Molecular characterization of rabbit scavenger receptor class B types I and II: portal to central vein gradient of expression in the liver

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Abstract **To further elucidate the role of scavenger receptor class B type I (SR-BI) in reverse cholesterol transport and in atherogenesis, we performed studies in the rabbit, an animal model displaying a lipoprotein profile similar to that of human, expressing cholesteryl ester transfer protein in plasma and having been demonstrated to be susceptible to atherosclerosis. In this report, we describe for the first time the isolation and characterization of rabbit cDNA fragments encoding SR-BI and scavenger receptor class B type II (SR-BII). Development of an isoform-specific Taqman Real Time PCR system and generation of isoform-specific polyclonal antibodies allowed us to measure SR-BI and SR-BII expression in various rabbit organs on mRNA and protein levels, respectively. We found the highest expression of SR-BI in adrenal gland, liver, and proximal intestine; lesser expression was found in appendix and spleen. Immunohistochemical staining of frozen sections showed SR-BI expression in the cortex but not in the medulla of adrenal gland. An increasing portal to central vein gradient of expression was found within the hepatic lobule. As shown in this report, identification and characterization of SR-BI expression in the rabbit affords a powerful tool to elucidate the role of SR-BI in cholesterol homeostasis and atherogenesis in human.**—Ritsch, A., I. Tancevski, W. Schgoer, C. Pfeifhofer, R. Gander, P. Eller, B. Foeger, U. Stanzl, and J. R. Patsch. **Molecular characterization of rabbit scavenger receptor class B types I and II: portal to central vein gradient of expression in the liver.** ***J. Lipid Res.*** **2004.** 45: **214–222.**

Supplementary key words antibody • atherosclerosis • cDNA cloning • enzyme-linked immunosorbent assay • high density lipoprotein • immunohistochemical staining • real-time polymerase chain reaction • reverse cholesterol transport

The delivery of HDL-cholesterol to cells via the scavenger receptor class B type I (SR-BI) is fundamentally distinct from the well characterized endocytotic pathway mediated by the LDL receptor. SR-BI mediates high affinity binding of HDL and the selective uptake of HDL-derived lipids into cells (1). Scavenger receptor class B type II (SR-BII), an isoform of SR-BI containing a distinctly different cytoplasmic tail, also mediates selective lipid transfer from HDL to cells, but with a lower efficiency than the variant previously characterized (2). The SR-BI cDNA was identified by expression cloning (3), and SR-BI homologs of several species have been cloned. The human homolog of SR-BI has been identified as CLA-1 and mapped to chromosome 12 (4, 5).

In rodents, the selective lipid uptake represents the major pathway for delivering cholesteryl esters to steroidogenic tissues and the liver. A series of in vitro and in vivo studies clearly demonstrated SR-BI to function as a physiologically and pathophysiologically relevant receptor for HDL metabolism in vivo (6–11). Furthermore, SR-BI was shown to play a role in the development of atherosclerosis in these animal models (12–15). However, data from these studies are not fully applicable to the situation in humans for the following reasons. First, the lipoprotein pattern of rodents, with HDL particles being the most prominent lipoprotein class and with the very low abundance of LDL particles, is totally different from that found in humans. Second, no plasma activity of cholesteryl ester transfer protein, one of the key proteins of reverse cholesterol transport, is found in mice and rats. Finally, development of atherosclerosis is nearly absent in wild-type rodents. To circumvent these hindrances, we decided to extend our research to another animal model, i.e., the rabbit. The lipoprotein profile of rabbits is much more similar to that of humans compared with rodents, and cholesteryl ester transfer protein mass and activity are present in rabbit plasma. Additionally, rabbits are well established models for atherosclerosis inducible by a high-cholesterol diet (16).

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Abbreviations: DIG, digoxigenin; HRP, horseradish peroxidase; RACE, rapid amplification of cDNA ends; SR-BI, scavenger receptor class B type I; SR-BII, scavenger receptor class B type II.

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In this report, we describe the cloning and characterization of SR-BI and SR-BII cDNAs from rabbits and their abundance in various tissues. We also describe the development of isoform-specific antibodies and their application in Western blot analysis and immunohistochemical techniques.

METHODS

Animals

All Chinchilla Bastard rabbits were purchased from Charles River Laboratories (Kißlegg, Germany) and housed in temperature- and humidity-controlled rooms with a 12 h light/dark

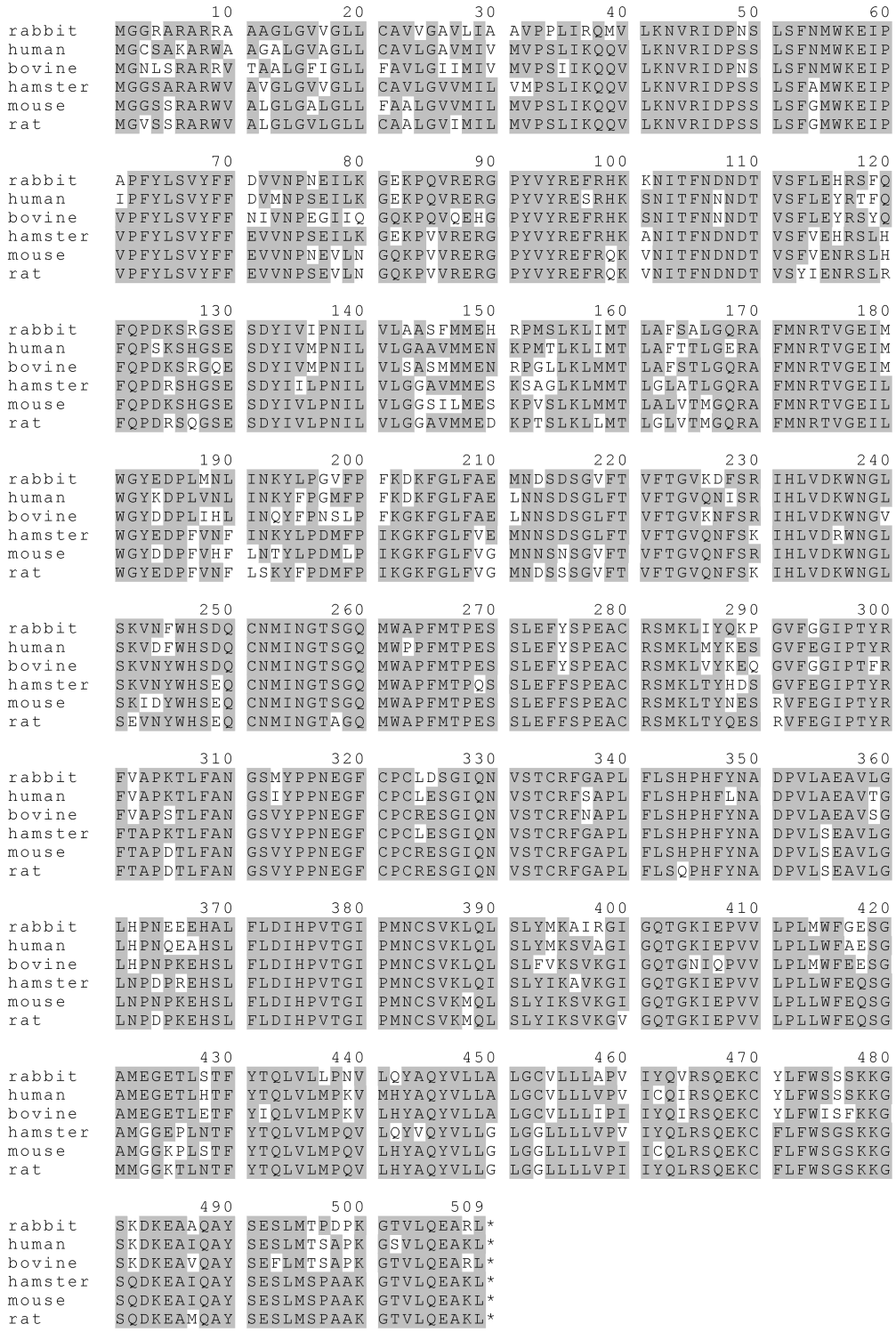

Fig. 1. Amino acid sequence alignment of scavenger receptor class B type I (SR-BI) from six species. SR-BI sequences are shown in one-letter code. Differences are highlighted white, and the numbering is according to the amino acid sequence. In-frame stop codons are indicated by asterisks.

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          468                                                  506
rabbit    GRRQS-----LAAGPEQPAGPHTPLLQDSPCGPPAGPTA*
human     GPEDTVSQPGLAAGPDRPPSPYTPLLPDSPSRQPPSPTA*
bovine    GSKDAISQPCLAAGPDQLPSPYTPLLQDSLRGQPTSPKA*
hamster   GPKDTTSPPNLIAWSDQPSSPYTPLLEDSLSGQSΔ
mouse     GPEDTISPPNLIAWSDQPPSPYTPLLEDSLSGQPTSAMA*
rat       GPEDTTSPPNLIAWSDQPPSPYTPLLEDSLSGQPASATS*
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Fig. 2. Amino acid sequence alignment of C-terminal ends unique to scavenger receptor class B type II (SR-BII) from six species. SR-BII sequences are shown in one-letter code. Differences are highlighted white, and the numbering is according to the amino acid sequence. In-frame stop codons are indicated by asterisks.

cycle and fed a chow diet at the Central Laboratory Animal Facilities in Innsbruck under protocols approved by the Austrian Animal Care and Use Committee. Before taking tissue specimens, rabbits were killed by a 3-fold overdose of pentobarbital (Nembutal; Abbot Laboratories, Abbot Park, IL).

PCR and cDNA cloning

A 530 bp fragment of rabbit SR-BI and SR-BII cDNA was amplified using redundant oligonucleotide primers deduced from published sequences of human, mouse, rat, bovine, and hamster SR-BI (GenBank accession numbers Z22555, U37799, AB002151, NM174597, and U11453, respectively) and reverse-transcribed rabbit liver total RNA. After sequencing, divergently directed primers were designed for 5′ and 3′ rapid amplification of cDNA ends (RACE). All RACE reactions were performed as described by Sambrook and Russell (17). PCR fragments were subcloned into vector pCR2.1 (Invitrogen, Carlsbad, CA) and sequenced with the Thermo Sequenase fluorescently labeled primer cycle sequencing kit with 7-deaza-dGTP (Amersham, Little Chalfont, UK) using the Automated DNA Sequencer 2000l (Li-Cor Inc., Lincoln, NE) according to the user's manual. For expression studies, full-length cDNAs of rabbit SR-BI and SR-BII were subcloned into vector pcDNA3.1 (Invitrogen). cDNA and amino acid sequence comparison was performed using DNAsis software (MBI, Cascade, CO). The nucleotide sequences of SR-BI and SR-BII have been deposited in the GenBank database under accession numbers AY283277 and AY283278, respectively.

HDL uptake

HEK 293 cells were maintained in DMEM containing 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate. HDL was isolated by zonal ultracentrifugation as described (18). [^{3}H]HDL was prepared as described previously (19). One day before transfection, cells were plated out into six-well plates to reach 70% confluence after 24 h of incubation. $CaCl_2$ transfection was performed using 4 μg/well of vectors pcDNA3.1/SR-BI, pcDNA3.1/SR-BII, and pcDNA3.1/green fluorescent protein (GFP). HDL uptake assays were carried out essentially as described (20). Briefly, 2 days after transfection, the medium was changed to medium B [DMEM without glutamine containing 0.2% fatty acid-free BSA (ICN, Aurora, OH)] and cells were incubated with 10 μg/well [^{3}H]HDL for 5 h in the absence (total uptake) or presence of a 20-fold excess of unlabeled HDL (nonspecific binding). Cells were washed three times with buffer A (50 mM Tris, 0.9% NaCl, and 0.2% serum albumin, pH 7.4) followed by three washes with buffer B (50 mM Tris and 0.9% NaCl, pH 7.4) and solubilized in 0.1 N NaOH for 20 min at room temperature before protein and radioactivity quantitation.

Quantitative real-time PCR

The extent of differential gene expression of SR-BI and SR-BII in various tissues was investigated using quantitative real-time Taqman PCR using the ABI Prism 7700 Sequence Detector system (Applied Biosystems, Foster City, CA) according to the user's manual. Primers and Taqman probes were designed using Primer Express software (Applied Biosystems). The forward primer, reverse primer, and Taqman probe sequences for SR-BI were 5′-CCCAAGGGCACTGTGCTC-3′, 5′-GGCGGCCAGGCTCTG-3′, and 5′-CGGCGACCCTACAGCCTTGCTTCT-3′, respectively, and the corresponding sequences for SR-BII were 5′-TCGCCCCTGTCATCTACCA-3′, 5′-GGCGGCCAGGCTCTG-3′, and 5′-TCCGCAGCCAGGGTCGCC-3′, respectively.

18S rRNA was used as reference gene (human 18S rRNA; Applied Biosystems). Average cycle threshold (Ct) for duplicate standards and samples was calculated by Sequence Detector version 1.7 software (Applied Biosystems). Standard curve equations were calculated by regression analysis of the average Ct of duplicate standards and samples versus the log10 of the relative standard concentration. The relative concentration of SR-BI and SR-BII was corrected for the corresponding 18S rRNA concentration.

Antibodies

Peptides were synthesized according to the C-terminal sequences of rabbit SR-BI (495–509) and SR-BII (486–501) (Genxpress, Vienna, Austria). Chinchilla Bastard rabbits were immunized with 100 μg of peptide conjugated to keyhole limp hemocyanin in Freund's incomplete adjuvant subcutaneously at five to eight sites. The injection procedure was repeated after 2 and 4 months. Whole IgG-type antibodies were isolated from the antiserum using a Protein G4 Fast Flow column (Amersham, Uppsala, Sweden) and applied to an affinity column prepared by coupling 2 mg of SR-BI peptide to 3 g of cyanogen bromide-activated Sepharose 4 B (Amersham).

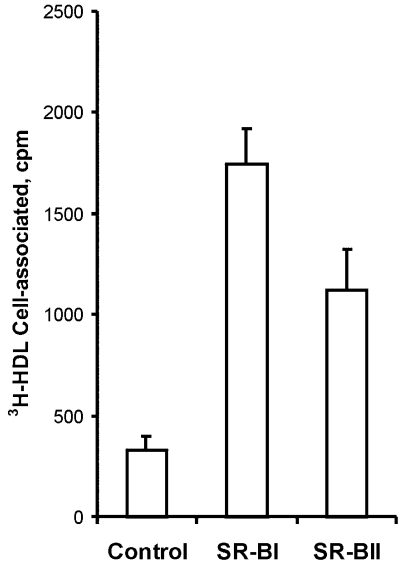

Fig. 3. HDL cholesterol uptake mediated by SR-BI and SR-BII. HEK 293 cells were transiently transfected with pcDNA3.1 expression vectors containing SR-BI, SR-BII, and green fluorescent protein (Control) cDNAs, respectively. Two days after transfection, cells were incubated with [^{3}H]HDL for 5 h and cell-associated label was quantified as described in Materials and Methods. Data are means ± SD of triplicate measurements. Similar results were obtained in two additional experiments.

Western blot analysis

Membrane extracts of rabbit tissue specimens were prepared as described (21). For Western blot analysis, 30 μg of protein of membrane extracts were analyzed by 10–20% gradient SDS-PAGE and transferred to polyvinylidene difluoride membranes. Immunodetection of SR-BI and SR-BII was carried out using our digoxigenin (DIG)-labeled isoform-specific antibodies described above. In peptide competition experiments, SR-BI and SR-BII antibodies were blocked with 5-fold excesses of peptides SR-$BI_{495–509}$ and SR-$BII_{486–501}$, respectively. After immunodetection with a secondary horseradish peroxidase (HRP)-conjugated anti-DIG antibody (Dako, Glostrup, Denmark) and chemoluminescent reaction using Super Signal West Dura Extended Duration Substrate (Pierce, Rockford, IL), the blots were exposed to XAR films (Kodak, Rochester, NY) or visualized with a Fluor-S-Imager using Quantity One version 4.1 software (Bio-Rad, Hercules, CA).

Immunohistochemical staining

Sections (5 μm) of snap-frozen tissue specimens were mounted on poly-L-lysine (Sigma, St. Louis, MO)-coated slides, air dried, and fixed with acetone. SR-BI was visualized by incubating with our polyclonal DIG-labeled anti-SR-BI antibody. In peptide competition experiments, a 5-fold excess of peptide SR-$BI_{495–509}$ was used to block the SR-BI antibody. The primary antibody was detected using a monoclonal mouse anti-DIG antibody (Roche, Mannheim, Germany) followed by a HRP-conjugated goat anti-mouse Ig antibody (Pierce). 3-Amino-9-ethyl-carbozole (Sigma) was used as a substrate, and sections were counterstained with Harris's hematoxylin. In double-staining experiments, the primary anti-SR-BI antibody was detected by a HRP-linked polyclonal anti-DIG antibody (Dako). Monoclonal antibodies specific for rabbit leukocyte antigen (RLA)-DR (Southern Biotechnology, Birmingham, AL) and CD31 (clone JC 70 A; DAKO) were detected by a biotinylated species-specific anti-mouse Ig (Amersham) followed by alkaline phosphatase-conjugated Extravidin (Sigma) using 4-nitroblue tetrazolium chloride as a substrate. All sections were mounted using an aqueous mounting medium (Dako).

RESULTS

Cloning of full-length rabbit SR-BI and SR-BII cDNAs

Rabbit full-length SR-BI and SR-BII cDNAs were isolated in two steps. First, a set of degenerate primers designed based on highly conserved regions of Chinese hamster,

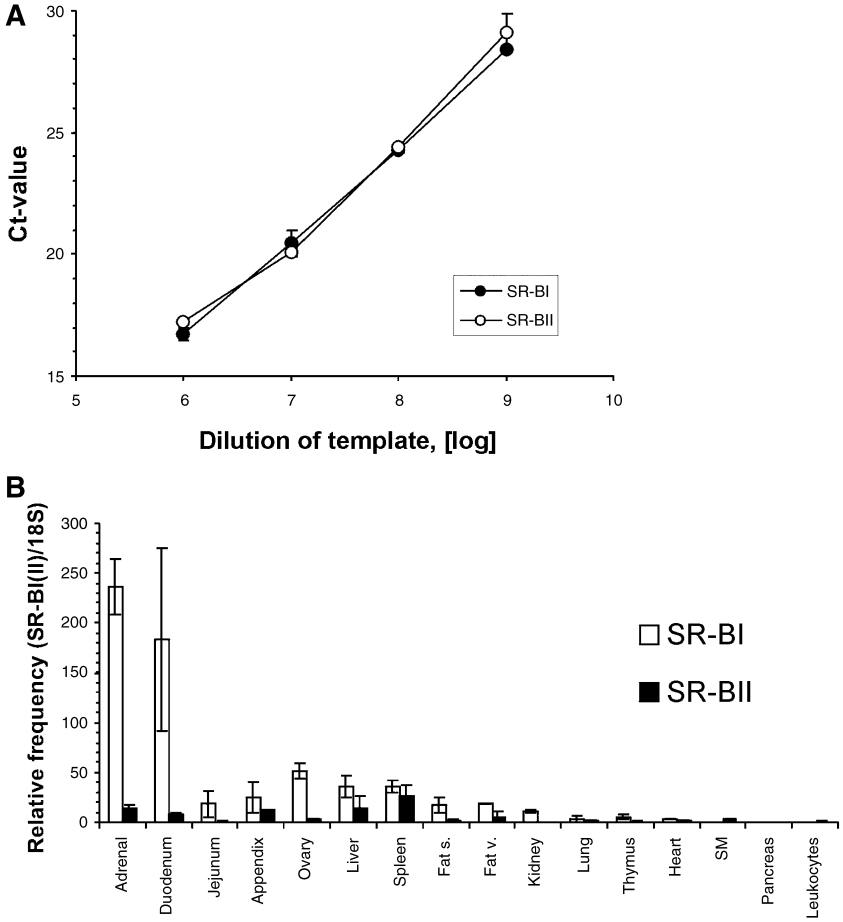

Fig. 4. Isoform-specific SR-BI and SR-BII Taqman Real Time PCR. A: Representative Taqman PCR standard curves specific for SR-BI (closed circles) and SR-BII mRNA (open circles). Ten-fold dilution series of samples containing standardized amounts of SR-BI and SR-BII PCR fragments were used as templates. Data are means ± SD of triplicate measurements. B: Isoform-specific Taqman PCR measurements of SR-BI and SR-BII mRNA distribution in various rabbit tissues. All data are corrected for 18S RNA frequency. Data are means ± SD of three independent experiments. Ct, cycle threshold; Fat s., subcutaneous fat; Fat v., visceral fat; SM, skeletal muscle.

mouse, rat, bovine, and human SR-BI were used to amplify a 530 bp fragment from reverse-transcribed total rabbit liver RNA. Subcloning of this fragment and subsequent sequence analysis showed an overall high similarity to the corresponding hamster, rat, murine, bovine, and human SR-BI sequences. Based on the sequence data of the 530 bp fragment, new divergently directed primers were designed and used for amplification of the remaining 5′ and 3′ cDNA fragments by RACE-PCR, respectively. One unique 5′ cDNA fragment and two different 3′ fragments were amplified covering the complete rabbit SR-BI and SR-BII cDNA sequences of 2,400 and 2,271 bp, respectively.

Characterization of rabbit SR-BI and SR-BII cDNAs

Our rabbit SR-BI cDNA clone consisted of a 100 bp 5′-untranslated region, the entire coding region followed by a 770 bp 3′-untranslated region including the poly(A)$^+$ tail. The open reading frame of 1530 bp predicts a protein of 509 amino acids. A 129 bp fragment of SR-BI cDNA was missing in the SR-BII cDNA fragment. Because this 129 bp fragment, which we assume to be a single exon, contained the stop codon of SR-BI, the first 105 bp of the 3′ noncoding region of SR-BI represent the last part of the coding region of SR-BII. The same situation has been found in all other species whose SR-BI and SR-BII cDNAs have been cloned.

Rabbit SR-BI cDNA showed a similarity of 84.1% to the human homolog, much higher than the corresponding fragments of rodents (79.8%). The predicted SR-BI translation products of all known sequences also exhibited a high degree of sequence similarity (74.0–88.1% identity compared with the human sequence). Again, the amino acid sequence of rabbit SR-BI showed a higher degree of similarity to the human sequence than the SR-BI sequences of rodents. A comparison of SR-BI amino acid sequences from six different species is shown in **Fig. 1**. The corresponding C-terminal ends unique to SR-BII showed a lower degree of similarity (62–67%), as shown in **Fig. 2**.

HDL uptake

To demonstrate the ability of rabbit SR-BI and SR-BII to mediate selective uptake, we performed HDL uptake assays in HEK 293 cells, which are an established SR-BI-deficient cell model (22). Transient expression of both rabbit SR-BI and SR-BII in these cells led to a marked increase of cell-associated label after 5 h of incubation with [^{3}H]HDL compared with cells transfected with the control vector containing GFP cDNA (**Fig. 3**).

Isoform-specific quantification of SR-BI and SR-BII mRNA in various tissues

Based on our rabbit SR-BI and SR-BII cDNA sequence data, we designed isoform-specific Taqman Real Time PCR systems. Both systems showed the same amplification efficiency as shown by overlaying the corresponding stan-

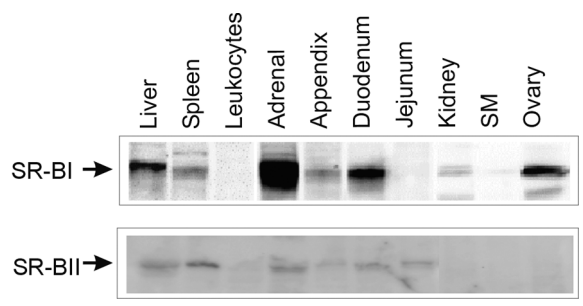

Fig. 5. Western blot analysis showing tissue-specific expression of SR-BI and SR-BII in rabbits. Twenty micrograms of protein of membrane extracts from various rabbit tissues was analyzed by SDS-PAGE, blotted onto polyvinylidene difluoride membranes, and stained using antibodies specific for SR-BI and SR-BII. SM, skeletal muscle.

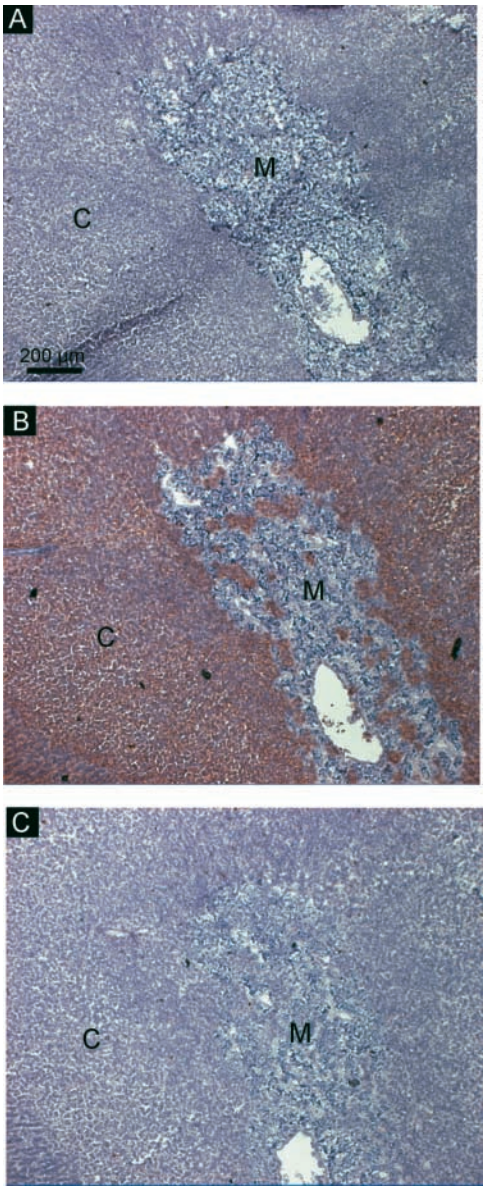

Fig. 6. Immunostaining of rabbit adrenal sections for SR-BI. Frozen sections of rabbit adrenal gland were stained with our anti-rabbit SR-BI antibody (B and C). Control experiments were performed without primary antibody (A) or using competing peptide SR-BI$_{495–509}$ (C). Nuclei were stained with Harris's hematoxylin. All sections were photographed at ×50 magnification. C, cortex; M, medulla.

dard curves for SR-BI and SR-BII (**Fig. 4A**). This enabled direct comparison of SR-BI and SR-BII mRNA frequencies determined in various tissues of three female Chinchilla Bastard rabbits. All data were normalized to the respective 18S RNA concentrations. We consistently found the highest values of SR-BI expression in adrenal gland and in the proximal part of the intestine (duodenum) and considerable amounts in liver and ovary. In addition to the findings of published results from other species, significant expression of SR-BI mRNA was also detected in appendix and spleen (Fig. 4B). Marked differences were detected in the expression ratios of SR-BI/SR-BII. Levels of SR-BII were less than 10% of the corresponding SR-BI values in most tissues except for appendix, liver, and spleen, with 26, 37, and 77%, respectively.

Expression of SR-BI protein in various tissues

Isoform-specific antibodies were developed by immunization of Chinchilla Bastard rabbits with peptides corresponding to amino acids 495–509 and 486–509 of the C-terminal ends of rabbit SR-BI and SR-BII, respectively. The specificity of the antibodies was proved by ELISA and Western blot analyses, including peptide competition experiments (data not shown).

Expression profiles of SR-BI and SR-BII protein levels were examined in various tissue specimens of three Chinchilla Bastard rabbits. We found the highest levels of SR-BI protein in adrenal gland and duodenum and high levels in liver, ovary, appendix, and spleen (**Fig. 5**). In agreement with the results from the Taqman PCR measurements, SR-BII was highest in membrane extract of spleen and also significantly expressed in liver, adrenal gland, and intestine. No significant expression of SR-BII was found in membrane extracts of skeletal muscle, kidney, leukocytes, and ovary (Fig. 5).

Immunohistochemical staining

To further characterize SR-BI expression in rabbit tissue, we performed immunohistochemical staining of frozen sections from different organs. High expression of SR-BI was found in the cortex but not in the medulla of adrenal gland (**Fig. 6B**). This expression pattern favors the cholesteryl uptake function of SR-BI, as cells within the adrenal cortex require high amounts of cholesterol for biosynthesis of steroid hormones. This is not the case for cells within the adrenal medulla, which mainly produce catecholamines. The specificity of the SR-BI antibody was demonstrated by peptide competition (Fig. 6C).

As expected, we also found high SR-BI expression in the liver. Within the hepatic lobules, we noticed a gradient of expression from portal to central veins indicating high expression at the regions of endocrine liver functions, including lipid metabolism (**Fig. 7A,** C). This expression pattern is distinct from that of the LDL receptor, in which no such gradient could be detected, and in contrast to the expression gradient of apoE, which is involved in lipoprotein synthesis (23, 24).

Immunostaining of sections from rabbit intestine showed high SR-BI expression in the duodenum but only low expression in the jejunum (data not shown), in good

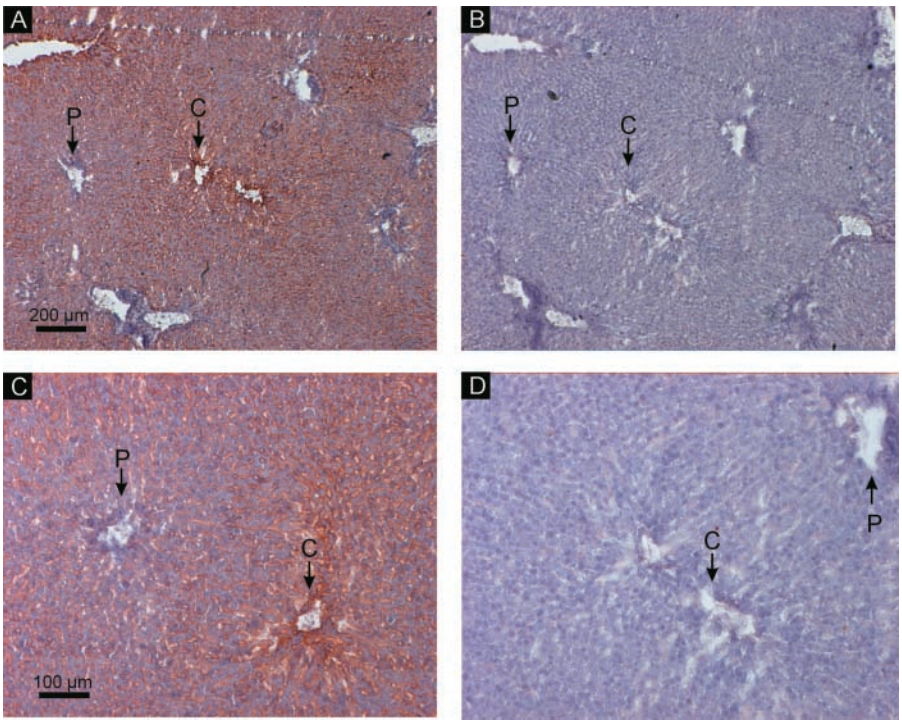

Fig. 7. Immunostaining of rabbit liver sections for SR-BI. Frozen sections of rabbit liver were stained with our anti-rabbit SR-BI antibody (A and C). Control experiments without primary antibody are shown in B and D. Nuclei were stained with Harris's hematoxylin. Sections were photographed at ×50 (A and B) and ×100 (C and D) magnification. C, central vein; P, peripheral vein.

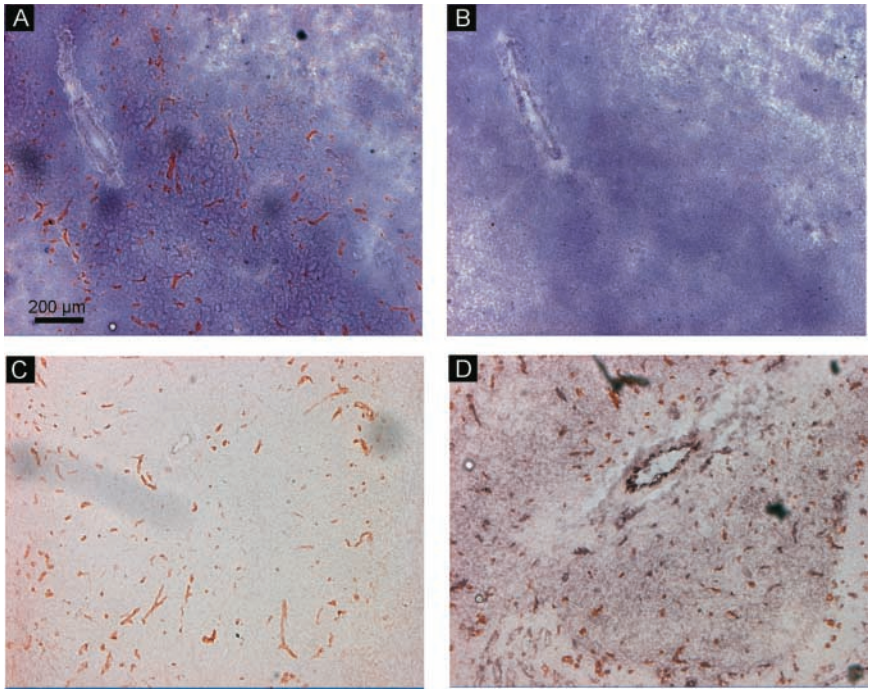

Fig. 8. Immunostaining of rabbit spleen sections for SR-BI. Frozen sections of rabbit spleen were stained with our anti-rabbit SR-BI antibody (A and C). A control experiment without primary antibody is shown in B. Nuclei were stained with Harris's hematoxylin in A and B. C: Shows SR-BI staining of spleen without Harris's hematoxylin staining. A double-staining experiment is shown in D, in which SR-BI staining as in C was followed by incubation with anti-CD31 antibody. All sections were photographed at ×50 magnification.

agreement with published data from mice and humans (25–28).

Next, we tried to confirm data from our real-time PCR and Western blot experiments reported above indicating significant SR-BI expression in the spleen. SR-BI expression was found only in cells within lymphatic nodules of the periarterial lymphatic sheath (**Fig. 8A**). In double-staining experiments, most of these cells were recognized by an endothelial cell-specific antibody (Fig. 8D) but not by an RLA-DR-specific antibody (data not shown). Given their characteristic shape, these cells may be endothelial cells of capillaries within the peripheral white pulp.

We were not able to detect significant amounts of SR-BII expression in any of our immunohistochemical experiments with various tissue specimens (data not shown). However, the SR-BII antibody served as an excellent control for our experiments using the SR-BI antibody, as both antibodies were raised, purified, and labeled using the same protocols.

In summary, these immunohistochemical experiments not only confirmed the results from real-time PCR and Western blot analysis but also provided additional information regarding the expression pattern of SR-BI within single organs.

DISCUSSION

In numerous studies, SR-BI was shown not only to function as a physiologically relevant receptor for HDL-derived cholesterol but also to play an important role in the development of atherosclerosis. These studies were performed exclusively in mouse and rat. However, these animal species are not ideal models to study atherosclerosis, mainly for two reasons. First, the lipoprotein profile of rodents is different from that of humans, with HDL being the predominant lipoprotein particle, probably attributable to the lack of cholesteryl ester transfer protein activity in the plasma of rodents. Second, mice and rats are not prone to the development of atherosclerosis. To study the role of SR-BI in the development of atherosclerosis, we searched for an animal model displaying cholesteryl ester transfer protein activity in the plasma, showing a lipoprotein pattern similar to that of humans, and developing atherosclerosis in response to a cholesterol-rich diet. Rabbits match all of these criteria and can be expected to express SR-BI, as early studies indicated the presence of a pathway for cellular uptake of high density cholesterol and thus the expression of SR-BI in these animals (29, 30).

In this report, we present, for the first time, cloning and characterization of cDNAs encoding SR-BI and SR-BII in rabbits. Rabbit SR-BI cDNA shared high overall homology with that of human, higher than that of any of the homologs identified to date in other species. This high homology not only confirmed the isolation of rabbit SR-BI but also promised a similar function compared with the human homolog.

To further elucidate this issue, we performed HDL cholesteryl ester uptake assays in HEK 293 cells, an estab-

lished SR-BI-deficient cell model (22). Transient expression of rabbit SR-BI and SR-BII led to 5.3- and 3.4-fold increases in HDL uptake, respectively, compared with cells transfected with the control vector. The apparent lower efficiency of SR-BII is in good agreement with published data from mice in which SR-BII mediated selective cellular uptake of cholesteryl ether from HDL with approximately 4-fold lower efficiency than SR-BI (2). However, results from our transfection experiments demonstrating the ability of both rabbit SR-BI and SR-BII to mediate selective lipid uptake strongly suggest an HDL receptor function for both isoforms.

Next, we developed an isoform-specific Taqman PCR system to quantify SR-BI and SR-BII mRNAs in various rabbit tissues. Both systems showed the same PCR efficiency, allowing direct comparison of the respective expression levels. As seen in other species, we found high levels of SR-BI mRNA in rabbit adrenal gland, liver, and the proximal part of the intestine (duodenum). These assays additionally indicated SR-BI expression in appendix and spleen. Expression of SR-BII was rather low compared with the respective levels of SR-BI, indicating that splice variant SR-BII may play a minor role for cholesterol homeostasis.

The development and application of isoform-specific antibodies raised against peptides corresponding to the C-terminal ends of rabbit SR-BI and SR-BII allowed us to confirm studies of SR-BI expression in other species and also to confirm our results from Taqman Real Time PCR measurements. In addition to the well-known sites of SR-BI expression as adrenal gland, liver, and duodenum, we again found SR-BI expression in membrane extracts of appendix and spleen. Western blot experiments using the SR-BII-specific antibody were in good agreement with the results of real-time PCR measurements, as in both assays the highest levels of SR-BII were found in the spleen.

As we were interested to investigate the function of SR-BI in certain tissues, we examined the expression of SR-BI in more detail by immunohistochemical staining of frozen sections. In adrenal gland, high expression of SR-BI was found in the cortex, with no detectable staining in the medulla. This is consistent with the proposed function of SR-BI in cholesterol uptake, as cells within the cortex require high amounts of cholesterol for the biosynthesis of steroid hormones, whereas this is not the case for cells of the medulla, which mainly produce adrenaline and noradrenaline.

Immunohistochemical staining of liver sections also yielded interesting results. We found an increasing portal to central vein gradient of expression within liver lobules. High expression of SR-BI in the liver generated by infection of mice with an adenoviral vector containing SR-BI cDNA led to increased HDL cholesterol uptake in the liver, decreased levels of HDL cholesterol, and increased bile cholesterol, favoring SR-BI to function in cholesterol uptake in the liver (7). The gradient of expression may be important for this function, as it is opposed to a decreasing portal to central vein gradient of expression of apoE involved mainly in the synthesis and secretion of lipoproteins in the liver (24). Interestingly, the LDL receptor did not show differentiated expression within the liver lobule, suggesting a somehow more universal function for this receptor (23).

As we were able to detect expression of SR-BI in the spleen by real-time PCR and Western blot experiments, we further investigated this issue by performing immunohistochemical staining of frozen sections in which we found staining of cells within the peripheral white pulp. These cells, however, looked like endothelial cells of capillaries. In double-staining experiments, the majority of SR-BI-positive cells were recognized by an endothelial cell-specific anti-CD31 antibody but not by an anti-RLA-DR antibody specific for major histocompatibility complex class II cells. Therefore, SR-BI is not likely to exert immunological functions in these tissues. Expression in endothelial cells of spleen capillaries suggests a role in cellular cholesterol uptake. Cholesteryl ester deposition in the spleen of patients with impaired cholesterol degradation capacities, i.e., cholesteryl ester storage disease and Wolman disease patients displaying acid lipase deficiency, demonstrated the presence of cholesterol uptake processes within the spleen.

In conclusion, we clearly identified the presence of a rabbit homolog of SR-BI. Characterization of SR-BI expression in various tissues at the mRNA and protein levels confirmed data from other species and suggested SR-BI to be mainly involved in cellular cholesterol uptake. This view could be internally confirmed by immunohistochemical experiments. We feel that these studies provide additional information regarding the role of SR-BI in reverse cholesterol transport. In addition, the characterization of SR-BI in rabbits should lead to an entirely novel set of studies to investigate the role of SR-BI in atherosclerosis.

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REFERENCES

1. Krieger, M. 2001. Scavenger receptor class B type I is a multiligand HDL receptor that influences diverse physiologic systems. *J. Clin. Invest.* **108:** 793–797.
2. Webb, N. R., P. M. Connell, G. A. Graf, E. J. Smart, W. J. de Villiers, F. C. de Beer, and D. R. van der Westhuyzen. 1998. SR-BII, an isoform of the scavenger receptor BI containing an alternate cytoplasmic tail, mediates lipid transfer between high density lipoprotein and cells. *J. Biol. Chem.* **273:** 15241–15248.
3. Acton, S. L., P. E. Scherer, H. F. Lodish, and M. Krieger. 1994. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J. Biol. Chem.* **269:** 21003–21009.
4. Calvo, D., and M. A. Vega. 1993. Identification, primary structure, and distribution of CLA-1, a novel member of the CD36/LIMPII gene family. *J. Biol. Chem.* **268:** 18929–18935.
5. Cao, G., C. K. Garcia, K. L. Wyne, R. A. Schultz, K. L. Parker, and H. H. Hobbs. 1997. Structure and localization of the human gene encoding SR-BI/CLA-1. Evidence for transcriptional control by steroidogenic factor 1. *J. Biol. Chem.* **272:** 33068–33076.
6. Temel, R. E., B. Trigatti, R. B. DeMattos, S. Azhar, M. Krieger, and D. L. Williams. 1997. Scavenger receptor class B, type I (SR-BI) is the major route for the delivery of high density lipoprotein choles-

terol to the steroidogenic pathway in cultured mouse adrenocortical cells. *Proc. Natl. Acad. Sci. USA.* **94:** 13600–13605.
7. Kozarsky, K. F., M. H. Donahee, A. Rigotti, S. N. Iqbal, E. R. Edelman, and M. Krieger. 1997. Overexpression of the HDL receptor SR-BI alters plasma HDL and bile cholesterol levels. *Nature.* **387:** 414–417.
8. Rigotti, A., B. L. Trigatti, M. Penman, H. Rayburn, J. Herz, and M. Krieger. 1997. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc. Natl. Acad. Sci. USA.* **94:** 12610–12615.
9. Varban, M. L., F. Rinninger, N. Wang, V. Fairchild-Huntress, J. H. Dunmore, Q. Fang, M. L. Gosselin, K. L. Dixon, J. D. Deeds, S. L. Acton, A. R. Tall, and D. Huszar. 1998. Targeted mutation reveals a central role for SR-BI in hepatic selective uptake of high density lipoprotein cholesterol. *Proc. Natl. Acad. Sci. USA.* **95:** 4619–4624.
10. Wang, N., T. Arai, Y. Ji, F. Rinninger, and A. R. Tall. 1998. Liver-specific overexpression of scavenger receptor BI decreases levels of very low density lipoprotein ApoB, low density lipoprotein ApoB, and high density lipoprotein in transgenic mice. *J. Biol. Chem.* **273:** 32920–32926.
11. Ueda, Y., L. Royer, E. Gong, J. Zhang, P. N. Cooper, O. Francone, and E. M. Rubin. 1999. Lower plasma levels and accelerated clearance of high density lipoprotein (HDL) and non-HDL cholesterol in scavenger receptor class B type I transgenic mice. *J. Biol. Chem.* **274:** 7165–7171.
12. Trigatti, B., H. Rayburn, M. Vinals, A. Braun, H. Miettinen, M. Penman, M. Hertz, M. Schrenzel, L. Amigo, A. Rigotti, and M. Krieger. 1999. Influence of the high density lipoprotein receptor SR-BI on reproductive and cardiovascular pathophysiology. *Proc. Natl. Acad. Sci. USA.* **96:** 9322–9327.
13. Arai, T., N. Wang, M. Bezouevski, C. Welch, and A. R. Tall. 1999. Decreased atherosclerosis in heterozygous low density lipoprotein receptor-deficient mice expressing the scavenger receptor BI transgene. *J. Biol. Chem.* **274:** 2366–2371.
14. Ueda, Y., E. Gong, L. Royer, P. N. Cooper, O. L. Francone, and E. M. Rubin. 2000. Relationship between expression levels and atherogenesis in scavenger receptor class B, type I transgenics. *J. Biol. Chem.* **275:** 20368–20373.
15. Kozarsky, K. F., M. H. Donahee, J. M. Glick, M. Krieger, and D. J. Rader. 2000. Gene transfer and hepatic overexpression of the HDL receptor SR-BI reduces atherosclerosis in the cholesterol-fed LDL receptor-deficient mouse. *Arterioscler. Thromb. Vasc. Biol.* **20:** 721–727.
16. Kolodgie, F. D., A. S. Katocs, Jr., E. E. Largis, S. M. Wrenn, J. F. Cornhill, E. E. Herderick, S. J. Lee, and R. Virmani. 1996. Hypercholesterolemia in the rabbit induced by feeding graded amounts of low-level cholesterol. Methodological considerations regarding individual variability in response to dietary cholesterol and development of lesion type. *Arterioscler. Thromb. Vasc. Biol.* **16:** 1454–1464.
17. Sambrook, J., and W. R. Russell. 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
18. Patsch, J. R., S. Sailer, G. Kostner, F. Sandhofer, A. Holasek, and H. Braunsteiner. 1974. Separation of the main lipoprotein density classes from human plasma by rate-zonal ultracentrifugation. *J. Lipid Res.* **15:** 356–366.
19. Kaser, S., C. F. Ebenbichler, H. J. Wolf, A. Sandhofer, U. Stanzl, A. Ritsch, and J. R. Patsch. 2001. Lipoprotein profile and cholesteryl ester transfer protein in neonates. *Metabolism.* **50:** 723–728.
20. Stangl, H., M. Hyatt, and H. H. Hobbs. 1999. Transport of lipids from high and low density lipoproteins via scavenger receptor-BI. *J. Biol. Chem.* **274:** 32692–32698.
21. Jokinen, E. V., K. T. Landschulz, K. L. Wyne, Y. K. Ho, P. K. Frykman, and H. H. Hobbs. 1994. Regulation of the very low density lipoprotein receptor by thyroid hormone in rat skeletal muscle. *J. Biol. Chem.* **269:** 26411–26418.
22. Lambert, G., M. B. Chase, K. Dugi, A. Bensadoun, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1999. Hepatic lipase promotes the selective uptake of high density lipoprotein-cholesteryl esters via the scavenger receptor B1. *J. Lipid Res.* **40:** 1294–1303.
23. Fong, L. G., E. Bonney, J. C. Kosek, and A. D. Cooper. 1989. Immunohistochemical localization of low density lipoprotein receptors in adrenal gland, liver, and intestine. *J. Clin. Invest.* **84:** 847–856.
24. Massimi, M., S. R. Lear, D. L. Williams, A. L. Jones, and S. K. Erickson. 1999. Differential expression of apolipoprotein E messenger RNA within the rat liver lobule determined by in situ hybridization. *Hepatology.* **29:** 1549–1555.
25. Cai, S. F., R. J. Kirby, P. N. Howles, and D. Y. Hui. 2001. Differentiation-dependent expression and localization of the class B type I scavenger receptor in intestine. *J. Lipid Res.* **42:** 902–909.
26. Schulthess, G., S. Compassi, M. Werder, C. H. Han, M. C. Phillips, and H. Hauser. 2000. Intestinal sterol absorption mediated by scavenger receptors is competitively inhibited by amphipathic peptides and proteins. *Biochemistry.* **39:** 12623–12631.
27. Voshol, P. J., M. Schwarz, A. Rigotti, M. Krieger, A. K. Groen, and F. Kuipers. 2001. Down-regulation of intestinal scavenger receptor class B, type I (SR-BI) expression in rodents under conditions of deficient bile delivery to the intestine. *Biochem. J.* **356:** 317–325.
28. Werder, M., C. H. Han, E. Wehrli, D. Bimmler, G. Schulthess, and H. Hauser. 2001. Role of scavenger receptors SR-BI and CD36 in selective sterol uptake in the small intestine. *Biochemistry.* **40:** 11643–11650.
29. Mackinnon, M., J. Savage, R. Wishart, and P. Barter. 1986. Metabolism of high density lipoproteins by the perfused rabbit liver. *J. Biol. Chem.* **261:** 2548–2552.
30. Goldberg, D. I., W. F. Beltz, and R. C. Pittman. 1991. Evaluation of pathways for the cellular uptake of high density lipoprotein cholesterol esters in rabbits. *J. Clin. Invest.* **87:** 331–346.