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Alternative forms of the scavenger receptor BI (SR-BI)

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Abstract The class B, type I scavenger receptor has been implicated as a receptor for high density lipoprotein (HDL). We have isolated a murine cDNA clone encoding an alternative form of SR-BI that differs in the putative cytoplasmic domain of the receptor. This variant form, likely the result of alternative mRNA splicing, is designated SR-BI.2. SR-BI.2 mRNA was detected in mouse tissues known to express SR-BI and tissue-specific differences in the relative abundance of SR-BI.2 were apparent. In mouse adrenal glands, SR-BI.2 represented approximately one-third of total SR-BI mRNA, whereas in mouse testes, SR-BI.2 represented the major mRNA species (79% of total). SR-BI.2 was also detected in the human cell lines examined, namely HeLa, HepG2, and THP-1 cells. CHO cells transfected with the mouse SR-BI.2 cDNA expressed significant levels of SR-BI.2 protein and acquired the ability to take up fluorescent lipid (DiI) from DiI-HDL. Alternative splicing of SR-BI represents a potentially important process for the regulation of SR-BI expression and function.—**Webb, N. R., W. J. S. de Villiers, P. M. Connell, F. C. de Beer, and D. R. van der Westhuyzen.** Alternative forms of the scavenger receptor BI (SR-BI). *J. Lipid Res.* 1997. **38:** 1490–1495.

Supplementary key words high density lipoprotein receptor • alternative splicing

The risk of coronary artery disease is inversely correlated with the plasma level of HDL cholesterol (1). The protective effect of HDL may be mediated by its role in reverse cholesterol transport, whereby plasma cholesteryl ester is transported to the liver for secretion. A candidate cell surface receptor for this process has recently been identified (2). This receptor, the scavenger receptor class B type I (SR-BI), mediates high-affinity, saturable binding of HDL and the selective uptake of HDL cholesteryl ester by cells (2). SR-BI mRNA and protein levels are highest in steroidogenic tissues and the liver, which are known to exhibit selective lipid uptake from HDL. SR-BI expression in steroidogenic cells is regulated by physiological conditions that alter cholesterol metabolism and cell requirements for cholesterol (3–5). Taken together, the evidence suggests that SR-BI may be a physiologically important HDL receptor. We now describe a new form of human and murine SR-BI, designated SR-BI.2, which probably results from alternative mRNA splicing.[2] SR-BI.2, which encodes a SR-BI with a different cytoplasmic domain, represents a significant portion of SR-BI mRNA in all murine tissues examined and is the major form present in mouse testes. CHO cells transfected with SR-BI.2 cDNA and incubated with DiI-HDL accumulate fluorescent lipid in plasma membranes, indicating that SR-BI.2 functions as an HDL receptor.

EXPERIMENTAL PROCEDURES

Library screening and analysis of cDNAs

A B6CBA mouse liver cDNA library (Stratagene, La Jolla, CA) was screened with a degenerate oligonucleotide probe with the sequence 5′-CCRTTRATCATRTTR CAYTG-3′ as described previously (6). DNA sequencing was performed using a Sequenase 2.0 kit (United States Biochemical Corp., Cleveland, OH). Surveys of sequence data bases were performed using BLAST (basic

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[2]In the nomenclature used in this paper, SR-BI.1 and SR-BI.2 are the alternatively spliced products of a single gene, *Srb1*. SR-BI.1 is identical to the protein that was first identified as an HDL receptor by Acton et al. (2) and designated SR-BI.

Abbreviations: HDL, high density lipoprotein; SR-BI, scavenger receptor class B type I; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; CHO, Chinese hamster ovary; RT-PCR, reverse transcriptase-polymerase chain recation; hCG, human chorionic gonadotropin.

local alignment search tool) from NCBI (National Center for Biotechnology Information) (7) and transmembrane regions were delineated using the program TMAP from EMBL (European Molecular Biology Laboratory).

Cell lines

Human HeLa, HepG2, and THP-1 cells were obtained from the ATCC. For the production of stable transfectants, SR-BI.2 coding sequences were cloned into the expression vector pCMV5 (8) and transfected into CHO ldlA cells (9) using an MBS transfection kit (Stratagene, La Jolla, CA). SR-BI.2-expressing cells were isolated by selection in 0.5 mg/ml G-418. For DiI-HDL experiments, cells were incubated for 1 hr in Ham's F-12 media containing 5% lipoprotein-deficient human serum and 1 μg/ml DiI-HDL prepared by the method of Pitas et al. (10). Flow cytometry was performed as described by Geng and Hansson (11).

RNA preparation and reverse transcription-polymerase chain reaction

Total RNA was isolated from C57BL/6J mouse tissues and mouse and human cell lines using the TRIzol™ reagent (Life Technologies, Grand Island, NY). For each preparation of adrenal RNA, the glands from three mice were pooled. The sequences of the primers used in the amplification of the human cDNAs were 5′-TTCTGCCCGTGCCTGGAGTC-3′ (forward primer) and 5′-GCTGTCTGCTGGGAGAGTC (reverse primer). DNA fragments generated by RT-PCR were sequenced using an AmpliCycle™ sequencing kit (Perkin Elmer, Branchburg, NJ).

S1 nuclease analysis

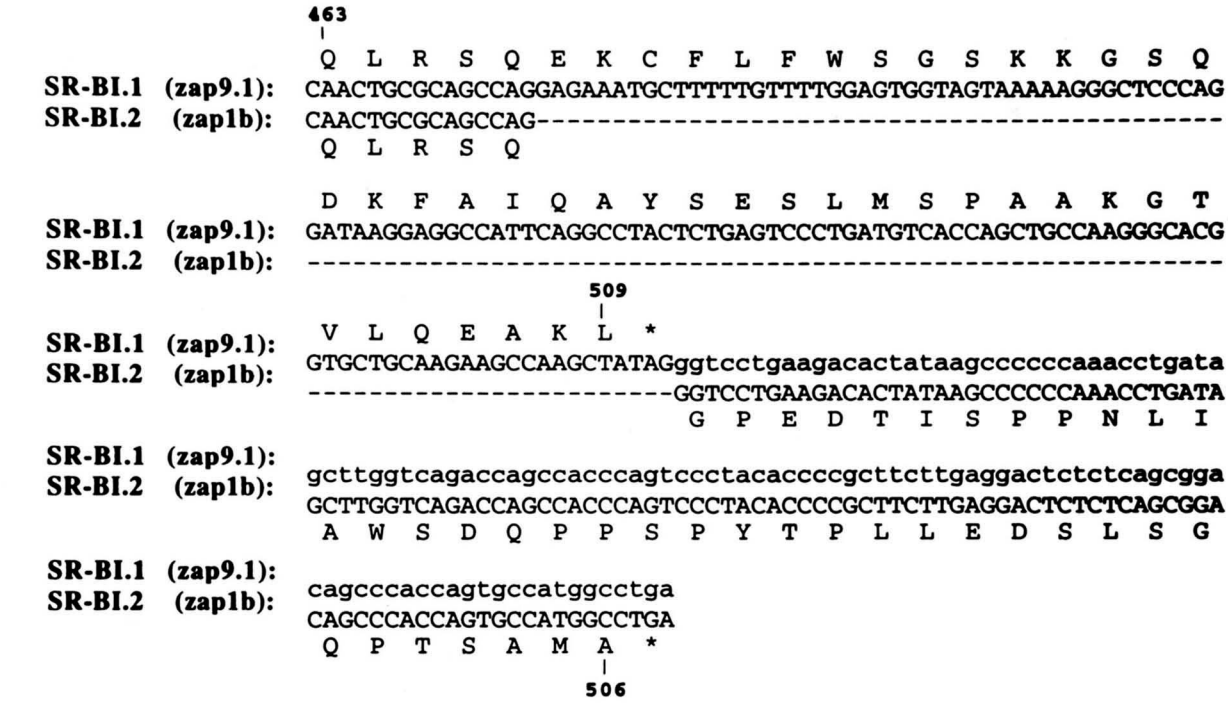
A DNA probe was end-labeled with the Klenow enzyme and [α-^{32}P]dCTP. For S1 nuclease analysis, 50 μg of liver or adipose RNA or 10 μg of testes or adrenal RNA was assayed. Fifty μg of RNA (a mixture of specific RNA and carrier tRNA) was hybridized with 2–10 ng probe (approx. 30,000 cpm) overnight at 48°C in a 50-μl reaction containing 10 mM PIPES, pH 6.5, 1 mM EDTA, 80% formamide and 0.4 M NaCl (12). The hybridization mixture was digested for 30 min at 37°C with 60 units of S1 nuclease (Pharmacia, Piscataway, NJ). The protected fragments were separated on 6% acrylamide/7 M urea gels and quantified using an Ambis Radioanalytic Imaging System.

RESULTS

Degenerate oligonucleotides based on the published sequences for the human and hamster SR-BI cDNAs (13, 14) were used to probe a mouse liver cDNA library. Four hybridizing clones were chosen for further analysis. Nucleotide sequence analysis of the cDNAs showed that two of the clones (zap9.1 and zap2.1) were identical to mouse SR-BI (2) whereas two other clones (zap16.2 and zap1b) lacked 129 nucleotides at the extreme 3′-end of the SR-BI coding sequences. We have designated the alternative forms "SR-BI.1" (2) and "SR-BI.2" (this report; see footnote 2). **Figure 1, top** shows the nucleotide and predicted amino acid sequences of these clones starting with amino acid 463, which corresponds to the beginning of the putative C-terminal cytoplasmic domain of SR-BI.1. The deleted portion in zap1b begins immediately after a CAG codon (encoding the fifth residue of the SR-BI.1 cytoplasmic domain) and extends through the stop codon of the SR-BI.1 open reading frame. This deletion shifts the open reading frame to the non-translated sequence of the SR-BI.1 cDNA (lower case letters in Fig. 1, top) and results in a predicted translation product extending 39 amino acid residues beyond the deleted portion. Thus, the predicted cytoplasmic domain of SR-BI.1 contains 47 amino acid residues, whereas the cytoplasmic tail of SR-BI.2 contains 44 amino acids (Fig. 1, bottom). The amino acid sequence of the SR-BI.2 C-terminal domain exhibited no significant similarity to any sequence in the existing gene databases.

To determine whether SR-BI.2 is present in human cells, we performed RT-PCR on RNA isolated from HeLa, THP-1, and HepG2 cells using primers specific for the human gene. For each of the human lines, the PCR products migrated at positions expected for SR-BI.1 and SR-BI.2 (data not shown). Nucleotide sequencing of the smaller fragment amplified from HepG2 RNA confirmed that this cDNA encodes SR-BI.2. Comparison of the mouse SR-BI.2 cytoplasmic domain with the same region of the human sequence showed that the open reading frame encoded by the human sequence is precisely the same length as the mouse SR-BI.2 (data not shown). The amino acid sequences are 67% identical in this region, and the nucleotide sequences exhibit 77% identity over this stretch of 120 bp. In contrast, the human and mouse nucleotide sequences in the next 85 bp are only 25% identical.

The relative amounts of SR-BI.1 and SR-BI.2 mRNAs in selected mouse tissues were determined using an S1 nuclease protection assay. A 621 nucleotide S1 nuclease probe spanning the deleted sequences protects a 441 nucleotide SR-BI.1 fragment and a 154 nucleotide SR-BI.2 fragment. **Figure 2, left** shows the results of S1 nuclease protection assays of various mouse tissues known to express SR-BI (2, 14). Both SR-BI transcripts were detected in mouse liver, testes, adipose tissue, and adrenal glands. However, the relative amounts of SR-



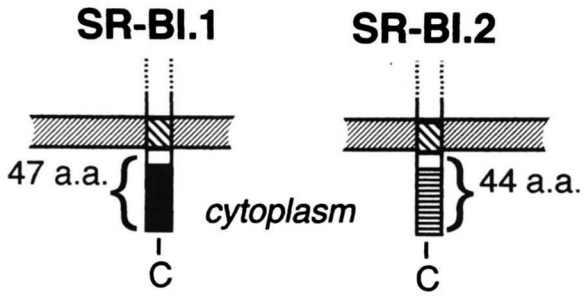


Fig. 1. Top: Nucleotide sequence and predicted amino acid sequence of cDNA clones (zap9.1 and zap1b) encoding murine SR-BI.1 and SR-BI.2 starting at amino acid 463, which corresponds to the beginning of the putative cytoplasmic domain of SR-BI. Coding and non-coding sequences are denoted by upper case and lower case letters, respectively. The dashed line indicates the 129 bp not present in zap1b. Bottom: Schematic diagram showing the proposed structures of the C-terminal region of SR-BI.1 and SR-BI.2. SR-BI.1 contains a cytoplasmic domain 47 amino acids in length; SR-BI.2 contains a 44-amino acid cytoplasmic domain. The N-terminal and transmembrane regions and membrane-proximal five amino acids of the cytoplasmic tail are identical in the two forms.

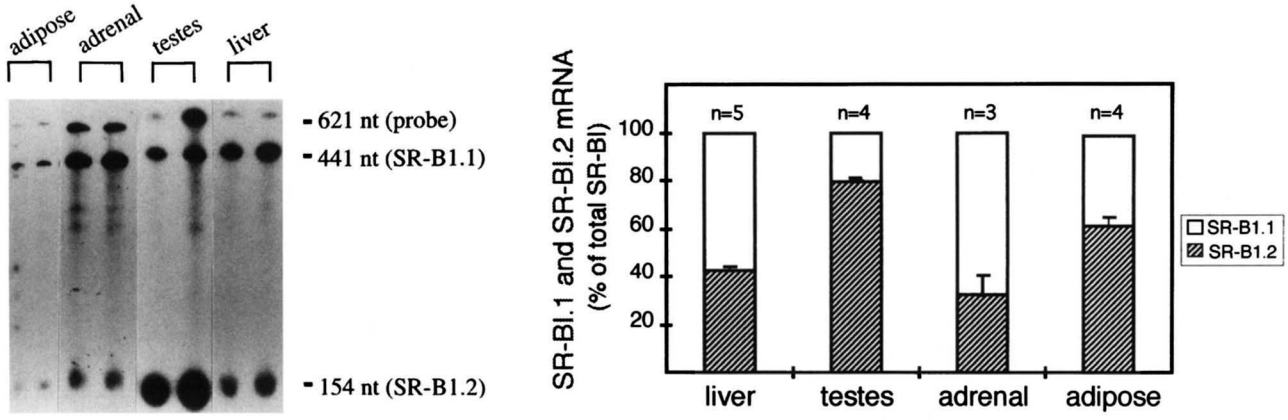


Fig. 2. Left: S1 protection analyses of adipose (50 μg), adrenal (10 μg), testes (10 μg), or liver (50 μg) RNA. Duplicate lanes are depicted. The positions of the probe and protected fragments are shown. Right: Relative expression of SR-BI.1 and SR-BI.2 in selected mouse tissues. The bars (mean ± SE) represent the values of assays performed in duplicate. The number of animals assayed is indicated. In the case of the adrenals, three glands were pooled for each RNA sample.

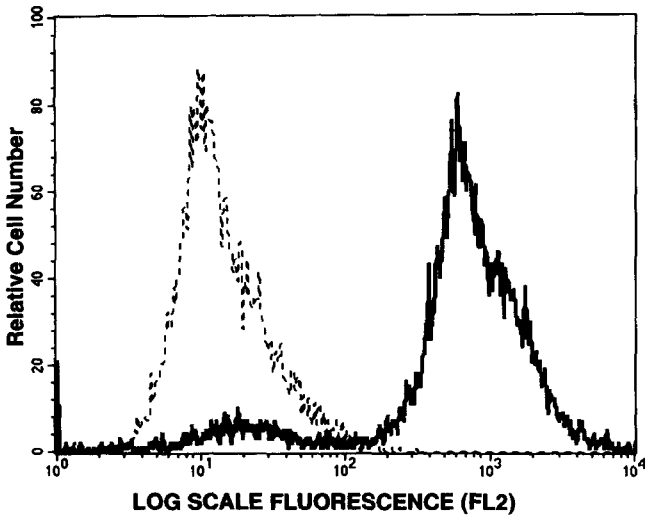


Fig. 3. Analysis by flow cytometry of untransfected (dashed line) and SR-BI.2-transfected (bold line) CHO ldlA cells incubated with DiI-HDL. Cells were fixed in 4% (w/v) paraformaldehyde in PBS and analyzed on a FACScan® (Becton Dickinson) using CELLQUEST software. Emitted fluorescence was collected at 563–607 nm (FL2 photomultiplier).

BI.1 and SR-BI.2 differed substantially among these tissues (Fig. 2, right). Whereas SR-BI.1 and SR-BI.2 levels were similar in mouse liver (43% ± 1.8% SR-BI.2), the amount of SR-BI.2 in mouse testes was approximately 4-fold higher than SR-BI.1 (79% ± 1.1% SR-BI.2). SR-BI.2 in mouse adipose tissue and mouse adrenal glands represents 61% (±4%) and 32% (±8.5%) of total SR-BI mRNA, respectively.

To determine whether SR-BI.2 functions as an HDL receptor, we stably transfected CHO ldlA cells with the SR-BI.2 cDNA. Appreciable levels of SR-BI.2 were detected in these cells by immunoblot analysis using a polyclonal antisera raised against amino acids 230–380 of SR-BI (data not shown). Analysis by flow cytometry of transfected cells incubated with DiI-HDL showed high levels of fluorescence staining, indicating that SR-BI.2 is expressed on the cell surface and can mediate the transfer of lipid from HDL (**Fig. 3**). Fluorescence microscopy of the DiI-HDL-treated cells indicated that the fluorescent lipid was delivered to the plasma membrane (data not shown) in a manner similar to that described for SR-BI.1 (2).

DISCUSSION

The scavenger receptor class B, type I (SR-BI) is a receptor for HDL. In this report, we describe an alternative form of SR-BI that differs in its intracellular domain. This form, designated SR-BI.2, likely results from alternative splicing of a single SR-BI precursor RNA. The sequences of the two SR-BI forms were identical except for a 129 nucleotide deletion in SR-BI.2 and furthermore, Southern blot analysis of genomic DNA indicated the presence of a single gene for SR-BI (C. L. Welch, Y. Xia, L. Gu, D. Machleder, M. Mehrabian, P. Wen, N. Webb, W. J. S. de Villiers, D. R. Van der Westhuyzen, and A. J. Lusis, unpublished results). The adopted nomenclature for SR-BI.1 and SR-BI.2, in our opinion, most clearly conveys that these two proteins are not encoded by two distinct genes in a class B family of scavenger receptors, but are rather the product of a single gene, *Srb1*. Several lines of evidence suggest that SR-BI.2 may be biologically important. First, SR-BI.2 mRNA represented a significant portion (>30%) of the total SR-BI mRNA in all mouse tissues examined. In testes, SR-BI.2 mRNA was the predominant form (79% of total). Second, RT-PCR of three human cell lines produced a cDNA analogous to the mouse SR-BI.2, indicating that this mRNA is also expressed in human cells. Finally, CHO cells stably transfected with the mouse SR-BI.2 cDNA showed that this protein is expressed on the cell surface and functions as an HDL receptor that mediates lipid uptake.

In the case of human SR-BI, an additional alternative form affecting the extracellular domain has been reported (13). These authors isolated a cDNA clone containing a 300-nucleotide deletion 126 nucleotides downstream of the SR-BI translation initiation codon. SR-BI is a member of a family of membrane proteins which also includes CD36 and rat lysosomal integral membrane protein II (LIMPII) (13, 15). The excised segment corresponds exactly to sequences within exons IV and V of the CD36 gene, suggesting that the genomic organization of these two family members may be conserved (13, 16). We are not aware of published data regarding the tissue distribution or level of expression of this alternative form of SR-BI.

To date, the SR-BI gene has not been characterized and the precise nature of the alternative splicing of the precursor transcript is not known. Interestingly, the analysis of cDNAs encoding CD36 identified an alternative form containing a variant 3′-untranslated region that started immediately after the TAA stop codon (17). Analysis of the CD36 gene showed that this variant form results from the use of an internal 5′-splicing site within exon XIV of the CD36 gene. We do not believe that alternative splicing of SR-BI involves usage of an internal 5′ splice site because, unlike CD36, the nucleotide sequence at this site (CAG ↓ GAG) does not conform to a consensus donor sequence (18).

The presence of alternative forms of SR-BI has important implications for studies of SR-BI regulation. In a

recent study, antiserum raised against the C-terminal 14 amino acids of SR-BI.1 was used to measure the effect of estrogen and hCG administration on SR-BI expression in rats (3). We have shown (data not shown) that a similarly prepared antisera, as would be predicted, does not detect SR-BI.2 and would therefore only indicate SR-BI.1 levels. In the same study hCG treatment significantly increased the level of SR-BI.1 protein detected in the Leydig cells of rat testes. SR-BI.2 represents 79% of SR-BI transcripts in mice testes. As alternative splicing of SR-BI could serve as a regulatory mechanism for SR-BI expression, it would be interesting to determine whether hCG increases SR-BI transcription per se, or shifts splicing of the SR-BI precursor mRNA to the SR-BI.1 form. Tissue-specific, post-transcriptional regulation of SR-BI has been proposed to account for the lack of SR-BI.1 protein in adipose tissue despite the presence of appreciable amounts of SR-BI mRNA (3). We have shown that SR-BI.2 represents approximately 60% of total SR-BI mRNA in adipose tissue. However, immunoblotting using an antibody raised against a portion of the extracellular domain (amino acids 230–380) failed to detect appreciable levels of SR-BI.2 protein in this tissue (data not shown), indicating that alternative splicing is not the only mechanism by which SR-BI expression is regulated in cells.

The functional significance of alternative forms of SR-BI that diverge at the C-terminal cytoplasmic domain is not known. Although SR-BI and the other members of the CD36 family exhibit approximately 33% sequence similarity overall, the length and sequence of their putative C-terminal cytoplasmic tails are diverse. For LIMPII and CD36, the C-terminal domains probably contribute to differences in the topology and function of these proteins. In the case of LIMPII, the C-terminal cytoplasmic tail and/or transmembrane region may serve as a targeting signal to transport this molecule to the lysosome (15). Huang et al. (19) have shown that CD36 expressed on the surface of platelets associates with three non-receptor protein tyrosine kinases, *fyn*, *lyn*, and *yes;* and this association is presumably mediated by the short (9 amino acid) C-terminal cytoplasmic domain. We have shown that SR-BI.2 is produced as a cell surface protein in transfected CHO cells and mediates the uptake of lipid from HDL. Further studies are necessary to define the precise functional significance of alternative forms of SR-BI.

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