

# Class A scavenger receptors: Recent advances in elucidation of structure-function relationships and their role in atherosclerosis

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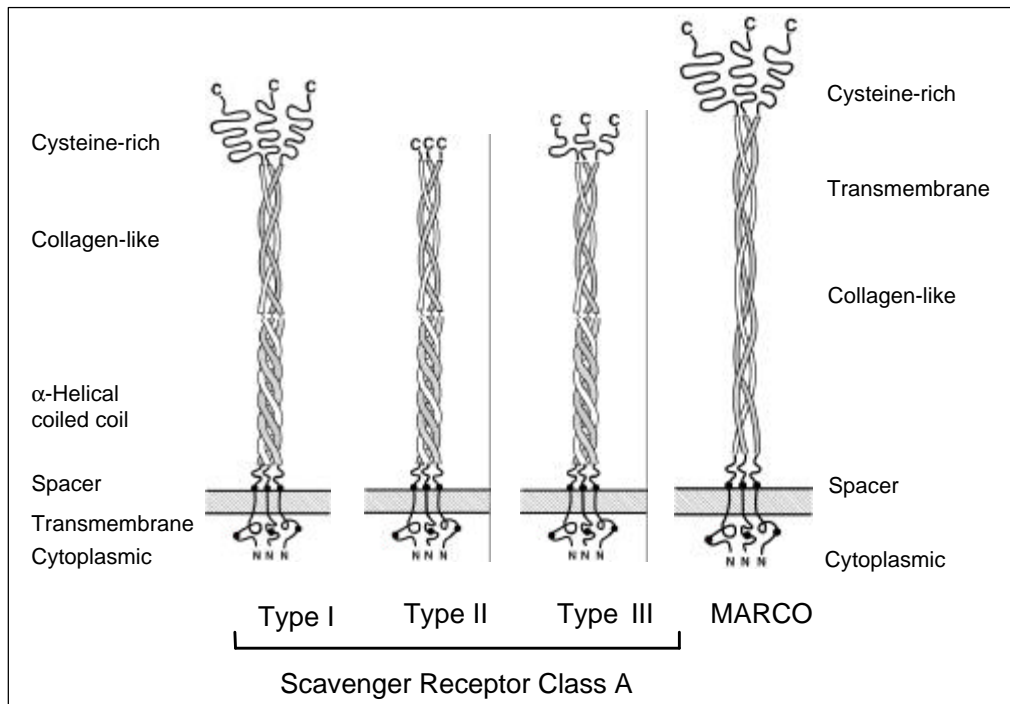
## Introduction

Atherosclerosis is a disease process in which monocytes are attracted to specific regions of arterial endothelium, adhere, and migrate through an intact endothelial layer. Once within the subendothelial space, macrophages ingest large amounts of lipid and are often referred to by the descriptive term of foam cells.

With the discovery and characterization of low density lipoprotein (LDL) receptors, a highly regulated

mechanism was described for the maintenance of cellular cholesterol homeostasis. Although individuals with highly elevated concentrations of LDL cholesterol were at greater relative risk of developing cardiovascular diseases, the determination of LDL receptor regulation abrogated the thought that native LDL was responsible for the formation of macrophage foam cells. Instead, it was hypothesized that LDL became damaged and that these modified particles were responsible for the disease. Acetylation was the first modification that was found to promote interactions of LDL with macrophages. The interaction of acetylated LDL (AcLDL) with macrophages had characteristics of a specific receptor, that was termed the scavenger receptor. The scavenger receptor was originally purified and cloned from cow liver [1•,2••], and has now been cloned from mouse [3,4••,5], rabbit [6], and human [4••]. Subsequently, many diverse proteins have been discovered that have the common ability to bind modified forms of LDL [7]. This has led to the classification of scavenger receptors in an alphabetic scheme, in which the originally characterized receptor has been included in the 'A' class. Currently, proteins in the 'A' class are scavenger receptors class A (SR-A) subtypes of I, II, and III and a structurally similar molecule termed MARCO [8] (Figure 1).

Figure 1. Proposed structure of the current scavenger receptor class A family members.



As noted above, SR-A were originally purified from macrophages. The extent of SR-A expression within macrophages varies with phenotype and may depend on the tissue of residence and the local milieu. For example, within the spleen, there is modest expression of SR-A in red pulp macrophages, but intense expression within macrophages in the outer marginal zone [11]. In addition to macrophages, SR-A have been identified in rabbit smooth muscle cells, although the detection in human cells has not been consistent [12]. Compelling evidence that SR-A is expressed in smooth muscle cells comes from the cloning of the rabbit cDNA from cultured smooth muscle cells [6]. SR-A have also been identified on specific forms of endothelium, including liver sinusoids and aorta [13].

## Ligands

SR-A was originally identified through its ability to recognize AcLDL. However, it is now established that SR-A is a promiscuous receptor that interacts with many diverse ligands. The only common characteristic of all SR-A ligands is that they are polyanionic, although not all polyanions are ligands (Table 1). The ability to bind multiple ligands indicates that SR-A may be involved in many physiological and disease processes. Its ability to interact with modified forms of lipoproteins could indicate its involvement in atherosclerotic and renal diseases. The recognition of phosphatidylserine has been related to the phagocytosis of cells undergoing apoptosis [9]. Recognition of both lipopolysaccharide and lipoteichoic acid infers a function in host defense of both gram-negative and -positive bacteria, respectively [14,15]. Finally, the interaction with crocidolite asbestos may infer a role in specific pulmonary disease [16].

## Structure and function of SR-A

The cDNA for SR-A was originally isolated from cow liver and was subsequently harvested from the human macrophage cell line, THP-1 [17], rabbit (smooth muscle cells and lung) [4,6], and mouse (P388D1 cells) [3,4]. Within species, the only polymorphism that has been described is for mice, in which the C57BL/6 strain differs from the sequence derived from DBA/2 mice [5]. It is not known whether there is any functional significance to this polymorphism, although it imparts the important characteristic of ablating reactivity with one of the most commonly used anti-SR-A monoclonal antibodies, 2F8 [18].

**Table 1. Ligands for SR-A.**

Groups	Examples of specific compounds
Modified lipoproteins and proteins	Acetylated LDL MDA-LDL Copper oxidized LDL MDA-albumin
Polyanions	Polyinosine Polyguanosine
Polysaccharides	Fucoidan Carageenan Dextran
Phospholipids	Phosphatidylserine
Bacterial products	Lipopolysaccharides Lipoteichoic acid
Miscellaneous	Crocidolite asbestos

SR-A are derived from a single gene containing 11 exons [17]. SR-A subtypes, I, II, and III are formed as a consequence of alternative splicing. SR-AI is a six domain protein that exists in a trimeric form as illustrated in Figure 1 [2]. These domains from intracellular to extracellular are: cytoplasmic, membrane, spacer,  $\alpha$ -helical coiled-coil, collagen-like, and cysteine-rich. The only difference between the three subtypes exists in the cysteine-rich region. In SR-AI, this domain comprises 110 amino acids and is truncated to 6 amino acids in SR-AII [19]. SR-AIII has a truncation of this region that leads to a domain of approximately half the length of that in SR-AI [20].

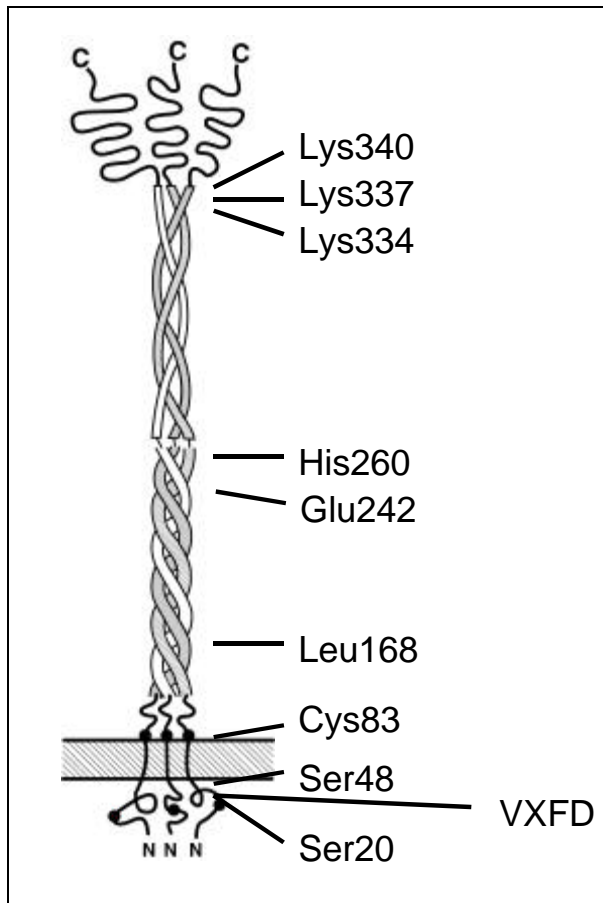
There have been specific functions assigned to several of the domains of SR-A. The following is a summary of the potential functions from the intercellular amino terminus to the extracellular carboxyl portion of the molecule:

**Domain I - Cytoplasmic** (50 amino acids in human, bovine and rabbit, 55 amino acids in mouse). The internalization of SR-A ligands appears to occur predominantly via endocytosis of clathrin-coated pits. In many receptor types that are trafficked through the same system, there are specific domains in the cytoplasmic tail that are required for an endocytic function. SR-A appears to have a unique internalization sequence of VXFD (figure 2) [21]. The deficiency of this motif leads to both a decrease in cell surface expression, in addition to an inability to permit endocytosis of ligands.

Many receptor systems lack an internalization sequence, but are endocytosed via clathrin-coated pits due to phosphorylation in the cytoplasmic domain. Following AcLDL binding, the cytoplasmic tail of SR-A becomes phosphorylated [22,23]. The cytoplasmic domain of each mature SR-A monomer contains three conserved serine residues and one conserved threonine residue. Two of these conserved phosphorylation sites are present in the same functionally important region defined by deletion mutants [21,24]. Pharmacological modulation of phosphorylation has generated somewhat conflicting data on SR-A expression and function. The serine/threonine phosphatase inhibitor, okadaic acid, reduced AcLDL-induced cholesterol esterification due to an inhibition of AcLDL binding to SR-A in the absence of any change in SR-A expression [22]. The protein kinase inhibitor, staurosporine, also inhibited AcLDL uptake, but due to an inhibition of internalization [22]. Furthermore, staurosporine increased SR-A expression. Some of these inconsistencies may relate to the potentially complex control of SR-A depending on the specific phosphorylation site. Site-directed mutagenesis demonstrated a conversion of Ser20→Ala20 increased SR-A expression associated with increased cell surface expression, whereas Ser48→Ala48 inhibited AcLDL uptake due to reduced kinetics of internalization associated with no change in expression [23].

Binding of modified-LDL to SR-A initiates a number of classic pertussis toxin-sensitive signaling cascades, implicating a Gi/o-protein signaling mechanism [25,26]. This stimulation has functional consequences on lipoprotein metabolism. Pertussis toxin treatment of peritoneal macrophages inhibits a component of AcLDL uptake [27]. This is not a generalized effect on internalization and processing of lipoproteins since pertussis toxin had no effect on LDL receptor-mediated uptake of  $\beta$ -VLDL [28]. Recent evidence has also suggested that

**Figure 2.** Location of residues and motifs with identified functions in SR-A. The proposed function of the identified residues or motifs in the figure are described in the text.



binding of modified LDL to SR-A will do more than facilitate entry of the lipoprotein. Incubation of macrophages with modified LDL stimulates a number of signaling cascades that promote effects such as upregulation of urokinase-type plasminogen activator expression [29], release of tumor necrosis factor (TNF)- $\alpha$  [25], and growth [26,30]. While not all of these responses have been defined as being directly mediated by SR-A, it demonstrates the possibility that the cytoplasmic domain of this receptor may have a wide array of properties that have only been recognized recently.

**Domain 2 - Transmembrane** (22 amino acids). At present there is no specific function assigned to this region other than membrane anchoring.

**Domain 3 - Spacer** (32 amino acids). One of the major functions of this domain may reside in the cysteine residues that are predicted to be present close to the cell membrane. The trimeric structure of SR-A was thought to be a result of disulfide bonding between the cysteines present at position 83. However, isolation of the protein in the presence of cysteine trapping agents demonstrated that the native protein exists as a disulfide-linked dimer and a non-covalently linked monomer [31].

**Domain 4 -  $\alpha$ -Helical coiled coil** (163 amino acids). Specific regions of the  $\alpha$ -helical coiled coil participate in the dissociation

of the ligand to permit its subsequent processing by the lysosome while the receptor is recycled to the cell surface. A specific histidine residue at position 260, close to the junction of the  $\alpha$ -helical coiled coil and the collagen-like region, is responsible for this pH-dependent dissociation of ligands from SR-A. Substitution of His260 with leucine did not influence the binding activity but led to an inability of the ligand to dissociate from the receptor [32]. Therefore, there is an allosteric dissociation of ligand from the collagen-like region as described below that is dependent on the structure of the  $\alpha$ -helical coiled coil domain.

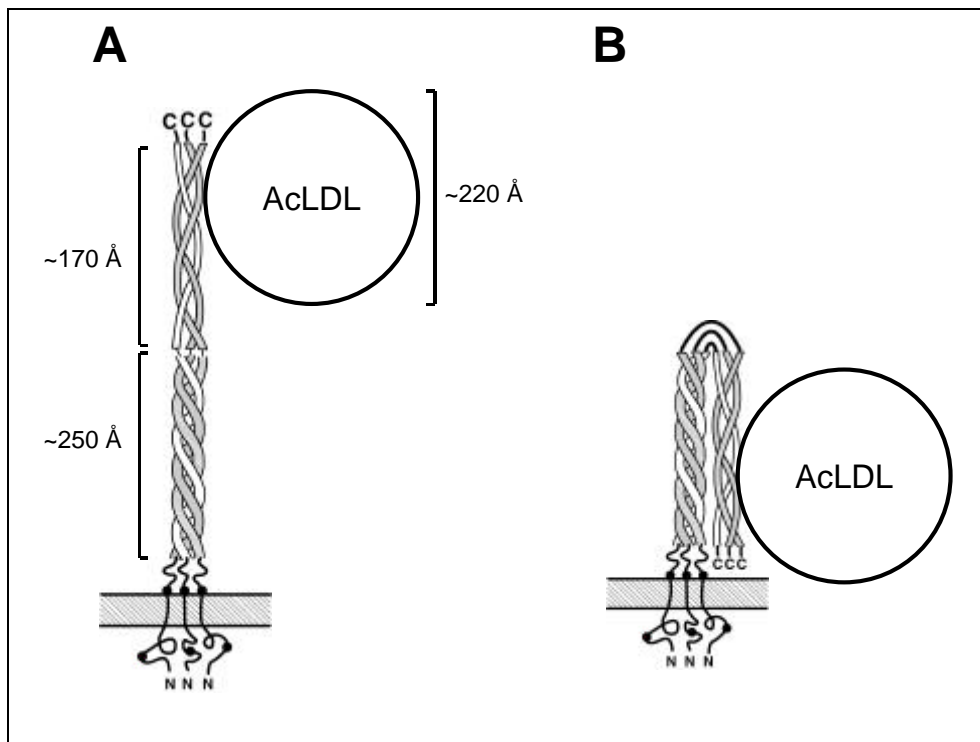
The conformation of this domain is pH-dependent, being a random coil at pH 7.0 and a  $\alpha$ -helical coiled coil structure at pH 5 [33]. The role of His260 has not been defined, but this pH-dependent conformational change is indicative of a participation of acidic residues. Indeed, it appears that this conformational change is due to a buried glutamate residue in the carboxyl end of this domain [34].

**Domain 5 - Collagen-like** (72 amino acids). This domain contains 24 triplet repeats of Gly-X-Y in all species cloned to date, except human, which has 23. Generation of truncated mutants that only contain part of the collagen-like domains antagonized the binding of AcLDL to SR-A through a dominant negative mechanism. This provided initial evidence that the lipoprotein binding area was within the collagen-like domain [35]. Synthetic peptides of the collagen-like domain are able to bind to AcLDL, but only when peptides are in a cross-linked tripeptide form [36].

Site-directed mutagenesis studies have further localized the binding region to a positively charged coiled groove in the carboxyl end of the collagen-like region. Substitution of Lys337 $\rightarrow$ Ala337 abolished the binding of AcLDL to SR-A at physiological temperature (37°C) but not at 4°C [4••]. This aberrant binding at 4°C may be a result of conformational changes in both the ligand and the receptor. Furthermore, substitution of Lys337 only partially decreased the degradation of copper-oxidized LDL. Substitution of a second lysine (either 334 or 340 in addition to 337) was needed to substantially reduce oxidized LDL degradation. As with AcLDL, there was a disparity of the results of binding assays when performed at either 37 or 4°C. AcLDL binding to SR-A is also inhibited by a number of mutations of the collagen-like region that are not in this lysine cluster. These mutations may lead to currently undefined conformational changes that inhibit ligand interactions, rather than interfere with direct ionic receptor-ligand interactions [37].

**Domain 6 - Cysteine-rich** (110 amino acids) This domain is a highly conserved region that is present in a large number of proteins, many having functions related to the immune system [38]. SR-AI contains a class A cysteine-rich domain that is characterized by the presence of six cysteines. The functional significance of this domain in SR-AI has not been defined. The only function for this domain that has been described in SR-AI of some species is its ability to interact with lipopolysaccharide [3]. Although the absence of this domain does not impart any detectable differences in the cellular processing of SR-A forms I and II, the truncated form of this domain in SR-AIII, leads to retention of the protein in the endoplasmic reticulum and has a dominant negative effect.

**Figure 3. Possible conformations of SR-A and its interaction with modified forms of LDL. A represents the originally proposed structure of SR-A and the site of AcLDL interaction, while B represents a more recently proposed modification.**



**Other structural considerations**

SR-A is usually represented in a straight stalk that has a calculated length of approximately 444 Å protruding from the membrane. However, the region between the α-helical coiled coil and the collagen-like domain is highly flexible. At physiological pH, electron microscopy studies have demonstrated that these two regions are juxtaposed as represented in Figure 3 [39]. This structure would explain the apparent paradox of the inhibition of AcLDL-induced cholesterol esterification by the monoclonal antibody, 2F8 [8,19]; since 2F8 binds to the α-helical coiled coil domain, it may be assumed that this antagonism is due to steric

hindrance which would be consistent with a lateral apposition of these domains [5,18•].

Data from adhesion assays are also consistent with SR-A being present in a folded form. The adhesion properties of SR-A were discovered through the generation of 2F8 [18•]. The epitope recognized by 2F8 is in the α-helical coiled coil domain, based on the identification of an SR-A polymorphism at Leu168 [5]. However, antibodies to the collagen-like region inhibit SR-A-mediated adhesion to glycosylated collagen [40]. Therefore, while there is no direct comparison, like the effects of 2F8 on AcLDL degradation, this

**Table 2. Examples of regulation of SR-A in cultured cells.**

Upregulation	Downregulation
<b>Macrophage</b>	
Lymphokines [68]	Lymphokines [69]
IFNγ [43-45]	IFNγ [42,70]
Lipopolysaccharide [71]	Lipopolysaccharide [50]
Phorbol esters [72]	Phorbol esters [41]
Dexamethasone [73]	Dexamethasone [74]
GM-CSF [45]	GM-CSF [75]
IL-4 [45]	IL-6 [76]
IL-1α [44]	TGF-β [77,78]
Platelet-derived products [79]	PPAR agonists [80]
M-CSF [81]	TNF-α [51,82]
Cytomegalovirus infection [83]	α-Tocopherol [84]
OxLDL and AcLDL [48,49]	Retinoic acids [72]
<b>Smooth muscle cell</b>	
Protein kinase C stimulation [85]	TGF-β [86]
Serum [12]	
Platelet-derived growth factor [87]	
TNF-α [52]	
IFNγ [52]	

antibody may be inhibiting adhesion by steric hindrance rather than acting directly on an adhesion motif.

### Regulation

SR-A is regulated by many substances in cultured cells (Table 2), although there are many conflicting reports in the published literature. The most extensively studied cell type in which SR-A regulation has been studied is those of macrophage lineage. There has been a particular interest in the role of selected cytokines in SR-A regulation. One of the cytokines that exemplifies the inconsistency in the published literature is IFN $\gamma$ , which has been reported to have an inhibitory [41,42], stimulatory [43-45], or absent effect on SR-A [46,47]. In part, some of the inconsistency may be due to the differing culture conditions that may impart phenotypical differences. Also, one pragmatic consideration is that several of the cytokines lead to considerable difference in cell protein content and consequently the normalization of SR-A to cell protein or number is an important consideration.

Unlike LDL receptors, SR-A are not downregulated by intracellular accumulation of cholesterol. On the contrary, it has been recently described that SR-A can be upregulated by cholesterol-containing ligands such as AcLDL and oxidized LDL [48,49].

The regulation of SR-A in smooth muscle cells has not been studied as extensively. The most striking difference is the effect of tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), which is inhibitory in macrophages [50,51], but was markedly stimulatory in smooth muscle cells [52]. Currently, there are no data on the regulation of SR-A in cultured endothelial cells.

There are limited data on the regulation of SR-A *in vivo* [53]. However, the relative expression of SR-A differs considerably among different macrophage populations [11]. There appears to be some local regulation of SR-A in macrophages of atherosclerotic tissue [54], although more studies are needed on the expression of SR-A in the complex milieu of atherosclerotic lesions.

### Function in atherosclerosis

Since scavenger receptors are thought to play a major role in the atherogenic process, this disease has been the focus of many of the studies in which SR-A activity was either deleted or increased by genetic engineering (Table 3). While an initial report demonstrated an important atherosclerotic role of SR-A, subsequent data has also ascribed an anti-atherosclerotic effect.

**Table 3. Effects of genetic manipulation of SR-A.**

Mouse strain/background	SR-A expression	Effect
<b>Atherosclerosis studies</b>		
No genetic manipulation	SR-A <sup>-/-</sup> after bone marrow transplantation	20% Higher plasma cholesterol concentrations, 60% reduction in lesions [55]
	YAC SR-A transgenic	Increased AcLDL induced cholesterol deposition in macrophages [56]
ApoE <sup>-/-</sup>	SR-A <sup>-/-</sup>	46% Increase in plasma cholesterol concentrations, 58% decrease in lesion size
	YAC SR-A transgenic after bone marrow transplantation	No effect on lesion size [58]
LDL receptor <sup>-/-</sup>	SR-A <sup>-/-</sup>	21% Decrease in plasma cholesterol concentrations, 20% reduction in lesions [59]
	SR-A <sup>-/-</sup> after bone marrow transplant	No effect on plasma cholesterol concentrations, 60% reduction in lesions [58]
	SR-A <sup>-/-</sup> transgenic	27% Reduction in lesion size [60]
ApoE3Leiden transgenic	SR-A <sup>-/-</sup>	No reduction in lesion size but changes in cellular characteristics
	YAC SR-A transgenic after bone marrow transplantation	35% Reduction in lesion size [57]
<b>Non-atherosclerosis studies</b>		
Mixed ICR and C57BL/6	SR-A <sup>-/-</sup>	Decrease in granuloma size [61]
C57BL/6	SR-A transgenic - lysozyme promoter	Increase in granuloma size [62]
C57BL/6	SR-A transgenic - transferrin promoter	42% Reduction in apoB containing lipoproteins in plasma, 43% increase in HDL cholesterol [63]

There have been no reports of the effects of SR-A deficiency alone on the development of atherosclerosis. More commonly, the effect of SR-A deficiency is examined in mice that have an additional genetic manipulation that promotes lesion susceptibility. The only atherosclerosis study in which SR-A deficiency alone has been investigated was in the setting of chimeric mice created by bone marrow transplantation (BMT). Deficiency of SR-A in bone marrow derived cells in C57BL/6 mice caused a 60% decrease in lesions in the aortic root, despite a 20% increase in plasma cholesterol concentrations [55]. Overexpression of SR-A has also been described in mice with the use of a yeast artificial chromosome (YAC) in which peritoneal macrophages had greater cholesterol ester accumulation in response to AcLDL [56]. However, the effect of the YAC SR-A transgenic expression has only been reported on atherosclerosis when combined with other genetic manipulations as described below.

The initial observation of the anti-atherogenic effects of SR-A deficiency was demonstrated in SR-A<sup>-/-</sup> mice that were also deficient in apoE. The absence of SR-A led to a 58% decrease in the size of atherosclerotic lesions in the aortic root, even in the face of a 46% increase in plasma cholesterol concentrations [57]. Conversely, overexpression of SR-A in transgenic mice using the YAC system failed to show any effect on lesion progression in apoE<sup>-/-</sup> mice [58].

The results of SR-A regulation in LDL receptor<sup>-/-</sup> mice has been mixed. The initial publication demonstrated that SR-A<sup>-/-</sup> x LDL-receptor<sup>-/-</sup> mice had a 20% reduction in atherosclerosis, although there was also a 21% reduction in plasma cholesterol concentrations [59]. A striking 60% reduction in aortic lesions was noted in LDL receptor<sup>-/-</sup> mice that were irradiated and repopulated with bone marrow from congenic SR-A<sup>-/-</sup> mice [55]. In this study, there was also no significant change in plasma cholesterol concentrations. In contrast to studies that imply a detrimental effect of SR-A in LDL receptor<sup>-/-</sup> mice, another group demonstrated that overexpression of SR-A by the use of a YAC led to 27% reduction in disease in these mice [60].

Another animal model that has been used to define the role of SR-A in atherosclerosis is the apoE3Leiden transgenic mouse. Deficiency of SR-A in these mice has no effect on the size of lesions formed. However, the lesions formed in SR-A-deficient mice contained fewer foam cells and relatively more fibrotic components. Furthermore, the generation of chimeric apoE3Leiden transgenic mice by BMT demonstrated that overexpression of SR-A reduced lesion severity by 35% [60].

Overall, there is no obvious consensus in the literature regarding the role of SR-A in the atherogenic process. However, while the effects of changes in SR-A have been mixed, the fact that there has consistently been an effect on atherogenesis lends support to an important role of SR-A in the disease process. Therefore, further studies will have to address whether SR-A has specific effects that depend on the stimulus for lesion formation and on the stage of the disease.

Since a ubiquitously described event in atherogenesis is monocyte recruitment, studies on granuloma formation

have been used as a surrogate for this specific aspect of the disease. Deficiency of SR-A decreased hepatic granulomas formed by the injection of *Corynebacterium parvum* and was associated with decreased expression of monocyte chemoattractant protein (MCP)-1, TNF- $\alpha$ , and IFN- $\gamma$  [61]. Macrophage overexpression of SR-A increased the formation of subcutaneous carrageenan granulomas in C57BL/6 mice fed a lipid-enriched diet [62]. However, this increase in size of granuloma was not associated with any increase in the lipid content of the tissue, but rather was associated only with an increase in the number of cells present.

Genetic regulation of SR-A in mice has also produced the surprising result that this receptor type influences plasma concentrations of cholesterol. Generation of transgenic mice in which SR-A was overexpressed in hepatocytes under the control of the transferrin promoter demonstrated a significant reduction on apoB-containing lipoproteins [63]. There have been no reports of SR-A interacting with native lipoproteins, so this effect may be due to interactions with modified lipoproteins. Whether modified lipoproteins exist in the plasma is contentious. However, the presence of modified lipoproteins would only be a small contribution of overall plasma cholesterol concentrations [64], and the mechanisms underlying these changes are not readily apparent.

## Antagonists

There are several antagonists of SR-A, including polyanionic molecules such as fucoidin, dextran sulphate, polyinosine, and polyguanosine [65]. These compounds have been used in numerous cell culture experiments to antagonize the effects of modified forms of LDL and some have also been used in whole animal experiments. At present, the specificity of these polyanions is not known. Indeed, there is reason to question the specificity since polyinosine antagonizes the clearance of AcLDL from plasma even in mice that are deficient in SR-A [66].

A small molecule, non-peptide antagonist of the binding of AcLDL to SR-A has been reported recently by SmithKline Beecham [67]. The molecule is (E)-methyl-4-chloro- $\alpha$ -[4-(4-chlorophenyl)-1,5-dihydro-3-hydroxy-5-oxo-1-(2-thiazolyl)-2H-pyrrol-2-ylidene]benzene acetate. This compound is a non-competitive SR-A antagonist that inhibits the binding of AcLDL to a wide variety of cell types, including cells transfected with SR-A, human monocyte-derived macrophages, and rat peritoneal macrophages. In an analogous manner to the data described for the antagonism of the monoclonal antibody, 2F8, it is also not clear that this molecule is exerting its actions directly on the lysine cluster in the collagen-like domain that has been described to interact with AcLDL [4••].

## Conclusion

Since the existence of SR-A was first hypothesized in the 1970s, there has been considerable progress on the molecular characterization of these proteins, which has revealed functions that extend beyond its initially proposed role that was limited to lipoprotein metabolism. The demonstration that SR-A has many biological effects, in addition to its

originally described property of facilitating endocytosis of modified lipoproteins, will require the determination of the relative importance of these properties on atherogenesis. Genetic manipulation of SR-A in atherosclerosis-susceptible mice has revealed that this receptor has significant effects on the evolution of the characteristics and size of lesions. However, the specific impact may depend on factors that are currently unknown, since there has been a range of responses to over- and underexpression of this molecule in animal models of the disease. It is provocative to suggest that inhibition of SR-A would have a beneficial effect on the atherogenic process in humans, but further studies will be needed to determine whether the complex biological properties of SR-A translate into a useful therapeutic target.

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