Differentiation-dependent expression and localization of the class B type I scavenger receptor in intestine

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Abstract **The current study used the human Caco-2 cell line and mouse intestine to explore the topology of expression of the class B type I scavenger receptor (SR-BI) in intestinal cells. Results showed that intestinal cells expressed only the SR-BI isoform with little or no expression of the SR-BII variant. The expression of SR-BI in Caco-2 cells is differentiation dependent, with little or no expression in preconfluent undifferentiated cells. Analysis of Caco-2 cells cultured in Transwell porous membranes revealed the presence of SR-BI on both the apical and basolateral cell surface. Immunoblot analysis of mouse intestinal cell extracts demonstrated a gradation of SR-BI expression along the gastrocolic axis of the intestine, with the highest level of expression in the proximal intestine and decreasing to minimal expression levels in the distal intestine. Immunofluorescence studies with SR-BI-specific antibodies also confirmed this expression pattern. Importantly, the immunofluorescence studies also revealed that SR-BI immunoreactivity was most intense in the apical membrane of the brush border in the duodenum. The crypt cells did not show any reactivity with SR-BI antibodies. The localization of SR-BI in the jejunum was found to be different from that observed in the duodenum. SR-BI was present on both apical and basolateral surfaces of the jejunum villus. Localization of SR-BI in the ileum was also different, with little SR-BI detectable on either apical or basolateral membranes. Taken together, these results suggest that SR-BI has the potential to serve several functions in the intestine. The localization of SR-BI on the apical surface of the proximal intestine is consistent with the hypothesis of its possible role in dietary cholesterol absorption, whereas SR-BI present on the basolateral surface of the distal intestine suggests its possible involvement in intestinal lipoprotein uptake.**—Cai, S. F., R. J. Kirby, P. N. Howles, and D. Y. Hui. **Differentiation-dependent expression and localization of the class B type I scavenger receptor in intestine.** ***J. Lipid Res.*** **2001.** 42: **902–909.**

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The class B type I scavenger receptor (SR-BI) is an 82-kDa membrane protein responsible for the selective cellular uptake of neutral lipids from lipoproteins (1–3) and the binding of anionic phospholipids (2). The binding of HDL and anionic phospholipids to SR-BI on the cell surface may also promote cholesterol efflux from cells (4, 5). An alternative form of SR-BI, derived from alternative splicing of its mRNA, is also present on cell surfaces and capable of mediating selective uptake of neutral lipids from HDL (6, 7).

The expression of SR-BI is most abundant in the liver and steroidogenic tissues, and in macrophages within the vascular wall (1, 5, 8, 9). In the liver, SR-BI is expressed primarily in the parenchymal cells under normal conditions. However, pharmacological doses of estrogen or chronic feeding of rats with a cholesterol-enriched diet has been shown to reduce SR-BI expression in hepatocytes while inducing its expression in the Kupffer cells (8, 10). In hamsters, hepatic expression of SR-BI is resistant to dietary cholesterol feeding, but can be induced by a PUFA-enriched diet (11, 12). Both estrogen- and diet-induced hepatic SR-BI expression correlate positively with increased HDL cholesteryl ester transport to the liver and reduction of plasma HDL cholesterol concentration (8, 11). A physiological role of hepatic SR-BI in mediating uptake of HDL-associated neutral lipids was demonstrated in genetically modified animals. Overexpression of SR-BI in the liver, either by adenovirus-mediated gene transfer or tissue-specific transgenic overexpression, increased hepatic clearance of HDL-associated free cholesterol and facilitated its transport to the bile (13–16). In contrast, SR-BI-deficient mice were shown to be defective in transporting HDL cholesterol to the bile, and as a consequence displayed elevated plasma HDL cholesterol (16–18).

SR-BI in steroidogenic tissues facilitates HDL cholesterol uptake and its utilization for steroidogenic hormone synthesis. Its expression in these tissues is modulated by cellular cholesterol availability and by the demand for steroid hormone production. For example, SR-BI expression in adrenals is induced by adrenocorticotropin or by the depletion of intracellular cholesterol stores (9, 19–21). In contrast, adrenal SR-BI expression is suppressed after glucocorticoid treatment (19, 21). Likewise, in ovarian

Abbreviations: SR-BI, class B type I scavenger receptor; SR-BII, an alternatively spliced isoform of SR-BI; SSC, buffer containing 150 mM NaCl and 15 mM sodium citrate.

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cells and Leydig cells of the testis, SR-BI levels are elevated after hormone desensitization (8, 22, 23). SR-BI in these cells is localized to surface microvilli and in complex channel systems within the cytoplasm, which are specialized components for cholesterol uptake by steroidogenic cells (22–24).

The expression of SR-BI was also detected in undifferentiated monocytes as well as in differentiated macrophages (5, 25, 26). Its level of expression in cultured macrophages showed a positive correlation with the rate of cholesterol efflux from these cells (5). The latter observation, along with demonstration of the presence of SR-BI in caveolae (27), which is the site of cholesterol efflux (28), suggests that SR-BI may participate in cellular cholesterol efflux as well as in cholesterol uptake. This hypothesis was supported by experiments showing increased cholesterol efflux from cultured cells overexpressing SR-BI (5).

More recently, SR-BI was shown to be present in the intestine (8) and was postulated to be the cholesterol transporter in mediating dietary cholesterol absorption (29). However, possible regulation of SR-BI expression in the intestine has not been addressed. Although SR-BI was reported to be present in the brush border membrane (29), the immunohistochemistry data showed low reactivity, which may be due either to a low level of SR-BI expression or to the low sensitivity of the procedure employed. There is also no current information regarding the distribution of SR-BI within the intestinal mucosa. Moreover, whether SR-BI or its alternatively spliced form SR-BII is present in the intestine is also unknown. The current study was undertaken to determine whether intestinal SR-BI expression is differentiation dependent. In addition, we provide additional data about SR-BI expression along the villus-crypt axis and along the longitudinal axis of the intestine.

MATERIALS AND METHODS

Mice and tissue culture

The Caco-2 human colonic adenocarcinoma cell line, the hepatoma cell line HepG2, and the monkey kidney cell line Cos-1 were obtained from the American Type Culture Collection (Rockville, MD: ATCC HTB 37) and cultured in DMEM containing glucose (4.5 g/l), 20% FBS, 2 mM L-glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37°C in 10% CO_2. Stock cultures were maintained in 75-cm^2 flasks and media were changed every 2–3 days. For experiments, the Caco-2 cells were seeded in 24-well culture dishes or on polycarbonate semipermeable Transwell membranes with 0.4 μm pores (Costar, Cambridge, MA) in either 24- or 6-well culture dishes, seeded at a density of 10^5 or 10^6 cells per well, respectively.

C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). The mice were housed in a temperature- and humidity-controlled room with a 12-h light:dark cycle and fed Purina mouse chow before tissue collection. Mice between the ages of 6 and 8 weeks were used for all experiments.

RNA isolation and analysis

The Caco-2 cells were cultured on Transwell membranes in six-well culture dishes. When the cells reached confluency, they were harvested or allowed to differentiate by culturing for an additional 10-day period. The cells were washed with PBS, scraped from the Transwell membranes, and centrifuged at 1,000 *g* for 10 min. Small intestine was excised from anesthetized mice, flushed with cold 0.9% saline, and sectioned into duodenum (first 25%), jejunum (middle 50%), and ileum (last 25%). Each section was processed immediately as described below.

Total cellular RNA was isolated with RNA Stat-60 (Tel-Test, Friendswood, TX) and quantitated by absorbance at 260 nm. Complementary DNA was synthesized by denaturing the RNA at 65°C followed by reverse transcription with SuperScript RT (Life Technologies, Rockville, MD), using oligo$(dT)_{16}$ as the primer. The cDNA product was amplified with a set of SR-BI-specific primers, 5′-TTCTGCCCGTGCCTGGAGTC-3′ and 5′-GCTGTCTGCTGGGAGAGTC-3′, which overlap nucleotides 1,027 to 1,699 of the human SR-BI mRNA sequence (7) and include the region that is divergent for SR-BI and SR-BII isoforms. Amplification was performed in 80 μl containing 2 mM $MgCl_2$, a 0.15 μM concentration of each oligonucleotide primer, and 2.5 units of *Taq* DNA polymerase. Thirty-five cycles of PCR amplification were performed with denaturation set at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 90 s. The PCR products were separated on a 1% agarose gel and visualized by staining with ethidium bromide. Resulting bands were excised from the gel and sequenced by the method of Sanger, Nicklen, and Coulson (30).

Northern blot hybridization was carried out by denaturing 20 μg of total cellular RNA in 20 μl of sample buffer containing 50% formamide and 16.5% formaldehyde for 10 min at 65°C, followed by electrophoresis on a 1% agarose gel in MOPS buffer containing 10% formaldehyde. The samples were transferred onto nitrocellulose paper, baked at 80°C in a vacuum oven, and then prehybridized for 2 h with buffer containing 5× SSC buffer (150 mM NaCl and 15 mM sodium citrate), 5× Denhardt's solution, 0.1% SDS, 50% formamide, and denatured salmon sperm DNA (2 mg/ml). Hybridization was carried out by incubating the nitrocellulose paper overnight at 50°C with the same buffer containing either ^{32}P-labeled human SR-BI or glyceraldehyde-3-phosphate dehydrogenase cDNA probe (1×10^6 cpm/ml). The membranes were washed twice for 20 min at 23°C with 2× SSC buffer containing 0.1% SDS, and then twice for 20 min at 50°C with 0.2× SSC buffer containing 0.1% SDS. Hybridization signals were detected by exposing the nitrocellulose paper to a phosphoimager screen and then analyzed with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The image was captured with Adobe (San Jose, CA) Photoshop.

Membrane preparation and Western blot analyses

Centrifuged Caco-2 cell pellets and segments of mouse intestine were homogenized at 4°C in 10 ml of buffer [10 mM HEPES, pH 7.4; 1 mM PMSF; 1% protease inhibitor cocktail (Sigma, St. Louis, MO)]. Total membrane proteins were prepared by adding 1.25 ml of 2 M sucrose followed by centrifugation at 700 *g* for 10 min. The supernatant was transferred to a fresh tube and centrifuged again at 700 *g* for 10 min. Supernatant was then centrifuged at 100,000 *g* for 30 min. Membrane pellets were resuspended in 0.5 ml of buffer A containing 25 mM Tris-HCl (pH 7.4), 140 mM NaCl, and 1 mM $CaCl_2$. Protein concentration was determined by the method of Lowry et al. (31).

For Western blot analysis of SR-BI in the various samples, 50 μg of proteins was solubilized in gel electrophoresis buffer containing 62.5 mM Tris-HCl (pH 8), 3% SDS, 10% glycerol, and 0.1% bromophenol blue (32), electrophoresed on 10% SDS-polyacrylamide gels, and transferred to nitrocellulose paper. Immunoblot analysis was performed as described previously (33), using polyclonal rabbit antibodies raised against residues 495 to 509 of the extracellular domain of human SR-BI or the cytoplasmic domain

of SR-BI (Novus Biologicals, Littleton, CO). Immunoreactive proteins were visualized by enhanced chemiluminescence (ECL; Amersham, Arlington Heights, IL). Liver homogenates were used as positive control. Linearity of the chemiluminescence detection was established by varying concentrations of membrane proteins applied to the gels. The results were scanned into the computer and relative intensities of the bands were determined by image analysis with the Scion Image program (Scion, Frederick, MD).

Determination of SR-BI orientation in Caco-2 cell surface

The Caco-2 cells cultured to 12–15 days postconfluency on Transwell membranes were washed twice with serum-free DMEM and then incubated overnight at 37°C in serum-free medium containing either a 1:1,000 dilution of antiserum raised against the extracellular domain of human SR-BI or a 1:200 dilution of antiserum prepared against an irrelevant antigen. The antibodies were added to medium at either the apical or the basolateral compartment of the Transwell chamber. At the end of the incubation period, the cells were washed twice with serum-free medium and then incubated for 2 h with horseradish peroxidase-conjugated goat anti-rabbit IgG at a dilution of 1:5,000 in serum-free medium. Controls were incubated either with secondary antibodies only or with serum-free medium without antibodies. Cells were washed twice with PBS and then incubated with peroxidase substrate (Sigma). Reaction product was detected by spectrophotometry at 450 nm, using a microtiter plate reader. Background reactivity due to nonspecific binding of secondary antibodies was subtracted from reactivity observed in the presence of primary antibodies. An additional control, determination of alkaline phosphatase activity in the top and bottom chambers of the Transwell, was performed with phosphatase substrates (Sigma) to verify cell polarity and integrity of the monolayer.

Immunohistochemical staining of intestinal tissue

The small intestine was removed from mice immediately after sacrifice and short lengths (5–10 mm) from midduodenum, jejunum, and ileum were quickly placed in molds, covered with freezing compound (Histoprep™; Fisher Scientific, Pittsburgh, PA), wrapped in foil, and submerged into liquid nitrogen. Blocks were stored at −80°C until use. Tissue sections (8 μm) were cut and placed onto positively charged microscope slides and then stored at −80°C with desiccant. All subsequent reactions were performed at room temperature. Sections were fixed and permeabilized by covering the tissue for 1 min each with 5% formalin in PBS; followed by 5% formalin and 10% methanol in PBS; then 30% methanol in PBS; and finally twice with 50% methanol in water. Slides were blocked with 1% BSA in PBS for 30 min. SR-BI- and SR-BII-specific antibodies (Novus Biologicals) was diluted 1:500 with 1% BSA and placed on the sections for 1 h, followed by three 5-min washes with PBS. An unrelated antibody (anti-S-100) at similar concentration was used as a negative control. Fluorescein-conjugated, goat anti-rabbit antibody (Sigma) was diluted to 4.4 μg/ml with 1% BSA in PBS and placed on the sections for 1 h, and then washed as described above. A minimal amount of VECTASHIELD™ mounting medium (Vector Laboratories, Burlingame, CA), which includes propidium iodide (1.5 mg/ml), was placed on the sections before applying the coverslip. Sections were viewed with an Olympus (Norwood, MA) fluorescence photomicroscope system with automatic exposure control. Excitation was with 455- to 490-nm incident light. Emitted and reflected light that passed through a 500-nm bandpass filter and a 515-nm barrier filter constituted the images presented. Equal exposure times were used for all photographs.

RESULTS

The human colonic adenocarcinoma cell line Caco-2 was used as a model with which to investigate SR-BI expression in undifferentiated and differentiated intestinal cells. Total RNA was isolated from pre- and postconfluent Caco-2 cells for RT-PCR amplification with oligonucleotide primers corresponding to the 3′ terminus of human SR-BI mRNA. Human HepG2 liver cells, which express high levels of SR-BI (6), and Cos-1 monkey kidney cells, which express low levels of SR-BI (34), were used as positive controls for the RT-PCR. Results showed that RT-PCR of liver and Cos-1 RNA with SR-BI-specific primers yielded two products with sizes of 672 and 542 bp (**Fig. 1**). These are consistent with the predicted sizes of products amplified from SR-BI and SR-BII mRNA (6). Nucleotide sequencing of these two RT-PCR products confirmed their identity as SR-BI and SR-BII amplification products (data not shown). Interestingly, amplification of SR-BI mRNA isolated from pre- and postconfluent Caco-2 cells showed only the higher molecular weight band (Fig. 1), suggesting that Caco-2 cells expressed only SR-BI isoform.

The level of SR-BI expression in undifferentiated and differentiated Caco-2 cells was assessed by Northern blot hybridization of total cellular RNA. Undifferentiated Caco-2 cells expressed only low levels of SR-BI mRNA, which was detectable with the more sensitive RT-PCR procedure but not detectable by Northern blot hybridization (**Fig. 2**). The differentiation of Caco-2 cells to enterocytes was associated with the increased expression of SR-BI, in which SR-BI mRNA signal could be visualized by Northern blot hybridization (Fig. 2). The differentiation-induced SR-BI expression was accompanied by a concomitant increase in the level of SR-BI protein detected in cell membranes. Immunoblot analysis revealed that SR-BI protein was not present in membranes of preconfluent Caco-2 cells, and the level of SR-BI in membranes increased ap-

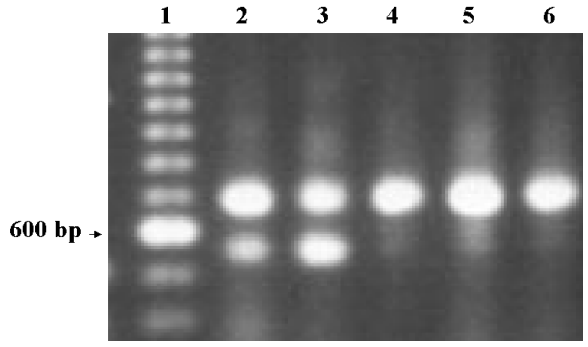

Fig. 1. Detection of SR-BI mRNA in Caco-2 cells by RT-PCR. Total RNA was isolated from human HepG2 liver cells (lane 2), Cos-1 cells (lane 3), and 10-day postconfluent (lanes 4, 5) or preconfluent (lane 6) Caco-2 cells for reverse transcription with oligo(dT)$_{16}$ and amplification with oligonucleotide primers specific to the 3′ terminus of SR-BI mRNA. The products of the reactions were electrophoresed in 1% agarose gels. The sizes of the reaction products were identified by comparison with the migration profile of a standard 100-bp DNA ladder (lane 1). The 600-bp band in the standard is identified as indicated.

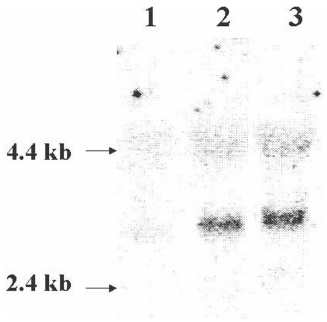

Fig. 2. Detection of SR-BI mRNA in Caco-2 cells by Northern blot hybridization. Total RNA was isolated from preconfluent (lane 1), 5-day postconfluent (lane 2), and 10-day postconfluent (lane 3) Caco-2 cell culture and used for Northern blot hybridization with a human SR-BI cDNA probe. The size of the RNA bands was determined on the basis of the migration of the 18S and 28S RNA as indicated.

proximately 8-fold as Caco-2 cell differentiation proceeded from 1 to 10 days postconfluency (**Fig. 3**).

The topology of SR-BI on the surface of Caco-2 cells was investigated by culturing the cells to postconfluency on polycarbonate membrane inserts to establish polarity of the cells. Under these culturing conditions, the cells form a tight junction separating the apical compartment from the basolateral compartment (35). Antibodies specific for the extracellular domain of SR-BI were then added to either the top chamber or the bottom chamber for incubation with the cells. At the end of the incubation period, the cells were washed extensively to remove unbound antibodies. The amount of SR-BI-specific rabbit polyclonal antibody bound to the cells at either the apical or basolateral surface was then determined by adding a peroxidase-conjugated anti-rabbit IgG followed by incubation with a peroxidase substrate. A significant amount of SR-BI antibody was found to be bound to both the apical and the basolateral membrane surfaces (**Fig. 4**). The amount of nonspecific antibody adhesion to cell membranes was evaluated with an irrelevant antibody, the anti-pancreatic lipase (Fig. 4).

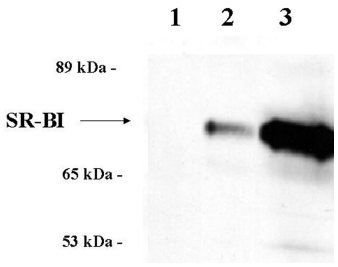

Fig. 3. SR-BI protein expression in Caco-2 cells. Total membrane proteins prepared from preconfluent Caco-2 cells (lane 1) and 1-day postconfluent (lane 2) or 10-day postconfluent (lane 3) Caco-2 cell cultures were subjected to SDS-PAGE and then transferred to nitrocellulose membranes for immunoblot analysis with rabbit polyclonal antibodies against the extracellular domain of human SR-BI. Immunoreactivity was visualized by ECL chemiluminescence. The identification of the immunoreactive protein as authentic SR-BI was based on its migration in comparison with molecular weight standards as indicated in the margin.

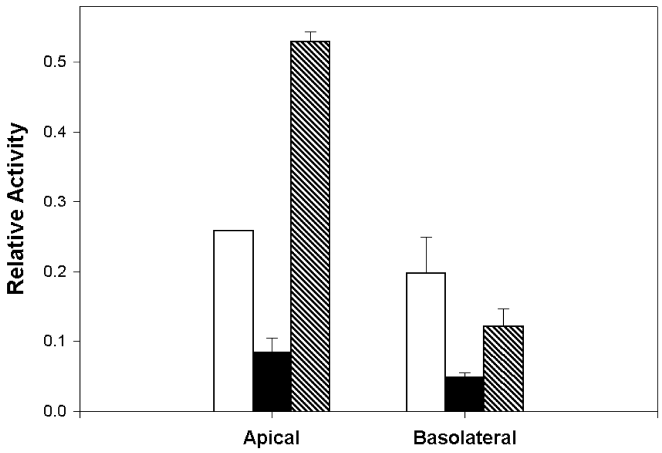

Fig. 4. Analysis of SR-BI expression on Caco-2 cell surface. Human Caco-2 cells were cultured on Transwell membranes to 15 days postconfluency before experimentation. The extracellular domain-specific SR-BI antibodies (open columns) or an irrelevant antibody (solid columns) were added to either the top chamber or the bottom chamber of the Transwell apparatus and incubated overnight at 37°C. The cells were washed and then incubated for an additional 2 h with horseradish peroxidase-conjugated goat anti-rabbit IgG. The amount of antibodies bound to the apical cell surface facing the top chamber or the basolateral cell surface facing the bottom chamber was then determined by incubation with peroxidase substrates. Reaction product was assayed spectrophotometrically at 450 nm. Alkaline phosphatase activity on the apical and basolateral surfaces (hatched columns) was determined on the basis of the hydrolysis of phosphatase substrates (Sigma) as described by the manufacturer. The data are presented as means ± SD from triplicate determinations in five different experiments.

The difference in the amount of SR-BI- and pancreatic lipase-specific antibody bound to cell membranes suggested the specific interaction of SR-BI antibody with the receptor on both membrane surfaces. Monolayer integrity and cell polarity on the Transwell were confirmed by positive intestinal alkaline phosphatase reactivity in the apical compartment and its absence in the basolateral compartment (Fig. 4).

The distribution of SR-BI along the gastrocolic axis of intestine was assessed by immunoblot analysis of protein extracts prepared from duodenum, jejunum, and ileum sections of mouse intestine. The results showed that anti-SR-BI IgG reacted with a single band in all three sections of the mouse intestine, with the most intense reaction detected in the duodenal preparation (**Fig. 5**). The expression of SR-BI in the duodenum was found to be 2-fold higher than that observed in the jejunum and the ileum segments of the intestine (Fig. 5).

The pattern of SR-BI expression along the gastrocolic axis as determined by immunoblot analysis was confirmed by immunofluorescence studies. The most intense fluorescent staining of mouse intestine with SR-BI antibodies was detected in the duodenum. Considerably less staining was found in the jejunum and even less was detected in the ileum (compare **Fig. 6A**, **C**, and **E**). In addition, consistent with results observed in vitro with Caco-2 cells in Transwell membranes, SR-BI expression was observed on both the apical and basolateral surfaces of mouse en-

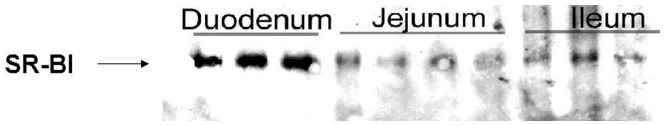

Fig. 5. Expression of SR-BI in mouse intestine. Mouse intestine was divided into midduodenum, jejunum, and ileum segments and then homogenized in HEPES buffer (pH 7.4) containing a cocktail of protease inhibitors. Membrane proteins from three separate mouse preparations were then electrophoresed in SDS-polyacrylamide gels, transferred to nitrocellulose membranes, and used for hybridization with antibodies against the extracellular domain of SR-BI. Immunoreactivity was visualized by ECL chemiluminescence. The identification of the immunoreactive protein as authentic SR-BI was based on its migration in comparison with molecular weight standards

terocytes. In the duodenum, SR-BI immunofluorescence was somewhat more intense in the apical membrane than in the basolateral membrane (Fig. 6A and B). In contrast, more anti-SR-BI-derived immunofluorescence was detected on the basolateral surfaces of jejunal enterocytes (Fig. 6C). The ileum displayed only weak immunofluorescent staining with the anti-SR-BI antibodies and most of this appeared diffuse and intracellular (Fig. 6D). Crypt cells were also inspected to determine whether SR-BI expression was affected by the differentiation state of intestinal epithelial cells. As shown in Fig. 6A and E, only minimal staining of duodenal crypt cells (marked with asterisks) was observed as compared with mature enterocytes. Similarly, no staining above background was observed in crypt cells of the jejunum and ileum (not shown). In parallel experiments with antibodies specific to the SR-BII isoform, dramatically less immunofluorescent staining was detected. The immunofluorescence detected with the SR-BII antibodies was minimally above background fluorescence and was detectable only at high magnification. A similar gastrocolic gradient of expression appeared to exist for the low level of SR-BII and it was found predominantly in intracellular locations (Fig. 6F, G, and H). Incubations with a nonspecific antibody (S-100, a

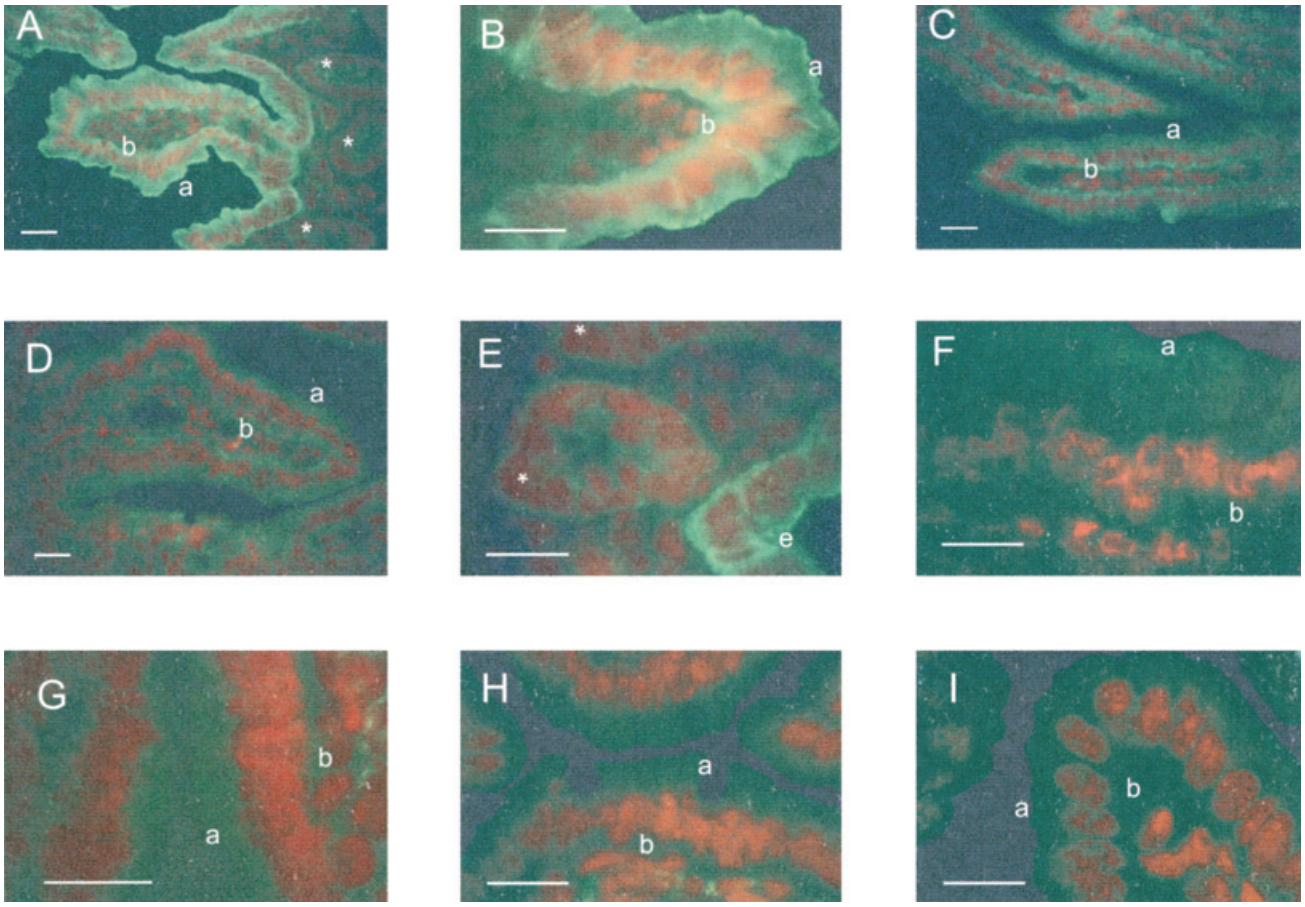

Fig. 6. Immunofluorescence studies of SR-BI and SR-BII expression in mouse intestine. Tissue sections (8 μm) prepared from mouse duodenum, jejunum, or ileum were incubated with rabbit polyclonal antibodies against the C-terminal sequence of SR-BI or SR-BII followed by a fluorescein-conjugated goat anti-rabbit IgG. A minimum amount of mounting medium containing propidium iodide (1.5 mg/ml) was used for identification of cell nuclei. Sections were viewed under an Olympus fluorescence microscope with excitation at 455–490 nm, a band pass of 500 nm, and a 515-nm barrier filter. Equal exposure time was used for all the images. The letters a and b indicate apical and basolateral surfaces of enterocytes. A and B: Low and high magnifications of duodenum stained for SR-BI; asterisks denote crypt cells. C and D: Jejunum and ileum stained for SR-BI. E: High magnification of duodenal crypt area stained for SR-BI; asterisks denote crypts and e denotes enterocytes. F–H: Duodenum, jejunum, and ileum stained for SR-BII. I: A duodenum section incubated with a primary antibody against neural protein S-100 as a negative control. Magnification bar: (A–I) 20 μm.

neural protein) or with nonimmune rabbit serum did not result in significant immunofluorescent staining (Fig. 6I). These results indicated that enterocytes express predominantly the SR-BI isoform and only a minor amount, if any, of the SR-BII isoform. These results demonstrated the specificity of the immunofluorescence studies for SR-BI and further confirmed the in vitro data from the Caco-2 cells.

DISCUSSION

The absorption of dietary and biliary cholesterol is a multistep process initiated by the transfer of unesterified cholesterol from bile salt mixed micelles into brush border membranes of the intestine. Originally, this was thought to be mediated by simple passive diffusion of cholesterol from micelles to the enterocyte membrane. However, detailed kinetics analysis revealed that cholesterol transport from micelles to brush border membranes follows second-order reaction kinetics (36). Importantly, proteolytic digestion of brush border membrane proteins converted this second-order reaction to one that follows first-order reaction kinetics (36). These data suggested that cholesterol absorption may be a protein-mediated process (36). This hypothesis was strengthened by the ability to reconstitute cholesterol transport activity in liposomes with proteins extracted from intestinal brush border membranes (37). Unfortunately, attempts to identify the cholesterol transport protein in intestine remain futile. Earlier studies by Hauser and colleagues (38) suggested that the sterol carrier protein 2 (SCP_2) is the cholesterol transporter in the brush border membrane responsible for intestinal absorption of cholesterol from the lumen. This hypothesis was challenged because SCP_2 could not be localized to the brush border membrane (39).

More recently, Hauser and colleagues (29) proposed that SR-BI may be the transporter responsible for dietary cholesterol absorption in the intestine. Their studies showed that cholesterol uptake by Caco-2 cells was minimized in the presence of SR-BI antibodies. However, it was unclear from that study whether SR-BI inhibited cellular cholesterol uptake from the apical or the basolateral side of the enterocytes. Although SR-BI antibodies also inhibited cholesterol uptake by rabbit brush border membranes in vitro (29), the purity of the membrane preparation was not described. Immunolocalization studies suggested only low levels of SR-BI along the villus brush border (29).

The current study used Caco-2 cells cultured on porous membranes to assess SR-BI expression during cell differentiation as well as its distribution between apical and basolateral membranes. Our studies showed that SR-BI expression in intestinal cells is differentiation dependent, with the highest level of expression detected in highly differentiated enterocytes. Importantly, SR-BI can be found to be present on both the apical and basolateral cell surface. Additional studies with mouse intestine revealed that the apical expression was most prominent on proximal intestinal villus and less apical expression was detectable on the villus of distal intestinal epithelium. These observations, along with the preferential absorption of cholesterol in the villi of the proximal intestine (40), are consistent with the hypothesis that SR-BI expressed in the intestine may play a role in mediating dietary and biliary cholesterol absorption in vivo. However, it should be noted that SR-BI also mediated cholesteryl ester and triglyceride uptake by brush border membrane vesicles in vitro (29). Because these neutral lipids are not absorbed as intact molecules to any significant extent in vivo, the physiological function of intestinal SR-BI, especially that present in the apical membrane, needs additional clarification. It is possible that the structure of the cholesteryl ester and triglyceride carrier in the intestinal lumen precludes their interaction with SR-BI on the brush border. Alternatively, SR-BI on brush border membrane may serve a different function independent of dietary cholesterol absorption and transport. The assessment of cholesterol absorption efficiency in SR-BI gene knockout mice will be able to shed light on this issue (17, 18).

The current study revealed that SR-BI is also present on the basolateral surface of enterocytes. Although the exact function of the basolateral SR-BI is unknown at this time, it is possible that SR-BI may also be responsible for uptake of neutral lipids from HDL in circulation. Previous studies demonstrated that Caco-2 cells can selectively remove cholesteryl esters from HDL and also exhibit cholesterol efflux in the presence of apolipoprotein A-I and apolipoprotein A-II (41, 42). As reviewed in the Introduction, these are the same properties ascribed to the function of SR-BI in liver, steroidogenic tissues, and macrophages. Thus, the basolaterally expressed SR-BI may serve similar functions in the intestine in vivo. The colocalization of HDL_3 to the basolateral membrane of enterocytes (43) is supportive of this hypothesis.

In summary, the results of the current study showed that SR-BI is present on both the apical and the basolateral surface of well-differentiated enterocytes. SR-BI may participate in dietary and biliary cholesterol uptake by intestinal cells, as postulated by Hauser and colleagues (29). SR-BI on the basolateral membrane may participate in selective uptake of cholesteryl esters in HDL or for direct cholesterol secretion to acceptor lipoproteins. There may also be additional functions of SR-BI on the apical and basolateral surface that are required for intestinal physiology and lipid metabolism. These functions remain to be identified.

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