Scavenger Receptor Activity Is Increased in Macrophages From Rabbits With Low Atherosclerotic Response: Studies in Normocholesterolemic High and Low Atherosclerotic Response Rabbits

Daniel Teupser, Olga Stein, Ralph Burkhardt, Klaus Nebendahl, Yechezkiel Stein, Joachim Thiery

Abstract—We have previously described 2 strains of New Zealand White rabbits with a high (HAR) or low (LAR) atherosclerotic response to hypercholesterolemia. In the present study, we focused on class A scavenger receptor (SR-A) activity and ApoE expression in macrophages from both rabbit strains. These parameters play a crucial role in maintaining cholesterol homeostasis in the arterial wall and may be involved in the development of atherosclerosis. SR activity, as measured by uptake of DiI-labeled acetylated LDL, was significantly higher in macrophages from LAR rabbits (2177 ± 253 ng/mg cell protein) than in macrophages from HAR rabbits (1153 ± 200 ng/mg cell protein). The higher SR activity was caused by a greater number of SRs (apparent V_max, 4100 ng/mg in LAR and 1980 ng/mg in HAR rabbits). The high SR activity in macrophages from LAR rabbits was associated with a significantly higher expression of SR-A mRNA compared with macrophages from HAR rabbits. However, the latter finding could not be explained by differences in the activity of transcription factor–activating protein 1 (AP-1), which was comparable in macrophages from both strains of rabbits. Because under certain circumstances SR-A mRNA expression is regulated in parallel with ApoE expression, we also evaluated this parameter. Although ApoE mRNA was 74% higher in macrophages from LAR rabbits, the difference did not reach statistical significance. In conclusion, the increased expression of SR-A in macrophages in the presence of adequate amounts of ApoE may play a role in attenuating atherosclerosis in LAR rabbits. (Arterioscler Thromb Vasc Biol. 1999;19:1299-1305.)

Key Words: atherosclerosis ■ scavenger receptor ■ ApoE ■ hypercholesterolemia ■ rabbit macrophages

In our laboratory, 2 strains of New Zealand White rabbits were developed in which the atherosclerotic response to hypercholesterolemia was either high (HAR) or low (LAR). This difference in the propensity to atherosclerosis development, in view of similar plasma cholesterol and lipoprotein levels as well as their composition, seems to be genetically determined because it is still evident after 11 generations. In a previous study, we compared several putative factors that could influence lesion induction at the level of the arterial wall. No difference was seen in the enhancement of monocyte adhesion to arterial endothelial cells with β-VLDL derived from LAR and HAR rabbits. However, in the aortae of HAR rabbits, in which more atherosclerotic lesions were found than in the aortae of LAR rabbits, there was more expression of vascular cell adhesion molecule-1 (VCAM-1), but only associated with the lesions. In addition, the peroxidative capacity of macrophages and smooth muscle cells (SMCs) of both rabbit strains toward LDL was similar. However, the responsiveness of cultivated aortic SMCs to induction of scavenger receptor (SR) activity when challenged with either phorbol 12-myristate 13-acetate (PMA) or platelet-derived growth factor-BB was significantly lower in LAR than in HAR rabbits.

Since during the initial stages of atherogenesis cholesterol esters accumulate primarily in macrophages, the aim of the present study was to compare certain aspects of cholesterol metabolism in macrophages derived from normocholesterolemic LAR and HAR rabbits. Because the propensity to develop atherosclerosis in pigeons has been attributed to impaired hydrolysis of cholesteryl ester in macrophages, we also examined this parameter in LAR and HAR rabbits. We studied the uptake of 1,1'-dioctadecyl-3,3',3'-tetramethyl-indocarboxyanine perchlorate (DiI)-labeled acetylated LDL (DiI-acLDL) by cultured macrophages and the plasma clearance of intravenously injected DiI-acLDL. In addition, we also compared the expression of class A scavenger receptor type I and II (SR-A/II) and ApoE in peritoneal macrophages isolated from the 2 strains of rabbits.
Methods

Animals
The rabbits used in the present study are from the 2 strains established previously. One strain was designated LAR, with a low atherosclerotic response to cholesterol feeding; the other strain, HAR, had a high atherosclerotic response. The rabbits were kept on regular chow diet and used when 12 months old. Animal care and experimental procedures involving animals conformed to the position of the American Heart Association on research animal use and were approved by the Regierung von Oberbayern.

Isolation of Rabbit Peritoneal Macrophages
For isolation of peritoneal macrophages, rabbits were anesthetized with ketamine-HCl/xylazine-HCl and were injected with 40 mL of mineral oil, through a 16-gauge catheter. After 3 days, the rabbits were injected intravenously with 500 U heparin and then given an overdose of pentobarbital. The peritoneal cavity was washed with 1000 mL of a 154 mmol/L NaCl solution. The cell suspension was collected through a catheter and placed in 50 mL Falcon tubes containing EDTA (0.1 g/L). All the solutions used contained 100 µg/mL of gentamycin. The tubes were centrifuged at 200g for 10 minutes, the supernatant discarded, and the cell pellet resuspended in 10 mL of Hanks’ balanced salt solution containing 0.1 g/L EDTA. Cell suspensions from 4 tubes were pooled and recentrifuged as above. The cell pellet was washed with DMEM (Sigma) and resuspended in DMEM containing 10% FBS; 2×10^6 cells were plated on 35-mm culture dishes. After 2 hours, the nonadherent cells were removed and the cell layer washed with serum-free medium. The cells were cultivated in DMEM containing 10% FBS.

Lipoprotein Isolation and Labeling
LDL was prepared from freshly drawn CPD plasma from normolipidemic human blood donors. To prevent oxidation, 0.1 g/L EDTA was present during the whole isolation procedure. Plasma density was adjusted to 1.063 g/mL with crystalline NaCl. After ultracentrifugation at 150 000g for 24 hours at 10°C, the supernatant was collected, pooled, and adjusted with distilled water to d = 1.019 g/mL. It was recentrifuged as before and the infranatant was collected and adjusted to d = 1.063 g/mL. LDL (d = 1.019 to 1.063 g/mL) was collected from the supernatant after ultracentrifugation (150 000g, 24 hours, 10°C). LDL preparations were extensively dialyzed against 5 mmol/L Tris, 154 mmol/L NaCl, 0.1 g/L EDTA, pH 7.4, at 4°C. LDL was labeled with the fluorescent probe DiI (Molecular Probes) as previously described. LDL and DiI-LDL were acetylated according to Basu et al. All lipoprotein preparations were stored at 4°C in sterile containers after filtration sterilization (0.45 µm). Protein concentrations were determined by the method of Lowry et al. by using BSA as a standard. Total cholesterol was determined by using an enzymatic procedure (Boehringer Mannheim).

Clearance of DiI-acLDL From Plasma In Vivo
The rabbits were anesthetized with ketamine-HCl and xylazine-HCl, and 1 mg of DiI-acLDL protein (1 mL) was injected into the marginal ear vein. Blood was drawn from the artery of the contralateral ear before and 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 45, and 60 minutes after injection. Fluorescence intensity was measured in 1:20 dilutions of plasma with 154 mmol/L NaCl by using a microtiter plate fluorescence reader (Fluorolite 1000, Dynatech) with emission and excitation wavelengths set at 520 and 580 nm, respectively. The fluorescence reader (Fluorolite 1000, Dynatech) with emission and excitation wave-lengths set at 520 and 580 nm, respectively. The results were expressed as nanograms of cell-associated DiI-acLDL per milligram of cell protein. Scatchard plot analysis was used for the calculation of K and Vmax. All incubations were performed in triplicate, and values agreed within 10% of the mean.

Nuclear Blot Analysis
Total RNA was extracted from 3×10^7 freshly isolated peritoneal macrophages, using the monophasic phenol-guanidine isothiocyanate Trizol reagent (Life Technologies). RNA (15 µg) was electrophoresed on 1% agarose gels containing 2.2 mol/L formaldehyde, transferred to a Nytan-N membrane (Schleicher & Schuell), and cross-linked by UV irradiation. The membranes were prehybridized for 2 hours at 42°C, followed by an overnight hybridization with a fragment of rabbit class A SR cDNA common to both type I and type II receptors or a cDNA probe specific for rabbit ApoE (Table), radiolabeled with 32P by random-primer (Multiprime, Amersham). The membranes were washed and exposed to storage phosphor screens. In addition, the membranes were stripped and rehybridized with a rabbit β-actin probe to control for any variation in RNA loading (Table). The blots were quantified by phosphorimager densitometry (Storm 860, Molecular Dynamics). SR-A and ApoE mRNA were expressed relative to β-actin mRNA.

Preparation of Nuclear Extracts
Nuclear extracts were prepared from 7×10^7 freshly isolated rabbit peritoneal macrophages as described by Schreiber et al. with modifications. In brief, the cells were washed once with 10 mL of ice-cold PBS and resuspended in 250 µL of ice-cold buffer containing 10 mmol/L HEPES, pH 7.9, 10 mmol/L KCl, 0.1 mmol/L EDTA, 1 mmol/L DTT, 1 mmol/L PMSF, 1% protease inhibitor cocktail, and 0.4% NP-40. One milliliter of protease inhibitor cocktail consisted of 500 µg of antipain, 500 µg of aprotinin, 500 µg of leupeptin, 50 µg of Pepstatin, 750 µg of bestatin, 400 µg of phosphoramidon, and 500 µg of trypsin inhibitor (Boehringer Mannheim). The cell suspension was pipetted up and down, to lyse the cells, transferred to an Eppendorf tube, incubated on ice for 10 minutes, and centrifuged at 12 000g for 5 minutes at 4°C. The nuclear pellet was resuspended in 100 µL of buffer containing 20 mmol/L HEPES, pH 7.9, 0.4 mol/L NaCl, 1 mmol/L EDTA, 1 mmol/L DTT, 1% Triton X-100, 0.1 mmol/L EDTA, 0.1 mmol/L PMSF, 1% protease inhibitor cocktail, and 0.4% NP-40.

Determination of SR Activity
SR activity was determined 18 hours after isolation of peritoneal macrophages. The cells were incubated with 10 µg DiI-acLDL protein/mL at 37°C for 5 hours. The specificity of DiI-acLDL uptake was determined in the presence of 50-fold excess of unlabeled lipoproteins. In some experiments, the concentration-dependent uptake was measured in the presence of 5 to 50 µg DiI-acLDL protein/mL. After incubation with DiI-acLDL, the cells were washed twice with 0.4% BSA in PBS and were lysed with 0.1 mol/L NaOH/0.1% SDS as previously described. Fluorescence intensity was determined with a microtitr reader (Fluorolite 1000, Dynatech) with emission and excitation wavelength set at 520 and 580 nm, respectively. The results were expressed as nanograms of cell-associated DiI-acLDL per milligram of cell protein. Scatchard plot analysis was used for the calculation of K and Vmax. All incubations were performed in triplicate, and values agreed within 10% of the mean.

Characterization of cDNA Probes Used for Northern Hybridization

<table>
<thead>
<tr>
<th>Probe</th>
<th>Length of Fragment</th>
<th>Primers Used for Amplification (Forward: Upper Lane; Reverse: Lower Lane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit SR-AI, II</td>
<td>225 bp</td>
<td>5’-GCTCCTCTGCCGACCCCATGAAA-3’, 5’-CAGATGCTTCCATCGCTCT-3’</td>
</tr>
<tr>
<td>Rabbit ApoE</td>
<td>508 bp</td>
<td>5’-GACATTGGAGAAGCTGCTGCAACC-3’, 5’-ACTGCGCTCAGTCTCTCC-3’</td>
</tr>
<tr>
<td>Rabbit β-actin</td>
<td>435 bp</td>
<td>5’-AACGTGAGGATGGAGTACCGAGAC-3’, 5’-TGAACGTGCTGCTGAGTAGCAGAC-3’</td>
</tr>
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Probes) as previously described. LDL and DiI-LDL were acetylated according to Basu et al. All lipoprotein preparations were stored at 4°C in sterile containers after filtration sterilization (0.45 µm). Protein concentrations were determined by the method of Lowry et al. by using BSA as a standard. Total cholesterol was determined by using an enzymatic procedure (Boehringer Mannheim).
1 mmol/L PMSF, and 1% protease inhibitor cocktail and rocked vigorously on a shaking platform for 15 minutes. After centrifugation (12,000g, 5 minutes, 4°C) the supernatant containing the nuclear extracts was frozen at −70°C. Protein content of the nuclear extracts was determined with the Bradford assay (Coomassie Plus, Pierce).

DNA Binding Assay

Double-stranded activating protein-1 (AP-1) consensus oligonucleotide (Promega) was labeled with [γ-32P]ATP (Amersham), using T4 polynucleotide kinase and purified on G-25 columns (ProbeQuant G-25 microcolumn, Pharmacia). For DNA binding assays, 0.8 μg of nuclear protein was used and diluted with buffer containing 20 mmol/L HEPES, pH 7.9, 0.4 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L DTT, 1 mmol/L PMSF, and 1% protease inhibitor cocktail on ice to give a final volume of 9 μL per sample. After addition of 2 μL H2O and 3 μL 5× EMSA-binding buffer [containing 20% glycerol, 5 mmol/L MgCl2, 2.5 mmol/L EDTA, 2.5 mmol/L DTT, 250 mmol/L NaCl, 50 mmol/L Tris-HCl, pH 7.5, 0.25 mg/mL poly(dI–dC), 50 mmol/L Tris-HCl, pH 7.5, 0.25 mg/mL poly(dI–dC), the mixture was incubated on ice for 10 minutes. The radiolabeled AP-1 probe (50,000 cpm/μL) was then added and the mixture was incubated on ice for another 30 minutes. After addition of 2 μL of 50% glycerol, the samples were loaded on a nondenaturing 5% acrylamide/bisacrylamide (29:1) gel. DNA–protein complexes were fractionated by electrophoresis at 20 V (4°C) for 16 hours in 0.25× Tris–borate–EDTA buffer. Gels were dried, exposed to storage phosphor screens, and quantified by phosphorimage analysis (Storm 860, Molecular Dynamics).

[3H]Cholesteryl Ester Hydrolysis

Peritoneal macrophages were incubated between days 1 and 2 after plating for 24 hours in DMEM containing 10% FBS, [3H]cholesterol 1.5 μCi/mL (Amersham), and acLDL 50 μg of protein/mL. Thereafter, the cells were washed as described above, triplicate dishes were added to wash the DNA, and 0.25 mg/mL apoproteins derived from HDL2 and 3 bands of SR-AI and II, namely, 1.8, 2.8, and 3.8 kb, could be visualized. A significantly higher signal was determined by the uptake of acLDL in peritoneal macrophages from HAR and LAR rabbits. The cells were incubated with 10 μg DiI-acLDL/mL for 5 hours. Nonspecific uptake was determined in experiments, using triplicate dishes in each experiment. For specific uptake, P<0.01; nonspecific uptake, P=NS.

Figure 1. Clearance of [3H]cholesteryl ester from peritoneal macrophages of HAR and LAR rabbits. Peritoneal macrophages were labeled with [3H]cholesterol in the presence of acLDL for 24 hours. Thereafter, the cells were washed and incubated for up to 48 hours in the presence of 40 μg/mL of HDL apoproteins and 20 μg/mL of phosphatidylcholine liposomes. Data are mean±SEM values of triplicate dishes, using cells derived from 3 HAR and 3 LAR rabbits.

In the next experiments, we compared SR activity as determined by the uptake of acLDL in peritoneal macrophages from HAR and LAR rabbits. Figure 2 shows the results obtained from cells of 13 HAR and 12 LAR rabbits. Macrophages from LAR rabbits had a significantly higher specific uptake of acLDL than macrophages from HAR rabbits. There was no difference in nonspecific uptake. In several experiments, saturation kinetics of uptake of acLDL by peritoneal macrophages from HAR and LAR rabbits were analyzed (Figure 3). In experiments with macrophages from LAR rabbits, the apparent Vmax was significantly higher whereas no difference was found in Km or slope. Thus, the higher uptake of acLDL by macrophages isolated from LAR rabbits is apparently caused by a higher receptor number rather than by an increase in affinity.

To determine whether the high expression of SRs in macrophages of LAR rabbits is regulated at the level of transcription, we determined SR-A mRNA in macrophages of rabbits. The membranes were exposed to probes complementary to sequences in the collagen-like domain of the SR and thus the 3 major bands of SR-A1 and II, namely, 1.8, 2.8, and 3.8 kb, could be visualized. A significantly higher signal was
obtained with mRNA isolated from LAR rabbits. The membranes were rehybridized with a rabbit β-actin cDNA probe (Figure 4b). The intensity of the bands was quantified and the data were expressed as SR-A mRNA relative to β-actin mRNA. The results (mean±SE values of 3 animals per strain) show a significantly higher expression of SR-AI/II in the macrophages of LAR rabbits, a finding that is consistent with the higher uptake of DiI-acLDL by these cells.

SR gene expression is regulated via a signal transduction pathway involving AP-1 among other transcription factors. To learn more about the regulatory processes involved in the SR activity and expression in HAR and LAR macrophages, we studied AP-1 activity in these cells. It is noteworthy that we found a time-dependent effect, with low AP-1 in freshly isolated peritoneal macrophages and a substantial increase of AP-1 activity during the first 2 hours of cultivation at 37°C, when the cells became adherent to culture dishes. AP-1 activity was highest after 24 hours; however, there was no significant difference between AP-1 activity in macrophages from both rabbit strains (Figure 5).

To learn whether a difference in SR activity between LAR and HAR rabbits could also be detected in vivo, we determined the clearance of DiI-acLDL from plasma after intravenous injection in 6 LAR and 6 HAR rabbits. Figure 6 depicts a representative experiment in a pair of LAR and HAR rabbits in which the decay curves of fluorescence intensity are superimposed. There was no difference in the t_1/2 of DiI-acLDL clearance between the HAR and LAR rabbits, suggesting that there is no difference in hepatic SR activity in the 2 strains of rabbits.

Under certain circumstances, such as in the presence of macrophage colony-stimulating factor (M-CSF), high expression of SR mRNA in macrophages was accompanied by high expression of ApoE. Therefore, we also examined the expression of ApoE in macrophages of LAR and HAR rabbits. Membranes were hybridized with a rabbit ApoE cDNA probe, stripped of the probe, and rehybridized with a rabbit β-actin probe. The expression of ApoE in macrophages of LAR rabbits was 74% higher than in HAR rabbits, but the difference did not reach statistical significance (Figure 7).

**Discussion**

The aim of this study was to explore if certain factors of macrophage function involved in cholesterol balance are related to the attenuated atherosclerotic response of LAR rabbits to hypercholesterolemia. Because of the paucity of blood monocytes in normocholesterolemic rabbits, we chose to study peritoneal macrophages as substitutes for ‘lesion macrophages.’ Investigations in pigeons that differed in their susceptibility to develop atherosclerosis when fed cholesterol-rich diets have shown an impairment in cholesteryl ester hydrolysis in peritoneal macrophages derived from the susceptible birds. In the present study, we did not find any difference in the rates of hydrolysis of cholesteryl ester by macrophages derived from the 2 strains of rabbits. Thus, the

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**Figure 3.** Saturation kinetics of uptake of DiI-acLDL by peritoneal macrophages of HAR and LAR rabbits. Data are mean±SEM values of 5 HAR and 6 LAR rabbits. The difference in apparent V_max, P<0.05; K_m, P=NS.

**Figure 4.** Expression of SR-AI and SR-AII mRNA in peritoneal macrophages of HAR and LAR rabbits. (a) Northern blot analysis, using 15 μg of RNA from peritoneal macrophages. SR-A mRNA was detected by hybridization with a rabbit-specific 32P-labeled cDNA probe that recognizes both type I and type II receptors. The lower panel shows rehybridization of the same blots with control probes for rabbit β-actin. Data are mean±SEM values of 3 HAR and 3 LAR rabbits; P=0.014.

**Figure 5.** Quantitation of AP-1 activity in peritoneal macrophages from HAR and LAR rabbits at the indicated time points after adhesion to cell culture dishes. Data are mean±SEM values of 4 rabbits from each strain; differences, NS.

**Figure 6.** Clearance of DiI-acLDL from plasma of 1 HAR and 1 LAR rabbit. The rabbits were injected with 1 mg of DiI-acLDL protein, and the change in fluorescence intensity of plasma was determined at the indicated time points for up to 60 minutes. All fluorescence data are expressed as percentages of the first 1-minute time point (initial concentration). The t_1/2 values of DiI-acLDL clearance determined in 6 HAR and 6 LAR rabbits were 7.4±1.8 and 8.2±1.6 minutes (mean±SD), respectively.
mechanism responsible for the difference in aortic cholesterol accumulation found in the above-mentioned pigeons is apparently not operative in the LAR and HAR rabbits.

The major new finding of the present study is that the SR activity in macrophages of normocholesterolemic LAR rabbits was consistently higher than in the HAR rabbits. At first glance such a result might seem contradictory to our previous findings, which showed that induction of SR in aortic SMCs of LAR rabbits was highly attenuated when compared with HAR rabbits. However, there are significant differences in SR regulation in SMCs and in macrophages. First, the SR in macrophages is expressed constitutively and its activity is high, whereas in SMCs this receptor is hardly expressed unless stimulated. Second, the regulation of SR in macrophages and SMCs is rather different, especially in response to various effectors. Thus, although platelet secretory products were shown to inhibit SR activity in macrophages, they potently stimulate SR activity in rabbit and human SMCs. Among the active factors in the platelet secretory products was transforming growth factor-β1, which enhanced SR activity in SMCs ≈2-fold, and when combined with platelet-derived growth factor-BB and epidermal growth factor, the activation was ≈12-fold. On the other hand, transforming growth factor-β1 was shown to inhibit SR activity in THP-1 macrophages. The other cytokines that have an opposite influence on SR in macrophages and SMCs are tumor necrosis factor-α and interferon-γ (IFN-γ). Both cytokines cause a reduction in SR expression in macrophages but upregulate the SR expression in SMCs.

Recently, IFN-γ has been implicated to potentiate atherosclerosis in ApoE knockout mice. In that study, IFN-γ receptor knockout mice were crossed with ApoE knockout mice and fed a western diet for 12 weeks. There was a significant reduction in atherosclerotic lesion size and a 60% reduction in lesion lipid accumulation in these double knockout mice. In the above-mentioned study, disruption of the IFN-γ receptor gene also resulted in marked induction of hepatic ApoA4 and an increase in plasma phospholipid–ApoA4–rich particles, which are potentially atheroprotective. One could also expect in these double knockout mice an increase in macrophage SR activity because of the absence of IFN-γ receptor.

Augmentation of SR expression in macrophages, which results in an increase in the number of SR, was reported after treatment with macrophage colony-stimulating factor (M-CSF) or dexamethasone. It is noteworthy that M-CSF prevented atherosclerosis in Watanabe Heritable Hyperlipidemic rabbits, without reducing plasma cholesterol levels, and dexamethasone treatment suppressed atherosclerosis in cholesterol-fed rabbits. These 2 independent studies provided evidence, contrary to the prevailing opinion, that an increase in SR-A activity in macrophages can be associated with attenuation of atherosclerosis. On the other hand, accelerated atherosclerosis, which was ascribed to an increase in macrophage SR activity, was found in mice lacking tumor necrosis factor receptor p55.

The role of the macrophage SR in atherosclerosis was further investigated in a recent publication in which the creation of SR-AI/II knockout mice was reported by Suzuki et al. When these mice were fed a high-fat diet containing 1.25% cholesterol for 11 weeks, plasma cholesterol levels rose to >300 mg/dL in both SR-A knockout mice and controls, but no data concerning development of atherosclerosis were given. When SR-A knockout mice were crossed with ApoE knockout mice, the lesion size in the female double knockout mice at 5 months of age was significantly smaller than in ApoE knockout mice. However, in the double knockout mice, plasma cholesterol levels were 50% higher than in the ApoE knockout mice. Thus, there could have been an increase in size of the atherogenic lipoprotein particles in the double knockout mice, in which case one might expect a reduction in atherosclerosis despite higher plasma cholesterol levels. The latter possibility is further strengthened by the finding in a recent study in which SR-A knockout mice were crossed with ApoE3Leiden transgenic mice. In these mice fed an atherogenic diet, serum cholesterol, triglycerides, and ApoE levels were the same as in controls (SR-A+/+; ApoE3Leiden). However, contrary to expectations, SR-A deficiency did not result in reduction of atherosclerosis in this mouse model and even more severe complex lesions were found in the SR-A−/−; ApoE3Leiden mice.

In another study with SR-AI/II knockout mice it was reported that the serum decay and liver uptake were the same in control and knockout mice irrespective whether a high or low dose of acLDL was injected. The authors concluded that additional receptors (other than SR-AI/II) participate in the uptake of acLDL in the liver. Presently, there is no difference in the plasma decay when HAR and LAR rabbits are injected with Dil-acLDL, and thus our findings would agree with these conclusions.

How do we envisage the putative role of high SR expression of LAR rabbit macrophages in attenuation of atherosclerosis? Because SR expression is not downregulated by cholesterol, a macrophage expressing more SR could be better equipped to metabolize modified lipoproteins efficiently. In human monocytes stimulated with M-CSF, an increase in macrophage SR occurred in parallel with a rise in ApoE mRNA, a major participant in reverse cholesterol transport. The importance of macrophage ApoE in the reduction of atherosclerosis (without reduction of plasma cholesterol levels) accrued from studies on ApoE-deficient mice that were transplanted with normal bone marrow. Moreover, macrophage-derived ApoE, which comprised <7% of normal
plasma levels, restored the cholesterol efflux capacity of ApoE-deficient plasma.  

The conclusion of the latter study was that low-dose expression of macrophage ApoE protected ApoE-deficient mice from atherosclerosis.  

Moreover, increased atherosclerosis was found in irradiated mice, transplanted with ApoE null bone marrow from ApoE knockout mice, compared with mice transplanted with normal bone marrow.  

In the present study, ApoE mRNA in macrophages of LAR rabbits was 1.7-fold higher than in those of HAR rabbits, but the difference did not reach statistical significance (P = 0.09). Thus, it appears that in the presence of normal or moderately increased ApoE, macrophages with high SR activity could contribute to lesser cholesterol accumulation in the aortae of LAR rabbits.  

In a recent study on human monocyte-derived macrophages, higher uptake of modified lipoproteins and higher expression of ApoE mRNA were found in healthy old subjects than in young individuals.  

These results supported the notion that higher expression of SR and ApoE in monocyte-derived macrophages could be beneficial, because these old males (mean age, 84 years) had no clinical coronary heart disease. In another study, a remarkable pedigree of a Canadian family was described, in which 2 members had coronary heart disease. In another study, a remarkable pedigree of a Canadian family was described, in which 2 members had coronary heart disease. In another study, a remarkable pedigree of a Canadian family was described, in which 2 members had coronary heart disease. In another study, a remarkable pedigree of a Canadian family was described, in which 2 members had coronary heart disease. In another study, a remarkable pedigree of a Canadian family was described, in which 2 members had coronary heart disease. In another study, a remarkable pedigree of a Canadian family was described, in which 2 members had coronary heart disease. In another study, a remarkable pedigree of a Canadian family was described, in which 2 members had coronary heart disease.

Prominent coronary heart disease was not found in any member of the family. In fact, not 1 in the whole family of 13 members, 31 to 90 years of age, died of coronary heart disease, although in 6, plasma cholesterol levels were >200 mg/dL. The authors suggest that the overexpression of SR and CD36 in monocytes may have a protective effect on atherosclerosis; this is in accord with the findings of our study.

In conclusion, the increased expression of SR-A in macrophages in the presence of adequate amounts of ApoE may play a role in attenuation of atherosclerosis in LAR rabbits. This postulate could be in accord with the accepted protective role of SR-A in host defense against lethal endotoxic shock induced by Gram-negative bacterial infections.

Acknowledgments  

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References  


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