Scavenger Receptor BI (SR-BI) Promotes High Density Lipoprotein-mediated Cellular Cholesterol Efflux*

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Scavenger receptor BI (SR-BI) binds high density lipoproteins (HDL) with high affinity and mediates the selective uptake of HDL choleseryl ester. We examined the potential role of SR-BI in mediating cellular cholesterol efflux. In Chinese hamster ovary cells stably transfected with murine SR-BI, overexpression of SR-BI resulted in a 3–4-fold stimulation of initial cholesterol efflux rates. Efflux rates correlated with SR-BI expression in cells and HDL concentration in the medium. When incubated with synthetic cholesterol-free HDL, SR-BI-transfected cells showed ~3-fold increases in initial rates of efflux compared with control cells, indicating that SR-BI expression enhances net cholesterol efflux mediated by discoidal HDL. In six different cell types, including cultured macrophages, the rate of efflux of cholesterol mediated by HDL or serum was well correlated with cellular SR-BI expression level. In addition, in situ hybridization experiments revealed that SR-BI mRNA was expressed in the thickened intima of atheromatous aorta of apolipoprotein E knockout mice. Thus, SR-BI is an authentic HDL receptor mediating cellular cholesterol efflux. SR-BI may facilitate the initial steps of HDL-mediated cholesterol efflux in the arterial wall as well as later steps of reverse cholesterol transport involving uptake of HDL cholesterol in the liver.

The levels of plasma high density lipoproteins (HDL) are inversely related to the incidence of atherosclerosis and coronary artery disease (1, 2). The protective effect of HDL is thought to involve the reverse transport of cholesterol from cells in the arterial wall to the liver for disposal (3, 4). The transfer of cholesterol from cells to HDL may result from aqueous diffusion (5, 6) and/or the interaction between a cell surface receptor and HDL (7). A number of HDL-binding proteins have been described (5, 7) but none has been shown to be an authentic HDL receptor mediating cholesterol efflux. Recently a member of the scavenger receptor family, scavenger receptor type B class I (SR-BI), was shown to bind HDL with high affinity and to mediate the selective cellular uptake of HDL choleseryl ester (CE) (8). SR-BI is highly expressed in steroidogenic tissues and the liver (8–11), and in vivo evidence suggests that SR-BI expression is under feedback regulation (10). While these results show that SR-BI is an HDL receptor that is likely to provide sterol for steroidogenesis, the exact role of SR-BI in the regulation of HDL metabolism and the maintenance of general cholesterol homeostasis is unknown.

In the present study we used SR-BI-transfected cells to evaluate a possible role of SR-BI in HDL-mediated cellular cholesterol efflux. We also sought to establish a relationship between cholesterol efflux and the level of SR-BI expression in a variety of cell types. The results, together with our finding that SR-BI mRNA is expressed in the thickened intima of atheromatous aorta, suggest a potentially important role of SR-BI in the initial steps of cholesterol efflux in the arterial wall.

EXPERIMENTAL PROCEDURES

Transfection of SR-BI and Selection of SR-BI-overexpressing Cells—The murine SR-BI cDNA (10) was subcloned into a mammalian expression vector pRS/CMV (Invitrogen) and transfected into Chinese hamster ovary (CHO) cells by electroporation. Stable transformants were selected with 0.8 mg/ml and maintained with 0.3 mg/ml of G418 (Life Technologies, Inc.), respectively, in Ham's F-12 medium containing penicillin (100 units/ml), streptomycin (100 μg/ml), and 2 mM glutamine (medium A) supplemented with 5% fetal bovine serum. This procedure yielded clones with moderate SR-BI overexpression (“low expression”). To obtain high expressing cells, the original G418-resistant pool was further screened with fluorescence-activated cell sorting using HDL labeled with 1,1'-di-octadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (Molecular Probes) (12). Cells thus obtained (“high expression”) were used in all experiments except for phosphatidylcholine (PC) and sphingomyelin (SM) uptake experiments. Y1-B51 cells were kindly provided by Dr. David L. Williams (State University of New York).

Hybridization of HLd and Reconstituted HDL—Human plasma HDL was prepared by sequential ultracentrifugation. Cholesterol-free reconstituted HDL was prepared with the sodium cholate method from purified human apolipoprotein (apo) A-I and egg yolk PC (13). The initial molar ratio of PC:apoA-I:sodium cholate was 80:1:80, and sodium cholate was removed by exhaustive dialysis.

Cholesterol Efflux and Lipid Uptake—Cell monolayers were labeled with either [7,7'-dihydrocholesterol (11,12)-]H]cholesterol or [4-14C]FC and [cholesteryl-1,2,6,7-3H]cholesterol oleate (19) or t-1-1-palmitoyl-2-oleoyl-[oleoyl-1-14C]PC and [N-palmitoyl-9,10-3H]SM (prepared from [9,10-3H]palmitic acid) (20). After incubation with labeled HDL, cells...
were washed with medium A containing 0.5% fatty acid-free bovine serum albumin, and cell-associated radioactivity was measured.

**Immunoblot Analysis and RNase Protection Assay**—Cells were homogenized in the presence of protease inhibitors (0.5 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin A, 0.2 mM phenylmethylsulfonyl fluoride, and 1 mM EDTA). Cell membrane protein was collected by ultracentrifugation and separated by SDS-polyacrylamide gel electrophoresis. Immunoblotting was performed using anti-SR-BI antiserum (10) and chemiluminescence detection (Amersham Corp.). RNase protection analysis was carried out as described previously (10). The expression levels of SR-BI mRNA and protein were determined by densitometric quantitation of radiographic and chemiluminescence images, respectively.

**In Situ Hybridization and Immunohistochemistry**—The cRNA probes for in situ hybridization were produced by in vitro transcription from a 290-base pair murine SR-BI cDNA encoding amino acids 315–412 and labeled with digoxigenin 11-UTP (Boehringer Mannheim). Nonisotopic in situ hybridization and hematoxylin counterstaining were performed as described previously (21). The frozen tissue sections were probed with sense or antisense riboprobe at 10 ng/ml. Anti-digoxigenin antibody and alkaline phosphatase-conjugated secondary antibody were used to detect the hybridized riboprobes. A monoclonal antibody against murine MAC-3 (PharMingen) was biotinylated and used for immunohistochemistry (21).

**RESULTS**

To evaluate a potential role of SR-BI in HDL-dependent cholesterol efflux, we stably transfected CHO cells with the murine SR-BI cDNA (10). Compared with control CHO cells, incubation of SR-BI-expressing cells with HDL resulted in a marked 3–4-fold stimulation of initial cholesterol efflux rate; this effect was completely dependent on the presence of HDL in the medium (Fig. 1A). Over a range of HDL concentrations from 75 to 300 μg/ml, SR-BI expression resulted in a 4–5-fold stimulation of the initial rate of HDL-mediated cholesterol efflux from cells (Fig. 1B). The cellular cholesterol efflux mediated by HDL correlated with the expression level of SR-BI in cell membranes (Fig. 1C). CHO cells with a relatively low level of SR-BI overexpression displayed a 1.5-fold increase in cholesterol efflux, while CHO cells with high expression of SR-BI exhibited a 3-fold increase in cholesterol efflux. Western blots of cellular membranes showed that SR-BI was present in control CHO cells; low expression transfected cells had ~2-fold higher expression than control while high expression cells had ~10-fold higher expression (Fig. 2).

Further characterization of SR-BI overexpressing cells revealed an increase in the initial rate of cellular uptake of HDL FC (~3-fold) as well as CE (~6-fold) (Fig. 3A). Using HDL containing radiolabeled PC and SM, we observed that SR-BI overexpression resulted in ~2–3-fold stimulation of initial lipid cellular uptake, respectively. Together the data in Figs. 1 and 3A indicate that SR-BI stimulates the bi-directional exchange of FC between spherical plasma HDL and cells. To determine if SR-BI is capable of mediating net cholesterol efflux from cells, we used synthetic cholesterol-free HDL (13) as a cholesterol acceptor in the medium (14, 22). These discoidal particles resemble nascent HDL formed in the blood stream as a by-product of lipolysis, remodeling, or secretion (3, 4, 23). When incubated with discoidal HDL, SR-BI-transfected cells exhibited an ~3-fold increase in initial rates of cholesterol efflux compared with control cells (Fig. 3B). Since in this case cholesterol-free acceptor was added to the medium, this experiment indicated a marked stimulation of net cholesterol efflux as a result of HDL-SR-BI interaction. In contrast to these findings, SR-BI-expressing CHO cells showed no increase in efflux of cellular cholesterol mediated by free apoA-I (over a range of 2–8 μg/ml) (24–26), suggesting that SR-BI is not responsible for the efflux of cellular cholesterol mediated by free apolipoproteins (7).

Even though SR-BI stimulated cellular uptake of both HDL-free and esterified cholesterol, efflux of cellular cholesterol was limited to free sterol. Thus, in an experiment similar to Fig. 1A, almost all (~99%) of the cholesterol transported to the medium was found to be in the unesterified form even though ~65% of cellular [3H]cholesterol was found to be esterified. When the cellular acyl-CoA:cholesterol acyltransferase activity was abolished by Sandoz 58035 during cholesterol labeling, similar effects of SR-BI on the HDL-mediated [3H]cholesterol efflux to that shown in Fig. 1A were subsequently observed (data not shown). These data indicate that SR-BI stimulates the efflux of FC but not CE from cells. Thus, cellular CE stores appear to be in a compartment not directly accessible to SR-BI.

To explore the physiological significance of the SR-BI-medi-
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Fig. 3. Effect of SR-BI on bi-directional cholesterol exchange between HDL and cells. Values are means ± S.D., n = 3. A, SR-BI mediated uptake of HDL FC and CE. Cells were incubated with HDL (2.2 µg of CE/ml) labeled with [14C]FC and [3H]cholesteryl oleate, and cell-associated radioactivity was measured. B, SR-BI-mediated cholesterol efflux as stimulated by reconstituted HDL. The cholesterol-free discoidal HDL was prepared with the sodium cholate method from egg PC and human apoA-I (80:1, mol/mol). Cells were incubated with HDL (50 µg/ml of HDL protein) for the indicated time. Net cholesterol mass efflux was calculated from the radioactivity released to the culture medium using the initial cholesterol specific activity.

ated cholesterol efflux, we examined the expression level of SR-BI in a variety of murine cell types and then correlated expression levels with the rate of HDL-mediated cholesterol efflux. SR-BI expression varied greatly from well above that of murine liver (Fu5AH cells) to almost undetectable (Y1 cells) (Fig. 2). SR-BI was expressed in several types of macrophages as well as control CHO cells (Fig. 2). The abundance of SR-BI mRNA in these cells was determined by RNAse protection assay and was found to be highly correlated with protein expression levels (r = 0.99, p = 0.0001) except for Fu5AH cells, which had disproportionately high protein expression.

The ability of HDL and serum to stimulate cholesterol efflux in different types of cells was correlated with their expression level of SR-BI (Fig. 4). The rate of efflux to HDL was determined at three different concentrations for each of the cell types shown. The rate of efflux per microgram of HDL/ml was well correlated with the expression level of SR-BI in cellular membranes (A, note that the x axis is a log scale). The rate constant for efflux to 5% human serum was also correlated with SR-BI expression level (B). In contrast, there was no correlation between SR-BI expression and efflux to cyclodextrins, synthetic cage-like molecules that markedly stimulate cholesterol efflux (17, 27) (not shown). The response of J774 macrophages and control CHO cells was in the intermediate region but appeared to be greater than cells with very low SR-BI expression (Fig. 4).

The CE laden foam cells in atheromatous lesions arise from macrophages or smooth muscle cells (28). To evaluate possible expression of SR-BI in atheroma, in situ hybridization experiments were carried out on complex atheromatous lesions from apoE knockout mice (29, 30) (Fig. 5A, hematoxylin stain). This revealed a strong signal with an antisense probe (Fig. 5C) not seen with the sense strand control (Fig. 5B). The signal was present throughout the foam cell-containing region of thickened intima (I, Fig. 5C) and was strong in the cellular layer immediately beneath the endothelial surface of the vessel; however, the signal was weak in the arterial media (M, Fig. 5C). A monoclonal antibody against MAC-3, a macrophage surface marker, gave a signal in the same region as the SR-BI mRNA signal (not shown). These observations demonstrate that SR-BI mRNA is expressed in atheromatous lesions, probably in macrophages foam cells.

DISCUSSION

In this study we showed that SR-BI overexpression markedly stimulated HDL-mediated cholesterol efflux in CHO cells. This appears to be the first demonstration of an authentic HDL receptor mediating cellular cholesterol efflux. The good correlation of the rate of HDL-mediated efflux with SR-BI expression levels in six different cell types suggests a physiological role of SR-BI in mediating this process in a variety of cell types. Expression of SR-BI in the atheromatous lesions raises the possibility that HDL uses this receptor to promote cholesterol efflux from atheromatous arterial walls.

Our results suggest that the HDL receptor, SR-BI, may play a critical role in reverse cholesterol transport, a pathway by which cholesterol is transported from peripheral cells to the liver (31). Numerous in vitro studies have demonstrated that HDL can remove cholesterol from cells through either a process of aqueous diffusion (5, 6) or as a result of the interaction of HDL with cell surface binding sites (7), depending on the cell...
types and other experimental conditions. Although a number of HDL-binding proteins have been described (5, 7), their physiological role in reverse cholesterol transport is doubtful since none has been shown to be a functional HDL receptor that facilitates cholesterol efflux. Our results show that cellular cholesterol efflux is greatly enhanced by SR-BI overexpression and well correlated with SR-BI expression levels in different cells. This strongly suggests that SR-BI may have a physiological role in cellular cholesterol efflux, although our studies by no means preclude the probable role of other receptors or processes in HDL-mediated cellular cholesterol efflux. SR-BI has been shown to mediate the selective uptake of cholesteryl ester from HDL without taking up or degrading HDL protein (8). This is important in steroidogenic tissues and liver, where SR-BI is highly expressed (8–11). Our studies indicate that SR-BI also promotes uptake of HDL FC and CE-poor pre-β HDL (similar to the discoidal HDL in Fig. 3B) and other forms of phospholipid-rich HDL are the preferred acceptors of cellular cholesterol (5). Our data suggest that such particles may use SR-BI as a receptor to mediate cholesterol efflux; the expression of SR-BI mRNA in atheromatous lesions of aorta suggests that optimal HDL particles may also mediate cholesterol efflux via SR-BI in the arterial wall. Although our SR-BI antibodies were found to be unsuitable for immunohistochemistry, the high correlation between cellular SR-BI mRNA and protein suggest that SR-BI protein would also be expressed in atheroma. Adenovirus-mediated overexpression of hepatic SR-BI leads to decreased HDL and increased biliary cholesterol (32), providing strong support for the notion that SR-BI may play a physiological role in promoting the transport of HDL FC and CE across the liver. Thus, SR-BI may be involved in the initial steps of removal of cholesterol from foam cells in the arterial wall, as well as the final stage of reverse cholesterol transport in the liver.

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