Scavenger receptors: diverse activities and promiscuous binding of polyanionic ligands

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Scavenger receptors are a diverse family of proteins that share a common property – the binding of modified lipoprotein – but they have recently been shown to recognise a diverse range of ligands. Understanding the molecular interaction of receptor–ligand binding should provide insight into how scavenger receptors contribute to important biological processes.

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Introduction

Scavenger receptors are defined by their ability to bind modified lipoprotein. In particular, a combination of two discoveries — the isolation of new receptors and the identification of their broad ligand-binding — has intensified research interest in scavenger receptors. Recent reviews have concentrated on the general properties and structures of scavenger receptors [1,2], and their contributions to lipoprotein metabolism [3] and host defense [4,5]. In this article we will attempt to relate the biology of scavenger receptors to their structure and possible mechanisms of their unique ligand-binding properties. A greater knowledge of the molecular basis of the receptor–ligand interaction might help us to understand the specific biological contributions of scavenger receptors, identify potential targets and aid the design of therapeutic molecules.

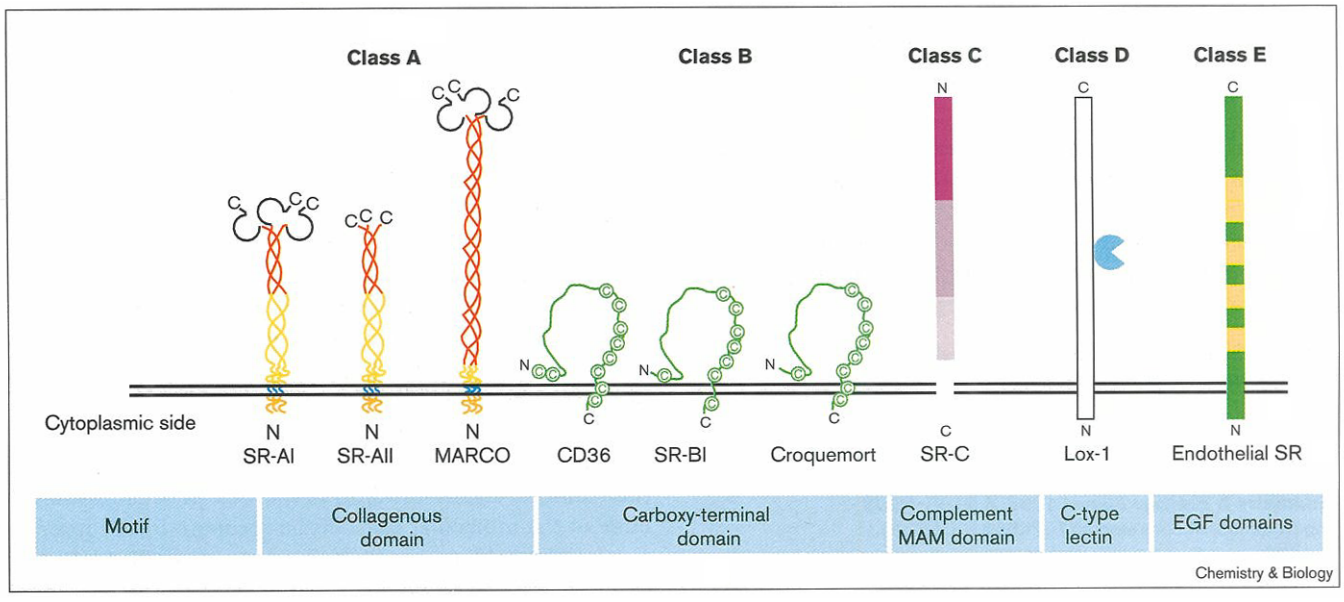
Scavenger receptors – an historical perspective

The family of proteins known as scavenger receptors was originally identified by the Nobel laureates Michael Brown and Joseph Goldstein [6] in their seminal investigations of endocytic uptake of lipoproteins by cells, a key aspect of lipid deposition in artery walls during the development of atherosclerotic lesions. They showed that the low-density lipoprotein (LDL) receptor was not responsible for the accumulation of cholesterol esters, and proposed and demonstrated the existence of a distinct receptor-mediated mechanism. These receptors, originally called acetylated LDL receptors, now termed scavenger receptors, were capable of endocytosing large quantities of chemically modified LDL, culminating in the conversion of macrophages into cells packed with esterified cholesterol that resemble the foam cells characteristic of the atherosclerotic plaque [7]. Unlike LDL receptors, scavenger receptors are not down-regulated by high intracellular levels of cholesterol. Although the involvement of scavenger receptors in the metabolism of modified lipoproteins remains the major focus of current research, other developments, including the recent isolation of several new receptors, strongly suggest scavenger receptors play important biological roles in addition to their role in vascular disease.

Scavenger receptors are multifunctional

The identification of several scavenger receptor genes that fall into at least five distinct classes (Figure 1), and the availability of specific reagents with which to analyse them has facilitated studies of the distribution and activities of scavenger receptors. All are plasma membrane proteins that are expressed on a number of cell types, in particular cells of the immune system. Some, such as class A scavenger

Figure 1



Structures of the five classes of scavenger receptors. Five classes of scavenger receptors have been recognised on the basis of structural homologies and ligand-binding properties. Class A are trimeric glycoproteins; three members have been identified [1,2]. SR-AI and SR-AII are alternative transcripts from the same gene. They differ from each other by the presence of the carboxy-terminal scavenger-receptor cysteine-rich domain (black, SRCR) in SR-AI. SR-AI and SR-AII have four other domains, a cytoplasmic tail (orange), a transmembrane domain (blue), an α-helical coiled coil (yellow) and a collagenous domain (red) that includes the binding site for acetylated LDL. The third member of class A, MARCO, has cytoplasmic, transmembrane and spacer domains that are not related to those of SR-AI, but a collagenous domain (red) and SRCR (dark blue) that are homologous to SR-AI [60]. Class B scavenger receptors [1,8,43] all have a single extracellular domain with a carboxy-terminal region that has conserved cysteine residues (green, enclosed c). Class C is currently represented by a single molecule, SR-CI [23] isolated from *Drosophila*, that is composed of multiple domains that include regions homologous to vertebrate proteins, including complement control protein (dark purple), the MAM (meprin-A5 antigen-receptor tyrosine phosphatase μ) family (pale purple) and a mucin-like domain (pink). Class D and class E scavenger receptors are both represented by single species that have either a C-type lectin structure (blue, Lox-1 [65]) or multiple epidermal growth factor (EGF) repeats (pale yellow, endothelial scavenger receptor [66]). The protein motif characteristic of each class in indicated beneath. Redrawn from [30].

receptors (SR-As) are essentially restricted in expression to a single cell lineage, the macrophages [1]; others, like the receptor CD36, are expressed on several cell types, including macrophages, platelets and endothelia [8]. These different patterns of expression are consistent with discrete, and sometimes overlapping, biological activities. Experimental evidence supports important contributions by scavenger receptors to a number of biological processes and we shall briefly outline each of these with appropriate examples.

Scavenger receptors and the endocytosis of modified lipoproteins

By definition, scavenger receptors are able to endocytose chemically modified lipoproteins. Typically, this has been determined *in vitro* by measuring either the binding and uptake or the degradation of labelled lipoprotein [1,3]. Other ligands for scavenger receptors have been identified by their ability to inhibit lipoprotein endocytosis. The contribution of a specific scavenger receptor to lipoprotein endocystosis has been difficult to define *in vivo* because of the presence of a multitude of different receptors with apparently overlapping activities. This can now be addressed through the recent generation of mice that are genetically deficient in certain scavenger receptors. Mice that lack type I and type II SR-A (SR-AI and SR-AII) were crossed with animals genetically deficient in apolipoprotein E, which develop atherosclerotic lesions when fed a cholesterol-rich diet. Atherosclerotic plaques were about 40% smaller in the double knockout than in the single knockout animal [9]. This work confirms the contribution of SR-AI and SR-AII to atherogenesis, but implies other receptor mechanisms are involved in the pathology. The theme of receptor redundancy or compensatory mechanisms is supported by analyses of the clearance of modified lipoproteins in the SR-A-deficient animal. Although Kupffer cells and endothelial cells isolated from the liver of the SR-A null mice show reduced endocytosis *in vitro*, there is no difference in the profile of lipoprotein clearance in these cells from that of the background strain when oxidised lipoprotein is injected into the mutant mouse [10]. It is suggested that the failure to observe a phenotype in the SR-A null mouse is due to an uncharacterised ligand-binding site in the liver. A role for the class B receptor SR-BI in high density lipoprotein (HDL) metabolism has

been confirmed by the demonstration that mice in which the gene for SR-BI has been disrupted have increased concentrations of plasma cholesterol because of a reduction in selective cholesterol uptake from the tissues [11].

Studies of human subjects and monocytes and blood-derived macrophages in tissue culture suggest that the class B receptor CD36 is involved in lipoprotein metabolism. Expression cloning of molecules able to endocytose oxidised LDL resulted in the isolation of CD36 [12]. The expression of the receptor is upregulated during the maturation of monocytes into macrophages [13]; transcriptional induction of the gene can occur via activation of the nuclear peroxisome-proliferator-activated receptor γ [14]. Finally monocyte-derived macrophages from patients that lack CD36 have reduced, but not absent, endocytosis of oxidised LDL *in vitro* [15]. These studies highlight that multiple scavenger receptors are likely to function in the uptake of modified lipoproteins *in vivo*.

Scavenger receptors as adhesion molecules

The search for scavenger receptor activities besides endocytosis has highlighted adhesion as a major function for scavenger receptors. This has been deduced largely from investigations that have used blocking reagents, such as antibodies, that are able to inhibit adhesive interactions. Specific antibodies that recognise CD36 are capable of preventing the adherence of platelets to collagen and thrombospondin-mediated binding of monocytes and macrophages to platelets and at various inflammatory sites [8]. In addition to the adherence of platelets in the normal host, studies using antibodies, supported by work using purified CD36 protein and transfected cells, have demonstrated that CD36 is the receptor on endothelium to which erythrocytes infected with the malarial parasite *Plasmodium falciparum* adhere [8,16]. This sequestration is an essential stage in the life cycle of the parasite.

A similar experimental approach has demonstrated an adhesive activity for SR-AI and SR-AII. A monoclonal antibody directed against the receptor can inhibit a component of cation-independent adhesion of macrophages on tissue culture plastic surfaces in the presence of serum [17], which has significance for the contribution of SR-A to atherogenesis. SR-A might not only bring about the conversion of macrophages into foam cells through endocytic uptake of lipoprotein, but might be responsible for the retention of circulating cells at the site of the developing plaque. SR-A can also mediate adhesion to glucose-modified collagen IV, which could explain the accelerated development of vascular lesions in diabetics [18]. Other populations of macrophages also express SR-A, including activated cells [19], and it is possible that the class of receptor is responsible for retention of macrophages within tissues and at sites of infection, such as in granulomata. Although peritoneal macrophages derived from mice lacking SR-A display delayed adherence and spreading in culture [9], initial investigations have failed to reveal a deficit in macrophage adherence *in vivo* (R. Haworth, N.P. and S.G., unpublished observations). Again, it is very likely that other receptors are involved and it therefore might be necessary to block these receptors in order to reveal the role of SR-A.

Attachment not only brings cells into intimate contact with other cells or the extracellular matrix, but signals changes in the biological properties of the adherent cell. Platelets are activated following CD36-mediated adhesion to collagen [8]. Interactions with ligands for scavenger receptors are known to induce proinflammatory cytokines and growth factor production by macrophages, thereby altering the microenvironment of the vascular lesion to increase the influx of monocytes and promote the proliferation of smooth muscle cells [1].

SR-A-mediated adhesion is believed to initiate a sequence of damaging inflammatory events that contribute to the pathology of Alzheimer's disease. Senile plaques contain both microglia that express SR-A and glycated matrix proteins that are scavenger receptor ligands [20]. El Khoury *et al.* [21] demonstrated that SR-A can mediate the adhesion of microglia to Aβ1-42 amyloid fibrils, which are found in plaques, and this stimulates the production of reactive oxygen species and cytokines that could damage neighbouring neurons. Furthermore, because isolated murine microglia can endocytose Aβ amyloid aggregates via scavenger receptor(s), the disease process might be accentuated through the accumulation of fibrils [22].

Scavenger receptors and host defence

The ability of some scavenger receptors to bind to the microbial surface, combined with their expression on relevant immune cells, suggest that they act as pattern recognition molecules and contribute to the innate immune system [4,5]. This is an apparently ancestral activity because specific scavenger receptors have been identified in hemocytes of simple animals, such as *Drosophila* [23]. SR-A can also recognise lipopolysaccharide, a component of gram-negative bacteria, and bind lipoteichoic acid that is present on gram-positive bacteria [4,5,24]. Evaluation of the SR-A role in host defence has been possible from analyses of SR-A-deficient mice. These mice are more susceptible to *Listeria* infection than are the wild type [9], and isolated macrophages from these animals have a reduced capacity to phagocytose *Escherichia coli* (R. Haworth, N.P. and S.G., unpublished observations). Hypersensitivity of the SR-A knockout mice to endotoxin challenge confirmed that the receptor is involved in lipid clearance and protects against lipopolysaccharide-induced toxic shock [24]. Other potentially hazardous environmental particulates, such as asbestos and silica, can also bind to and be internalised by scavenger receptors [25,26]. Scavenger receptors are therefore important for the functioning of the

innate immune system because of their capacity to recognise 'non-self'. They might also participate in the acquired immune response. SR-AI and SR-AII are expressed on blood-derived dendritic cells (R. Haworth, N.P. and S.G., unpublished observations) and could mediate antigen uptake for subsequent processing and presentation to T and B cells [27]. Maleylation of certain proteins, which converts them to scavenger receptor ligands, enhances their ability to stimulate antibody and T cell responses through increased antigen uptake [28].

Scavenger receptors and the phagocytosis of apoptotic cells

The large number of unwanted host cells that die by apoptosis are rapidly phagocytosed by neighbouring cells (most frequently macrophages) before they lyse in order to prevent tissue damage and inflammation, which is now recognised as a vital and conserved stage in programmed cell death [29]. Identification of receptors on the phagocyte that are able to mediate recognition and engulfment of dying cells has confirmed that many scavenger receptors have this property [30]. For example, antibody blocking of CD36 inhibits the uptake of apoptotic neutrophils by blood-derived human macrophages [31] and transfection of this scavenger receptor into cells can confer the capability to ingest apoptotic cells [32], as can a *Drosophila* homologue, *croquemort*. [33]. Thymic macrophages lacking SR-A show a 50% reduction in the phagocytosis of apoptotic thymocytes *in vitro* [34]. A characteristic feature of cells undergoing apoptosis and aging of erythrocytes is the loss of phospholipid asymmetry and the exposure of phosphatidylserine on the outer leaflet of the plasma membrane [35]. Experimental evidence suggests that exposure of phosphatidylserine triggers recognition and uptake by certain populations of macrophages [36]. Although the identity of the phosphatidylserine receptor on the phagocyte remains unknown, CD36 and SR-BI can bind liposomes containing anionic phospholipids [37]. Evidence for scavenger receptor involvement in cell clearance *in vivo* has yet to be demonstrated, however. A failure to clear apoptotic cells might have implications for health; impaired phagocytosis is now considered to be an important contributor to the development of autoimmune conditions, such as systemic lupus erythematosis [38]. Other modifications of the surface of dying cells, such as oxidation, might generate ligands for scavenger receptors [39]. Scavenger receptors can therefore distinguish 'altered self' from 'self', as well as non-self, which might mean that scavenger receptors represent an evolutionary step in the development of the immune system.

Scavenger receptor classes

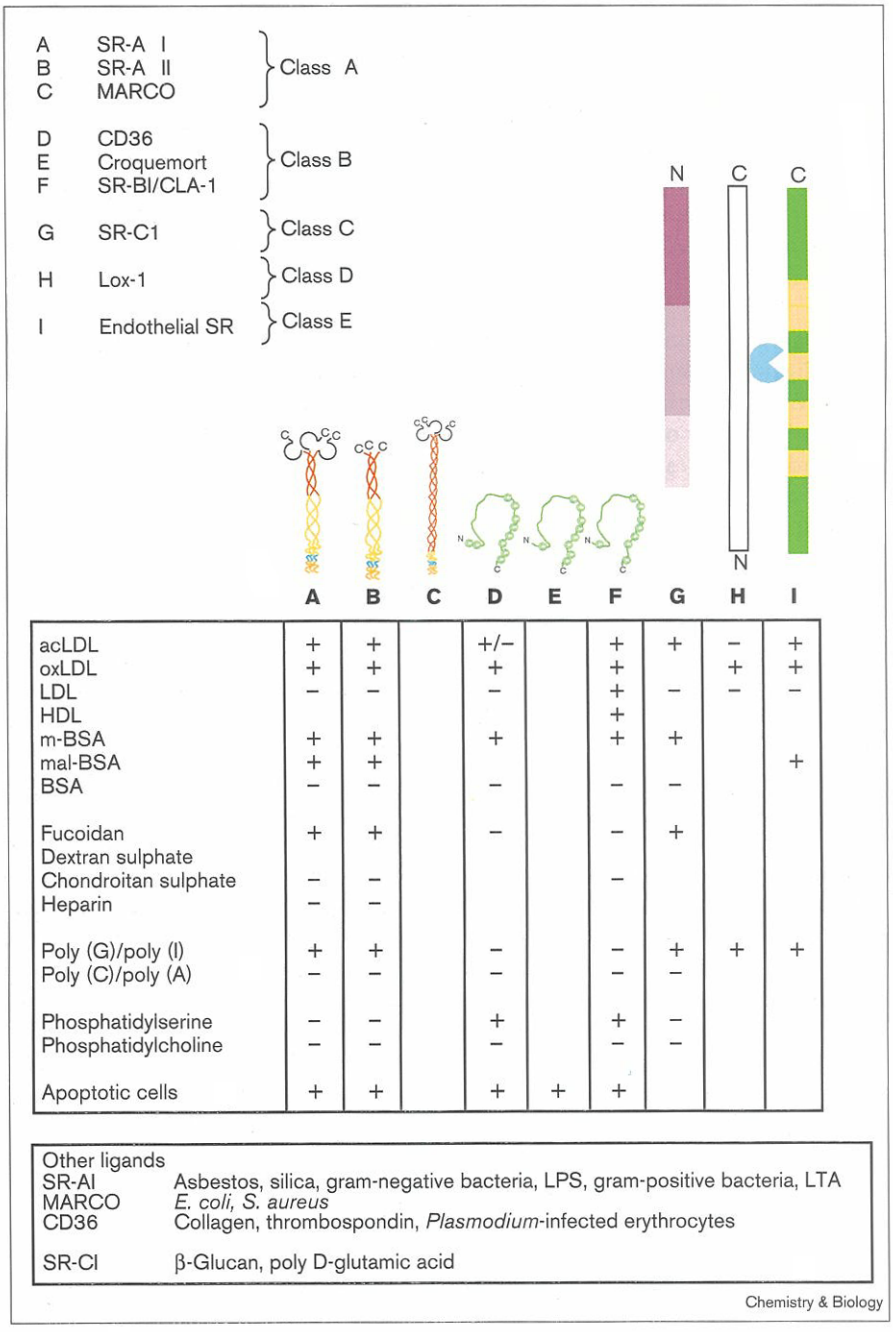
During the past three years, several new genes that encode molecules that can bind modified lipoproteins have been isolated (Figure 1). The ligand-binding properties of these proteins suggest that there are at least five classes of scavenger receptors (Figure 2). There is no sequence motif that is characteristic of all scavenger receptors. Receptors of a particular class may share a specific protein domain; for example, the macrophage type I and II receptors and the molecule MARCO that comprise class A all have a collagenous domain that includes the binding site for acetylated LDL and possible microbial ligands [1,2]. The collectin C1q also has a collagenous domain that displays similar ligand-binding properties to SR-AI, but because C1q cannot bind acetylated LDL it does not qualify as a scavenger receptor [40]. Similarly, CD14 is best known as a physiologically important receptor for lipopolysaccharide (which is a ligand for SR-A) and has also been shown recently to bind to apoptotic B cells [41], but its potential for binding to modified lipoproteins has not yet been reported. SR-AI and MARCO both contain a carboxy-terminal domain, the scavenger receptor cysteine-rich domain, that is found in many proteins which are not scavenger receptors [42]. Whether there is also sequence conservation within all classes of scavenger receptor will require the identification of additional members of those classes that are currently represented by only single species (Figure 1). The most intriguing question that the diversity of scavenger receptors begs is how do apparently dissimilar receptors bind very similar ligands? Clearly data that define the quaternary nature of scavenger receptors, for example, the analysis of the crystallisation of different scavenger receptors combined with the same ligand, will be crucial to resolving this problem.

Scavenger receptors bind a multitude of ligands

Scavenger receptors are defined by their property of binding modified, but not native, LDL (with exception of SR-BI) [43]. Modifications that include oxidation and acetylation convert LDL into a ligand for scavenger receptors and abolish binding to the LDL receptor. Chemical modification of about 15% of the lysine residues of LDL is sufficient to convert it into a ligand for SR-AI [44]. The feature of scavenger receptors that underlies their contribution to multiple biological processes is their broad, high-affinity ligand-binding activities (Figure 2). This property (that of 'one receptor–many ligands') sets them apart from the vast majority of membrane receptors that can only bind a single ligand. They have been described as 'molecular flypaper' [3]. The range of ligands comprises a diverse array of molecules that includes endogenous molecules generated during metabolism, such as chemically modified lipoproteins, nonphysiological compounds derived from the environment, (e.g., asbestos and silica) and molecules that are restricted to prokaryotic cells, such as bacterial lipopolysaccharide [1,3–5]. Although the precise structural requirements for binding to scavenger receptors have not been fully determined (see below), all scavenger receptor ligands are macromolecular and polyanionic, but the latter property alone is insufficient to confer receptor binding,

Figure 2

Scavenger receptors ligands. The ligand-binding profiles of members of the five classes of scavenger receptors are shown. (+) Indicates the ability of the receptor to recognise the molecule, either by direct binding or by the ability to inhibit modified lipoprotein endocytosis. (–) Indicates when the molecule is unable to bind or compete. A space indicates the combination has not been reported. The binding of acetylated LDL to CD36 is controversial and might be dependent on species and cell. LDL, low density lipoprotein; acLDL, acetylated low density lipoprotein; oxLDL, oxidised low density lipoprotein; HDL, high density lipoprotein; poly(G), polyguanylic acid; poly(I), polyinosinic acid; poly(C), polycytidylic acid; poly(A), polyadenylic acid; m-BSA, maleylated bovine serum albumin; mal-BSA, malondialdehyde bovine serum albumin; LPS, lipopoylsaccharide; LTA, lipoteichoic acid; *E. coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*.



because many polyanionic molecules are not ligands. It is important to stress that although these polyanionic ligands can be used to identify scavenger receptors as a group, typically they cannot be used to discriminate individual members. This can be achieved using more specific probes, such as antibodies [17].

It is unlikely that the list of scavenger receptor ligands in Figure 2 is complete; for example, the endogenous serum ligand required for cation-independent adhesion of macrophages by SR-A is still unidentified [17]. Class A receptors show 'nonreciprocal cross-competition' — that is, although one ligand can compete for binding of a second ligand, the latter cannot compete for binding by the former [1]. This observation can be explained by several potential mechanisms, including the presence of multiple binding sites on single receptors or different conformations of the receptor that have distinct binding properties, and could

have significant bearing on scavenger receptor function in biological situations. Because nearly all ligands for SR-A have been identified through their inhibition of acetylated LDL endocytosis, ligands that bind at sites distinct from that for the lipoprotein would be excluded. An alternative strategy could employ soluble forms of scavenger receptors as an affinity matrix to purify potential ligands. In addition, the way in which potential ligands are presented to the receptor might be important, albeit as a soluble ligand, as high-density ligand on a particle or in the context of the surface of a cell.

Scavenger receptors within a class can also display differential ligand binding. The receptor SR-BI differs significantly from other class B scavenger receptors because it can bind not only oxidised LDL and acetylated LDL but also native LDL and HDL [43]. Expression of mutant receptors might be useful for examining these differences in ligand binding. Truncated receptors could determine the location and extent of the binding site for particular ligand(s). Chimeric receptors created by the exchange of protein domains could be informative about specificity for the binding of multiple ligands.

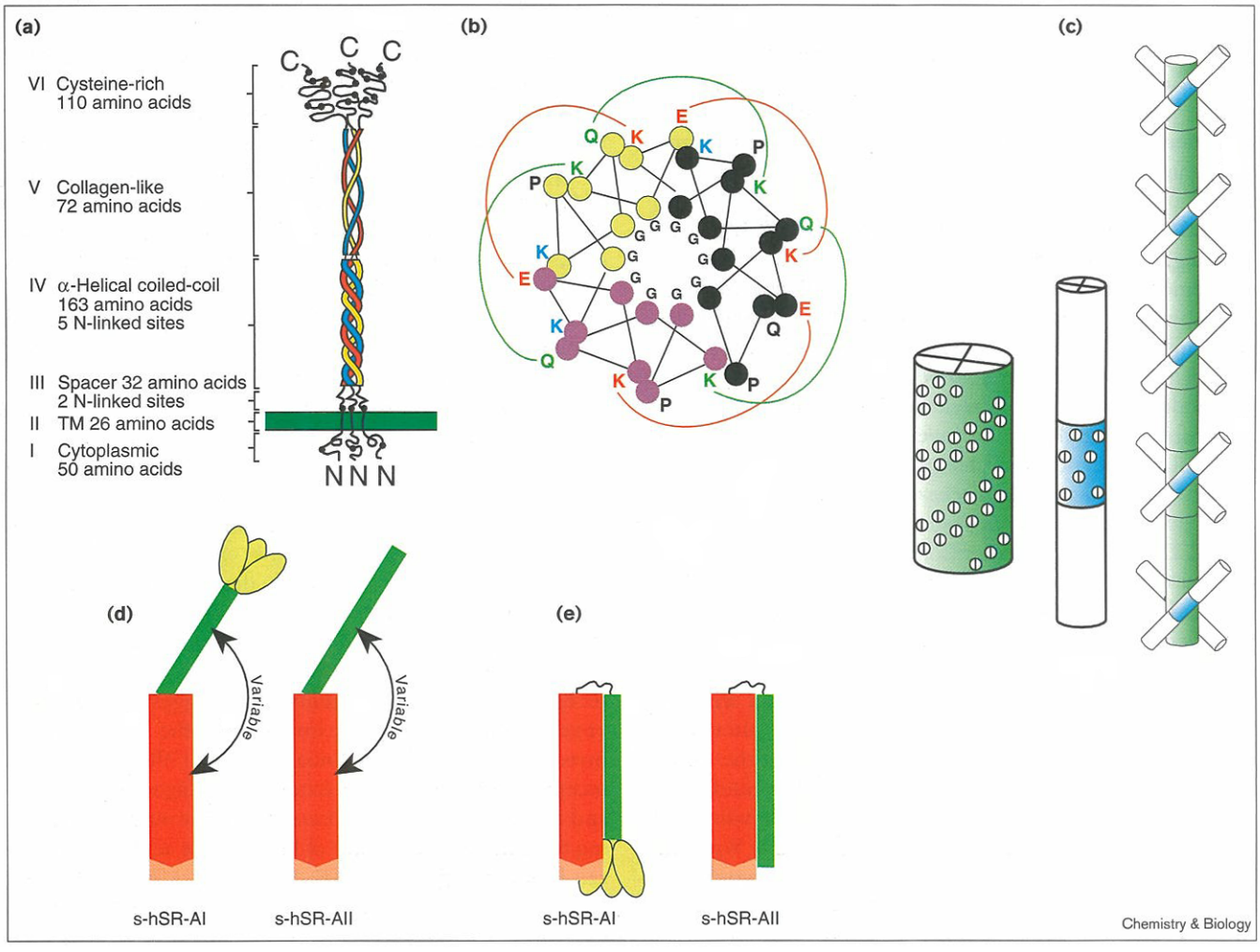
Structure–activity studies of SR-A

Since SR-A types I and II, alternative spliced transcripts from a single gene, were the first scavenger receptors to be cloned and have their binding properties characterised, there have been several reports detailing receptor structure and its relationship to biological activity. As shown in Figure 1, the predicted protein structures for SR-AI and SR-AII are trimeric, multi-domain glycoproteins that differ only by the presence of an additional cysteine-rich domain in the type I molecule. The remainder of the extracellular part of each receptor is identical and is composed of a short spacer domain, an α-helical coiled-coil domain necessary for trimer formation and a collagen-like domain [45]. When this predicted sequence was obtained two questions were raised: which of these extracellular domains is responsible for the binding of ligand and what is the structural basis that determines the unusual specificity? The striking feature of SR-A is the collagenous domain, formed by Gly–X–Y amino acid repeats (where X and Y are any, but different, amino acids), that is highly conserved across species and includes a completely conserved amino acid sequence with repeated lysine residues — Lys–Gly–Gln–Lys–Gly–Glu–Lys–Gly–Ser. Truncation mutagenesis of the receptor reveals that the carboxy-terminal 22 amino acid region of the collagen domain contains the site of acetylated LDL binding [46,47] and mutation of Lys337 in bovine SR-A prevents all acetylated LDL binding. Interestingly, oxidised LDL binding was not completely abolished unless an adjacent lysine residue was also substituted, implying differential binding requirements. The finding that this cluster of positively charged lysine residues is an essential part of the ligand-binding site was not entirely unexpected because all the ligands for SR-A are negatively charged macromolecules (Figure 2).

The demonstration that synthetic trimerised peptides could mimic the core binding site of the bovine receptor has facilitated studies of molecular recognition and binding [48–50]. A triple-stranded 18 amino acid peptide, but not the single-stranded peptide, formed a collagen-like structure and bound acetylated LDL, binding that could be inhibited by maleylated bovine serum albumin [48]. A trimeric peptide in which Ly337 was changed to a neutral charged alanine residue showed no binding activity, as was observed when the same mutation was introduced into the intact receptor. This small synthetic ligand-binding site has been used for biophysical measurements and molecular modelling of the collagen domain. Circular dichroism (CD) spectroscopy measurements confirmed the predicted triple-helical conformation [49] (Figure 3), and its dependence on the strong electrostatic interactions between three Lys–Glu residues (intramolecular ion pairs) and Lys–Gln residues (by hydrogen bonding). Unpaired lysine residues that are at the outer edge of the helical wheel would be available for intermolecular interactions with negatively charged ligands (Figure 3b). The stability of the triple helix was significantly reduced at a $pH < 4.5$ and is likely to be important for the dissociation of ligand from the receptor that occurs in acidic endosomes [50]. A pH-dependent change in the conformation of triple-stranded peptides derived from the carboxyl terminus of the α-helical coiled-coil domain has been described [51], which might also be involved in ligand release.

Although studies of these peptides have demonstrated that the peptides have properties very similar to those of the native receptor, such as the binding of acetylated LDL, there is evidence that they do not have all the discrimination of ligands of the full-length receptor. Pearson *et al.* [52] demonstrated that SR-A expressed in Chinese hamster ovary (CHO) cells can interact with the polyribonucleotides poly(I) and poly(G), but not poly(C) or poly(A). This is because poly(G) and poly(I) form quadruplexes on whose surface the stereospecific distribution of phosphate groups generates a negative charge that is complementary to the lysine cluster of the collagenous domain. Mielewezyk *et al.* [50] tested the interactions between 30-mer trimeric peptide from the ligand-binding site and tetraplex nucleic acids. As expected the peptide bound poly(I) (see Figure 3c for proposed model of binding), but there was no perturbation of the CD spectra with poly(G) or other polyoligonucleotides that are known to bind to the complete receptor, indicating that short peptide models may not be adequate to explain fully all requirements for receptor-ligand interaction. Peptides that mimic binding sites could be useful for selecting small

Figure 3



Organisation of SR-A type I. **(a)** Protein domains (I–VI) of the predicted structure of SR-A type I [45,46]. TM, transmembrane. **(b)** Helical wheel model of a triple-stranded peptide derived from the collagen-like domain that can bind acetylated low-density lipoprotein. The three peptides are indicated by yellow, purple and black spheres. The unpaired lysine residues are blue; lysine–glutamic acid that form hydrogen bonds are red; lysine–glutamine that form ion pairs are green. Single-letter amino-acid code is used; glycine residues are marked as G [49]. **(c)** Model (right) for the binding of polyinosinic acid (left, shaded green) to a triple peptide (centre, shaded blue) derived from the collagen-like domain [50]. Extended **(d)** and compact **(e)** forms of soluble human SR-AI and SR-AII, as seen by rotary shadowing and negative staining [53]. Scavenger receptor cysteine-rich domain (SRCR), yellow; collagen-like domain, green; coiled-coil domain, red; spacer, orange. s-hSR, soluble human scavenger receptor.

molecule agonists or antagonists. Perhaps studies with larger fragments of SR-A would identify sequences outside of the core ligand-binding domain that affect the interaction. Techniques such low angle X-ray scattering could be used to measure changes in conformation and domain organisation of such fragments of receptor complexed with ligand. Nuclear magnetic resonance analysis of a receptor domain and labelled ligand might be an intermediate, yet informative, approach. The definitive data will be obtained when SR-A is crystallised and analysed in the presence of ligand, but it is acknowledged that attempts to produce collagen crystals have so far been unsuccessful.

The other major drawback with the studies of peptide–ligand interactions is the exclusion of the other receptor domains that, although they do not bind ligand directly, might significantly influence the interaction. Resnick *et al.* [53] have produced quantities of soluble SR-AI and SR-AII, sufficient to initiate studies of their covalent structure using electron microscopy. Rotary shadowing and negative staining of the proteins revealed that the two fibrous domains, the α-helical coiled coil and the collagenous triple helix, are joined by a flexible hinge with a variable angle between the two domains (Figure 3d). This extended form is very similar to that predicted from the

primary amino acid sequence of the receptors. Compact forms were frequently seen (Figure 3e), however, in which the collagen domain is bent back on the coiled coil with a hinge angle of 0°. This form was dominant at neutral pH, establishing the occurrence of this structure under physiological conditions and within an integral membrane and, subsequently, determining the functional relevance of the compact form is an exciting prospect.

Structure–activity relationships of SR-A ligands

Although all SR-A ligands are negatively charged macromolecules, this property in itself is not sufficient to determine binding. Because SR-A is involved in atherogenesis, the minimum modification requirements for the endocytosis of LDL have been examined. Dose-dependent chemical modification of lysine residues by oxidation increased lipoprotein degradation by over tenfold [54]. Although removal of the positive charge of the lysine residue is required, the magnitude of charge change is not the only factor involved, because the rate of degradation of succinylated LDL was not different from acetylated LDL, despite a larger net charge, and both were less than that of malondialdehyde-modified lipoprotein [44]. One possibility is that modifications of particular lysine residues on the LDL molecule are more important than others. An LDL peptide rich in modified lysine residues that can interact with the collagenous domain of SR-A might be an alternative approach to solving this problem. It is clear that the oxidation of apoprotein B plays a major role in the binding of oxidised LDL to SR-A, but a recent study showing that the lipid moiety of oxidised LDL can compete with the binding of oxidised red blood cells for mouse macrophages, indicates that this component of the lipoprotein might also be important for the interaction [55].

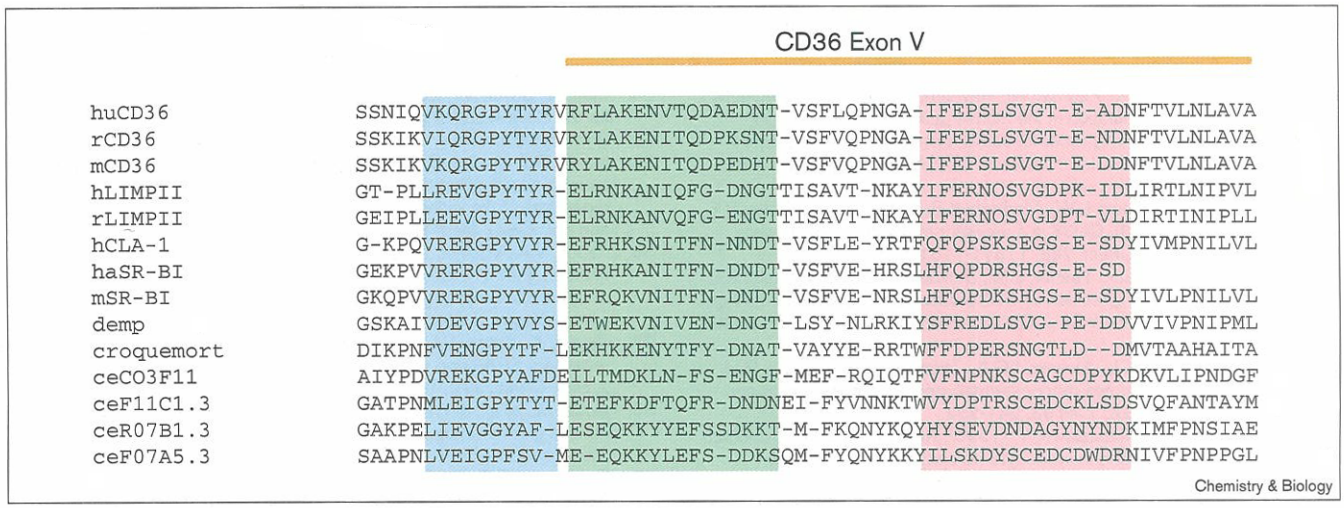
We have stated that all scavenger receptor ligands are negatively charged polyanions, but not all polyanions can bind these receptors, presumably because of specific requirements that are currently not well understood. It is very likely that particular stereo organisation and density of charge are essential. Design and manipulation of model ligands has, and will be, of use, but it would, perhaps, be more interesting to define physiologically relevant ligands. For example, although the adhesion of microglia to surfaces coated with fibrous Aβ1-42 amyloid peptide could be inhibited by poly(I) [21], it is unclear if this molecule is a polyanion and whether it is the form of the protein that is important *in vivo*.

Identifying scavenger receptor ligands on microorganisms is important for defining the role of the receptors in host defence. It would help to identify those pathogens with which appropriate immune cells bearing scavenger receptors might be able to interact. SR-A can bind gram-positive bacteria, possibly through recognition of lipoteichoic acid [56]. Gram-positive bacteria can essentially be divided into two subgroups: those in which lipoteichoic acid is the major macroamphiphile anchored to the cytoplasmic membrane and those in which lipoteichoic acid is largely replaced by lipoglycans [57]. Greenberg *et al.* [58] have investigated a series of lipoteichoic acid and lipoglycan species for their ability to compete with poly(G) for the binding of type I bovine SR-A. Many, but not all, lipoteichoic acids were able to bind, the strength of binding being dependent upon the size of the negative charge of the sidechain phosphate groups. The classical lipoteichoic acid, from *Staphyloccus aureus*, showed the highest affinity binding, which was increased when the molecule was made even more negatively charged. Lipoteichoic acid from *Enterococcus faecalis* and *Listeria monocytogenes*, amongst others, also bound to SR-A, but those of *Streptococcus pneumonia* and *Clostridium innocuum*, which have lipoteichoic acid substituted with positive sugar charges (the Forssman antigen), failed to bind. The lipoglycan of *Bifidobacterium bifidium* bound as effectively as some lipoteichoic acid species, whereas that of *Micrococcus luteus* did not, even though it has comparable negative charge. It would seem that the distribution as well as the density of charge is critical. The latter observation might be of some significance, the lipoglycans of *Mycobacterium tuberculosis* and *M. leprae* have similar sidechain ester substitutions, yet preliminary receptor-blocking experiments have suggested that scavenger receptors might be involved in the interaction of mycobacteria and human macrophages [59]. The class A receptor MARCO has an extended collagen-like domain and can bind *E. coli* and *S. aureus* [60]; it would be interesting to compare the breadth of lipoteichoic acid and lipoglycan binding with that of SR-AI and SR-AII. That certain lipoteichoic acid species and lipoglycans are SR-A ligands suggests that soluble receptor or small molecules that mimic the collagenous domain might provide effective protection against septic shock.

Structure–activity relationships of CD36

Human CD36 has been shown to be an adhesion receptor for thrombospondin, type I and IV collagen and for malaria-infected erythrocytes [8], and be involved in the endocytosis of long-chain fatty acids, oxidised lipoproteins and anionic phospholipids [37] and the phagocytosis of apoptotic cells [32]. A series of monoclonal antibodies and peptides has been used to investigate whether these diverse ligands interact with the same region of the receptor or whether there are multiple sites. The sites of receptor–ligand binding were investigated by testing the ability of the antibodies to block binding and adhesion [61–63]. In summary, a single domain that extends from amino acids 155–183 mediates the interactions with thrombospondin, oxidised LDL and apoptotic neutrophils. Chimeric receptors, created by swopping regions between human and murine CD36 and receptor-derived peptides confirmed the antibody results, but revealed species specificity. For both oxidised LDL endocytosis and

Figure 4



Sequence homologies that identify the CLESH-1 (CD36, LIMPII, *emp*, SR-BI Homology sequence 1) motif amongst CD36-related proteins. hu, human; r, rat; m, mouse; ha, hamster; d, *Drosophila*; ce, *C. elegans*. Regions of homology are shaded in blue, green and pink. Redrawn from [64].

human neutrophil ingestion, replacement of human 155–183 with the murine counterpart (which differs only by two amino acids) significantly reduced both lipoprotein binding and cell phagocytosis [62,63]. The binding of thrombospodin to CD36 has been shown to be a stepwise, conformation-dependent event and this might be true for the other ligands. Crombie and Silverstein [64] identified a region in the mammalian molecule lysosomal integral membrane protein II (LIMPII) that is structurally related to the 155–183 binding domain of CD36, which also bound thrombospondin. Using the consensus sequence between these two molecules for database searching, they found 14 proteins related to CD36 that contain the CLESH-1 (CD36, LIMPII, *emp*, SR-BI homology sequence 1) motif (Figure 4). This offers the prospect to study the structure–properties of this domain across species and investigate its evolution.

Concluding remarks

Studies of scavenger receptors essentially began with investigations of a clinical pathology. An ongoing period of receptor isolation, determination of structure and properties has followed, providing the opportunity to exploit these approaches, not only for beneficial therapy for health conditions in which scavenger receptors are involved, but also to understand their many biological contributions. Several areas will require further examination, however. Our understanding of the molecular criteria that define a scavenger receptor ligand remains inadequate and we certainly cannot predict which molecules will or will not bind. The nature and extent of the chemical alterations that induce ligand formation and whether proteins or lipids are of more or equal importance need to be more fully explored. It is likely that in the complex situations in which scavenger receptor–ligand interactions occur (such as on the surface of apoptotic cells or the extracellular matrix), there will be a number of ligands that might sequentially bind to allow for a more complex biological outcome. Finally, which characteristics of ligand–receptor interactions mediate different responses of scavenger receptor positive cells, be they adhesion, endocytosis or phagocytosis, awaits investigation.

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