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Hepatic Scavenger Receptor BI Protects Against Polymicrobial-induced Sepsis through Promoting LPS Clearance in Mice*

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Background: We utilized Scarb1^{I179N} mice, a model deficient in hepatic SR-BI, to determine the role of hepatic SR-BI in sepsis.
Results: Upon cecal ligation and puncture (CLP), Scarb1^{I179N} mice had a 3.5-fold increase in fatality associated with an impaired LPS clearance.
Conclusion: Hepatic SR-BI protects against sepsis through promoting LPS clearance.
Significance: Promoting hepatic SR-BI-mediated LPS clearance may provide a therapeutic approach for sepsis.

Recent studies revealed that scavenger receptor BI (SR-BI or Scarb1) plays a critical protective role in sepsis. However, the mechanisms underlying this protection remain largely unknown. In this study, using Scarb1^{I179N} mice, a mouse model specifically deficient in hepatic SR-BI, we report that hepatic SR-BI protects against cecal ligation and puncture (CLP)-induced sepsis as shown by 75% fatality in Scarb1^{I179N} mice, but only 21% fatality in C57BL/6J control mice. The increase in fatality in Scarb1^{I179N} mice was associated with an exacerbated inflammatory cytokine production. Further study demonstrated that hepatic SR-BI exerts its protection against sepsis through its role in promoting LPS clearance without affecting the inflammatory response in macrophages, the glucocorticoid production in adrenal glands, the leukocyte recruitment to peritoneum or the bacterial clearance in liver. Our findings reveal hepatic SR-BI as a critical protective factor in sepsis and point out that promoting hepatic SR-BI-mediated LPS clearance may provide a therapeutic approach for sepsis.

Sepsis is a major health issue, which claims over 215,000 lives and costs $16.7 billion per year in the United States (1–3). The death rate of sepsis is high, exceeding 30%, because of the poor understanding of the disease (4). Identifying molecules involved in sepsis, especially endogenous protective modulators, is of great importance not only in understanding the mechanisms, but also in providing new insights for efficient therapies.

Scavenger receptor BI (SR-BI or Scarb1)[2] is a HDL receptor predominately expressed in the liver and steroidogenic tissues (5–12). It regulates HDL cholesterol content through reverse cholesterol transport in which SR-BI selectively uptakes cholesteryl ester from HDL (10–13). Mice deficient in SR-BI display abnormally large HDL particles associated with a 180% increase in plasma HDL cholesterol concentrations (14–17). SR-BI$^{-/-}$ mice are susceptible to atherosclerosis (18–22). Emerging evidence indicates that SR-BI is a multi-functional protein. In addition to mediating intracellular cholesterol uptake from HDL, SR-BI has been shown to activate eNOS in endothelial cells (23–26), induce apoptosis (27, 28), modulate erythrocyte development (29, 30), and platelet function (31–34) mediate the entrance of hepatitis C virus (35), regulate lymphocyte homeostasis and autoimmunity (36), and regulate thymocyte apoptosis in sepsis (37). Mutations in human SR-BI induce a number of pathological phenotypes observed in SR-BI-null mice, indicating the importance of SR-BI in human (38–47).

Recent studies revealed an important function of SR-BI, namely, protection against sepsis, as shown by a marked increase in fatality in mice deficient in SR-BI upon lipopolysaccharides (LPS) or cecal ligation and puncture (CLP) challenge (48–51). Available evidence suggests that SR-BI may exert its protection via multiple mechanisms. Macrophage SR-BI suppresses inflammatory response by modulating LPS-TLR4 signaling in macrophages, which contributes to protection against septic animal death (50, 52); Adrenal SR-BI is a key determinant of inducible glucocorticoid (GC) generation in response to stress (49, 50, 53), and SR-BI null mice are completely deficient in inducible GC generation in CLP-induced sepsis (50); A number of studies suggest that SR-BI may provide protection against sepsis through detoxifying LPS. SR-BI binds to LPS and mediates the uptake of LPS *in vitro* (54), and SR-BI-null mice display impaired LPS clearance in circulation in LPS-induced endotoxemia or CLP-induced sepsis (49, 50). Liver is the most important organ for LPS detoxification. In sepsis, the majority of LPS target to Kupffer cells and hepatocytes (55–57). However, little is known about how the liver detoxifies LPS. It has been speculated that Kupffer cells and hepatocytes participate in uptake and clearance of LPS via a receptor-mediated mech-

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[2] The abbreviations used are: SR-BI, scavenger receptor BI; HDL, high density lipoprotein; CLP, cecal ligation and puncture; LPS, lipopolysaccharide; ALT, alanine aminotransferase.

anism. Given the abundant expression of SR-BI in hepatocytes (9) and the role of SR-BI in LPS uptake and clearance, we hypothesized that hepatic SR-BI provides protection against sepsis by promoting LPS clearance.

Huby *et al.* developed loxp-floxed SR-BI mice but the mice exhibited hypo-phenotypes as shown by a marked reduction in SR-BI expression in all tissues (58). Overexpression of SR-BI by adenoviral vector is a useful and widely used animal model to elucidate the function of hepatic SR-BI in regulating HDL metabolism (20, 59, 60). Unfortunately, adenovirus induces host immune response, which may alter the outcomes of sepsis (61). This presents a barrier to use these animal models for septic study. Stylianou *et al.* recently reported an interesting hepatic specific SR-BI deficient mouse model, Scarb1^{I179N} mice (62). The Scarb1^{I179N} mutant mice had a 90% decrease in hepatic SR-BI protein expression, resulting in a 170% increase in plasma cholesterol concentrations compared with C57BL/6J control mice. However, the Scarb1^{I179N} mice had normal SR-BI expression in non-hepatic tissues such as adrenal gland and ovary (62). In this study, we utilized this unique animal model to assess the role of hepatic SR-BI in sepsis. We demonstrate that hepatic SR-BI protects against CLP-induced septic death by promoting LPS clearance. Our findings reveal hepatic SR-BI as a critical protective factor in sepsis and point out that promoting hepatic SR-BI-mediated LPS clearance may provide a therapeutic approach for sepsis.

EXPERIMENTAL PROCEDURES

Materials—Anti-SR-BI serum was custom made by Sigma-Genosys using a 15 amino acid-peptide derived from the C terminus of human SR-BI. LPS (*Escherichia coli* serotype K12) was from InvivoGen. The endotoxin low FBS was from Hyclone. ELISA kits for quantifying TNF-α and IL-6 were from eBioscience. The alanine aminotransferase (ALT) kit was from BQ Kits, Inc. (San Diego, CA). The competitive ELISA kit for quantifying mouse corticosterone was from Cayman. The ELISA kit for quantifying LPS was from Charles River.

Mice Specifically Deficient in Hepatic SR-BI (Scarb1^{I179N})—Scarb1^{I179N} mice in C57BL/6J background were obtained from the Jackson Laboratory. The mice have an I179N point mutation resulting in a 90% decrease in liver SR-BI expression, but the mice have normal SR-BI expression in non-liver tissues (62). Thus, this is a unique animal model to elucidate the role of hepatic SR-BI in sepsis. C57BL/6J mice were used as controls. The animals were fed with a standard laboratory rodent diet. Animal care and experiments were approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

PCR Genotyping of Scarb1^{I179N} Mice—The SR-BI I179N mutation is caused by a T to A transversion which eliminates a BstYI site. Utilizing this character, we developed a simple PCR genotyping method to replace TaqMan SNP genotyping (62). Forward primer: 5′-GCC TGA GCT CTT TGC CTG AA; reverse primer: 5′-CCC AAC AAA CAG GCC AAA. PCR was run for 35 cycles using standard conditions. Upon BstYI digestion of the PCR product, a single 294 bp band for Scarb1^{I179N} mice, and 96 bp and 198 bp bands for C57BL/6J controls were obtained.

CLP Septic Animal Model—CLP was performed on 10–12-week-old Scarb1^{I179N} and C57BL/6J mice as described previously (50). Given the C57BL/6J background of Scarb1^{I179N} mice, a mild CLP (21-gauge needle, half ligation) was employed. Survival was monitored for a 7-day period.

Gram-positive Bacteria Infection—8–10-week old Scarb1^{I179N} and C57BL/6J mice were *intraperitoneal* injected with 7×10^8 CFU/mouse *Staphylococcus aureus* (ATCC 25923) and survival was monitored for 7 days.

Analysis of Leukocyte Recruitment to Peritoneum—leukocyte recruitment to peritoneal cavity was analyzed by flow cytometry as described (63–65). Briefly, 8–10-week old Scarb1^{I179N} and C57BL/6J mice were treated with/without CLP for 6 h and the peritoneal fluids were collected. Peritoneal neutrophils (PMN, CD11b^{hi} Ly6C^{hi}) and inflammatory monocytes (IM, CD11b^{int} Ly6C^{hi}) were gated by CD11b, and Ly6C expression on CD45$^+$ cells. Ly6G expression was confirmed to be high in gated PMNs and low in gated IMs.

Biochemical Assays—10–12-week-old mice were euthanized by CO_2 inhalation 6 and 20 h following CLP. The blood was obtained by cardiac puncture and stored at −80 °C. Serum ALT levels were quantified with the ALT kit and were used as an indicator for liver damage; the serum TNF-α, IL-6, corticosterone, and LPS levels were quantified with corresponding ELISA kits.

Quantification of Cytokine Generation in LPS-stimulated Macrophages—Bone marrow-derived macrophages were cultured as described previously (50). Briefly, bone marrow cells were cultured in a 12-well plate at 1.5×10^6 cells/well in RPMI1640 medium containing 20% FBS and 15% supernatant of L929 cell culture for 5 days. For measurement of cytokines, the cells were incubated in PBS for 4 h and treated with LPS at 0.5 ng/ml for 20 h in RPMI1640 medium containing 20% FBS. Cytokines in the culture supernatant were quantified with corresponding ELISA kit. Of note, LPS from *E. coli* k12 strain, is much more potent than LPS from other commonly used strains with respect to the activity of stimulating inflammatory cytokine production.

Assay for Bacterial Load—Bacterial load in liver and spleen was analyzed as previously described (50).

Assay for SR-BI Expression—Tissue or cells were homogenized in lysis buffer containing 1% proteinase inhibitor mixture (Sigma), and subjected to Western blot analysis against SR-BI as described previously (50). The expression of SR-BI was quantified with Fuji LAS-4000 and normalized to actin expression.

Statistical Analysis—The survival assay was analyzed by Log-Rank x^2 test using SAS software. Significance in experiments comparing two groups was determined by 2-tailed Student's t test. Significance in experiments comparing more than two groups was evaluated by One Way ANOVA, followed by post hoc analysis using Tukey's test. Means were considered different at $p < 0.05$.

RESULTS

Hepatic SR-BI Protects against CLP-induced Septic Death—The Scarb1^{I179N} mutation is caused by a T to A transversion in exon 4 which eliminates a BstYI site (R/GATCY). Based on this character, we designed a PCR method to genotype the mutant

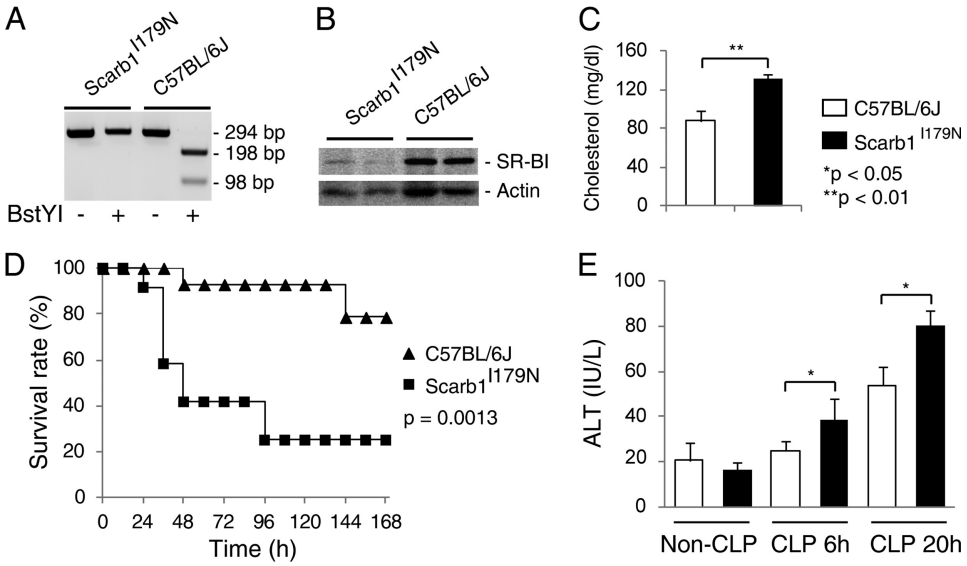

FIGURE 1. **Mice deficient in hepatic SR-BI are susceptible to CLP-induced septic death.** *A*, PCR genotyping of Scarb1^{I179N} mice. The PCR products were digested with/without BstYI and subjected to 2.5% agarose gel electrophoresis; *B*, Western blot analysis of SR-BI expression in the liver; *C*, serum cholesterol concentrations. The Scarb1^{I179N} and C57BL/6J mice were fasted for 4 h. $n = 6$ each group with duplicate measurements, mean ± S.E.; *D*, survival analysis. Scarb1^{I179N} ($n = 12$) and C57BL/6J mice ($n = 14$) were treated with CLP and survival was observed for 7 days. The data were expressed as the percentage of mice surviving at indicated times, and survival was analyzed by Log-Rank x^2 test. *E*, CLP induced liver injury. Scarb1^{I179N} and C57BL/6J mice were treated with CLP for the indicated times, and liver injury was assessed by measuring serum ALT levels. $n = 6$ - 11 each group with duplicate measurements, mean ± S.E.

mice. As shown in Fig. 1*A*, PCR generated a 294-bp product. BstYI digestion of the PCR product yielded a single 294 bp band for Scarb1^{I179N} mice, but 96 bp and 198 bp bands for C57BL/6J controls. The Scarb1^{I179N} mice had a 90% decrease in SR-BI expression in the liver (Fig. 1*B*) and a 150% increase in serum cholesterol concentrations (Fig. 1*C*), which was consistent with the previous report (62). Thus, the Scarb1^{I179N} mice presented a unique animal model to elucidate the role of hepatic SR-BI in sepsis. As shown in Fig. 1**D**, CLP induced 21.4% fatality in C57BL/6J control mice, but 75% fatality in Scarb1^{I179N} mice, indicating that hepatic SR-BI provides significant protection against CLP-induced septic death. CLP-induced liver injury was assessed by measuring serum ALT levels. Compared with wild type controls, Scarb1^{I179N} mice had a significant increase in serum ALT levels at 6 and 20 h following CLP (Fig. 1*E*). These findings indicate that hepatic SR-BI protects against polymicrobial sepsis.

Exacerbated Innate Immune Response in Scarb1^{I179N} Mice during Sepsis—To understand why Scarb1^{I179N} mice were susceptible to septic death, we assessed inflammatory cytokine production at 6 and 20 h following CLP. Upon CLP, C57BL/6J control mice exhibited a typical acute phase response as shown by a rapid and strong induction of TNF-α and IL-6 in the early stage of sepsis (6 h) and significant decreases in TNF-α and IL-6 levels by 20 h (Fig. 2, *A* and *B*). Scarb1^{I179N} mice also had a rapid and strong induction of TNF-α and IL-6 in the early stage of sepsis (6 h); however, Scarb1^{I179N} mice displayed uncontrolled inflammatory cytokine generation as shown by high serum concentrations of TNF-α and IL-6 20 h after CLP (Fig. 2, *A* and *B*).

Hepatic SR-BI Is Required for LPS Clearance in Sepsis—Macrophages are one of the major types of cells that generate inflammatory cytokines during sepsis. SR-BI is moderately expressed in macrophages and recent studies demonstrated that macrophage SR-BI suppresses inflammatory response of

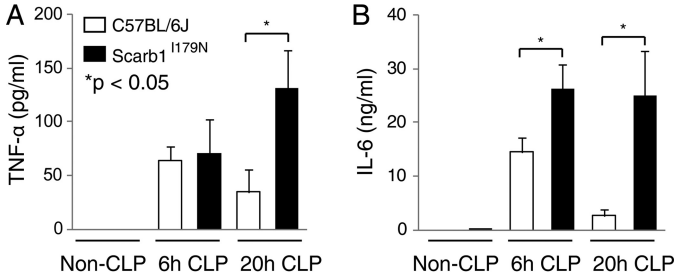

FIGURE 2. **Exacerbated inflammatory cytokine production in Scarb1^{I179N} mice in sepsis.** Scarb1^{I179N} and C57BL/6J mice were treated with/without CLP for the indicated times, and the serum concentrations of TNF-α (*A*) and IL-6 (*B*) were quantified. $n = 6$–11 each group with duplicate measurements, mean ± S.E.

macrophages to LPS (50, 52). To test whether a deficiency of hepatic SR-BI affects inflammatory response in macrophages, we utilized bone marrow-derived macrophages from Scarb1^{I179N} and control mice. Upon stimulation by 0.5 ng/ml of LPS for 20 h, macrophages from Scarb1^{I179N} mice produced similar levels of TNF-α and IL-6 compared with macrophages from the control mice (Fig. 3, *A* and *B*).

We then looked for an alternative explanation for hepatic SR-BI's protective role in sepsis. Endotoxemia is a hallmark of sepsis. LPS released by Gram-negative bacteria is a major stimulator for systemic inflammatory cytokine production. We speculated that the exacerbated inflammatory cytokine production observed in Scarb1^{I179N} mice is caused by elevated LPS in the circulation. To test this, we measured serum LPS levels in CLP-challenged mice. Scarb1^{I179N} mice had a 3-fold increase in serum LPS levels compared with wild type controls 6 and 20 h following CLP (Fig. 4*A*), suggesting that hepatic SR-BI is responsible for LPS clearance in sepsis.

SR-BI is abundantly expressed in adrenal glands. Adrenal SR-BI mediates the intracellular uptake of cholesterol ester from HDL, which provides a substrate for glucocorticoid syn-

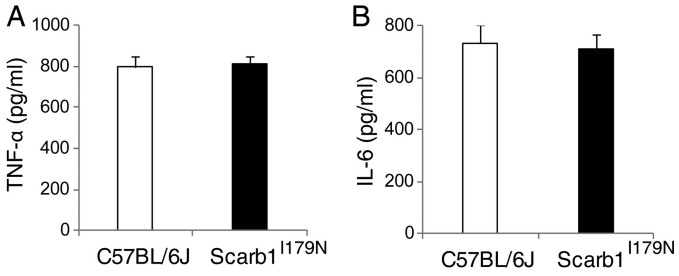

FIGURE 3. **Deficiency of hepatic SR-BI does not affect LPS-induced inflammatory cytokine production in macrophages.** Bone marrow-derived cells were isolated from Scarb1^{I179N} and C57BL/6J mice and cultured at 37 °C for 5 days. The cells were then treated with 0.5 ng LPS/ml for 20 h. The concentrations of TNF-α (*A*) and IL-6 (*B*) in the culture supernatant were quantified. $n = 3$ per group with triplicate measurements, mean ± S.E.

thesis (9, 12, 49, 66, 67). Given the strong effects of glucocorticoids in regulating inflammatory response, we tested whether the exacerbated inflammatory cytokine generation is caused by a disruption in adrenal glucocorticoid production. As shown in Fig. 4*B*, CLP induced a significant increase in corticosterone generation in both Scarb1^{I179N} mice and wild type mice 6 and 20 h following CLP. Unexpectedly, CLP-Scarb1^{I179N} mice actually had a moderate increase in corticosterone concentrations compared with CLP-wild type mice. It is unlikely that the exacerbated inflammatory cytokine generation in Scarb1^{I179N} mice is caused by altered adrenal glucocorticoid production.

SR-BI is capable of binding Gram-negative bacteria and facilitating the uptake of Gram-negative bacteria *in vitro* (54, 68). This raises a possibility that SR-BI might play a role in bacterial clearance in sepsis. Kupffer cells in the liver constitute a major pool of macrophages in the body. To determine whether hepatic SR-BI affects bacterial uptake by macrophages during sepsis, we isolated total DNA from the liver and quantified bacteria with qPCR using bacteria specific primers. No significant difference in the number of bacteria was observed between Scarb1^{I179N} mice and C57BL/6J control mice (Fig. 4*C*). We also quantified living bacteria in spleen, no significant difference in the number of living bacteria was observed between Scarb1^{I179N} mice and C57BL/6J mice ($1.5 \times 10^5 \pm 2.0 \times 10^5$/g tissue in Scarb1^{I179N} mice *versus* $1.2 \times 10^5 \pm 2.5 \times 10^5$/g tissue in C57BL/6J mice).

SR-BI is a well-established HDL receptor. Hepatic SR-BI mediates the uptake of cholesterol ester from HDL, which plays a critical role in regulating HDL metabolism. Stylianou *et al.* reported that Scarb1^{I179N} mice display moderately larger HDL particles. We had similar observations (data not shown). HDL regulates the expression of adhesion molecules (69), and a deficiency of HDL has been shown to impair the recruitment of leukocytes to peritoneal cavity following CLP (65). To elucidate if hepatic SR-BI affects leukocyte recruitment in sepsis, we quantified peritoneal neutrophils and inflammatory monocytes with flow cytometry in Scarb1^{I179N} mice and C57BL/6J mice. Almost no peritoneal neutrophils and inflammatory monocytes were observed in non-CLP animals (Fig. 5*A*). Six hours following CLP, a significant number of neutrophils and inflammatory monocytes were recruited to the peritoneum (Fig. 5*B*) in both Scarb1^{I179N} and C57BL/6J mice, but no difference in leukocyte recruitment was observed between these two strains (Fig. 5*C*).

Role of Hepatic SR-BI in Gram-positive Bacteria-induced Sepsis—The above findings suggest that hepatic SR-BI provides protection primarily through its role in promoting LPS clearance. To determine whether hepatic SR-BI provides protection other than LPS clearance, we infected Scarb1^{I179N} mice and C57BL/6J mice with Gram-positive bacteria *S aureus,* and monitored survival for 7 days. A moderate decrease in survival was observed in Scarb1^{I179N} mice compare with C57BL/6J mice ($p = 0.68$) (Fig. 6).

DISCUSSION

Using Scarb1^{I179N} mice, a unique hepatic specific SR-BI deficient mouse model, we demonstrate that hepatic SR-BI is a critical protective factor in sepsis. Scarb1^{I179N} mice had a 3-fold increase in CLP-induced septic fatality associated with exacerbated inflammatory cytokine production compared with wild type control mice.

To understand the mechanisms by which hepatic SR-BI protects against sepsis, we tested five possible effects of hepatic SR-BI: 1) on LPS clearance. SR-BI has been shown to bind LPS and mediate the uptake of LPS *in vitro* (54), and SR-BI-null mice display impaired LPS clearance in circulation in LPS-induced endotoxemia or CLP-induced sepsis (49, 50). These findings suggest that SR-BI plays a critical role in LPS clearance. Given that majority of LPS target to the liver in sepsis (55–57) and SR-BI is abundantly expressed in the liver, it is plausible that hepatic SR-BI functions to clear LPS. In this study, by measuring serum LPS levels, we found that Scarb1^{I179N} mice had a 3-fold increase in serum LPS concentrations compared with wild type control mice; 2) on macrophage inflammatory response. We found that macrophages from Scarb1^{I179N} and wild type control mice exhibit similar inflammatory cytokine production in response to LPS, suggesting that a deficiency of hepatic SR-BI does not alter the macrophage inflammatory response; 3) on adrenal steroidogenesis. We found that Scarb1^{I179N} mice have a moderate increase in corticosterone production compared with wild type control mice in sepsis. Given the potent suppressive effects of glucocorticoid on inflammation, it is unlikely that the increase in corticosterone production contributes to the elevated inflammatory cytokine production; 4) on bacterial clearance. We found no difference in the number of liver bacteria between Scarb1^{I179N} mice and wild type mice; 5) on leukocyte recruitment. Given that HDL regulates leukocyte recruitment, we assessed neutrophils and inflammatory monocytes in the peritoneum in response to CLP. We found no difference in the number of peritoneal neutrophils or inflammatory monocytes between Scarb1^{I179N} mice and wild type mice.

While LPS-induced inflammatory cell damage has been considered as a major cause of septic death, the failure of anti-LPS monoclonal antibodies in clinical trials questions the contribution of LPS to septic death. An issue associated with LPS neutralization is that the binding of antibody to LPS sequesters LPS, which delays LPS clearance (70), suggesting that simple neutralization of LPS may have limited effect on detoxifying LPS. It is worth noting that, different from neutralizing LPS, SR-BI detoxifies LPS through mediating the intracellular uptake of LPS and promoting LPS clearance (49, 50, 54). Com-

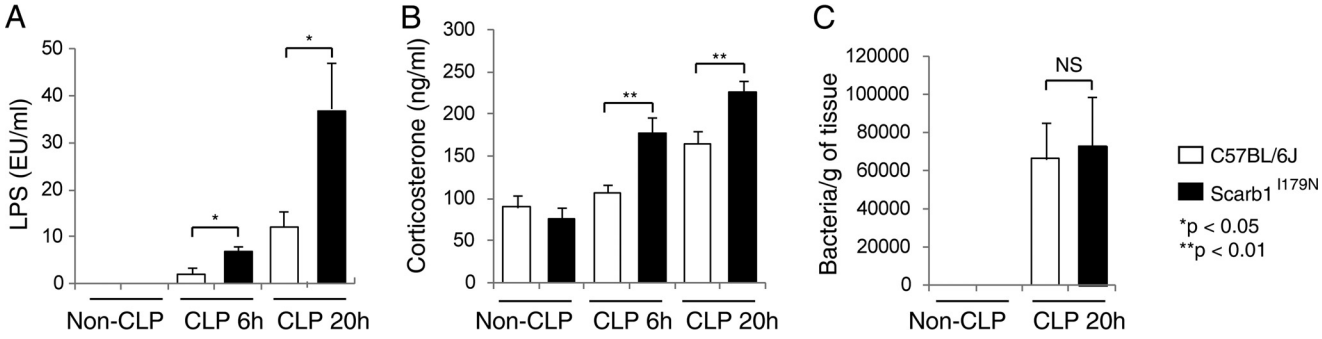

FIGURE 4. **Impaired LPS clearance in Scarb1^{I179N} mice in sepsis.** Scarb1^{I179N} and C57BL/6J mice were treated with CLP for 6 and 20 h, and the sera were collected for measurements of LPS (*A*) and corticosterone (*B*); the number of bacteria in the liver was analyzed by quantitative PCR (*C*). $n = 6–11$ each group with duplicate measurements, mean ± S.E.

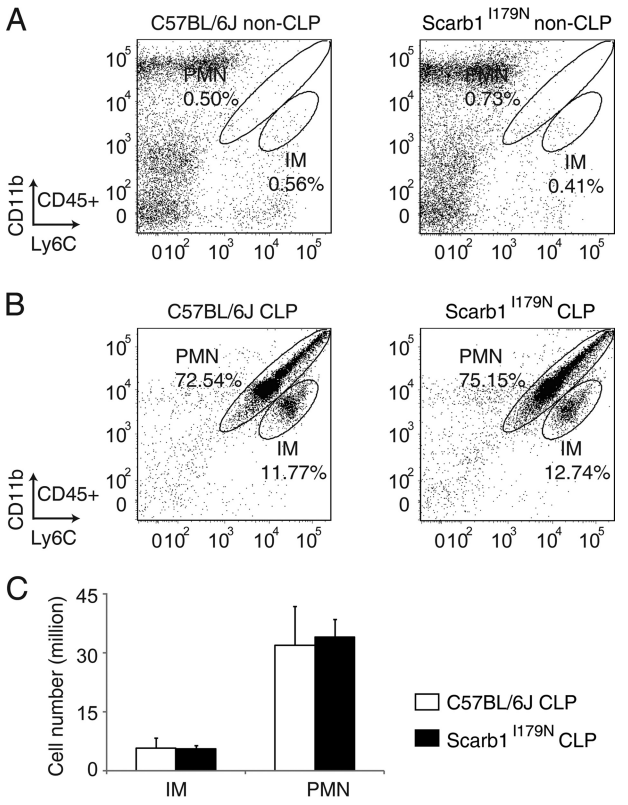

FIGURE 5. **Deficiency of hepatic SR-BI does not affect CLP-induced leukocyte recruitment to peritoneal cavity.** Scarb1^{I179N} and C57BL/6J mice were treated with/without CLP for 6 h, and the peritoneal fluid was collected. Peritoneal neutrophils (PMN, CD11b^{hi} Ly6C^{hi}) and inflammatory monocytes (IM, CD11b^{int} Ly6C^{hi}) were gated by CD11b, and Ly6C expression on CD45^{+} cells. *A*, non-CLP Scarb1^{I179N} and C57BL/6J mice. Almost no neutrophils and inflammatory monocytes were observed in the peritoneum of non-CLP mice. *B* and *C*, CLP Scarb1^{I179N} and C57BL/6J mice. Similar percentage of PMNs and IMs in CD45^{+} cells was detected in Scarb1^{I179N} and C57BL/6J mice. *C*, similar number of recruited PMNs and IMs was detected in CLP Scarb1^{I179N} and C57BL/6J mice. $n = 7$ per group, mean ± S.E.

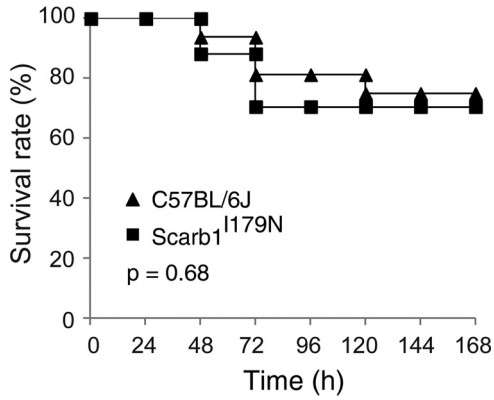

FIGURE 6. **Deficiency of hepatic SR-BI slightly affects Gram-positive bacteria-induced sepsis.** Scarb1^{I179N} ($n = 17$) and C57BL/6J ($n = 16$) mice were intraperitoneal injected with 7×10^8 CFU/mouse *Staphylococcus aureus*, and survival was monitored for 7 days. The data were expressed as the percentage of mice surviving at indicated times, and survival was analyzed by Log-Rank x^2 test.

bined with the observations that SR-BI-null mice are susceptible to LPS-induced endotoxic animal death (48, 49), SR-BI is abundantly expressed in liver (9), liver is a major organ for LPS clearance (55–57) and a deficiency of hepatic SR-BI leads to impaired LPS clearance, we get our conclusion that hepatic SR-BI-mediated LPS clearance contributes to protection against sepsis. To further clarify this issue, we administered polymyxin B (PMB, a potent LPS neutralizer (71), 0.6 mg/kg body weight, intraperitoneal) to Scarb^{1I179N} and C57BL/6J mice 2h post CLP challenge. PMB profoundly inhibited IL-6 production in Scarb^{1I179N} mice but not in B6 mice 18 h post CLP, compared with mice without PMB treatment (0.47 ± 0.08 ng/ml in CLP-Scarb1^{I179N} mice with PMB treatment *versus* 25.08 ± 8.32 ng/ml in CLP-Scarb1^{I179N} mice without PMB treatment; 5.22 ± 4.66 ng/ml in C57BL/6J mice with PMB treatment *versus* 2.66 ± 1.21 ng/ml in C57BL/6J mice without PMB treatment). We only observed a slight increase in survival in PMB-treated septic Scarb^{1I179N} (33.3% survival in CLP-Scarb1^{I179N} mice with PMB treatment *versus* 25% survival in CLP-Scarb1^{I179N} mice without PMB treatment). The PMB treatment actually decreased the survival in septic B6 mice (33.3% survival in CLP-C57BL/6J mice with PMB treatment *versus* 78.6% survival in CLP-C57BL/6J mice without PMB treatment). These suggest that simple neutralization of LPS by LPS neutralizer cannot provide efficient protection against septic death and promoting LPS clearance is a more efficient way for LPS detoxification.

Our earlier study indicated that mice with whole body SR-BI deficiency are susceptible to CLP-induced septic death (50). While both whole body SR-BI-null mice and the hepatic SR-BI-deficient mice are susceptible to CLP-induced sepsis, we have noticed an interesting difference in inflammatory cytokine generation between these two animal models. Compared with strain-marched wild type control mice, Scarb1^{I179N} mice had a rapid induction of TNF-α and IL-6 in the early stage of sepsis (6

h), and uncontrolled inflammatory cytokine generation in the late stage of sepsis (20 h). Meanwhile, whole body SR-BI-null mice displayed delayed inflammatory cytokine generation in the early stage of sepsis and uncontrolled inflammatory cytokine generation in the late stage of sepsis (50); interestingly, the difference in inflammatory cytokine generation patterns is in line with the LPS levels in the circulation. Scarb1^{I179N} mice had elevated serum LPS in both the early and late stage of sepsis; but, the whole body SR-BI-null mice had lower serum LPS in the early stage of sepsis and elevated serum LPS in the late stage of sepsis (50). These findings suggest that hepatic SR-BI is responsible for LPS removal and a defect in hepatic SR-BI-mediated LPS removal leads to endotoxemia, which likely accounts for exacerbated inflammatory cytokine production observed in Scarb1^{I179N} mice; These findings also imply that non-hepatic SR-BI may be required for the recruitment of LPS to circulation, and a deficiency of non-hepatic SR-BI leads to a delayed inflammatory response in the early stage of sepsis. Endothelial cells have been shown to uptake LPS in a receptor-dependent manner (72, 73). Interestingly, earlier studies showed that endothelial cells express high levels of SR-BI (23, 24, 74). It is likely that endothelial SR-BI is responsible for recruitment of LPS to circulation. Further study is warranted to test these speculations.

An earlier study pointed out that hepatic SR-BI expression is suppressed by LPS and by inflammatory cytokines such as TNF-α (75). Sepsis induces profound increases in circulating LPS and inflammatory cytokines, which likely causes the down-regulation of hepatic SR-BI. Indeed, we found that the hepatic SR-BI expression is down-regulated in C57BL/6J mice 20 h post CLP (data not shown). Combined with our finding that hepatic SR-BI is required for LPS clearance, these observations suggest that promoting hepatic SR-BI expression may provide a therapeutic approach for sepsis. Further study is needed to test this speculation.

In summary, using the unique hepatic specific SR-BI-deficient animal model, we demonstrate that hepatic SR-BI provides protection against sepsis through promoting LPS clearance. Given that the expression of SR-BI is down-regulated in sepsis, we propose that promoting hepatic SR-BI-mediated LPS removal may provide a novel therapeutic strategy for sepsis.

REFERENCES

1. Martin, G. S., Mannino, D. M., Eaton, S., and Moss, M. (2003) The Epidemiology of Sepsis in the United States from 1979 through 2000. *N. Engl. J. Med.* **348,** 1546–1554
2. Riedemann, N. C., Guo, R. F., and Ward, P. A. (2003) The enigma of sepsis. *J. Clin. Invest.* **112,** 460–467
3. Angus, D. C., and van der Poll, T. (2013) Severe sepsis and septic shock. *N. Engl. J. Med.* **369,** 840–851
4. Sessler, C. N., Perry, J. C., and Varney, K. L. (2004) Management of severe sepsis and septic shock. *Curr. Opin. Crit. Care* **10,** 354–363
5. Murao, K., Terpstra, V., Green, S. R., Kondratenko, N., Steinberg, D., and Quehenberger, O. (1997) Characterization of CLA-1, a human homologue of rodent scavenger receptor BI, as a receptor for high density lipoprotein and apoptotic thymocytes. *J. Biol. Chem.* **272,** 17551–17557
6. Landschulz, K. T., Pathak, R. K., Rigotti, A., Krieger, M., and Hobbs, H. H. (1996) Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat. *J. Clin. Invest.* **98,** 984–995
7. Calvo, D., and Vega, M. A. (1993) Identification, primary structure, and distribution of CLA-1, a novel member of the CD36/LIMPII gene family. *J. Biol. Chem.* **268,** 18929–18935
8. Acton, S. L., Scherer, P. E., Lodish, H. F., and Krieger, M. (1994) Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J. Biol. Chem.* **269,** 21003–21009
9. Acton, S., Rigotti, A., Landschulz, K. T., Xu, S., Hobbs, H. H., and Krieger, M. (1996) Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* **271,** 518–520
10. Holmes RS, and LA, C. (2012) Comparative studies of vertebrate scavenger receptor class B type 1: a high-density lipoprotein binding protein. *Res. Rep. Biochem.* **2,** 9–24
11. Mineo, C., and Shaul, P. W. (2012) Functions of scavenger receptor class B, type I in atherosclerosis. *Curr. Opin. Lipidol.* **23,** 487–493
12. Krieger, M. (1999) CHARTING THE FATE OF THE "GOOD CHOLESTEROL": Identification and Characterization of the High-Density Lipoprotein Receptor SR-BI. *Annu. Rev. Biochem.* **68,** 523–558
13. Lim, H. Y., Thiam, C. H., Yeo, K. P., Bisoendial, R., Hii, C. S., McGrath, K. C., Tan, K. W., Heather, A., Alexander, J. S., and Angeli, V. (2013) Lymphatic vessels are essential for the removal of cholesterol from peripheral tissues by SR-BI-mediated transport of HDL. *Cell Metab.* **17,** 671–684
14. Rigotti, A., Trigatti, B. L., Penman, M., Rayburn, H., Herz, J., and Krieger, M. (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. U.S.A.* **94,** 12610–12615
15. Brundert, M., Ewert, A., Heeren, J., Behrendt, B., Ramakrishnan, R., Greten, H., Merkel, M., and Rinninger, F. (2005) Scavenger receptor class B type I mediates the selective uptake of high-density lipoprotein-associated cholesteryl ester by the liver in mice. *Arterioscler. Thromb. Vasc. Biol.* **25,** 143–148
16. Brundert, M., Heeren, J., Bahar-Bayansar, M., Ewert, A., Moore, K. J., and Rinninger, F. (2006) Selective uptake of HDL cholesteryl esters and cholesterol efflux from mouse peritoneal macrophages independent of SR-BI. J. *Lipid Res.* **47,** 2408–2421
17. Ma, K., Forte, T., Otvos, J. D., and Chan, L. (2005) Differential Additive Effects of Endothelial Lipase and Scavenger Receptor-Class B Type I on High-Density Lipoprotein Metabolism in Knockout Mouse Models. *Arterioscler. Thromb. Vasc. Biol.* **25,** 149–154
18. Arai, T., Rinninger, F., Varban, L., Fairchild-Huntress, V., Liang, C. P., Chen, W., Seo, T., Deckelbaum, R., Huszar, D., and Tall, A. R. (1999) Decreased selective uptake of high density lipoprotein cholesteryl esters in apolipoprotein E knock-out mice. *Proc. Natl. Acad. Sci. U.S.A.* **96,** 12050–12055
19. Braun, A., Trigatti, B. L., Post, M. J., Sato, K., Simons, M., Edelberg, J. M., Rosenberg, R. D., Schrenzel, M., and Krieger, M. (2002) Loss of SR-BI expression leads to the early onset of occlusive atherosclerotic coronary artery disease, spontaneous myocardial infarctions, severe cardiac dysfunction, and premature death in apolipoprotein E-deficient mice. *Circ. Res.* **90,** 270–276
20. Kozarsky, K. F., Donahee, M. H., Glick, J. M., Krieger, M., and Rader, D. J. (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
21. Yu, H., Zhang, W., Yancey, P. G., Koury, M. J., Zhang, Y., Fazio, S., and Linton, M. F. (2006) Macrophage apolipoprotein E reduces atherosclerosis and prevents premature death in apolipoprotein E and scavenger receptor-class BI double-knockout mice. *Arterioscler. Thromb. Vasc. Biol.* **26,** 150–156
22. Van Eck, M., Twisk, J., Hoekstra, M., Van Rij, B. T., Van der Lans, C. A., Bos, I. S., Kruijt, J. K., Kuipers, F., and Van Berkel, T. J. (2003) Differential effects of scavenger receptor BI deficiency on lipid metabolism in cells of the arterial wall and the liver. *J. Biol. Chem.* **278,** 23699–23705
23. Li, X. A., Titlow, W. B., Jackson, B. A., Giltiay, N., Nikolova-Karakashian, M., Uittenbogaard, A., and Smart, E. J. (2002) High density lipoprotein binding to scavenger receptor, Class B, type I activates endothelial nitric-oxide synthase in a ceramide-dependent manner. *J. Biol. Chem.* **277,** 11058–11063

24. Yuhanna, I. S., Zhu, Y., Cox, B. E., Hahner, L. D., Osborne-Lawrence, S., Lu, P., Marcel, Y. L., Anderson, R. G., Mendelsohn, M. E., Hobbs, H. H., and Shaul, P. W. (2001) High-density lipoprotein binding to scavenger receptor-BI activates endothelial nitric oxide synthase. *Nat. Med* **7,** 853–857
25. Mineo, C., Yuhanna, I. S., Quon, M. J., and Shaul, P. W. (2003) High Density Lipoprotein-induced Endothelial Nitric-oxide Synthase Activation Is Mediated by Akt and MAP Kinases. *J. Biol. Chem.* **278,** 9142–9149
26. Gong, M., Wilson, M., Kelly, T., Su, W., Dressman, J., Kincer, J., Matveev, S. V., Guo, L., Guerin, T., Li, X. A., Zhu, W., Uittenbogaard, A., and Smart, E. J. (2003) HDL-associated estradiol stimulates endothelial NO synthase and vasodilation in an SR-BI-dependent manner. *J. Clin. Invest.* **111,** 1579–1587
27. Li, X. A., Guo, L., Dressman, J. L., Asmis, R., and Smart, E. J. (2005) A novel ligand-independent apoptotic pathway induced by scavenger receptor class B, type I and suppressed by endothelial nitric-oxide synthase and high density lipoprotein. *J. Biol. Chem.* **280,** 19087–19096
28. Feng, H., Guo, L., Gao, H., and Li, X. A. (2011) Deficiency of calcium and magnesium induces apoptosis via scavenger receptor BI. *Life Sci.* **88,** 606–612
29. Holm, T. M., Braun, A., Trigatti, B. L., Brugnara, C., Sakamoto, M., Krieger, M., and Andrews, N. C. (2002) Failure of red blood cell maturation in mice with defects in the high-density lipoprotein receptor SR-BI. *Blood* **99,** 1817–1824
30. Meurs, I., Hoekstra, M., van Wanrooij, E. J., Hildebrand, R. B., Kuiper, J., Kuipers, F., Hardeman, M. R., Van Berkel, T. J., and Van Eck, M. (2005) HDL cholesterol levels are an important factor for determining the lifespan of erythrocytes. *Exp. Hematol.* **33,** 1309–1319
31. Dole, V. S., Matuskova, J., Vasile, E., Yesilaltay, A., Bergmeier, W., Bernimoulin, M., Wagner, D. D., and Krieger, M. (2008) Thrombocytopenia and platelet abnormalities in high-density lipoprotein receptor-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **28,** 1111–1116
32. Valiyaveettil, M., Kar, N., Ashraf, M. Z., Byzova, T. V., Febbraio, M., and Podrez, E. A. (2008) Oxidized high-density lipoprotein inhibits platelet activation and aggregation via scavenger receptor BI. *Blood* **111,** 1962–1971
33. Brodde, M. F., Korporaal, S. J., Herminghaus, G., Fobker, M., Van Berkel, T. J., Tietge, U. J., Robenek, H., Van Eck, M., Kehrel, B. E., and Nofer, J. R. (2011) Native high-density lipoproteins inhibit platelet activation via scavenger receptor BI: role of negatively charged phospholipids. *Atherosclerosis* **215,** 374–382
34. Korporaal, S. J., Meurs, I., Hauer, A. D., Hildebrand, R. B., Hoekstra, M., Cate, H. T., Praticò, D., Akkerman, J. W., Van Berkel, T. J., Kuiper, J., and Van Eck, M. (2011) Deletion of the high-density lipoprotein receptor scavenger receptor BI in mice modulates thrombosis susceptibility and indirectly affects platelet function by elevation of plasma free cholesterol. *Arterioscler. Thromb. Vasc. Biol.* **31,** 34–42
35. Dao Thi, V. L., Dreux, M., and Cosset, F. L. (2011) Scavenger receptor class B type I and the hypervariable region-1 of hepatitis C virus in cell entry and neutralisation. *Exp. Rev. Mol. Med.* **13,** e13
36. Feng, H., Guo, L., Wang, D., Gao, H., Hou, G., Zheng, Z., Ai, J., Foreman, O., Daugherty, A., and Li, X. A. (2011) Deficiency of Scavenger Receptor BI Leads to Impaired Lymphocyte Homeostasis and Autoimmune Disorders in Mice. *Arterioscler. Thromb. Vasc. Biol.* **31,** 2543–2551
37. Guo, L., Zheng, Z., Ai, J., Howatt, D. A., Mittelstadt, P. R., Thacker, S., Daugherty, A., Ashwell, J. D., Remaley, A. T., and Li, X. A. (2014) Scavenger Receptor BI and High-Density Lipoprotein Regulate Thymocyte Apoptosis in Sepsis. *Arterioscler. Thromb. Vasc. Biol.* **34,** 966–975
38. Vergeer, M., Korporaal, S. J., Franssen, R., Meurs, I., Out, R., Hovingh, G. K., Hoekstra, M., Sierts, J. A., Dallinga-Thie, G. M., Motazacker, M. M., Holleboom, A. G., Van Berkel, T. J., Kastelein, J. J., Van Eck, M., and Kuivenhoven, J. A. (2011) Genetic variant of the scavenger receptor BI in humans. *N. Engl. J. Med.* **364,** 136–145
39. Chadwick, A. C., and Sahoo, D. (2013) Functional genomics of the human high-density lipoprotein receptor scavenger receptor BI: an old dog with new tricks. *Curr. Opin. Endocrinol. Diabetes Obes.* **20,** 124–131
40. Clifford, A. J., Chen, K., McWade, L., Rincon, G., Kim, S. H., Holstege, D. M., Owens, J. E., Liu, B., Müller, H. G., Medrano, J. F., Fadel, J. G., Moshfegh, A. J., Baer, D. J., and Novotny, J. A. (2012) Gender and single nucleotide polymorphisms in MTHFR, BHMT, SPTLC1, CRBP2, CETP, and SCARB1 are significant predictors of plasma homocysteine normalized by RBC folate in healthy adults. *J. Nutr.* **142,** 1764–1771
41. Brunham, L. R., Tietjen, I., Bochem, A. E., Singaraja, R. R., Franchini, P. L., Radomski, C., Mattice, M., Legendre, A., Hovingh, G. K., Kastelein, J. J., and Hayden, M. R. (2011) Novel mutations in scavenger receptor BI associated with high HDL cholesterol in humans. *Clin. Genet.* **79,** 575–581
42. Manichaikul, A., Naj, A. C., Herrington, D., Post, W., Rich, S. S., and Rodriguez, A. (2012) Association of SCARB1 variants with subclinical atherosclerosis and incident cardiovascular disease: the multi-ethnic study of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **32,** 1991–1999
43. Yates, M., Kolmakova, A., Zhao, Y., and Rodriguez, A. (2011) Clinical impact of scavenger receptor class B type I gene polymorphisms on human female fertility. *Hum. Reprod* **26,** 1910–1916
44. Naj, A. C., West, M., Rich, S. S., Post, W., Kao, W. H., Wasserman, B. A., Herrington, D. M., and Rodriguez, A. (2010) Association of scavenger receptor class B type I polymorphisms with subclinical atherosclerosis: the Multi-Ethnic Study of Atherosclerosis. *Circ Cardiovasc Genet* **3,** 47–52
45. Roberts, C. G., Shen, H., Mitchell, B. D., Damcott, C. M., Shuldiner, A. R., and Rodriguez, A. (2007) Variants in scavenger receptor class B type I gene are associated with HDL cholesterol levels in younger women. *Hum. Hered* **64,** 107–113
46. Edmondson, A. C., Braund, P. S., Stylianou, I. M., Khera, A. V., Nelson, C. P., Wolfe, M. L., Derohannessian, S. L., Keating, B. J., Qu, L., He, J., Tobin, M. D., Tomaszewski, M., Baumert, J., Klopp, N., Döring, A., Thorand, B., Li, M., Reilly, M. P., Koenig, W., Samani, N. J., and Rader, D. J. (2011) Dense genotyping of candidate gene loci identifies variants associated with high-density lipoprotein cholesterol. *Circ. Cardiovasc Genet* **4,** 145–155
47. Rejeb, J., Omezzine, A., Boumaiza, I., Rebhi, L., Kacem, S., Rejeb, N. B., Nabli, N., Abdelaziz, A. B., Boughzala, E., and Bouslama, A. (2012) Association of three polymorphisms of scavenger receptor class BI gene (exon8, exon1, intron5) with coronary stenosis in a coronary Tunisian population. *Gene* **511,** 383–388
48. Li, X. A., Guo, L., Asmis, R., Nikolova-Karakashian, M., and Smart, E. J. (2006) Scavenger receptor BI prevents nitric oxide-induced cytotoxicity and endotoxin-induced death. *Circ. Res.* **98,** e60–65
49. Cai, L., Ji, A., de Beer, F. C., Tannock, L. R., and van der Westhuyzen, D. R. (2008) SR-BI protects against endotoxemia in mice through its roles in glucocorticoid production and hepatic clearance. *J. Clin. Invest.* **118,** 364–375
50. Guo, L., Song, Z., Li, M., Wu, Q., Wang, D., Feng, H., Bernard, P., Daugherty, A., Huang, B., and Li, X. A. (2009) Scavenger Receptor BI Protects against Septic Death through Its Role in Modulating Inflammatory Response. *J. Biol. Chem.* **284,** 19826–19834
51. Guo, L., Zheng, Z., Ai, J., Huang, B., and Li, X.-A. (2012) Comment on "Class B Scavenger Receptor Types I and II and CD36 Targeting Improves Sepsis Survival and Acute Outcomes in Mice". *J. Immunol.* **189,** 501
52. Cai, L., Wang, Z., Meyer, J. M., Ji, A., and van der Westhuyzen, D. R. (2012) Macrophage SR-BI regulates pro-inflammatory signaling in mice and isolated macrophages. *J. Lipid Res.* **53,** 1472–1481
53. Hoekstra, M., Meurs, I., Koenders, M., Out, R., Hildebrand, R. B., Kruijt, J. K., Van Eck, M., and Van Berkel, T. J. (2008) Absence of HDL cholesteryl ester uptake in mice via SR-BI impairs an adequate adrenal glucocorticoid-mediated stress response to fasting. *J. Lipid Res.* **49,** 738–745
54. Vishnyakova, T. G., Bocharov, A. V., Baranova, I. N., Chen, Z., Remaley, A. T., Csako, G., Eggerman, T. L., and Patterson, A. P. (2003) Binding and internalization of lipopolysaccharide by Cla-1, a human orthologue of rodent scavenger receptor B1. *J. Biol. Chem.* **278,** 22771–22780
55. Freudenberg, N., Freudenberg, M. A., Bandara, K., and Galanos, C. (1985) Distribution and localization of endotoxin in the reticulo-endothelial system (RES) and in the main vessels of the rat during shock. *Pathol. Res. Pract.* **179,** 517–527
56. Ge, Y., Ezzell, R. M., Tompkins, R. G., and Warren, H. S. (1994) Cellular distribution of endotoxin after injection of chemically purified lipopolysaccharide differs from that after injection of live bacteria. *J. Infect. Dis.* **169,** 95–104

57. Mathison, J. C., and Ulevitch, R. J. (1979) The clearance, tissue distribution, and cellular localization of intravenously injected lipopolysaccharide in rabbits. *J. Immunol.* **123,** 2133–2143
58. Huby, T., Doucet, C., Dachet, C., Ouzilleau, B., Ueda, Y., Afzal, V., Rubin, E., Chapman, M. J., and Lesnik, P. (2006) Knockdown expression and hepatic deficiency reveal an atheroprotective role for SR-BI in liver and peripheral tissues. *J. Clin. Invest.* **116,** 2767–2776
59. Fu, T., Kozarsky, K. F., and Borensztajn, J. (2003) Overexpression of SR-BI by adenoviral vector reverses the fibrateinduced hypercholesterolemia of apolipoprotein E-deficient mice. *J. Biol. Chem.* **278,** 52559–52563
60. Yesilaltay, A., Morales, M. G., Amigo, L., Zanlungo, S., Rigotti, A., Karackattu, S. L., Donahee, M. H., Kozarsky, K. F., and Krieger, M. (2006) Effects of hepatic expression of the high-density lipoprotein receptor SR-BI on lipoprotein metabolism and female fertility. *Endocrinology* **147,** 1577–1588
61. Liu, Q., and Muruve, D. A. (2003) Molecular basis of the inflammatory response to adenovirus vectors. *Gene Therap.* **10,** 935–940
62. Stylianou, I. M., Svenson, K. L., VanOrman, S. K., Langle, Y., Millar, J. S., Paigen, B., and Rader, D. J. (2009) Novel ENU-Induced Point Mutation in Scavenger Receptor Class B, Member 1, Results in Liver Specific Loss of SCARB1 Protein. *PLoS ONE* **4,** e6521
63. Ocuin, L. M., Bamboat, Z. M., Balachandran, V. P., Cavnar, M. J., Obaid, H., Plitas, G., and DeMatteo, R. P. (2011) Neutrophil IL-10 suppresses peritoneal inflammatory monocytes during polymicrobial sepsis. *J Leukoc Biol* **89,** 423–432
64. Yurdakul, P., Dalton, J., Beattie, L., Brown, N., Erguven, S., Maroof, A., and Kaye, P. M. (2011) Compartment-specific remodeling of splenic microarchitecture during experimental visceral leishmaniasis. *Am. J. Pathol.* **179,** 23–29
65. Guo, L., Ai, J., Zheng, Z., Howatt, D. A., Daugherty, A., Huang, B., and Li, X. A. (2013) HDL protects against polymicrobial-induced sepsis in mice. *J. Biol. Chem.* **288,** 17947–17953
66. Han, A., Marandici, A., and Monder, C. (1983) Metabolism of corticosterone in the mouse. Identification of 11β, 20α-dihydroxy-3-oxo-4-pregnen-21-oic acid as a major metabolite. *J. Biol. Chem.* **258,** 13703–13707
67. Raubenheimer, P. J., Young, E. A., Andrew, R., and Seckl, J. R. (2006) The role of corticosterone in human hypothalamic-pituitary-adrenal axis feedback. *Clin. Endocrinol.* **65,** 22–26
68. Vishnyakova, T. G., Kurlander, R., Bocharov, A. V., Baranova, I. N., Chen, Z., Abu-Asab, M. S., Tsokos, M., Malide, D., Basso, F., Remaley, A., Csako, G., Eggerman, T. L., and Patterson, A. P. (2006) CLA-1 and its splicing variant CLA-2 mediate bacterial adhesion and cytosolic bacterial invasion in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* **103,** 16888–16893
69. Barter, P. J., Nicholls, S., Rye, K.-A., Anantharamaiah, G. M., Navab, M., and Fogelman, A. M. (2004) Antiinflammatory Properties of HDL. *Circulation Res.* **95,** 764–772
70. Van Amersfoort, E. S., Van Berkel, T. J., and Kuiper, J. (2003) Receptors, mediators, and mechanisms involved in bacterial sepsis and septic shock. *Clin. Microbiol. Rev.* **16,** 379–414
71. Mayumi, T., Takezawa, J., Takahashi, H., Kuwayama, N., Fukuoka, T., Shimizu, K., Yamada, K., Kondo, S., and Aono, K. (1999) Low-Dose Intramuscular Polymyxin B Improves Survival of Septic Rats. *Shock* **11,** 82–86
72. Kamimoto, M., Rung-Ruangkijkrai, T., and Iwanaga, T. (2005) Uptake ability of hepatic sinusoidal endothelial cells and enhancement by lipopolysaccharide. *Biomed. Res.* **26,** 99–107
73. Dunzendorfer, S., Lee, H. K., Soldau, K., and Tobias, P. S. (2004) TLR4 is the signaling but not the lipopolysaccharide uptake receptor. *J. Immunol.* **173,** 1166–1170
74. Yeh, Y. C., Hwang, G. Y., Liu, I. P., and Yang, V. C. (2002) Identification and expression of scavenger receptor SR-BI in endothelial cells and smooth muscle cells of rat aorta *in vitro* and *in vivo*. *Atherosclerosis* **161,** 95–103
75. Khovidhunkit, W., Moser, A. H., Shigenaga, J. K., Grunfeld, C., and Feingold, K. R. (2001) Regulation of scavenger receptor class B type I in hamster liver and Hep3B cells by endotoxin and cytokines. *J. Lipid Res.* **42,** 1636–1644