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Scavenger Receptor Expressed by Endothelial Cells I (SREC-I) Mediates the Uptake of Acetylated Low Density Lipoproteins by Macrophages Stimulated with Lipopolysaccharide*

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Scavenger receptor expressed by endothelial cells I (SREC-I) is a novel endocytic receptor for acetylated low density lipoprotein (LDL). Here we show that SREC-I is expressed in a wide variety of tissues, including macrophages and aortas. Lipopolysaccharide (LPS) robustly stimulated the expression of SREC-I in macrophages. In an initial attempt to clarify the role of SREC-I in the uptake of modified lipoproteins as well as in the development of atherosclerosis, we generated mice with a targeted disruption of the *SREC-I* gene by homologous recombination in embryonic stem cells. To exclude the overwhelming effect of the type A scavenger receptor (SR-A) on the uptake of Ac-LDL, we further generated mice lacking both SR-A and SREC-I ($SR\text{-}A^{-/-};SREC\text{-}I^{-/-}$) by cross-breeding and compared the uptake and degradation of Ac-LDL in the isolated macrophages. The contribution of SR-A and SREC-I to the overall degradation of Ac-LDL was 85 and 5%, respectively, in a non-stimulated condition. LPS increased the uptake and degradation of Ac-LDL by 1.8-fold. In this condition, the contribution of SR-A and SREC-I to the overall degradation of Ac-LDL was 90 and 6%, respectively. LPS increased the absolute contribution of SR-A and SREC-I by 1.9- and 2.3-fold, respectively. On the other hand, LPS decreased the absolute contribution of other pathways by 31%. Consistently, LPS did not increase the expression of other members of the scavenger receptor family such as CD36. In conclusion, SREC-I serves as a major endocytic receptor for Ac-LDL in LPS-stimulated macrophages lacking SR-A, suggesting that it has a key role in the development of atherosclerosis in concert with SR-A.

Scavenger receptors mediate the endocytosis of chemically modified lipoproteins such as acetylated low density lipoprotein (LDL),[1] thereby contributing to the development of atherosclerosis (1). The scavenger receptor gene family comprises a series of unlinked genes encoding membrane proteins with diverse ligand binding activity (2). The class A type I/type II scavenger receptor (SR-A) is the prototype receptor belonging to this family (3) and accounts for ~80% of the uptake of Ac-LDL in macrophages (4, 5).

Recently, we identified scavenger receptor expressed by endothelial cells I (SREC-I), which encodes a protein of 830 amino acids and binds fluorescent DiI-labeled Ac-LDL when expressed in Chinese hamster ovary cells (6), and its paralogous gene, *SREC-II* (7). The SREC-I protein is composed of an N-terminal extracellular ligand binding domain with seven epidermal growth factor receptor-like cysteine pattern signatures, a membrane-spanning domain, and an unusually long C-terminal cytoplasmic domain that includes a Ser/Pro-rich region followed by a Gly-rich region. SREC-II encodes an 834-amino acid protein with 35% homology to SREC-I. Although SREC-II has little activity to internalize modified LDL, SREC-I-expressing fibroblasts are intensely aggregated with SREC-II-expressing fibroblasts, indicating the association of SREC-I and SREC-II (7). However, the precise functions of these two proteins are currently unknown.

In atherosclerotic lesions, macrophages are laden with lipids and immunologically activated (8). In line with this, the development of atherosclerosis is accelerated by LPS (9), a major component of Gram-negative bacteria that stimulates the production of various cytokines *in vivo*, thereby contributing to the pathogenesis of endotoxin shock (10). Conversely, the absence of toll-like receptor 4, a receptor for LPS, inhibits its progression (11). These considerations have prompted us to examine the effects of LPS on the expression of SREC-I. In the present study, we show that LPS robustly stimulated the expression of

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[1] The abbreviations used are: LDL, low density lipoprotein; SR-A, class A type I/type II scavenger receptor; SR-BI, class B type I scavenger receptor; SREC, scavenger receptor expressed by endothelial cells.

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SREC-I in macrophages. In an initial attempt to clarify the role of SREC-I in the uptake of modified lipoproteins as well as in the development of atherosclerosis, we generated mice with targeted disruption of the *SREC-I* gene by homologous recombination in embryonic stem cells. To exclude the overwhelming effect of SR-A on the uptake of Ac-LDL, we further generated mice lacking both SR-A and SREC-I ($SR\text{-}A^{-/-};SREC\text{-}I^{-/-}$). By comparing the uptake and degradation of Ac-LDL in peritoneal macrophages isolated from these mice, we found that SREC-I plays a significant role in the uptake of Ac-LDL in the setting of SR-A deficiency, especially when stimulated with LPS. From these results, we propose that SREC-I contributes to the development of atherosclerosis in concert with SR-A.

EXPERIMENTAL PROCEDURES

General Methods—Standard molecular biology techniques were used (12). The current experiments were performed in accordance with institutional guidelines for animal experiments at the University of Tokyo and the Jichi Medical School.

SREC-I Antibody Preparation—Two milligrams of the carboxyl-end peptide of mouse SREC-I (amino acid residues 801–820, KEQEEPLY-ENVVPMSVPPQH) was conjugated with keyhole limpet hemocyanin using the Imject sulfhydryl-reactive antibody production kit (Pierce). The keyhole limpet hemocyanin-peptide was gel-purified and emulsified with an equal volume of complete Freund's adjuvant (Calbiochem). A female Wister rat was immunized with the emulsions. One week after the boost injection, blood was collected, and the antiserum was purified and eluted through an affinity column (Sulfolink coupling gel; Pierce) to which the antigen peptide was coupled.

Mice—SR-A knock-out mice were generated previously (4). ApoE knock-out mice were purchased from the Jackson Laboratory (Bar Harbor, ME) (13). Both mice had been back-crossed to C57BL/6J genetic background and fed a normal chow diet (MF diet from Oriental Yeast Co., Tokyo, Japan) that contained 5.6% (w/w) fat with 0.09% (w/w) cholesterol, and the mice were allowed access to water and food *ad libitum*.

Cells—Thioglycolate-elicited peritoneal macrophages (14) and mouse embryonic fibroblasts (15) were prepared as described previously. Cells were treated with varying concentrations of LPS (*Escherichia coli* O127:B8; Sigma) for 12 h before the experiments.

Northern Blot Analysis—For the SREC-I cDNA probe, two probes were prepared, namely Probe A, a 5′ 2.0-kb fragment spanning the extracellular and intracellular domains, and Probe B, a 0.1-kb fragment consisting of only the transmembrane domain. Poly $(A)^+$ RNA was purified using Oligotex-dT30™, an oligo(dT) latex (Roche Applied Science) from 100–150 μg of total RNA that was extracted by TRIzol reagent (Invitrogen) from either cultured cells or tissues. One to three milligrams of poly$(A)^+$ RNA was subjected to 1% agarose gel electrophoresis in the presence of formalin, transferred to Hybond N (Amersham Biosciences), and hybridized to the ^{32}P-labeled probes for SREC-I and other scavenger receptors as described previously (16).

Western Blot Analysis—Cells were lysed with 0.1% SDS. After centrifugation, 50 μg of the supernatant was subjected to SDS-PAGE and transferred to Hybond ECL™, a nitrocellulose membrane (Amersham Biosciences). After incubation with the anti-SREC-I antibody (1:400 dilution), the membrane was incubated with a goat anti-rat IgG conjugated with horseradish peroxidase (1:2000 dilution; Amersham Biosciences). The secondary antibody was visualized by an enhanced chemiluminescence kit (Amersham Biosciences).

Generation of the SREC-I Knock-out Mice—The *SREC-I* gene was cloned from the 129/Sv mouse genomic library (Clontech) using the mouse cDNA as a probe. A replacement-type targeting vector was constructed so that a 35-bp segment in exon 8, which encodes 3′ two-thirds of the transmembrane domain, was replaced with a polIIneo cassette (Fig. 5*A*). Long arm consists of a 10-kb NotI/KpnI fragment spanning the 5′ untranslated region and exon 8; short arm consists of a 0.9-kb SacI/XbaI fragment within intron 9. These were inserted together into the vector pPolIIshort-neobpA-HSVTK, as described previously (17). After digestion with SalI, the vector was electroporated into JH-1 embryonic stem cells (a generous gift from Dr. Herz at University of Texas Southwestern Medical Center at Dallas, TX). Targeted clones, which had been selected in the presence of G418 and 1-(2-deoxy, 2-fluoro-β-D-arabinofuranosyl)-5 iodouracil, were identified by PCR using the primers 5′-GATTGGGAAGACAATAGCAGGCATGC-3′ and 5′-CAGAGAGTGTCACCACAACAAGAGGA-3′ (Fig. 5*A*). Homologous recombination was verified by Southern blot analysis after digestion with EcoRI using a 0.5-kb SpeI/SmaI fragment, which was downstream of the short arm, as a probe (Fig. 5*A*). Targeted embryonic stem clones were injected into C57BL/6J blastocysts, yielding one line of chimeric mice that transmitted the disrupted allele through the germline.

Generation of the SR-A/SREC-I Double Knock-out Mice—The $SR\text{-}A^{-/-}$ mice were crossed with the $SREC\text{-}I^{-/-}$ mice, which were a C57BL/6J × 129/Sv hybrid, to obtain $SR\text{-}A^{+/-};SREC\text{-}I^{+/-}$ mice, which were interbred to obtain four types of mice, namely wild-type, $SR\text{-}A^{+/+};SREC\text{-}I^{-/-}$, $SR\text{-}A^{-/-};SREC\text{-}I^{+/+}$, and $SR\text{-}A^{-/-};SREC\text{-}I^{-/-}$ mice. Thus, the genetic background of these mice was 75% C57BL/6J and 25% 129/Sv. Littermates were used for the experiments.

Biochemical Analyses—Blood was collected from the retro-orbital venous plexus after a 12-h fast. Plasma glucose (ANTSENSE II, Bayer Medical, Tokyo, Japan), cholesterol (Determiner TC, Kyowa Medex, Tokyo), and triglycerides (TGLH; Wako Chemicals, Tokyo, Japan) were measured.

Histology—Mice were sacrificed by decapitation. Tissues were excised, fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with hematoxylin-eosin.

Preparation of Lipoproteins—LDL (d 1.019–1.063 g/ml) and lipoprotein-deficient serum (d >1.21 g/ml) were prepared by stepwise ultracentrifugation from plasma obtained from healthy volunteers. The lipoproteins and lipoprotein-deficient serum were dialyzed against 10 mM sodium phosphate, pH 7.4, 150 mM NaCl, 0.01%(w/v) EDTA, and 0.01% (w/v) NaN_3. LDL was acetylated with acetic anhydrate and radioiodinated by the iodine monochloride method as described (18). Protein concentrations were determined by the BCA protein assay reagent kit (Pierce).

Cellular Uptake and Degradation of ^{125}I-Ac-LDL—Peritoneal macrophages were plated in 12-well plates at a density of 1×10^6/well and treated with or without 100 ng/ml of LPS for 12 h. After stringent washing with PBS, the cells were incubated with a medium containing varying concentrations of ^{125}I-Ac-LDL and 5 mg/ml lipoprotein-deficient serum, with or without a 50-fold excess of unlabeled Ac-LDL, for 5 h at 37 °C. The amounts of ^{125}I-Ac-LDL either degraded by or associated with the cells were measured according to a modified method (19) of Goldstein *et al.* (18).

Statistics—The differences of the means were compared by Student's t test.

RESULTS

Tissue Distribution of mRNA Expression of SREC-I and -II—We performed Northern blot analyses to examine the expression of SREC-I and II in various organs of a mouse (Fig. 1). SREC-I was expressed in a wide variety of organs, most predominantly in liver, lung, kidney, and heart. On the other hand, the expression of SREC-II was restricted to lung and kidney.

LPS Stimulates the Expression of SREC-I in Peritoneal Macrophages—LPS robustly increased the mRNA expression of both SREC-I and SR-A in macrophages (Fig. 2). The peak of the stimulation was reached by the 12-h time point of the stimulation (Fig. 2*A*), and the maximal responses were obtained at the concentration of 10 ng/ml (Fig. 2*B*). The relative increase in the expression of SREC-I was 4-fold, which was more prominent than that of SR-A (1.8-fold) (Fig. 3). It is of note that the treatment with LPS did not significantly change the expression of MARCO (macrophage receptor with collagenous structure) (20) and SR-BI (21) and that it even decreased the expression of CD36 (22) and FEEL-1 (fasciclin, EGF-like, laminin-type EGF-like, and link domain-containing scavenger receptor-1) (23).

Expression of SREC-I in Aortas—We compared the mRNA expression levels of SREC-I in the atherosclerotic aortas, which were taken from 12-month-old apoE knock-out mice, with normal aortas, mouse embryonic fibroblasts, peritoneal macrophages, kidney, or lung from wild-type mice (Fig. 4). Normal and atherosclerotic aortas expressed 1.7- and 2.1-fold higher levels of SREC-I mRNA than the non-stimulated macrophages, respectively. The expression levels were comparable with those in the kidney, but much lower than those of the LPS-treated macrophages.

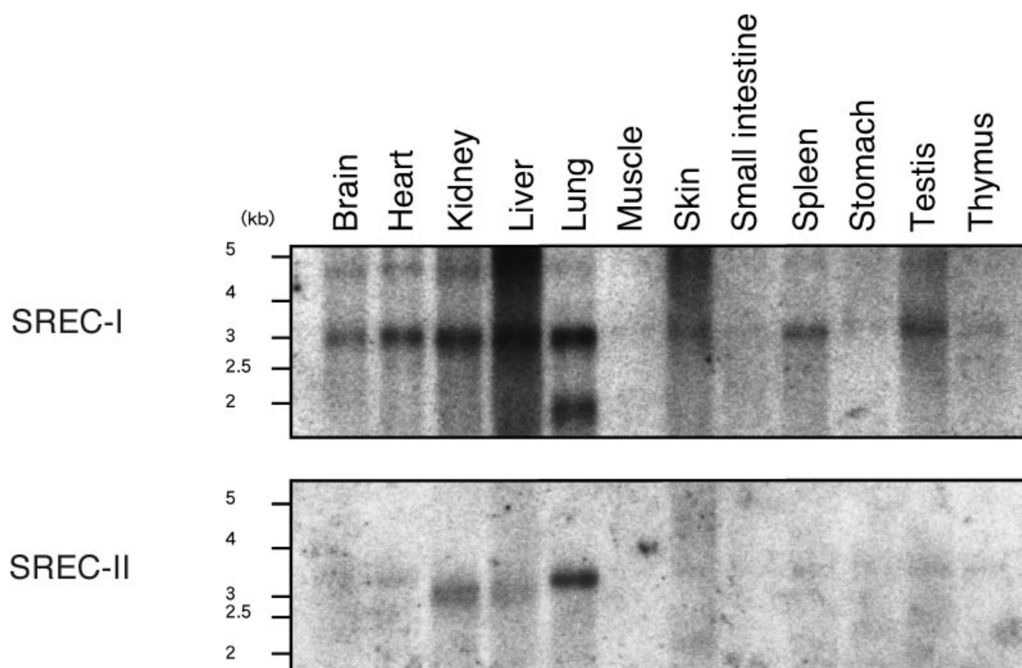


FIG. 1. **Northern blot analysis of SREC-I (*upper panel*) and SREC-II (*lower panel*) in various organs of a mouse.** Two micrograms of poly(A)$^+$ RNA was subjected to Northern blot analysis.

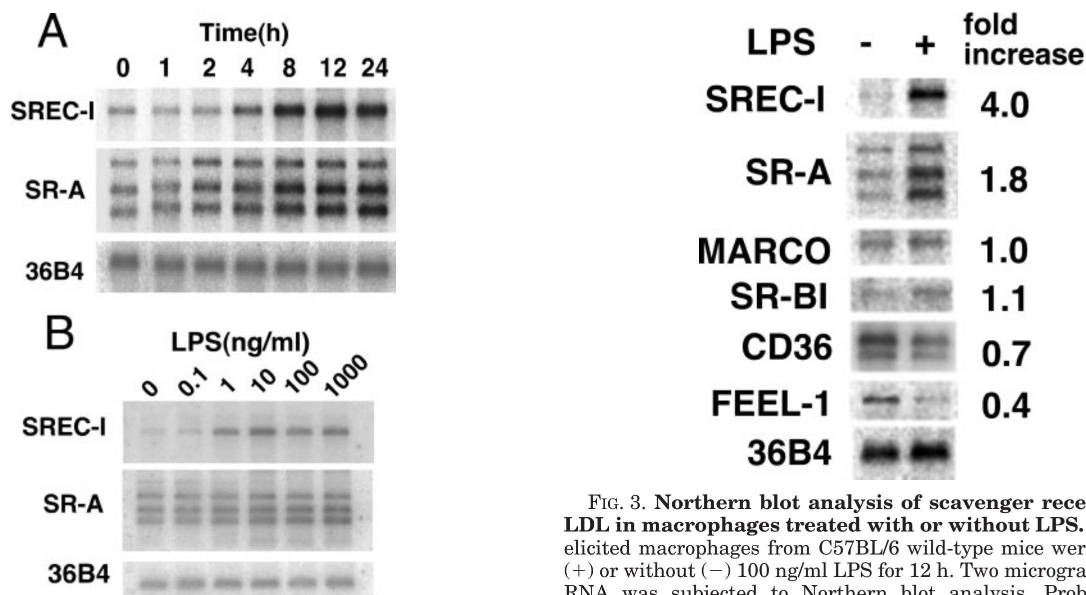


FIG. 2. **Northern blot analysis of SREC-I and SR-A in macrophages treated with LPS.** *A*, thioglycolate-elicited macrophages from C57BL/6 wild-type mice were treated with 100 ng/ml LPS for the indicated times. *B*, cells were treated with the indicated concentrations of LPS for 12 h. Two micrograms of poly(A)$^+$ RNA was subjected to Northern blot analysis. 36B4 was used as a loading control.

FIG. 3. **Northern blot analysis of scavenger receptors for Ac-LDL in macrophages treated with or without LPS.** Thioglycolate-elicited macrophages from C57BL/6 wild-type mice were treated with (+) or without (−) 100 ng/ml LPS for 12 h. Two micrograms of poly(A)$^+$ RNA was subjected to Northern blot analysis. Probes used were SREC-I, SR-A, MARCO, CD36, SR-BI, and FEEL-1. Signal intensity was corrected against the intensity of 36B4, and the relative signal increase ratio was calculated.

Generation of Mice Lacking the SREC-I and/or SR-A Gene—The intercross of the progeny (*SREC-I*$^{+/-}$) resulted in offspring of both sexes with all three genotypes at the SREC locus with the expected Mendelian ratios (105:191:85 $\chi^2 = 1.06$, $p > 0.05$) (Fig. 5*B*). Fig. 5*C* shows the results of Northern blot analyses of SREC-I in peritoneal macrophages. When hybridized with Probe A, which contains nearly the whole coding region of the SREC-I cDNA, Northern blot revealed a band with mRNA size of 2.9 kb in wild-type macrophages and a band of 2.4 kb in *SREC-I*$^{-/-}$ macrophages. When hybridized with Probe B, which contains the transmembrane domain, no band was detectable in *SREC-I*$^{-/-}$ macrophages. These results indicate that *SREC-I*$^{-/-}$ mice express a truncated transcript that lacks the transmembrane domain. *SREC-I*$^{-/-}$ mice were fertile and apparently normal. There were no significant differences in the growth curves of wild-type and *SREC-I*$^{-/-}$ mice. As shown in Table I, plasma levels of glucose, cholesterol, and triglycerides were not different between wild-type and *SREC-I*$^{-/-}$ mice. We failed to detect any pathological findings in the brain, lung, heart, liver, kidney, and testis of *SREC-I*$^{-/-}$ mice.

Northern blot analysis of four types of mice confirmed the absence of the expression of SR-A in both *SR-A*$^{-/-}$*;SREC-I*$^{+/+}$ and *SR-A*$^{-/-}$*;SREC-I*$^{-/-}$ mice as well as the expression of the disrupted allele of SREC-I in both *SR-A*$^{+/+}$*;SREC-I*$^{-/-}$ and *SR-A*$^{-/-}$*;SREC-I*$^{-/-}$ mice (Fig. 6*A*). Western blot analysis confirmed the absence of SREC-I protein (~140 and160 kDa) in both *SR-A*$^{+/+}$*; SREC-I*$^{-/-}$ and *SR-A*$^{-/-}$*;SREC-I*$^{-/-}$ mice (Fig. 6*B*).

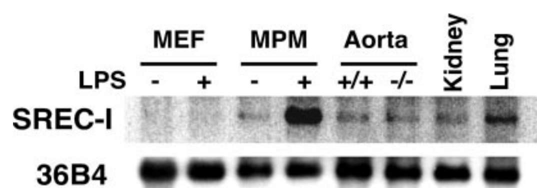


FIG. 4. **Northern blot analysis of SREC-I in normal and atherosclerotic aortas.** RNA were extracted from the following cells or organs: mouse embryonic fibroblasts (*MEF*) treated with (+) or without (−) 100 ng/ml LPS for 12 h; mouse peritoneal macrophages (*MPM*) treated with or without 100 ng/ml LPS for 12 h; kidney; lung; normal aortas from wild-type mice (*Aorta* +/+); and atherosclerotic aortas from apoE−/− mice (*Aorta* −/−). Aortic arches and the thoracic part of descending aortas with rampant visible plaques were excised from five 12-month-old mice. After adipose tissues surrounding the aortas were removed as much as possible, the aortas were used for the preparation of RNA. Three micrograms of poly(A)$^+$ RNA was subjected to Northern blot analysis of SREC-I. 36B4 was used as a loading control.

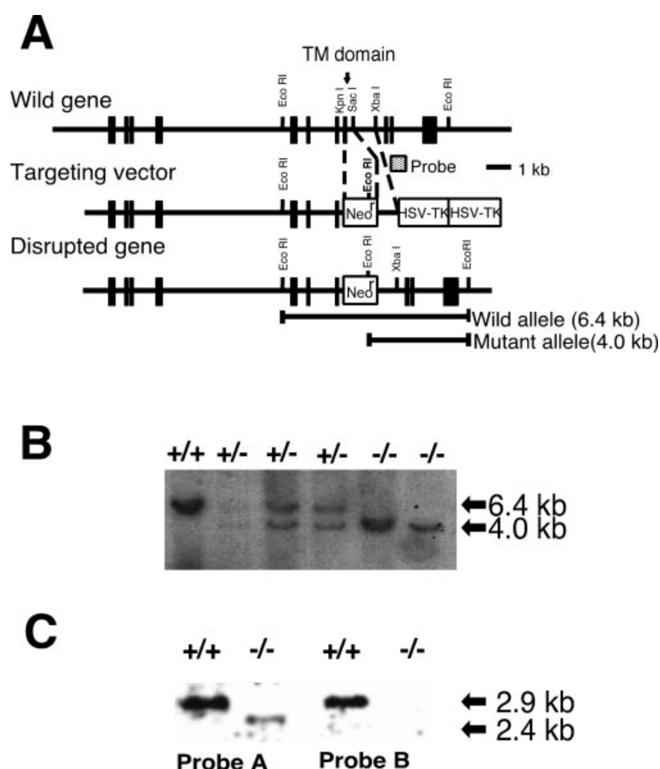


FIG. 5. **Targeted disruption of SREC-I gene.** *A*, map of the SREC-I gene and targeting construct. *Long boxes* represent exons. The exon coding transmembrane domain was replaced with the neomycin resistance gene (*Neo^r*) of targeting vector, which has a herpes simplex virus thymidine kinase (*HSV-TK*) cassette for a negative selection downstream of its short arm. A 0.5-kb fragment was used as a probe for Southern blot analysis (*shaded box*). *B*, Southern blot analysis. After digestion with EcoRI, tail DNA was used for Southern blot analysis. The size of the disrupted allele, 4 kb, was smaller than that of wild-type allele (6.4 kb). *C*, mRNA expression of SREC-I in peritoneal macrophage. Two micrograms of poly(A)$^+$ RNA from wild-type (+/+) and *SREC-I*$^{-/-}$ mice (−/−) macrophage was hybridized with two cDNA probes, namely Probe A, a 5′ 2-kb fragment spanning the extracellular and intracellular domains, and Probe B, a 0.1-kb fragment consisting of only the transmembrane domain.

LPS Stimulates Protein Expression of SREC-I—LPS significantly increased the SREC-I protein by ~2-fold in wild-type and *SR-A*$^{-/-}$*;SREC-I*$^{+/+}$ macrophages (Fig. 6*B*).

LPS Increases the Contribution of SREC-I to the Cellular Uptake and Degradation of the ^{125}*I-Ac-LDL*—In non-stimulated conditions (Fig. 7*A*) there was no significant difference in the specific uptake and degradation of ^{125}I-Ac-LDL between wild-type and *SR-A*$^{+/+}$*;SREC-I*$^{-/-}$ macrophages. Based on the values for 20 μg/ml degraded ^{125}I-Ac-LDL, *SR-A*$^{-/-}$*;SREC-I*$^{+/+}$ macrophages degraded significantly smaller amounts of ^{125}I-Ac-LDL (15%) than did wild-type macrophages, supporting the dominant role of SR-A in the uptake and degradation of ^{125}I-Ac-LDL in macrophages. Compared with *SR-A*$^{-/-}$*;SREC-I*$^{+/+}$ macrophages, *SR-A*$^{-/-}$*;SREC-I*$^{-/-}$ macrophages showed a further reduction in the specific uptake (21%) and degradation of ^{125}I-Ac-LDL (31%). Based on the values for 20 μg/ml degraded ^{125}I-Ac-LDL, the contribution of SR-A and SREC-I to the overall degradation of Ac-LDL was calculated to be 85 and 5%, respectively, in the non-stimulated condition.

LPS increased the uptake and degradation of Ac-LDL by 1.8-fold (Fig. 7*B*). In this condition, there was no significant difference in the specific uptake and degradation of ^{125}I-Ac-LDL between wild-type and *SR-A*$^{+/+}$*;SREC-I*$^{-/-}$ macrophages. Based on the values for 20 μg/ml degraded ^{125}I-Ac-LDL,

TABLE I
Plasma levels of glucose, total cholesterol, and triglycerides

After a 12-h fast, blood was collected from the retro-orbital venous plexus of mice aged 8 weeks. Plasma glucose, total cholesterol, and triglycerides were measured. All values are expressed as means ± S.E. No significant deference between wild-type and *SREC-I*$^{-/-}$ mice.

	Wild-type (+/+)	*SREC-I*$^{-/-}$
	mg/dl	
Glucose	61.1 ± 3.2 (n = 31)	63.2 ± 3.2 (n = 31)
Total cholesterol	89.7 ± 5.1 (n = 22)	82.0 ± 3.3 (n = 27)
Triglycerides	89.6 ± 9.4 (n = 22)	97.4 ± 10.3 (n = 27)

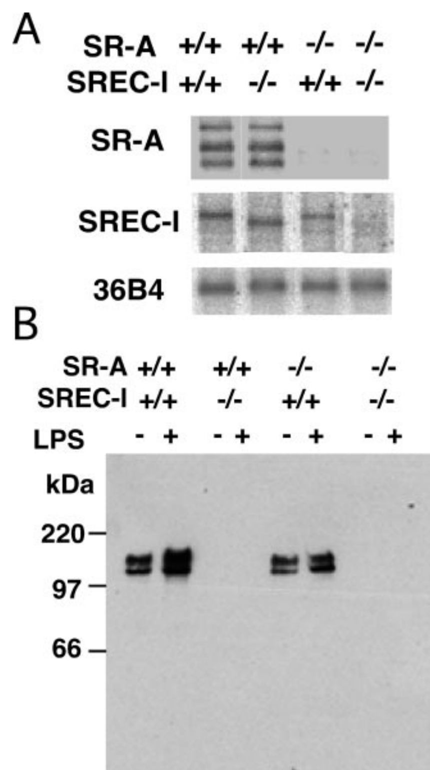
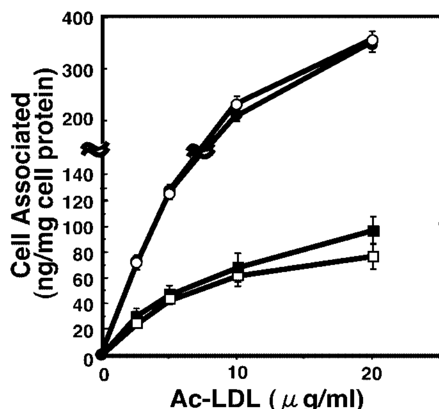
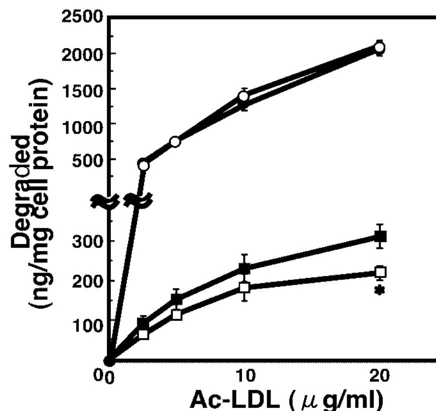


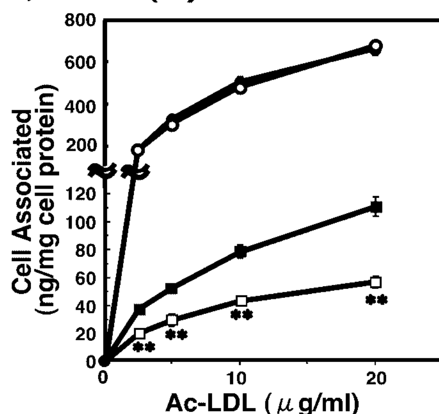
FIG. 6. **Expression of SR-A and SREC-I in macrophages isolated from wild-type, *SR-A*$^{+/+}$*;SREC-I*$^{-/-}$, *SR-A*$^{-/-}$*;SREC-I*$^{+/+}$, and *SR-A*$^{-/-}$*;SREC-I*$^{-/-}$ mice.** Thioglycolate-elicited peritoneal macrophages were prepared from wild-type, *SR-A*$^{+/+}$*;SREC-I*$^{-/-}$, *SR-A*$^{-/-}$*;SREC-I*$^{+/+}$, and *SR-A*$^{-/-}$*;SREC-I*$^{-/-}$ mice. *A*, Northern blot analysis of SR-A and SREC-I. One microgram of poly(A)$^+$ RNA from macrophages was hybridized with SR-A and SREC-I probes (Probe A). *B*, Western blot analysis of SREC-I in macrophages treated with or without LPS. After stimulation with (+) or without (−) 100 ng/ml LPS for 12 h, the cells were lysed with 0.1% SDS, and 50 μg of protein was subjected to SDS-PAGE. Immunoblotting was performed using the rat anti-mouse SREC-I antibody and an enhanced chemiluminescence kit.

A, LPS(-)





B, LPS(+)



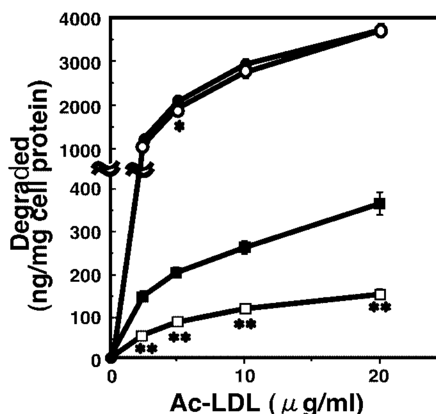


FIG. 7. **Cell association and degradation of ^{125}I-Ac-LDL by peritoneal macrophages isolated from wild-type, *SR-A$^{+/+}$;SREC-I$^{-/-}$*, *SR-A$^{-/-}$;SREC-I$^{+/+}$*, and *SR-A$^{-/-}$;SREC-I$^{-/-}$* mice.** Thioglycolate-elicited peritoneal macrophages were prepared from four types of mice (n = 5), namely wild-type (*solid circle*), *SR-A$^{+/+}$;SREC-I^{-}* (*open circle*), *SR-A$^{-/-}$;SREC-I$^{+/+}$* (*solid square*), and *SR-A$^{-/-}$;SREC-I$^{-/-}$* (*open square*). After treatment with (*panel B*) or without (*panel A*) 100 ng/ml LPS for 12 h, the cells were incubated with the indicated concentrations of ^{125}I-Ac-LDL with or without a 50-fold excess of unlabeled Ac-LDL at 37 °C. After 5 h, the amounts of ^{125}I-Ac-LDL associated or degraded were determined. Specific values were calculated by subtracting the nonspecific values from the total values. All values are expressed as means ± S.E. of five mice. *, $p < 0.05$ *versus SR-A$^{-/-}$;SREC-I$^{+/+}$* mice; **, $p < 0.01$ *versus SR-A$^{-/-}$; SREC-I$^{+/+}$* mice.

SR-A$^{-/-}$;SREC-I$^{+/+}$ macrophages degraded significantly smaller amounts of ^{125}I-Ac-LDL (10%) than did wild-type macrophages. Compared with *SR-A$^{-/-}$;SREC-I$^{+/+}$* macrophages, *SR-A$^{-/-}$;SREC-I$^{-/-}$* macrophages showed a further reduction in the specific uptake (49%) and degradation of ^{125}I-Ac-LDL (59%). Based on the values for 20 μg/ml degraded Ac-LDL, the contribution of SR-A and SREC-I to the overall degradation of ^{125}I-Ac-LDL was calculated to be 90 and 6%, respectively. LPS increased the absolute contribution of SR-A and SREC-I by 1.9- and 2.3-fold, respectively. On the other hand, LPS decreased the absolute contribution of other pathways by 31%.

DISCUSSION

In the present study, we have first shown that SREC-I, a novel member of the scavenger receptor family that recognizes modified lipoproteins, is expressed in a wide variety of tissues including macrophages and aortas, implicating its involvement in the development of atherosclerosis. The expression of SREC-I was not significantly different between normal and atherosclerotic aortas, although it was robustly induced by LPS in macrophages, a major cell type that is present in foam cell lesions. To define the precise role of SREC-I, we have generated wild-type, *SR-A$^{+/+}$;SREC-I$^{-/-}$*, *SR-A$^{-/-}$;SREC-I$^{+/+}$*, and *SR-A$^{-/-}$;SREC-I$^{-/-}$* mice and compared the uptake and degradation of Ac-LDL in macrophages between these mice. Results show that the contribution of SREC-I to the overall uptake and degradation of Ac-LDL was 5% in the non-stimulated condition and 6% in the LPS-stimulated condition. Although the involvement of SREC-I was relatively small compared with that of SR-A, LPS increased the SREC-I mediated degradation by 2.3-fold, which accounted for 60% of the amounts of Ac-LDL degraded by the pathway independent of SR-A.

Because the responses of scavenger receptors to LPS are variable, we did not expect that LPS induced the expression of SREC-I in macrophages. LPS induces SR-A expression in mouse macrophages, which was confirmed in our experiments (Figs. 2 and 3) but not in THP-1 monocyte/macrophages (24) and human monocyte-derived macrophages (25). Because SR-A is able to bind LPS (26), the induction of SR-A by LPS may have a protective role against endotoxemia. This notion is in line with the susceptibility of SR-A knock-out mice to endotoxin shock (4, 27, 28). On the other hand, LPS down-regulates SR-B1 expression (29), which was confirmed in our experiments (Fig. 2).

The mechanism by which LPS induces SREC-I is intriguing. It is well known that LPS modulates gene expression though the activation of NF-κB signaling (30), and some of the effects are mediated by proinflammatory cytokines, whose expression is stimulated by LPS. However, there is no NF-κB binding site in the 5′-flanking region of the human SREC-I gene 1 kb upstream of the transcription initiation site (31). Furthermore,

LPS did not induce SREC-I expression in human umbilical vein endothelial cells, and tumor necrosis factor-α did not have increasing effects on SREC-I expression in mouse macrophages (data not shown). Further studies are warranted to decipher how LPS induces SREC-I expression.

Although SREC-I is expressed in macrophages, particularly when stimulated with LPS, there was no significant difference in the expression levels between normal and atherosclerotic aortas (Fig. 4). This suggests that other cell types such as endothelial cells and smooth muscle cells in the aortas express comparable levels of SREC-I.

SR-A is the major pathway for the uptake and degradation of Ac-LDL, accounting for 80% of the total activity (4, 5). Because other scavengers are expressed in macrophages (Fig. 2) and are able to bind Ac-LDL (2), the question is which scavenger receptor is the second most important in the uptake and degradation of Ac-LDL. Our results have revealed that the role of SREC-I is relatively minor in the non-stimulated macrophages, which is largely consistent with the recent report by Kunjathoor *et al.* (22). According to them, SR-A and CD36 account for 75–90% of the total amounts of chemically modified LDL degraded by macrophages. In SR-A-deficient macrophages stimulated with LPS, however, the absolute contribution of SREC-I was significantly increased by 2.3-fold, accounting for 60% of the SR-A-independent uptake and degradation of Ac-LDL (Fig. 7*B*). The degree of increase in the SREC-I mediated uptake and degradation of Ac-LDL is largely comparable with that in SREC-I protein expression (Fig. 6*B*). It is interesting to note that LPS decreased the absolute contribution of the other endocytic pathway, which is independent of either SR-A or SREC-I, by 31%. This finding is consistent with the Northern blot results that show that LPS did not significantly increase the expression of the other members of scavenger receptor family such as MARCO, SR-BI, CD36, and FEEL-1 (Fig. 3). Thus, SREC-I is the second most important receptor mediating the uptake of Ac-LDL, at least in macrophages stimulated with LPS. Given the activated state of macrophages in rupture-prone unstable plaques (8), particularly in plaques infected with microorganisms such as *Chlamydia*, which is associated with an increased prevalence of coronary events (32), SREC-I may take a significant part in the foam cell formation in these pathological conditions. Other aspects of LPS may be involved in the atherogenesis. For example, Baranova *et al.* (29) and Khovidhunkit *et al.* (33) have recently reported that LPS inhibits high density lipoprotein-mediated cholesterol efflux via down-regulation of the expression of ABCA1 and ABCG1. These observations are consistent not only with the ability of LPS to stimulate lipid accumulation in macrophages *in vitro* (34) but also with the proatherogenic effects of LPS (9) and its cognate receptor, Toll-like receptor 4, *in vivo* (11).

Functions of adhesion molecules have been assigned to both SR-A and SREC-I/II. Chinese hamster ovary cells overexpressing SR-A have an increased ability to adhere to plastic surfaces (35). Likewise, intense aggregation was observed when SREC-I-expressing fibroblast L-cells were mixed with those expressing SREC-II (7). Thus, it is reasonable to speculate that *SREC-I*$^{-/-}$ mice have some phenotypes with regard to cell adhesion. However, there were no obvious abnormalities in the pathologies (data not shown).

The precise roles of scavenger receptors in atherogenesis have been tested only for SR-A and CD36. With regard to SR-A, we (4, 36, 37) and Babaev *et al.* (38) have reported that SR-A deficiency protects against the development of atherosclerosis in either apoE, LDL receptor-deficient, or wild-type mice. de Winther *et al.* (39), however, recently reported apparently opposite results, *i.e.* SR-A deficiency leads to more complex lesions in the APOE3Leiden mice. The same group reported the reduction in atherosclerosis in LDL receptor knock-out mice in which SR-A was overexpressed in a macrophage-specific manner (40). These contradictory results could be attributed to the broad repertoire of functions and the widespread expression of SR-A (41). With regard to CD36, Febbraio *et al.* have reported that CD36 deficiency protects against atherosclerosis in an apoE-deficient background (42). Availability of the *SR-A*$^{-/-}$*;SREC-I*$^{-/-}$ mice should allow us to determine the role of SREC-I in the development of atherosclerosis by crossing with the genetically hyperlipidemic mice, for example. If the hypothesis is correct, SREC-I should be a new target for preventing atherosclerosis.

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