[L. A. Reinke, D. R. Moore, C. M. Hague, P. B. McCay, *Free Radical Res.* **21**, 213 (1994)].
14. The signal attributable to DMPO-OH for A4V was 3.0 ± 1.1 times the WT signal (mean ± SEM, $n = 10$, $P < 0.005$ by unpaired, two-tailed t test; range, 2.1 to 5.7 times the WT signal), and that attributable to G93A was 2.1 ± 0.3 times the WT signal ($n = 5$, $P < 0.025$, range, 1.8 to 2.6 times the WT signal). Studies were also performed with WT and A4V enzymes at lower concentrations of H_2O_2. Marked DMPO-OH signals were observed at H_2O_2 concentrations of 3 to 30 mM; for A4V, the signal was more than five times that for the WT enzyme at an H_2O_2 concentration of 3 mM.
15. H. P. Misra, *J. Biol. Chem.* **254**, 11623 (1979).
16. D. A. Joyce, in *Variability in Response to Anti-Rheumatic Drugs*, P. M. Brooks, R. O. Day, G. G. Graham, K. M. Williams, Eds. (Birkhäuser, Basel, 1993), pp. 203–207.
17. Experiments were performed with conditions identical to those in Fig. 3, except that *d,l*-penicillamine was used in place of DDC. For both WT and A4V, the DMPO-OH signal was reduced by 60 to 95% in the presence of 13 μM penicillamine.
18. Both DDC and penicillamine inhibited the SOD activity of both WT and mutant CuZnSODs at high ratios of chelator to enzyme; however, the competing irreversible inhibition of CuZnSOD by H_2O_2 (*7*), the autooxidation of these sulfhydryl-containing Cu^{2+} chelators during the SOD assay, and the potential interference of these reagents with the assay itself (*24*) make conclusions concerning the concentrations required to inhibit the SOD activity of the enzymes and the mechanism of this activation uncertain (J. J. Goto, M. Wiedau-Pazos, E. B. Gralla, J. S. Valentine, unpublished data).
19. CSM14.1 is a conditionally immortalized rat nigral neural cell line [M. M. Durand, D. C. Chugani, M. Mahmoudi, M. E. Phelps, *Soc. Neurosci. Abstr.* **16**, 40 (1990); L. T. Zhong *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4533 (1993)].
20. Studies with penicillamine and cultured CSM14.1 cells were performed as described for DDC. *d,l*-Penicillamine (100 or 500 μM) was added to the cultures at the time of serum withdrawal. Penicillamine had no significant effect on survival of cells transfected with the vector alone (control) or with the WT CuZnSOD construct. In contrast, addition of penicillamine to cells expressing A4V, G41D, or G85R mutant enzymes increased survival significantly. Cells expressing the G37R mutant showed a small penicillamine-induced improvement in survival, but this effect did not achieve statistical significance.
21. J. S. Beckman, M. Carson, C. D. Smith, W. H. Koppenol, *Nature* **364**, 584 (1993).
22. The mechanism proposed (*7–9*) for the WT bovine enzyme (Enz) is as follows:
$Enz\text{-}Cu(II) + H_2O_2 \rightarrow Enz\text{-}Cu(I) + O_2^- + 2H^+$
$Enz\text{-}Cu(I) + HO_2^- + H^+ \rightarrow Enz\text{-}Cu(II)(\cdot OH) + OH^-$
$Enz\text{-}Cu(II)(\cdot OH) + DMPO \rightarrow Enz\text{-}Cu(II) + DMPO\text{-}OH$
23. L. A. Damani, Ed., *Sulphur-Containing Drugs and Related Organic Compounds: Chemistry, Biochemistry, and Toxicology* (Halsted, New York, 1989), vol. 3B; D. R. Gandara *et al.*, *J. Clin. Oncol.* **13**, 490 (1995).
24. R. E. Heikkila and F. Cabbat, *Anal. Biochem.* **75**, 356 (1976).
25. G. R. Buettner and L. W. Oberley, *Biochem. Biophys. Res. Commun.* **83**, 69 (1978).
26. E. Finkelstein, G. M. Rosen, E. J. Rauckman, *Arch. Biochem. Biophys.* **200**, 1 (1980).
27. We thank C. J. Epstein and T.-T. Huang for the WT CuZnSOD genomic clone; D. R. Borchelt, P. C. Wong, and S. S. Sisodia for the mutant CuZnSOD genomic clones; R. H. Brown Jr., T. B. Usdin, M. J. Brownstein, and D. McKenna-Yasek for mutant CuZnSOD complementary DNAs; K. F. Faull for assistance with electrospray mass spectrometry; E. M. Landaw for assistance with statistical evaluation of the data; M. Durand and D. C. Chugani for CSM14.1 cells; J. Shi for technical assistance; and R. Y. N. Ho for technical advice. Supported by NIH grants AG12282 (D.E.B.), GM28222 (J.S.V.), and DK46828 (J.S.V.) and grants from the Amyotrophic Lateral Sclerosis Association (E.B.G.) and VivoRx Corp. (S.R.).

19 July 1995; accepted 27 November 1995

Identification of Scavenger Receptor SR-BI as a High Density Lipoprotein Receptor

Susan Acton,* Attilio Rigotti, Katherine T. Landschulz, Shangzhe Xu, Helen H. Hobbs, Monty Krieger†

High density lipoprotein (HDL) and low density lipoprotein (LDL) are cholesterol transport particles whose plasma concentrations are directly (LDL) and inversely (HDL) correlated with risk for atherosclerosis. LDL catabolism involves cellular uptake and degradation of the entire particle by a well-characterized receptor. HDL, in contrast, selectively delivers its cholesterol, but not protein, to cells by unknown receptors. Here it is shown that the class B scavenger receptor SR-BI is an HDL receptor. SR-BI binds HDL with high affinity, is expressed primarily in liver and nonplacental steroidogenic tissues, and mediates selective cholesterol uptake by a mechanism distinct from the classic LDL receptor pathway.

The risk of developing atherosclerosis, the leading cause of death in Western industrialized countries, is directly related to plasma concentrations of LDL cholesterol and inversely related to concentrations of HDL cholesterol (*1*, *2*). Although receptor-mediated LDL metabolism is well defined (*3*), HDL metabolism is poorly understood (*1*, *2*, *4*). HDL delivers cholesteryl ester to nonplacental steroidogenic tissues (ovary, adrenal gland, and testis) for hormone synthesis (*5*) and transports cholesterol from extrahepatic tissues to the liver (reverse cholesterol transport) (*1*, *2*, *4*). Unlike the situation with LDL, delivery of the cholesterol of HDL to cells is generally not accompanied by degradation of the protein component of HDL; this process is known as selective lipid uptake (*6–13*). Although numerous ligand-blotting studies revealed a variety of HDL-binding proteins [58 to 140 kD, reviewed in (*4*)], cell surface receptors for selective lipid uptake have not been identified. Here we provide evidence that the class B scavenger receptor SR-BI (*14*) is an HDL receptor.

We previously showed that hamster SR-BI (haSR-BI) binds native LDL, chemically modified LDL, and anionic phospholipids (*14*). To further explore the lipoprotein-binding properties and functions of SR-BI, we cloned the complementary DNA (cDNA) of the murine homolog (mSR-BI) and transfected it into ldlA cells [LDL receptor–negative Chinese hamster ovary (CHO) cells (*15*)]. ^{125}I-labeled HDL (*16*) specifically associated with the stable transfectants (ldlA[mSR-BI]) with high affinity (dissociation constant, K_d, of ~30 μg of protein per milliliter) and saturability (Fig. 1A). Control (untransfected) ldlA cells exhibited substantially less ^{125}I-HDL association. Association reached a steady state in less than 1 hour (Fig. 1B) and was not affected by EDTA [1 to 10 mM (*17*)] or by the removal of the trace amounts of apolipoprotein E (apoE) present in the HDL (*16*, *17*). There was little effect of excess native LDL on ^{125}I-HDL association (*17*); thus, LDL in vivo would not be expected to significantly interfere with binding of HDL to mSR-BI. The ^{125}I-labeled protein components of HDL (apoAI and apoAII) were not degraded [Fig. 1C and (*17*)], which is in marked contrast to the lysosomal degradation of the protein component of LDL (apoB) after endocytosis via the LDL receptor (*3*).

We next compared the fates of the protein (labeled with ^{125}I) and lipid [labeled with [^{3}H]cholesteryl oleate or DiI (a fluorescent lipid)] (*16*) components of HDL in mSR-BI–transfected cells. The amount of cell-associated ^{125}I-HDL reached a steady state in less than 1 hour at 37°C with less than 0.5% of the total label bound to the transfected cells (Fig. 2A). In contrast, transfer of [^{3}H]cholesteryl oleate and DiI increased continuously, reaching ~18% of the total labeled HDL lipids after 5 hours of incubation at 37°C. DiI accumulation was saturable (*17*). Untransfected cells displayed little lipid or protein association (Fig. 2B). Thus, there was selective transfer of the lipid, but not the protein, components of HDL to cells expressing mSR-BI. Essentially identical results were observed with COS cells transiently transfected (*14*) with either the mSR-BI vector or a control vector (pcDNA1, no insert) and incubated with HDL labeled with [^{3}H]cholesteryl ether or ^{125}I [10 μg of HDL protein per milliliter (*16*)]. After 5 hours, there was 18% lipid and 0.3% protein association in cells expressing mSR-BI and very little of either label associated with the controls.

S. Acton, A. Rigotti, S. Xu, M. Krieger, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
K. T. Landschulz and H. H. Hobbs, Departments of Internal Medicine and Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX 75235, USA.
*Present address: Millennium Pharmaceuticals, Cambridge, MA 02139, USA.
†To whom correspondence should be addressed.

Similar selective transfer of HDL lipid has been reported for cultured cells (*6–9, 19*), perfused organs (*10*), and liver, adrenal gland, and ovary in vivo (*11, 12*).

To establish that the transfer of [^{3}H]cholesteryl ester from labeled HDL to cells reflected net transfer and not simply exchange, we compared the cholesterol contents of the cells after incubation with or without unlabeled HDL (20 μg of protein per milliliter for 5 hours) (*18*). In the presence of HDL, total cellular cholesterol increased by 20% (4.6 μg of free plus esterified cholesterol per milligram of cell protein) in mSR-BI–transfected cells. This corresponded to transfer of ~21% of the HDL cholesterol added to the incubation medium (*16*) and was comparable with results for the lipid-labeled HDL (Fig. 2A). In contrast, there was no significant HDL-dependent increase in the cholesterol content of untransfected cells (<0.2 μg of total cholesterol per milligram of cell protein). Thus, net transfer of HDL cholesteryl ester mediated by mSR-BI was quantitatively similar to that reported for a murine adrenal cell line (*9*). In addition, these results indicate that the fluorescent or radiolabeled lipids in HDL can be reliable reporters for total cholesterol transfer.

To examine the cellular pathway of selective lipid delivery mediated by mSR-BI, we compared the distribution of fluorescent lipid (DiI) delivered by the classic LDL receptor pathway with that of the mSR-BI pathway. A 1-hour incubation of LDL receptor–positive wild-type CHO cells with DiI-labeled LDL produced a receptor-dependent punctate pattern (Fig. 3A) that typifies receptor-mediated endocytosis from coated pits and vesicles to endosomes and lysosomes (*3, 20, 21*). DiI-HDL labeling of ldlA[mSR-BI] cells (Fig. 3B) produced a dramatically different pattern: There was diffuse staining over the entire cell surface (*22*), particularly at cell-cell interfaces, and even after 24 hours of incubation no punctate staining was seen (*17*). There also appeared to be accumulation of fluorescent lipid adjacent to the nucleus within the cells. Cultured human hepatocytes stained by DiI-LDL and DiI-HDL show similar patterns to those in Fig. 3 (*19*). Untransfected ldlA cells did not accumulate significant amounts of dye from DiI-HDL (Fig. 3C).

It is important to note that the initial distribution (≤1 hour) and the subsequent sites of accumulation of DiI, a positively charged lipid, may differ from that of cholesteryl ester, a neutral lipid. Indeed, after a 48-hour incubation with unlabeled HDL, neutral lipids (oil red O–positive) accumulated in transfected cells in small, well-defined cytoplasmic particles (*17*). Similarly, Reaven and colleagues (*23*) observed trans-

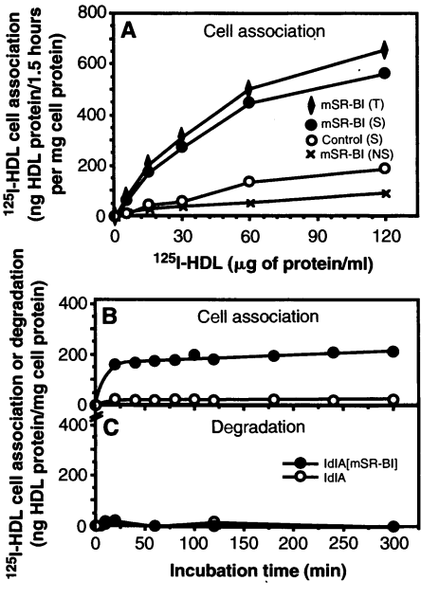

Fig. 1. Murine SR-BI mediates cell association (**A** and **B**) but not degradation (**C**) of ^{125}I-HDL. In (A) ldlA[mSR-BI] (solid symbols) and ldlA (open circles) cells were incubated for 1.5 hours at 37°C with the indicated amounts of ^{125}I-HDL, and cell-associated radioactivity (nanograms of ^{125}I-HDL protein per 1.5 hours per milligram of cell protein) was measured (*26*). The specific values [(S) open and solid circles] represent the differences between the total {(T) diamonds, ldlA[mSR-BI]} and nonspecific (NS) (40-fold excess of unlabeled HDL, crosses, ldlA[mSR-BI]) values. In (B) and (C) ldlA[mSR-BI] and ldlA cells were incubated for the indicated times with 20 (B) or 10 (C) μg of ^{125}I-HDL protein per milliliter at 37°C, and specific cell association (B) and specific cellular degradation (C) (nanograms of ^{125}I-HDL protein degraded per milligram of cell protein) were determined (*27*).

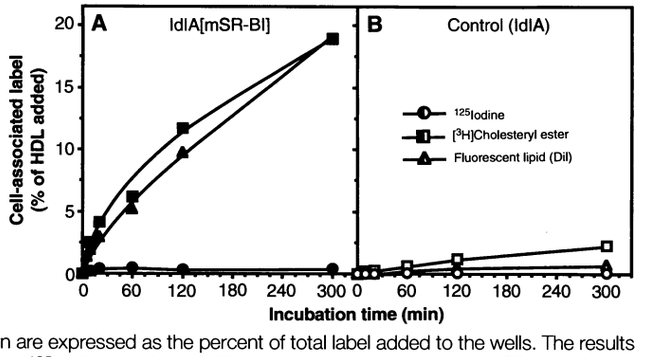

Fig. 2. Kinetics of selective uptake of HDL lipid by mSR-BI–transfected (**A**) and control, untransfected (**B**) cells. Cells (*26*) were incubated at 37°C with ^{125}I-HDL (10 μg of protein per milliliter, [^{3}H]cholesteryl oleate–labeled HDL (2.2 μg of cholesteryl ester per milliliter; ~8.8 μg of protein per milliliter), or DiI-labeled HDL (10 μg of protein per milliliter) (*16*), and cell-associated label was quantified (*26*). The specific values shown are expressed as the percent of total label added to the wells. The results in (A) (steady state of ~200 ng of ^{125}I-HDL protein per milligram of cell protein at 10 μg of ^{125}I-HDL protein per milliliter in less than 1 hour) are similar to those in Fig. 1B.

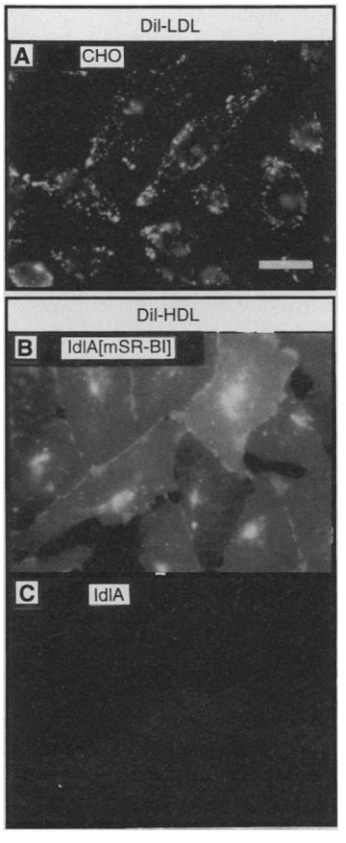

Fig. 3. Cellular distribution of fluorescent lipid (DiI) delivered to cells from DiI-LDL or DiI-HDL. On day 0, LDL receptor–positive wild-type CHO (**A**), ldlA[mSR-BI] (**B**), and receptor-negative ldlA (**C**) cells were plated in medium A–FBS (*26*) on cover slips coated with poly-D-lysine (molecular weight >300,000; Sigma). On day 1, the monolayers were re-fed with medium A containing 5% newborn calf lipoprotein-deficient serum. On day 3 the subconfluent cells were re-fed with the same medium containing either (A) DiI-LDL (10 μg of protein per milliliter) or [(B) and (C)] DiI-HDL (1 μg of protein per milliliter) and incubated for 1 hour at 37°C. The cover slips were then washed once with phosphate-buffered saline, and the cells were photographed with a fluorescence microscope with a rhodamine filter package. There was essentially no labeling by DiI-LDL of LDL receptor–negative ldlA cells (*17*). Scale bar = 25 μm.

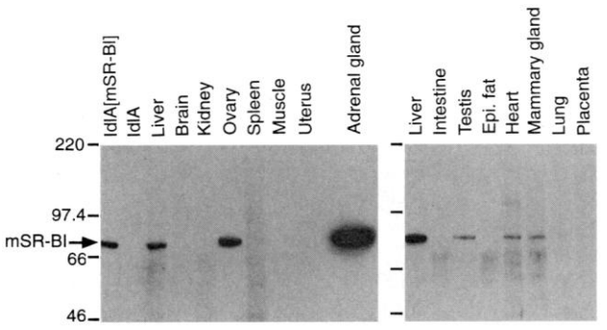

Fig. 4. Immunoblot analysis of the tissue distribution of mSR-BI. Extracts from the indicated cells and tissue membranes (postnuclear 100,000*g* membrane pellets) were reduced and analyzed by gel electrophoresis (50 μg of protein per lane) and immunoblotting with antibody to mSR-BI peptide (rabbit immunoglobulin G fraction, 1:5000 dilution) (*24, 28*). Ponceau S staining was used to control for loading and transfer efficiency (*17*). Epi., epididymal. Molecular sizes are indicated on the left in kilodaltons.

fer of a fluorescent cholesteryl ester derivative from HDL to cytoplasmic fat droplets in ovarian granulosa cells.

Taken together, these results indicate that the pathway by which mSR-BI mediates lipid transfer from HDL is distinct from the classic LDL receptor–mediated endocytic pathway and appears to involve initial transfer to the plasma membrane, as was previously proposed for selective HDL lipid uptake (*7, 9, 12*).

To determine the size of mSR-BI and its tissue distribution, we prepared a rabbit polyclonal antibody (*24*) and used it for immunoblot analysis (Fig. 4). This antibody specifically recognized mSR-BI, an ~82-kD protein in transfected, but not untransfected, cells (*25*). The mSR-BI was highly expressed in membranes from murine liver, ovary, and adrenal gland. Significantly less mSR-BI protein was detected in testis, in mammary gland, and occasionally in heart, and there was little expression in the other tissues examined (Fig. 4) (*24*). In earlier Northern (RNA) blotting studies (*14*) we observed that adipose tissue and cultured cells (3T3-L1) contain relatively high amounts of mSR-BI message. Nevertheless, in the present study these cells contained little immunoreactive mSR-BI protein [Fig. 4 and (*17*)], which raised the possibility that there may be posttranscriptional regulation of mSR-BI expression. The adrenal gland and ovary have the highest amounts of mSR-BI mRNA (*17*) and immunoreactive protein. Thus, mSR-BI is expressed most abundantly in those tissues that exhibit selective HDL lipid uptake in vivo (*11*).

Because mSR-BI binds HDL, mediates selective lipid transfer from HDL to cells, and is expressed most abundantly in liver and nonplacental steroidogenic tissues, it displays all the characteristics of a physiologically relevant HDL receptor. Additional molecular, physiological, and genetic studies of the function of mSR-BI should help to further our understanding of the mechanism by which HDL serves as a source of substrate for steroidogenesis and of the role of HDL in reverse cholesterol transport and protection against atherosclerosis.

REFERENCES AND NOTES

1. J. L. Breslow, in *The Metabolic and Molecular Bases of Inherited Disease*, C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Valle, Eds. (McGraw-Hill, New York, 1995), pp. 2031–2052.
2. S. M. Grundy, *J. Am. Med. Assoc.* **256**, 2849 (1986); A. R. Tall, *J. Clin. Invest.* **86**, 379 (1990).
3. J. L. Goldstein, H. H. Hobbs, M. S. Brown, in (*1*), pp. 1981–2030.
4. G. Assman, A. von Eckardstein, H. B. Brewer Jr., in (*1*), pp. 2053–2072; W. J. Johnson, F. H. Mahlberg, G. H. Rothblat, M. C. Phillips, *Biochim. Biophys. Acta* **1085**, 273 (1991); M. N. Pieters, D. Schouten, T. J. C. Van Berkel, *ibid.* **1225**, 125 (1994); C. J. Fielding and P. E. Fielding, *J. Lipid Res.* **36**, 211 (1995).
5. J. M. Andersen and J. M. Dietschy, *J. Biol. Chem.* **256**, 7362 (1981); M. S. Brown, P. T. Kovanen, J. L. Goldstein, *Recent Prog. Horm. Res.* **35**, 215 (1979); J. T. Gwynne and J. F. Strauss III, *Endocr. Rev.* **3**, 299 (1982); B. D. Murphy *et al.*, *Endocrinology* **116**, 1587 (1985).
6. R. Wishart and M. MacKinnon, *Biochim. Biophys. Acta* **1044**, 375 (1990); E. Leitersdorf, A. Israeli, O. Stein, S. Eisenberg, Y. Stein, *ibid.* **878**, 320 (1986).
7. T. P. Knecht and R. C. Pittman, *Biochim. Biophys. Acta* **1002**, 365 (1989); F. Rinninger, S. Jaeckle, R. C. Pittman, *ibid.* **1166**, 275 (1993); F. Rinninger *et al.*, *Hepatology* **19**, 1100 (1994); C. P. Sparrow and R. C. Pittman, *Biochim. Biophys. Acta* **1043**, 203 (1990); J. G. DeLamatre, R. M. Carter, C. A. Hornich, *J. Cell Physiol.* **146**, 18 (1991).
8. J. T. Gwynne and D. D. Mahaffee, *J. Biol. Chem.* **264**, 8141 (1989).
9. R. C. Pittman, T. P. Knecht, M. S. Rosenbaun, C. A. Taylor, *ibid.* **262**, 2443 (1987).
10. C. M. Arbeeny, V. A. Rifici, H. A. Eder, *Biochim. Biophys. Acta* **917**, 9 (1987); M. MacKinnon, J. Savage, R. Wishart, P. Barter, *J. Biol. Chem.* **261**, 2548 (1986); E. Reaven, Y. I. Chen, M. Spicher, S. Azhar, *J. Clin. Invest.* **74**, 1384 (1984).
11. C. Glass, R. C. Pittman, D. B. Weinstein, D. Steinberg, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5435 (1983); C. Glass, R. C. Pittman, M. Civen, D. Steinberg, *J. Biol. Chem.* **260**, 744 (1985); Y. Stein, Y. Dabach, G. Hollander, G. Halperin, O. Stein, *Biochim. Biophys. Acta* **752**, 98 (1983).
12. S. Jackle, F. Rinninger, T. Lorenzen, H. Greten, E. Windler, *Hepatology* **17**, 455 (1993).
13. P. T. Kovanen, W. J. Schneider, G. M. Hillman, J. L. Goldstein, M. S. Brown, *J. Biol. Chem.* **254**, 5498 (1979).
14. S. L. Acton, P. E. Scherer, H. Lodish, M. Krieger, *ibid.* **269**, 21003 (1994); A. Rigotti, S. Acton, M. Krieger, *ibid.* **270**, 16221 (1995).
15. A 600–base pair probe from the haSR-BI cDNA (*14*) was used to isolate an mSR-BI clone from a murine 3T3-L1 adipocyte pcDNA1 library: GenBank accession number U37799; 89% amino acid identity and 96% similarity to haSR-BI (*14*) and 79% identity and 91% similarity to the human sequence CLA1 [D. Calvo and M. A. Vega, *J. Biol. Chem.* **268**, 18929 (1993)]. The corresponding expression vector pmSR-BI-77 was transfected (*14*) into IdlA cells [D. M. Kingsley and M. Krieger, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5454 (1984)]. The receptor-positive cells, IdlA[mSR-BI] (clone 15), were isolated by selection and flow cytometry (*14*).
16. Human HDL and LDL were prepared by zonal centrifugation [B. H. Chung *et al.*, *Methods Enzymol.* **128**, 181 (1986); (*27*)]. The only major proteins in HDL were apoAI and apoAII (with a mass ratio of at least 3:1). ApoE, which was either undetectable or present in trace amounts, was removed by chromatography in some experiments [C. A. Converse and E. R. Skinner, Eds., *Lipoprotein Analysis: A Practical Approach* (Oxford Univ. Press, New York, 1992)]. The mass ratio of cholesterol to protein in HDL was assumed to be 1:4 (*13*). HDL was iodinated by the Pierce iodobead method (specific activities: 60 to 360 cpm per nanogram of protein). DiI (D-282; 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) from Molecular Probes (Eugene, OR) was used to prepare DiI-HDL (*20*), and [^{3}H]cholesteryl ether (Amersham) was used to label HDL (*8*).
17. S. Acton *et al.*, data not shown.
18. Cells were plated as in Fig. 1 in medium A (*26*) with 3% newborn calf lipoprotein-deficient serum. On day 2, cells were incubated with or without unlabeled HDL (20 μg of protein per milliliter for 5 hours at 37°C in medium B) and washed (Fig. 1); lipids were extracted twice with hexane: isopropanol (3:2; 3 ml for 30 min). Extracts were pooled, back-extracted with 1 ml of water, and dried, and total (free and esterified) cholesterol masses (averages of six replicates) were determined (Sigma Diagnostics, St. Louis, MO). The protein contents were estimated from replicate cultures. The values of total cholesterol (micrograms per milligram of cell protein, ± SEM) for cells incubated without HDL were 20.5 ± 0.3 (IdlA) and 23.0 ± 0.4 (IdlA[mSR-BI]).
19. D. Schouten *et al.*, *Arteriosclerosis* **10**, 1127 (1990).
20. R. E. Pitas, T. L. Innerarity, J. N. Weinstein, R. W. Mahley, *Arteriosclerosis* **1**, 177 (1981).
21. M. Krieger *et al.*, *J. Supramol. Struct.* **10**, 467 (1979).
22. R. E. Pagano and R. G. Sleight, *Science* **229**, 1051 (1985).
23. E. Reaven, L. Tsai, S. Azhar, *J. Lipid Res.* **36**, 1602 (1995).
24. A peptide containing residues 495 to 509 from mSR-BI plus an NH_2-terminal cysteine was coupled to keyhole limpet hemocyanin and used to generate mSR-BI^{495} antiserum in New Zealand white rabbits. We generated two additional antibodies that recognize different peptide domains in mSR-BI (*17*). When tested with liver and kidney, these antibodies gave the same relative tissue distribution as did antiserum to mSR-BI^{495}.
25. The difference between the predicted mass of mSR-BI (57 kD) and the observed mass (82 kD) is due primarily to glycosylation (J. Babitt, A. Rigotti, M. Krieger, unpublished results).
26. On day 0, cells were plated in six-well dishes (250,000 cells per well) in a 5% CO_2–humidified incubator 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 (A-FBS) without or with G418 (0.25 mg/ml) for IdlA and IdlA[mSR-BI], respectively. Assays were performed on day 2 in medium B [medium A containing 0.5% (w/v) fatty acid–free bovine serum albumin (FAF-BSA)]. After incubation with labeled HDL in the absence (duplicate incubations) or presence (single incubations) of a 40-fold excess of unlabeled HDL, cells were chilled and rapidly washed with tris buffer [twice with and once without BSA (2 mg/ml)], and radioactivity and protein determinations were made (*14*). [^{3}H]cholesteryl oleate was extracted with isopropyl alcohol (30 min at room temperature) and quantified by scintillation counting. DiI was extracted with dimethyl sulfoxide (DMSO) and its fluorescence (550-nm excitation, 565-nm emission) measured with DiI-HDL standards dissolved in DMSO.
27. M. Krieger, *Cell* **33**, 413 (1983).
28. E. V. Jokinen *et al.*, *J. Biol. Chem.* **269**, 26411 (1994).

29. We thank M. Christiansen and T. Hyatt for technical assistance and G. Baldini, H. Lodish, A. Tall, P. Kim, L. Liscum, and members of our laboratories for materials and advice. Supported by NIH grants HL41484, HL52212, HL09047, and HL20948; The Perot Family Fund; and a Howard Hughes Medical Institute postdoctoral fellowship (A.R.).

5 October 1995; accepted 7 December 1995