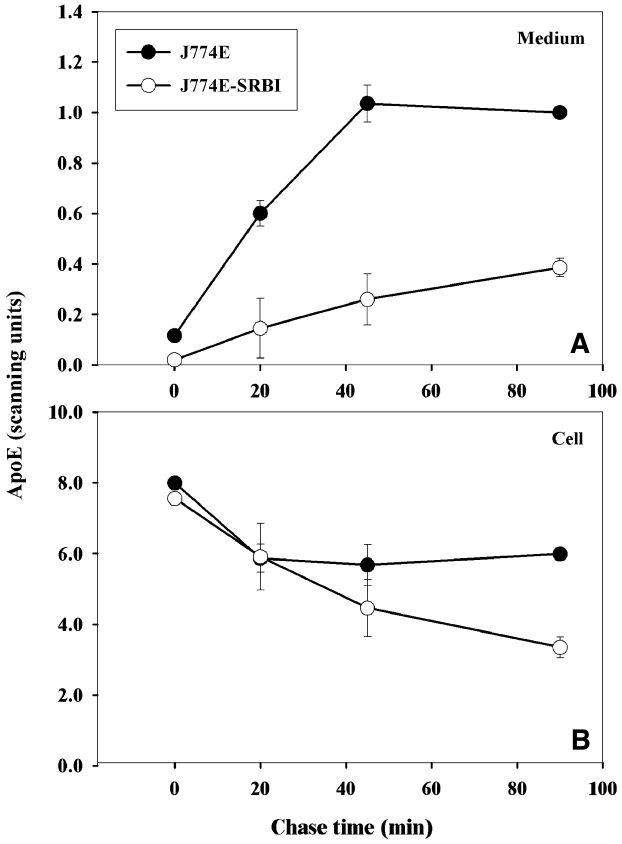

Fig. 6. Pulse-chase analysis of secreted and cellular apoE in J774E and J774E-SR-BI cells. J774E (apoE-expressing cells), or J774E cells with increased expression of SR-BI (J774E-SR-BI), were pulse-labeled with 100 μCi/ml [^{35}S]methionine for 30 min. At that time chase incubations were started, and cell culture medium and cell lysates were collected at the chase times indicated in the Figure. Cells and medium were utilized for quantitative immunoprecipitation of apoE as described in Materials and Methods. Values shown are the mean ± SD from triplicate wells.

analysis of apoE in cells and medium was performed. The results of a representative experiment are shown in **Fig. 6**. In J774E cells, apoE rapidly accumulated in the medium immediately after the start of the chase incubation (Fig. 6A), but the rapid rise of apoE in the medium was substantially attenuated by the increased expression of SR-BI. These kinetics for apoE accumulation in the medium indicate reduced apoE release from cells as a result of increased SR-BI expression. The apoE cellular decay curves for the pulse-chase analysis are shown in Fig. 6B. During the first 20 min of chase, while medium apoE is more rapidly accumulating from J774E cells than J774E-SR-BI cells, the rates of decay for cellular apoE are similar in the two cell types. This indicates that apoE not released into the medium from J774E-SR-BI cells is not retained in the cell and is, therefore, rapidly degraded. The enhanced degradation of apoE, resulting from increased SR-BI expression, is also supported by the observation that at the 90 min final chase time, there is significantly less apoE in the medium, and in the cells, as a result of SR-BI expression.

We have previously shown that a significant fraction of cellular apoE in macrophages is present on the cell surface (17, 23), and that this cell surface pool serves as the immediate precursor for secreted apoE (17). We have also shown that apoE in this cell surface pool plays a major role in facilitating the sterol efflux produced by the endogenous expression of apoE in macrophages (24). The cellular apoE measured in Fig. 6B included both intracellular and cell surface fractions. Based on the above results, that increased SR-BI expression reduced apoE secretion and reduced endogenous apoE-dependent sterol efflux, we predicted that less apoE would be present in the cell surface fraction as a result of SR-BI expression. To test this hypothesis, we directly measured cell surface apoE utilizing a cell surface biotinylation technique that we have previously validated for this purpose (17). In three separate experiments, increased SR-BI expression reduced the cell surface fraction of apoE. In the representative experiment shown in **Fig. 7**, the reduction in the cell surface fraction of apoE averaged 40% and was significant at the $P < 0.0005$ level.

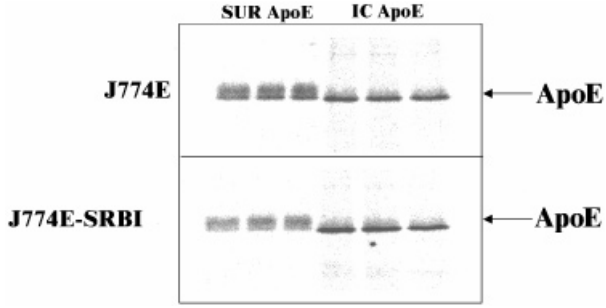

Fig. 7. Cell surface and intracellular fraction of apoE in J774E and J774E-SR-BI cells. J774E (apoE-expressing cells), and J774E cells with increased SR-BI expression (J774E-SR-BI), were labeled with [^{35}S]methionine for 18 h. At that time, cell surface apoE was biotinylated, and biotinylated apoE was separated from intracellular apoE as described in Materials and Methods. The results from a representative experiment with triplicate wells are shown. SUR = surface; IC = intracellular.

DISCUSSION

Further understanding of sterol efflux pathways in macrophages is important for understanding the role of these cells in preserving vessel wall homeostasis. Macrophages express a number of proteins shown to function in sterol efflux pathways, including ABCA1, SR-BI, and apoE (6–8, 10–13, 25, 26). It is important to investigate potential interactions of these pathways for modulating net sterol flux from macrophages. It might be reasonable to expect that these interactions could be complex and may occur at multiple levels. In this report, we have demonstrated an important post-transcriptional interaction between SR-BI expression and apoE expression in modulating sterol efflux from macrophages. Increasing expression of SR-BI, within its physiologic range in macrophages, increases sterol efflux from these cells when they do not express apoE (Fig. 2). This increase is observed in a number of structurally di-

verse extracellular acceptors, including apoA-I, hydroxypropyl β cyclodextrin (βCD), and PC liposomes. However, a similar increase in SR-BI level reduces efflux from macrophages that express endogenous apoE (Figs. 1 and 3). Importantly, this reduction remains intact in the presence of a number of extracellular sterol acceptors, including those that function through ABCA1 (apoA-I) and those that function independent of ABCA1 (βCD and PC iposomes) (25–27). In addition, the reduction in apoE-mediated efflux is specific for the endogenous expression of apoE. Increasing SR-BI level enhances efflux to exogenously added apoE (Fig. 4). The increased efflux to exogenous apoE after enhanced SR-BI expression provides useful mechanistic information. It argues against SR-BI-mediated reuptake of extracellular apoE-lipid complexes as a mechanism for reduced sterol efflux from J774E-SR-BI cells.

We investigated potential SR-BI-mediated changes in macrophage apoE as a mechanism for reduced efflux. These experiments led to the surprising finding that increased SR-BI expression decreases macrophage apoE, as detected by an approximate 50% decrease in secreted apoE (Fig. 5). Pulse-chase analyses suggested that the synthesis of apoE was not substantially changed by SR-BI expression; i.e., there were virtually identical amounts of newly synthesized apoE in cells and medium immediately after the 30 min pulse in J774E and J774E-SR-BI cells (see Fig. 6). However, the increased expression of SR-BI appeared to accelerate the degradation of newly synthesized apoE and, thereby, reduce the amount of apoE secreted from cells. We have previously shown that apoE in a macrophage cell surface pool is important for facilitating the sterol and phospholipid efflux produced by the endogenous expression of apoE (24). We, therefore, believe that the most likely mechanism for the reduced sterol efflux after increased SR-BI expression in apoE-expressing cells is the increased degradation of newly synthesized apoE leading to a reduced cell surface pool of apoE (Fig. 7).

Several mechanisms can be considered for the acceleration of endogenous apoE degradation by SR-BI expression. SR-BI could interact with the nascent apoE lipid-particle, either within the cell or on the cell surface, to prevent its secretion and enhance its degradation. Such an interaction could occur between SR-BI and apoE, or between SR-BI and the lipid associated with the apoE-lipid particle in macrophages. Alternatively, a direct interaction between the apoE-lipid particle and SR-BI may not be required. We have previously shown that a significant percentage of the newly synthesized apoE in macrophages can be degraded prior to secretion (28, 29). We have also shown that the amount of nascent apoE degraded can be modulated by the lipid composition of cellular membranes. SR-BI expression has been shown to alter the disposition and organization of lipid within cellular membranes (20–22). There is the intriguing possibility, therefore, that SR-BI-mediated changes in the disposition of cellular lipid could modulate the degradation rate of apoE in macrophages. As noted above, multiple interactions are possible between pathways modulating sterol balance in macrophages. Recently, an interaction has been noted in the expression of ABCA1 and apoE in macrophages (30). We have also noted an important interaction between the level of apoE and ABCA1 expression in the J774 macrophage model (31). The complex and, perhaps, redundant pathways available to defend macrophage sterol homeostasis underscore the importance of these cells for defending the vessel wall against lipoprotein-derived sterol deposition.

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