Scavenger receptor class B, type I (SR-BI)/ApoE double null mice develop severe atherosclerosis within 4 weeks, whereas ApoE null mice take several months to develop the disease, indicating that SR-BI plays a pivotal role in atherosclerosis. Importantly, SR-BI/ApoE double null mice have lower plasma cholesterol levels than ApoE null mice, suggesting involvement of a non-lipids mechanism. In the present study, we revealed a novel ligand-independent apoptotic pathway induced by SR-BI, and regulated by endothelial nitric-oxide synthase (eNOS) and high density lipoprotein (HDL). SR-BI significantly induces apoptosis in three independent cell systems. In contrast to known ligand-dependent apoptotic pathways, SR-BI-induced apoptosis is ligand-independent. We further showed that SR-BI-induced apoptosis is suppressed by eNOS and HDL. By using a single site mutation, we demonstrated that SR-BI induces apoptosis through a highly conserved CXXS redox motif. We finally demonstrated that SR-BI-induced apoptosis is via the caspase-8 pathway. We hypothesize that in healthy cells, the SR-BI apoptotic pathway is turned off by eNOS and HDL which prevents inappropriate apoptotic damage to the vascular wall. When HDL levels are low, oxidative stress causes the relocalization of eNOS away from caveolae, which turns on SR-BI-induced apoptosis and rapidly clears damaged cells to prevent further inflammatory damage to neighboring cells. The current studies offer a new paradigm in which to study the non-cholesterol effects of SR-BI, HDL, and eNOS on the development of atherosclerosis and potentially other cardiovascular diseases.

Scavenger receptor class B1 (SR-BI) is a physiological receptor for high density lipoprotein (HDL) and plays a key role in regulating plasma cholesterol levels (1, 2). SR-BI is expressed in a variety of tissues including the liver, endothelial cells, and ovaries (1). In the liver, SR-BI facilitates the selective uptake of cholesterol from circulating HDL, in which it is then processed and secreted as bile; however, the function of SR-BI in other tissues is unclear. Recently Braun et al. (3, 4) reported a surprising effect of SR-BI on the cardiovascular system. They showed that SR-BI/ApoE double null mice fed a standard chow diet developed severe occlusive coronary disease and atherosclerosis within 4 weeks. Other animal models such as ApoE null mice and ApoE/LDLR null mice fed a high fat diet develop occlusive coronary disease and atherosclerosis to a lesser extent than SR-BI/ApoE double null mice, and it takes considerably longer (3, 4). Importantly, the total plasma cholesterol levels in the ApoE or ApoE/LDLR mouse models are normally greater than 1500 mg/dl, whereas in SR-BI/ApoE null mice it is ~700–800 mg/dl (3, 4). These findings suggest that SR-BI has cholesterol-independent effects on the cardiovascular system. Interestingly, mice over-expressing SR-BI also develop atherosclerosis (5), suggesting again that SR-BI has multiple functions and that, depending on as yet unidentified factors, function in promoting or preventing the development of pathophysiological states such as atherosclerosis.

Endothelial nitric-oxide synthase (eNOS) is expressed in a variety of tissues and catalyzes the oxidation of arginine to produce nitric oxide, a small signaling molecule that plays a major role in the physiological function of the cardiovascular system (6). A decrease in the bioavailability of nitric oxide is thought to be involved in the development of atherosclerotic lesions and the development of hypertension (7). As was predicted, eNOS null mice develop atherosclerosis (8); however, more surprising was the finding that over-expression of eNOS also promotes the development of atherosclerosis (9). The mechanism(s) whereby eNOS promotes atherosclerosis is not understood, but these findings, like those for SR-BI, suggest that the roles of eNOS are more complex than originally proposed.

Apoptosis has at least two functions. One is to eliminate unneeded cells during development and another is to remove damaged cells to prevent further inflammatory damage (10). The process of apoptosis is especially important for vascular endothelial cells because vascular endothelial cells are constantly insulted by inflammatory factors such as oxidized LDL and superoxide (11–15). Damaged endothelial cells trigger inflammatory reactions that can subsequently damage surrounding cells. Fast clearance of the damaged endothelial cells by an apoptotic mechanism is helpful in maintaining normal vascular function. However, uncontrolled apoptosis is harmful, resulting in inappropriate damage to the tissue. Thus, a tightly regulated apoptotic process is required to maintain the normal function of cells and tissues.

In the current study we have described a novel ligand-independent apoptotic pathway that is induced by SR-BI and reg-

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ultated by eNOS and HDL. The physiological and clinical implications of this novel finding are discussed.

EXPERIMENTAL PROCEDURES

Materials—The cytotoxicity detection kit (lactate dehydrogenase (LDH) activity), the in situ cell death detection kit (TUNEL), and the apoptotic DNA ladder kit were from Roche Applied Science. The caspase-8 assay kit, caspase-9 assay kit, the anti-caspase-12 IgG, the cell-permeable caspase-3 inhibitor (DEVD), caspase-8 inhibitor (IETD), and caspase-9 inhibitor (LEHD) were from Calbiochem and Oncogene (San Diego, CA). The caspase-3 activity assay kit was from Sigma. The pLNCX2 vector was from Clontech Laboratories (Palo Alto, CA). The anti-human eNOS IgG was from BD Transduction Laboratories. The anti-mouse eNOS IgG and anti-VCAM-1 IgG were from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-SR-BI serum against the extracellular region of SR-BI was from Novus Biologicals (Littleton, CO). The anti-SR-BI serum against the extracellular region of SR-BI was from Calbiochem.

Generation of Mutant Construct and CHO Cell Lines—Human SR-BI cDNA was provided by Dr. Denyes van der Westhuyzen (University of Kentucky). A SR-BI point mutant (SRBI-C323G, cysteine to glycine mutant) was generated by PCR using wild type SR-BI as the template and subcloned into pLNCX2. The construct was transfected into CHO cells, and cell lines stably expressing the mutant were obtained by G418 selection as described previously (16). CHO cell lines stably expressing vector (pLNCX2), wild type SR-BI, wild type eNOS, or both of SR-BI and eNOS were established as described previously (16). The cells were cultured in Ham's F-12 medium containing 5% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin, and 0.3 mg/ml G418.

Knock-out Mice and Genotyping—SR-BI−/− and eNOS−/− mice were from the Jackson Laboratories (Bar Harbor, ME). The mice were backcrossed 10× onto a C57BL/6 background. SR-BI null mice were generated by breeding SR-BI−/− mice. Mouse tail DNA was used for genotyping by PCR following the methods provided by the Jackson Laboratories.

Primary Mouse Embryo Fibroblast Cell (MEF) and Primary Mouse Aortic Endothelial Cell Culture—MEFs were prepared as described previously (17). Briefly, SR-BI−/− females mated with SR-BI−/− males were euthanized at day 13.5 of gestation. Embryos were decapitated, digested in trypsin, and cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cells were genotyped by PCR and used at passage three. Mouse aortic endothelial cells were isolated from wild type, eNOS null and SR-BI null mice and cultured as described previously (16). The purity of endothelial cells was monitored with fluorescence-activated cell sorting using VCAM-1 antibody (data not shown).

Apoptosis Assay—Four different methods were employed to quantify cell death and to identify apoptosis. 1) Lactate dehydrogenase activity in the cell culture supernatant, which quantitatively monitors the degree of cell damage, was measured using the cytotoxicity detection kit following the instructions. Data were expressed as percentage of the total LDH activity. 2) TUNEL assay, which quantitatively measures cell death, was performed using the in situ cell death detection kit following the manufacturer's instructions. 3) Caspase activation is a biochemical marker of apoptosis (10). The caspase-3, caspase-8, caspase-9, and caspase-12 were measured as described below. 4) A DNA ladder assay, which is a biochemical hallmark of apoptosis (10), was used to identify and confirm apoptosis. The ladder DNA was isolated using the apoptotic DNA ladder kit from Roche Applied Science. Both adherent and detached cells were harvested. The isolated DNA was treated with RNase for 15 min at room temperature and applied to a 1% agarose gel electrophoresis.

Caspase Assay—Caspase-3 activity was measured using a colorimetric assay kit from Sigma. Caspase-8 and caspase-9 activities were measured using fluorometric assay kits from Oncogene. Activation of caspase-12 was detected by Western blot using an antibody against caspase-12. Briefly, 106 cells were plated in 100-mm tissue culture dishes, and the cells were cultured to 70–80% confluency in complete medium and then changed to serum-free medium. At the indicated times, the supernatants were collected for the LDH activity assay, and then the cells were scraped and lysed in 0.5 ml of lysis buffer (provided in the assay kit). The lysate was used for the caspase assays.

Electrophoresis and Immunobots—Cells were lysed at 4 °C for 20 min in lysis buffer consisting of 25 mM MESA, pH 6.5, 137 mM NaCl, 1% (v/v) Triton X-100, 60 mM octyl glucoside, and 0.1% (w/v) SDS. After centrifugation (12,000 rpm, 5 min), the supernatants were mixed with 1/5 volume of reduced sample buffer and heated at 95 °C for 5 min. Proteins were then separated by electrophoresis and detected by Western blot as described previously (16). Lipoproteins—Very low density lipoprotein (d < 1.006 g/ml), LDL (d = 1.019–1.05 g/ml), and HDL (d = 1.063–1.21 g/ml) were isolated from human plasma by sequential density gradient ultracentrifugation as described previously (19). SDS-PAGE and Coomassie Blue staining were used to assay the purity of each lipoprotein fraction.

Statistical Analysis—A paired Student t test was used to evaluate the data. Means were considered significant at p < 0.05.

RESULTS

SR-BI Induces Apoptosis, and eNOS Suppresses SR-BI-induced Apoptosis—To ensure that any measured effects of SR-BI were not cell line-specific, we used three independent cell systems. 1) We established CHO cell lines stably expressing vector cDNA, SR-BI, eNOS, or both of SR-BI and eNOS (Fig. 1a). 2) Primary MEFs express SR-BI but not eNOS; therefore it is a convenient model to examine the effects of SR-BI on apoptosis in the absence of eNOS using wild type and SR-BI−/− MEFs (Fig. 1b). 3) In vivo, endothelial cells express both SR-BI and eNOS; thus primary aortic endothelial cells isolated from wild type, SR-BI−/−, and eNOS−/− mice were used (Fig. 1c).

All of the CHO cell lines grew well in complete medium (5% serum) and displayed a healthy spindle-like morphology (Fig. 2a, Attached). However, a significant number of rounded cells were detected in the culture supernatant of cells expressing SR-BI (Fig. 2a, Floating). In contrast, few cells containing the vector appeared in the culture supernatant (Fig. 2a, Floating). The floating cells were collected and quantified by counting with a hemocytometer. Approximately 75,000 cells/10-cm culture dish were floating in the culture supernatant of CHO-SRBI cells compared with 9,000 cells for CHO-vector cells (Fig. 2b). To determine whether the floating cells were dead or dying a DNA ladder assay was conducted. Fig. 2c demonstrates that the floating cells harvested from the CHO-SRBI plates had fragmented DNA, whereas floating cells taken from the same number of plated cells for CHO-vector cells did not contain...
DNA ladders. To further confirm that SR-BI can induce cell damage we quantitatively measured LDH activity in the culture supernatant. CHO-SRBI cells had significantly more LDH leakage than CHO-vector cells (Fig. 2d).

Unlike ligand-dependent apoptosis, such as Fas-induced apoptosis, which needs a FasL ligand to stimulate apoptosis, SR-BI-induced apoptosis did not require a ligand (ligand-independent). Therefore, to prevent inappropriate apoptotic damage to the endothelial cells, a mechanism(s) must exist to prevent SR-BI-induced apoptosis in physiological condition. Several laboratories including ours recently demonstrated that eNOS and SR-BI are functionally coupled in caveolae (16, 20–22). Therefore, we tested whether eNOS can inhibit SR-BI-induced apoptosis using CHO-SRBI-eNOS cells expressing both SR-BI and eNOS. As shown in Fig. 2, the expression of eNOS completely inhibited SR-BI-induced apoptosis as revealed by morphological assay (Fig. 2a), floating cell counting (Fig. 2b), DNA ladder assay (Fig. 2c), and LDH assay (Fig. 2d).

Apoptosis is a physiological process to eliminate unneeded or damaged cells, but it must be tightly regulated because uncontrolled apoptosis will cause inappropriate damage to tissue. Although we demonstrated that eNOS effectively suppresses SR-BI-induced apoptosis, eNOS is not always co-expressed with SR-BI in all tissues. Therefore, we used serum starvation to further investigate whether there is an inhibitor(s) in the serum that suppresses SR-BI-induced apoptosis. Serum starvation is a classic method to enhance apoptosis (23). We also used this approach to further clarify whether SR-BI/eNOS are involved in apoptosis. After 24 h of serum starvation, CHO-SRBI cells released 30% of total cellular LDH into the culture medium, whereas the other cells released 8% of total cellular LDH into the culture medium (Fig. 3b). After 48 h of serum starvation, CHO-SRBI cells released more than 60% of total cellular LDH, whereas CHO-vector, CHO-eNOS, and CHO-SRBI-eNOS cells released 15.4, 12.9, and 14.1% of their total cellular LDH, respectively. To confirm that the LDH activity was due to apoptosis, we performed a TUNEL assay. After 24 h of serum starvation 12% of CHO-SRBI cells were TUNEL positive, whereas less than 0.5% of the other three cell lines were TUNEL positive (Fig. 3c). It is important to emphasize that the TUNEL assay detects dying cells attached to the culture dish and that cells that have already died will have detached from the dish and been washed away during the staining process. Thus, it is likely that the TUNEL assay values underestimated the actual number of dead/dying cells. Finally, we used the DNA ladder assay to determine whether the cells were dying by apoptosis. As shown in Fig. 3d, a clear DNA ladder was obtained.

**FIG. 2.** SR-BI induces apoptosis in the presence of serum. The CHO cell lines were grown in normal medium containing 5% serum to ~70% confluency. a, the floating cells were collected, and the floating cells and attached cells were visualized by phase-contrast microscopy (×10). Representative data are from four independent experiments. b, the number of floating cells was quantified for each cell type. **, *p < 0.01 with respect to vector. n = 4. c, DNA was isolated from floating cells (from 10 10-cm dishes of cells) and the extent of DNA fragmentation determined with a commercial apoptotic DNA ladder kit. Apoptotic DNA was not detected in DNA isolated from the attached cells (data not shown). Representative data are from three independent experiments. d, the extent of cytotoxicity was determined by measuring LDH activity in the culture medium. **, *p < 0.01 with respect to vector. n = 4.
for CHO-SRBI cells after 48 h of serum starvation, demonstrating that the SR-BI-induced cell death is by apoptosis. In contrast, only intact DNA was detected for the other cell lines. It is important to note that the cells expressing both SR-BI and eNOS did not undergo apoptosis, which is demonstrated by the morphological assay (Fig. 3a), LDL assay (Fig. 3b), TUNEL assay (Fig. 3c), and DNA ladder assay (Fig. 3d), indicating that eNOS effectively suppressed SR-BI induced apoptosis. The serum starvation greatly enhanced SR-BI-induced apoptosis, which suggested that a survival factor exists in serum. The identification of this factor will be discussed in the next section.

To ensure that the ability of SR-BI to induce apoptosis was not limited to CHO cells, we isolated wild type and SR-BI null mouse embryonic fibroblasts from the embryos obtained from breeding SR-BI+/H11001/H11002 mice. Mouse embryonic fibroblasts were isolated as described under “Experimental Procedures” and then placed in medium lacking serum for 24 h. The extent of apoptosis was measured by TUNEL assay. Fig. 4a demonstrates that wild type cells (containing SR-BI) underwent significantly more apoptosis than SR-BI null cells (lacking SR-BI). We next wanted to confirm that eNOS prevents SR-BI-associated apoptosis in cells that normally express eNOS. To this end we isolated mouse aortic endothelial cells from wild type mice, SR-BI null mice, and eNOS null mice. The endothelial cells were serum-starved for 24 h, and the extent of apoptosis was measured by TUNEL assay (Fig. 4b). Endothelial cells isolated from eNOS null mice (contain SR-BI) underwent significantly more cell death than cells from wild type or SR-BI null mice. These data are consistent with our findings in CHO and MEF cells that the apoptosis is SR-BI-dependent and the presence of eNOS prevents SR-BI-induced cell death.

**HDL Prevents SR-BI-induced Apoptosis**—The absence of serum greatly enhanced SR-BI-induced cell death, suggesting that a survival factor(s) exists in serum that prevented SR-BI-induced cell death. Because HDL is a physiological ligand of SR-BI, we tested whether the presence, absence, or amount of HDL influenced the ability of SR-BI to induce cell death. Therefore, various amounts of isolated HDL or lipoprotein-deficient serum were incubated with CHO-vector or CHO-SRBI cells for 48 h, the morphology of the cells was examined, and the amount of LDH activity was quantified (Fig. 5). Fig. 5a demonstrates that CHO-SRBI cells incubated in lipoprotein-deficient serum underwent a morphological change consistent with the onset of cell death and that the expression of eNOS along with SR-BI prevented the morphological change. However, CHO-SRBI cells incubated in 100 μg/ml HDL were healthy and looked similar to control cells. Lipoprotein-deficient serum was unable to prevent SR-BI-mediated cytotoxicity, as measured by LDH release, at any of the concentrations tested. In contrast, HDL provided significant protection against LDH release at 100 μg/ml and nearly completed protection at 150 μg/ml (Fig. 5b). Control experiments with LDL and very low density lipoprotein demonstrated that these lipoproteins were not able to prevent a change in cell morphology or LDH release (data not shown).

**A Highly Conserved CXXS Motif Is Required for the Apoptotic Activity of SR-BI**—We next explored the molecular mechanism of how SR-BI induces apoptosis. SR-BI contains a highly conserved putative redox motif at 322CXXS326 (Fig. 6a). CXXS...
proteins belong to the CXXC redox family and play important roles in preventing apoptosis caused by oxidative stress (24). To determine whether this putative redox motif is indeed responsible for the apoptotic activity of SR-BI, we generated a CHO cell line stably expressing C323G mutant SR-BI (CHO-SRBI-C<sup>323G</sup> mutant) (Fig. 6b) and tested whether this point mutant SR-BI can induce apoptosis. In contrast to CHO-SR-BI cells, the CHO-SRBI-C<sup>323G</sup> mutant cells maintained a healthy spin-
dle-like morphology in both complete medium and in serum-starving medium (Fig. 6c). The LDH assay demonstrated that this point mutant even had less LDH leakage than did CHO-vector cells in both nonstarving (Fig. 6d) and starving conditions (Fig. 6e). TUNEL assay revealed that this single point mutant eliminated the apoptotic activity of SR-BI completely (Fig. 6f). These data strongly suggest that the CXXS redox motif is the active site for the apoptotic activity of SR-BI.

**SR-BI and eNOS Regulate Apoptosis via a Caspase-8 Pathway**—We next explored the downstream signals of the putative SR-BI/eNOS apoptotic pathway. There are three main apoptotic pathways: 1) the membrane-mediated pathway that activates caspase-8; 2) the mitochondria-mediated pathway that activates caspase-9; and 3) the ER-mediated pathway that activates caspase-12 (10, 25). The activation of caspase-8 and caspase-9 was measured using a commercially available kit, and the activation of caspase-12 was measured with a commercially available antibody. Caspase-3, which is common to all of the apoptotic pathways, was measured with a commercial kit. CHO cells expressing vector, SR-BI, eNOS, or both SR-BI and eNOS were serum-starved for various times and the extent of specific caspase activity quantified. As illustrated in Fig. 7, serum starvation induced a dramatic increase in caspase-3, caspase-8, and caspase-9 activity in CHO-SRBI cells but not in CHO-SRBI-eNOS cells. An increase in caspase-8 activity was observed as early as 4 h after serum starvation (Fig. 7b), and caspase-3 and caspase-9 activity increased after 6 h of serum starvation. Caspase-12 activity was not observed in any of the cell lines within 48 h of serum starvation (data not shown).

We used specific caspase inhibitors to further confirm which caspase was activated by SR-BI. CHO cell lines expressing SR-BI, eNOS, or both SR-BI and eNOS were serum-starved for 24 h in the presence of 30 μM concentrations of inhibitors for caspase-3, caspase-8, or caspase-9. The caspase-8 inhibitor or
SR-BI Apoptotic Pathway

DISCUSSION

Numerous death receptor apoptotic pathways have been defined and consist of a ligand such as FasL or tumor necrosis factor binding to a receptor that triggers cell death (28, 29). The existence of a ligand-independent apoptotic pathway has not been demonstrated. The SR-BI/eNOS/HDL apoptotic pathway described in this study is a putative ligand-independent apoptotic pathway. In the absence of a survival factor (HDL) SR-BI induces apoptosis, and in the presence of HDL, SR-BI does not induce apoptosis. Five novel findings have been described in these studies: 1) SR-BI induces apoptosis in a ligand-independent manner; 2) HDL is a survival factor that prevents SR-BI-induced apoptosis; 3) eNOS suppresses SR-BI-induced apoptosis; 4) SR-BI induces apoptosis via a redox motif; and 5) SR-BI induces apoptosis via the caspase-8 pathway.

Three independent cell systems were used to demonstrate that SR-BI can induce apoptosis and that the effect is not cell line-specific. The first cell system used was CHO cells expressing SR-BI, which has been extensively used to study the role of SR-BI in the selective uptake of cholesteryl ester from HDL (1, 30–35). In the presence of serum, CHO-SRBI cells displayed a healthy spindle-like morphology. However CHO-SRBI cells showed significantly more floating cells than control cells (Fig. 2b). We collected the floating cells and demonstrated that these floating CHO-SRBI cells are apoptotic cells by using a DNA ladder assay (Fig. 2c). Floating cells are often thought to be dividing cells that temporarily detach from the culture dish. This may explain why this observation was not reported earlier. Importantly, CHO cells expressing both SR-BI and eNOS did not undergo apoptosis, which suggests that the SR-BI-induced apoptosis is not an artifact of SR-BI over-expression. A point mutation at \( \text{CXXS} \) redox motif completely abolished the apoptotic activity of SR-BI, further indicating that the SR-BI-induced apoptotic activity is not an artifact of SR-BI over-expression. However, all cell lines have the potential to misregulate protein function; therefore we isolated two types of primary cells from genetically manipulated mice (Fig. 1, b and c). Wild type fibroblasts expressed SR-BI and underwent significantly more apoptosis than SR-BI null fibroblasts, indicating that the SR-BI-induced apoptosis is not limited to CHO cells (Fig. 4e). Furthermore, endothelial cells isolated from eNOS null mice underwent more apoptosis than endothelial cell isolated from wild type mice, indicating that eNOS does indeed play a role in preventing SR-BI-induced cell death (Fig. 4f).

Investigation into the molecular mechanism whereby SR-BI induces apoptosis indicates that a highly conserved CXXS motif is responsible for the apoptotic activity of SR-BI (Fig. 6). This is surprising because it is generally believed that CXXC redox proteins prevent oxidative stress-induced apoptosis and that they do not induce apoptosis (36). More detailed studies are needed to define how SR-BI regulates the redox status of cells and how this putative change in redox status triggers apoptosis.

The binding of HDL to SR-BI greatly suppressed the ability of SR-BI to induce apoptosis. Experiments with lipoprotein-
deficient serum and other lipoproteins clearly demonstrated that HDL functions as a specific survival factor by preventing SR-BI-induced apoptosis. Interestingly, in our studies the concentration of HDL (100 μg/ml) that was needed to suppress SR-BI-induced apoptosis was higher than the reported $K_d$ (35 μg/ml) (1, 38). One possible explanation is that the inhibitory effect of HDL on apoptosis is not caused only by HDL binding; rather a component from HDL may be transferred to the cells, and this component may be responsible for the decrease in apoptosis. This general concept has been demonstrated previously whereby HDL-SR-BI interaction prevented oxidized LDL from destroying the integrity of caveolae by a mechanism involving SR-BI-mediated uptake of HDL cholesterol to caveolae (39). The mechanism(s) whereby HDL prevents SR-BI-induced apoptosis is not known and will require considerable more investigation. The relative contribution of SR-BI-induced apo-

**FIG. 8. Effects of caspase inhibitors on SR-BI-induced apoptosis.** The CHO cell lines were grown in normal medium containing 5% serum to ~70% confluency. The cells were then serum-starved for 24 h in the presence of caspase-3 (DEVD), caspase-8 (IETD), or caspase-9 (LEHD) inhibitors. a, morphology of the cells was visualized by phase-contrast microscopy (×10). Representative data are from three independent experiments. b, the extent of cytotoxicity was determined by measuring LDH activity in the culture medium. $p < 0.05 (*)$ and $p < 0.01 (**)$. with respect to vector. $n = 3$. 

![Diagram of SR-BI apoptotic pathway](image-url)
Fig. 9. Schematic model of the SR-BI-eNOS-HDL apoptotic pathway in healthy and damaged cells. SR-BI induces apoptosis in a ligand-independent manner via its CXRS redox motif through activation of caspase-8 (Casp-8) pathway. This apoptotic pathway is regulated by eNOS and HDL and functions differently in healthy and damaged cells. In damaged cells (right panel) in which HDL levels drop and oxidative stress causes the relocation of eNOS away from caveolae, SR-BI induces apoptosis via activating the caspase-8 pathway, which rapidly clears damaged cells to prevent further inflammatory damage to neighboring cells. In healthy cells (left panel), SR-BI-induced apoptosis is turned off by eNOS and HDL, which prevents inappropriate apoptotic damage to the vascular wall.

Apoptosis to the development of a human disease such as atherosclerosis has not been documented. However, it is tempting to speculate that low plasma HDL levels (risk factor for atherosclerosis) fail to prevent SR-BI from causing excessive apoptotic damage to endothelial cells, which could potentially contribute to the development of atherosclerosis.

Mice deficient in SR-BI or eNOS develop atherosclerosis (3, 40). However, mice over-expressing SR-BI or eNOS also develop atherosclerosis (5, 9). These phenomena are difficult to be explained by the known functions of SR-BI and eNOS. One of the major functions of apoptosis is the rapid removal of damaged cells to prevent further inflammatory damage to neighboring cells. This is especially important for vascular endothelial cells, which are constantly insulted by environmental factors such as oxLDL and superoxide (11). In fact, oxLDL-induced endothelial cell apoptosis has been well documented (12, 15, 41–45) (reviewed in Ref. 37). The mechanism by which induced endothelial cell apoptosis has been well documented factors such as oxLDL and superoxide (11). In fact, oxLDL-induced apoptosis in endothelial cells is not fully understood. We demonstrated previously that in the absence of HDL, oxLDL induces eNOS to translocate from caveolae to intracellular membranes (39). The absence of eNOS in caveolae may prevent SR-BI from causing excessive apoptosis to the vascular wall.

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