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# Conserved domains of the class A scavenger receptors: evolution and function

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Summary: The class A scavenger receptors are phagocytic pattern recognition receptors that are well represented in vertebrate genomes. The high level of conservation among vertebrates implies that this is an evolutionarily conserved family of receptors and indicates the presence of a common ancestral gene. The identity of this ancestral gene is not clear, as it appears that many of the domains of the scavenger receptors (e.g. collagenous, scavenger receptor cysteine rich) originated early in evolutionary history and are found in many combinations, often in genes of unknown function. These early receptors may function in cell-cell recognition, aggregation, or lipid recognition, and their involvement in pattern recognition, phagocytosis, and homeostasis may have been adaptations of such conserved patterns. Herein, we reclassify the class A scavenger receptors based on recent discoveries of new members of this family, describe the evolution of the various domains of the class A scavenger receptors, and discuss the appearance and function of these domains through evolutionary history.

Keywords: monocytes/macrophages, Toll-like receptors/pattern recognition receptors, comparative immunology/evolution, phagocytosis

#### Introduction

Since the introduction of the concept of pattern recognition by Janeway and Medzhitov (1), much has been learned concerning innate immune receptors and their role in cell activation, host defense, and induction of the adaptive immune response. The receptors are germ-line encoded, diverse in their structure and ligand recognition, and include a range of non-opsonic molecules, especially various scavenger receptors. The scavenger receptor family consists of structurally unrelated integral membrane molecules, with a common clearance function for endogenous modified host molecules or apoptotic cells, as well as exogenous microbial and foreign body particulates. Many of their ligands are polyanionic, although not all polyanions are ligands. These receptors vary in their expression by different myeloid cells of vertebrates, especially antigen-presenting cells (APCs), and in their regulation during differentiation and cell activation.

Macrophages and related professional migratory phagocytes appeared early in evolution and play a role in tissue remodeling and repair in invertebrates, as well as in host defense against infection. The evolution of the Toll-like receptors (TLRs) (2), the immunoglobulin (Ig) superfamily (3), major histocompatibility complex (MHC) family (4), and of complement proteins (5) has been well reviewed, but the scavenger receptor subclasses have not received comparable attention. Genomic and bio-informatic tools have begun to make it possible to assess the phylogeny of structurally related subclasses such as the class A subclass of scavenger receptors, to obtain a new perspective on their evolution.

#### Definition of the class A scavenger receptors

The macrophage scavenger receptors were initially discovered due to their ability to bind and take up selected polyanions, such as modified low-density lipoprotein (LDL) (e.g. acetylated or oxidized LDL). The ability to bind polyanions appears to be a widely conserved feature and, as a consequence, there are now eight classes of scavenger receptors (called classes A–H), with little structural and weak functional homology (reviewed in 6). Indeed this definition appears to be sufficiently flexible to include a broad range of receptors that, although able to bind modified LDL, do not appear to do this under physiological conditions. The presence of scavenger receptors is most often associated with phagocytosis and clearance of exogenous or modified endogenous ligands, although in some cases they are also believed to be involved in cell–cell recognition (7). Scavenger receptors have been identified in many classes of life. In mammals, scavenger receptors are expressed on several cell types including endothelial cells, B cells, monocytes, and, most significantly, on macrophages. In invertebrates, scavenger receptors are also expressed on specialized phagocytic cells. In single-cell organisms there is some evidence for expression on the plasma membrane, but very few functional studies have been performed.

This review will focus on the class A scavenger receptors. The original two members, scavenger receptor class A (SRA) and macrophage receptor with collagenous structure (MARCO) have the ability to bind to modified LDL and/or representative polyanions and thus are easily classified as scavenger receptors as defined by Brown and Goldstein (8). These receptors bind a range of overlapping but also distinct negatively charged molecules [e.g. fucoidin, dextran sulfate, and various endogenous and exogenous ligands (8, reviewed in 9, 10)]. Both MARCO and SRA are type II glycoproteins that are expressed on the plasma membrane and contain a scavenger receptor cysteine-rich domain (SRCR) domain and a collagenous domain (Fig. 1). There is, however, an additional, newly described member of the class A family, SCARA5, that has high sequence homology and a similar structure to MARCO and SRA, but does not bind modified LDL (11). Likewise, scavenger receptor with C-type lectin type I (SRCL) has high homology to MARCO and SRA but does not contain an SRCR domain (Fig. 1) and has not been shown to bind to modified LDL. Although homology indicates that SCARA5 and SRCL are clearly related to the class A scavenger receptors, it is justified to divide these receptors into subclasses



**Fig. 1. Mammalian class A scavenger receptors.** The class A scavenger receptors can be divided into two groups. Originally these were defined by the presence of a scavenger receptor cysteine-rich domain (SRCR) and the ability to bind to modified lipoproteins; however, homology searches led to the discovery of other proteins with high similarity. These may or may not contain the SRCR domain and instead are highly similar in the collagenous or coiled-coil domains. For example, SRCLI/II does not possess an SRCR domain, but rather a C-type carbohydrate recognition domain.

depending on domain composition. For example, MARCO, SRA, and SCARA5 could be designated class A SR-SRCR and SRCL could be designated class A SR-carbohydrate recognition domain (CRD).

For the purpose of this review, we propose that the definition of the class A scavenger receptors be expanded to include receptors expressed on the plasma membrane, that contain SRCR and collagenous domains, that are predicted to be phagocytic, and that recognize negatively charged and other ligands with lipid moieties such as lipoproteins and lipopolysaccharides. This definition may be flawed, as it infers much about function from studies in mammals, which may or may not be accurate across all species; preliminary studies indicate that there are indeed receptors that fit this description across many classes of life.

#### SRA

SRA I/II molecules are expressed mainly on macrophages and are involved in homeostatic functions such as lipid metabolism as well as recognition and clearance of modified host components, apoptotic cells, and pathogens (12-16). The SRAI/II isoforms (Fig. 1), which are usually co-expressed, respectively, contain an SRCR domain or not, but no functional difference has yet been described between them; a third isoform does not reach the cell surface and has no known function other than possibly as a dominant negative receptor (17). Artificial ligands for SRAI/II, shared for the most part with other SR, include acetylated LDL, oxidized LDL, AGE-modified and maleylated bovine serum albumin. Naturally occurring ligands include lipoteichoic acid (LTA) and the lipid A component of lipopolysaccharide (LPS). Selected bacterial protein ligands on Neisseria meningitidis have recently been identified (18), and it is likely that other bacteria bear uncharacterized protein ligands on their surface. The apoptotic cell ligands for SRA have not yet been identified but interact with a range of scavenger and other receptors in a complex fashion.

Most macrophage populations in tissues express SRAI/II, including cells in the outer marginal zone of mouse spleen. Macrophage colony-stimulating factor (CSF-I) is a potent inducer of SRAI/II in vitro and in vivo (19). Macrophages in atherosclerotic lesions and associated with tumor-stroma express this SR, perhaps due to local CSF-1 production, and the SRA may contribute to pathogenesis in these situations. Strikingly, polymorphonuclear leukocytes as well as circulating monocytes do not express SRAI/II. Its role in myeloid dendritic cells has not been studied in detail (20), but SRAI/II may contribute to immune peripheral tolerance. Selected sinusoidal endothelial cells, e.g. in liver, also express SRAI/II and contribute to their highly efficient endocytic clearance function. SRA-deficient mice are more susceptible to bacterial challenge, in part because of an enhanced pro-inflammatory cytokine response to LPS challenge, especially after priming by bacille Calmette–Guérin (BCG) and interferon- $\gamma$  (14).

#### MARCO

The structure of MARCO is similar to that of SRA (Fig. 1). The difference lies in the size of the collagenous structure and the absence of the alpha-helical coiled-coil domain (Fig. 1). The SRCR domain of MARCO and SRA differs in the distribution of cysteine residues (21). Unlike SRA, which binds to its ligands via the collagenous domain, MARCO requires the SRCR domain for ligand binding (21). MARCO is expressed constitutively on subsets of macrophages and is upregulated in response to TLR agonists and whole bacteria (22) but not by pro-inflammatory cytokines (23). In vivo expression increases on macrophages in response to infection or inflammatory conditions (21, 23-27), and, interestingly, this occurs on macrophages that are directly responsive to the stimuli as well as those distal to the initial infectious stimuli. Increased expression of MARCO may alter the function of MARCO-expressing macrophages by increasing bacterial binding and phagocytic capacity and by altering cytokine production (9). The induction of scavenger receptors by TLR stimuli contributes to increased phagocytic efficiency in terms of the percentage of macrophages engulfing bacteria and the number of bacteria engulfed by individual macrophages (28).

#### SCARA5

SCARA5 is expressed on the plasma membrane of selected epithelial cells, especially populations lining the testis, trachea, lung, bladder, and small intestine. It binds Gram negative (e.g. Escherichia coli) and Gram-positive bacteria (e.g. Staphylococcus aureus) and this binding is inhibited by maleylated BSA, polyG, polyI, but not polyC. It does not, however, endocytose oxLDL or acLDL, possibly because of sequence divergence from SRA in the collagenous domain (29). Sequence identity with the closely related SRA is the highest in the SRCR domain (59%) and in the collagenous domain (51%), and overall is 34% (11).

#### SRCL

SRCL is a membrane expressed scavenger receptor found on the surface of endothelial cells but not on the surface of macrophages. This receptor binds to both Gram-positive and Gram-negative organisms, but it is not clear if it binds to modified lipoproteins. The structure is homologous to SRA in both the coiled-coil regions and the collagenous region, but SRCL does not contain an SRCR domain (Fig. 1), instead containing a C-terminal C-type lectin/CRD (30, 31). The role of this receptor is not known.

#### Structural features of the class A scavenger receptors

#### SRCR domain

The SRA SRCR domain is the best characterized SRCR domain, although its function remains cryptic. It lends itself to bioinformatic analysis because of its conserved pattern of cysteine residues. Disulfide bond patterns are well conserved among protein domains because of their ability to form stable structures that are resistant to various biochemical and enzymatic stresses.

Although the SRCR domain is highly conserved, there are two distinct variants. The group A SRCRs have three disulfide bridges, whereas those of group B have an additional disulfide bridge (i.e. four in total). While the group A SRCRs are encoded by no fewer than two exons, the group B SRCRs are encoded by a single exon, raising the possibility that these members may have evolved from separate ancestral genes.

The crystal structure of the group A SRCR domain of M2BP (Mac-2-binding protein) was solved in 1997 (32). This domain can be used as a template for structures of other SRCR domains, as it has a fairly high sequence identity to the distantly related group B members (e.g. CD5 and CD6) and is more closely related to the class A members (approximately 50% sequence identity). The position of the conserved cysteine residues is well preserved among group A and B members. The structure revealed that the protein consists of three  $\beta$ -strands (labeled A–C), followed by an  $\alpha$ -helix and three additional  $\beta$  sheets (labeled D–F) (32). The structure also confirmed the previous observation that the pattern of cysteine bonds was Cys2–Cys7, Cys3–Cys8, Cys5–6, and in the case of the group B SRCRs, the additional bond was likely to be due to binding at Cys1–4 (33) (Fig. 1).

The crystal structure of the group B SRCR domain of CD5 indicates that most of the major elements of the structure are conserved with variation occurring at the loops between the  $\beta$  sheets (34). Despite the apparent structural homology, ligand binding is not conserved between the two SRCR groups or even between closely related members. The structure of the SRCR domain is conserved across multiple species by maintaining the conserved residues (cysteines) required to maintain protein folds, while other highly variable regions are

associated with ligand binding. Thus, although the structure may be loosely conserved, ligand specificity is not.

Despite the fact that the SRCR domain is the most highly conserved of the scavenger receptor domains, there is no consensus on its function. Putative functions for the SRCR domain include binding cell-associated ligands, exogenous ligands (as is the case in MARCO, which uses this domain to bind bacterial ligands, but not SRA, which utilizes its collagenous domains), and homo- or heterotypic interactions between cells. In the scavenger receptors, SRCR domains are often found in tandem repeats, similar to domains such as the Ig and epidermal growth factor domains. Remarkably, the CD163 antigen, a steroid-induced receptor for haptoglobin–hemoglobin complexes, consists almost entirely of multiple SRCR domains in its extracellular ligand-binding domain (35, 36).

#### $\alpha$ -Helical coiled-coil domain

The  $\alpha$ -helical coiled-coil domain is not a conserved feature of scavenger receptors in general but is found in SRAI/II/III and SRCL and is highly homologous between these two receptors. The  $\alpha$ -helical coiled-coil domain may add flexibility to surface expressed receptors. Negative staining and rotary metal-shadowed electron microscopy have demonstrated that there may be a 'hinge' region at the interface between the  $\alpha$ -helical coiled-coil and collagenous domains. Consequently, the surface expressed receptor is expected to have a 'jackknife' configuration, folding in on itself at this hinge (33). How this structure affects or contributes to the ligand binding, which is believed to be mediated through the collagenous domain, is not clear. This region may also be implicated in adhesion. A monoclonal antibody (2F8) towards the  $\alpha$ -helical coil-coil domain of mouse SRAI/II blocks macrophage adhesion to serum-coated tissue culture plastic (37, 38). This antibody has also been implicated in blocking acLDL binding and collagenmediated adherence, although these are not believed to be due to the involvement of the coil-coil domain per se but rather to alterations in the structure of the molecule, specifically the collagenous domain (38, 39).

#### Collagenous domain

The collagenous domain of SRAL/II has been implicated in binding to a number of ligands including denatured type I, III collagen, native type IV collagen (40), modified lipoproteins, and bacterial ligands. The exact region of this domain required for ligand binding is not clear, although multiple studies have demonstrated that charged residues are important. Subsequent studies have demonstrated that the residues required for binding are found throughout the collagenous domain (41) and are not limited to the lysine cluster at the C-terminus, as proposed originally (29). This domain is easily identified by bioinformatic analysis because of the Gly– Xaa–Yaa repeats, where Xaa is most often proline or lysine and Yaa is most often proline or lysine and less often arginine (42).

#### Cytoplasmic domain

The scavenger receptors are phagocytic receptors; however, it is not clear how they initiate phagocytosis. Presumably, activation of signaling pathways occurs either through recruitment of adapter proteins to the cytoplasmic tail or by activation of signaling due to protein-protein interactions between signaling effector molecules and the cytoplasmic tail. These putative interactions have been difficult to demonstrate. The best studied example is SRA, which, like the other class A scavenger receptors, has a very small cytoplasmic domain (between 40 and 55 amino acids, species dependent). Motifs within the cytoplasmic tail are associated with membrane trafficking and recycling, uptake of modified LDL, and adhesion (43-45). These are not conserved between even the closely related MARCO and other class A scavenger receptors. Although scavenger receptors from other species have been demonstrated to have short cytoplasmic tails (46), no function has been attributed to them.

#### The class A scavenger receptors as phagocytic receptors

Phagocytosis is an ancient function. In single-cell organisms, phagocytosis is required for ingestion of particulate matter as a source of food. Indeed, many of the cellular processes required for nutrient breakdown (e.g. proteolytic enzymes) are easily adapted to the destruction of pathogens. Macro-phage-like cells are evolutionarily ancient; single-cell organisms such as amoebae resemble macrophages closely in their ability to ingest foreign objects, to form tight, membrane-bound phagosomes, and to destroy or digest their contents.

In multicellular organisms, specialized phagocytic cells express specialized receptors to detect and ingest non-self molecules; however, many 'non-professional' phagocytes express a limited repertoire of phagocytic receptors and are capable of selective endocytosis. Phylogenetic analysis indicates that there are at least three evolutionarily ancient classes of phagocytic receptors: opsonic Fc and complement receptors and non-opsonic lectins and scavenger receptors. Of these three pathways, the Fc receptor pathway is the most evolutionarily recent, evolving in conjunction with the adaptive immune response. Although these receptors are best characterized in mammals, specifically in humans and mice, primitive orthologs are predicted to have arisen in jawed vertebrates (47).

A more ancient phagocytic pathway is mediated by lectin recognition. This pathway is characterized by the presence of lectin-like recognition molecules that bind and activate serine proteases [e.g. mannose-binding lectin (MBL)-associated serine protease (MASP)]. In vertebrates, the initial recognition molecules include ficolins and MBLs, which have a characteristic collagen-like domain and a carbohydrate domain which binds GlcNAc (48). Homologs of ficolin and MBL have been identified in urochordates and in amphioxus (49-51), and MASP activates the complement orthologs C4 and C2 in the absence of Igs (reviewed in 52). It appears that the transition between the exclusive use of the lectin pathway in urochordates and the addition of the classical complement pathway in the jawed vertebrates occurs in the jawless fishes (e.g. lamprey). An ortholog of the mammalian C1q has been discovered in the lamprey and functions as a lectin, although a cellular receptor for this has not been identified (53). Because the lectin pathway appears in the Urochordata (sea squirts), orthologs of the complement recognition molecules appear in the jawless vertebrates, and complement appears in conjunction with Igs in the jawed vertebrates, it appears likely that the lectin pathway is a precursor from which the complement pathway evolved.

The major difference between scavenger and Fc or complement receptors is that the scavenger receptors do not require opsonins for binding and recognition. Whether this observation is evidence that they are more primitive receptors is not clear. It is also difficult to determine how the phagocytic function of the scavenger receptors developed early in evolutionary history. Although certain well-characterized domains for the scavenger receptors (e.g. the SRCR domain) are found in virtually all classes of life, there is little evidence to suggest that they are expressed on the plasma membrane and even less to suggest that they function as phagocytic receptors in species other than the jawed vertebrates. Indeed, the role of the scavenger receptors in species such as the sea urchin has been suggested to be cell–cell adhesion and contact, rather than pattern recognition and homeostasis, as in mammals.

#### The class A scavenger receptors in cell-cell recognition

The scavenger receptors are generally thought to be macrophage receptors, whose primary role is uptake of modified endogenous and exogenous ligands; however, the expression of SCARA5 and SRCL on non-professional phagocytes, such as endothelial cells, indicates that they may also have a role in pathogen adherence or in detection at mucosal surfaces.

Both MARCO and SRA have been implicated in cell-cell recognition, and this may reflect an ancient function of scavenger receptors. It has been proposed that the earliest pattern recognition receptors evolved to distinguish 'self' from 'non-self' and that, in a single-cell world, self would have included other members of the same species. As organisms became multicellular this self-versus-non-self concept may have expanded to include cells of the same type (i.e. tissues). In sea sponges, proteins containing SRCR domains are associated with aggregation and association of similar cells (37, 46). This has been proposed to be a function of the SRCR in other classes of scavenger receptors (7). In mammals, the class A scavenger receptors are associated with heterotypic interactions between cells. For example, MARCO is associated with macrophage-B-cell interactions in the spleen (54), presumably by binding an unidentified B-cell ligand. SRA is associated with heterotypic interactions between dendritic cells or macrophages and malignant cells (55). It is not known which domain of the class A scavenger receptors mediates this function.

#### Evolution of SRA/MSR1 in eutherian species

SRA homologs have been identified in virtually all placental mammals (Eutheria). Although in some genomes that have not been fully covered the sequences are not complete (e.g. rabbit, cat, and others), sufficient coverage exists in databases to retrieve partial sequences. Fig. 2A illustrates the alignment of all available complete eutherian sequences. The fact that all placental mammals appear to have a SRA homolog and that this homolog is highly conserved implies that the ancestral gene existed before divergence of this family. No SRA/MSR1 homologs have been identified in more divergent genomes, although this is most likely because of incomplete coverage rather than an absence of the gene. The evolution of SRA appears to occur at a fairly consistent rate with minimal divergence in related species (e.g. primates) (Fig. 2B). Overall, SRA is highly conserved at the protein level, although sequence similarity ranges from 0% in the cytoplasmic domain to 80% in the collagenous domain (Fig. 2C). These numbers are misleading, however, as the annotated gene for some species (e.g. macaque) do not appear to have a cytoplasmic tail, whereas others (e.g. horse) do not appear to contain an SRCR domain or have a truncated version. When these outliers are removed, the sequence identity/similarity of the cytoplasmic region increases to 14%/30% in placental mammals and 8%/16% in all animals. The sequence identity/similarity of the SRCR domain is still relatively low at 7%/14% in placental mammals and 4%/6% in all species. As there are multiple isoforms of SRA in mammals (17, 56), which do not all contain the SRCR domain, it is not surprising that other species may also have lost or reduced this component of the receptor.

#### Evolution of MARCO/MARCO in eutherian species

It has been proposed that MSR1 is the ancestral gene for MARCO and SRCL, which are paralogs that arose by gene duplication (57). There is no convincing evidence that MSR1 is any more ancient than other class A scavenger receptors. Indeed orthologs of MARCO are found in more diverse genomes (e.g. chicken and zebrafish) in which there are no annotated orthologs of SRA and the SRCR domain of MARCO is conserved amongst many lower invertebrates [e.g. sea urchin (58)]. Until sequence data are available from a broader range of genomes, there are insufficient data to conclude which class A scavenger receptor arose first.

MARCO homologs exist in all placental mammals (Fig. 3A). The evolution of MARCO follows a similar trend to that of SRA, with little divergence in primates (Fig. 3B). Interestingly, areas of strong sequence identity between homologs of MARCO include the collagenous domains and also the SRCR domain (Fig. 2C), a region of significant divergence in SRA (Fig. 3C). One intriguing possibility for this difference may be that the SRCR domain of MARCO is the bacterial binding region (21), whereas this region appears to be dispensable for bacterial or modified LDL binding in SRA. Data in mouse models of infection imply that MARCO is primarily involved in host defense against infectious agents, whereas the role of SRA may be mainly in homeostasis. It would be of interest to determine if MARCO was involved in host defense against infectious disease in model species such as zebrafish.

It has been proposed recently that regulatory regions of genes may be more important in the evolution of differences among species than rare changes in coding regions. Changes and evolution in regulatory regions can be difficult to study, because sequences that define regulatory regions are less well understood than those that encode functional proteins. Nevertheless, regulatory regions in promoter sequences tend to have a higher level of conservation than surrounding DNA sequences. Regulation of MARCO has not been well studied, although it is clear that expression is clearly different than that of MSR1. Bioinformatic analysis of the promoter region of MARCO indicates that there are a number of potential



**Fig. 2. Scavenger receptor class A (SRA).** (A) CLUSTALW alignment of SRA protein sequences of placental mammals (Eutheria). Sequences of 10 eutherian species are ordered on the basis of similarity. Amino acids are colored based on their properties: hydrophobic (red), acidic (blue), basic (magenta), hydroxyl, amine, and basic (green). An asterisk (\*) indicates an amino acid that is conserved in all sequences, a colon (:) indicates a conserved substitution between amino acids with similar properties (i.e. within the same color scheme described above), and period (.) indicates the presence of a semi-conserved substitution. SRA has the highest degree of conservation within the collagenous domain and exhibits significant diversity in the cytoplasmic region. The scavenger receptor cysteine-rich (SRCR) domain is highly conserved within a subset of species (e.g. primates), whereas others (e.g. horse) diverge significantly. (B) CLUSTALW phylogram of SRA. All species with a complete sequence of SRA in the database are included. Branch lengths are proportional to the amount of inferred evolutionary change. As expected, divergence within primate species is relatively recent, whereas the non-eutherian mammals, opossum and platypus, have diverged considerably. (C) Sequence identity and similarity in the SRCR, collagenous, coiled-coil, and cytoplasmic domains of SRA. Sequence identity is defined as the percentage of amino acids that are conserved among all aligned sequences (Eutheria = human, macaque, orangutan, chimpanzee, lemur, dog, cow, horse, pika, mouse. All sequences include opossum, platypus in addition to eutherian sequences). Sequence similarity includes conserved and semi-conserved substitutions. The sequence identity/similarity in the cytoplasmic and SRCR domains is lower than might be expected (see text for details).

transcription factor-binding sites that are associated with inflammation (e.g. NF- $\kappa$ B), the cell cycle, and lipid metabolism (Fig. 4A). Alignments of this region indicate that there are

conserved regions of DNA indicating that elements of this region are evolutionarily important (Fig. 4B). Similar sequence data are not available for the promoter region of MSR1 across

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A Chimpanzee Human	MRNKKILKEDELL-SETQQAAFHQIAMEPFEINVPKPKRRNGVNFSLAVVVIYLILL 56 MRNKKILKEDELL-SETQQAAFHQIAMEPFEINVPKPKRRNGVNFSLAVVVIYLILL 56		Ch	— Maca	que	
Orangutan Macaque TreeShrew	MRNKKILKEDELL-SETQQTAFHQIAMEPFEINGKVRFPNNGIDQKCSLSASEIRKN 56 PKPKRRNGVNFSLAVVVIYLILL 23 MENQBILKEEEFLGSITGQAAFHQTRLPAMESFEINDPKPKKRKGMNCFVVMVAIYLVLL 60	Chimpanzee Human Tree shrew Mouse Lemur				
MouseLemur Cat	MENKEILKEGELLSSAKGPAAFDQTAMESFEISDPKPRKRSGVNFCIAMVVIYLILL 57 MENKEILKEDEFLASTGERETINQTMFPAMETFEINDPKSRKRKAVNCFTVMVAIYLVLL 60		—— Ca	t		
Mouse Horse Dog Cow	MGSKELLKEEDFLGSTEDRADFDQAMFPVMETEINDPVPKKRNGGTFCMAVMAIHLIL 60 MENKEILKEEESLNGPGERAALEPTMFTAMETFEINDPKPKKKYGMSCFLAMVSIYLVLL 60 MENEEILKEKELLASTEDGTALDQTMLSRMETFEVNDPKPKRRNGMNCIMVLVILYLVLL 60 MENEEILKEKELLASTEDGTALDQTMLSRMETFEVNDPKPKRRNGMNCIMVLVILYLVLL 60	4			Horse Mouse	,
Chimpanzee	TAGAGLLVVQVINLQARLRVL-EMYFINDTLAAEDSPSFSLLQSAHPGEHLAQGAWRLQV 115		Futh	peria	All som	1000005
Human Orangutan Macaque TreeShrew	TAGAGLLVVQVINLQARLKVL-EMYFLNDTLAAEDSLSFSILQSAHPGEHLAQGASRLQV 111 AVGWGPVLNLQARLKVL-EMYFLNDTLAAEDSLSFSILQSTHPGEHLAQGASRLQV 111 TAGAGLLVVQVINLQERLRVL-EMYLLNDTLSAEDSLPFSILRSTHSGERLAQASRLQV 82 TAGAGLLVVQVINLQERLQAM-EMYFYNGTLTADDSASFSILQLVSPKAHRALGMPGLQV 119	C Domain V	Sequence Identity 51%	Sequence Similarity 68%	Sequence Identity 35%	Sequence Similarity 60%
MouseLemur Cat Mouse Horse	TVGAGLLLVRVLNLQERLQVVEEMYFPNGTLATEDSFSFSLLQSAQPAAHLPPGAGSGLRI 117 TAGAGLLVVKVLNLQERLWAL-EMHFTNGTLAAEDSPSFSLLQSAHP-HLPGGALGLQV 117 TAGTALLLIQVLNLQEQLQML-EMCCGNGSLAIEDKPFFSLQWAPKTHLVPRAQGLQA 117 TAGAGLLVVKVLNLQKRLWAL-EMQFADETPATGDSPSFFSFQSAHAP-RLAGGAPRLQF 118	cysteine rich [SRCR])				
Dog Cow	TAGAGLLVIQVLNLQERLRVL-EMPYNNET 89 TAGAGLLVIQVLNLQERLRVL-EMPYNNET 89	8				
-1	:.* . ***** <b>:</b> * : ** : :	Domain IV (Co <b>l</b> lagenous)	29%	46%	22%	28%
Chimpanzee Human Orangutan Macaque TreeShrew	LQAQLTWVRVSHEHLLQRVDNFTQNPGMFGIKGEQGAPGLQGHKGAMGMPGA 167 LQAQLTWVRVSHEHLLQRVDNFTQNPGMFGIKGEQGAPGLQGHKGAMGMPGA 163 LQAQLTWVRVSHEHLLQRVDNFTQNPGMFGIKGEQGAPGLQGHKGAMGMPGA 163 LQAQLTWVRVSHEHLLQRVDNFTQNPDLLGFKGEPGVPDLSGQRGAPGMPGA 171	ß				
MouseLemur Cat Mouse Horse	LQAQLSQVRASQAHLLQRVDNFTRSPELFGIKGERGTTGLPGKR-ALGTPGI 168 LQTQITQVRTRQEHLLRKVDNFTRNPELFGIKGERGAPGLPGKS-GEGTSGI 168 LQAQLSWVHTSQEQLRQQFNNLTQNPELFQIKGERGSPGFKGAPGAPGI 166 LQAQLTDVRVRQEHLKQQVDNFTQSPERFQIRGEPGTPAQAHPPQPASP 167	Domain III (Spacer)				
Dog Cow	-QAQLTQVVTKQETLQQRMDNFTQIFQWSLPTEFGLFRENGSGGAACIFQSKGAQGGPRL 148 -QAQLTQVVTKQETLQQRMDNFIQIFQWSLPTEFGLFRENGSGGAACIFQSKGAQGGPRL 148 *:*::*::*:*:*:*:*:*:*:*	Domain II (Transmembrane) Domain I	4%	4%	4%	4%
Chimpanzee	PGPPGPPAEKGAKGAAGRDGATGPPGPOGPPGVKGEAG-LOGPOGAPGKOGATGTPGPOG 226	(Cytoplasmic)	.,.			
Human	PGPPGPPAEKGAKGAMGRDGATGPSGPQGPPGVKGEAG-LQGPQGAPGKQGATGTPGPQG 226					
Orangutan Magague	PGPPGPPAEKGAKGAVGRDGATGPPGPQGPPGVKGEAG-FQGPQGAPGKQGATGTPGPQG 222					
TreeShrew	PGLPGLPTEKGAKGNMGQGGATGLPGPQGPPGTKGEMG-LQGTKGAPGNQGATGAPGPQG 230					
MouseLemur	LGLPG-PAEKGAEGVR-CEGATGPPGPQGPPGIKGAAG-LQGPQGAPGKPGAAGAPGRQG 225					
Cat	PGLPRPPAERGTEGTETRQGATGLPGLQGPPGIKGEAG-LQGPSGTPGKQGTPGTPGRQG 227					
Horse	PGEPGPARERGERGARGENGERGVOGPOGPPGSRGERG-LOGELGAPGROGOGAPGPRG 223 PGFRTPDAEPOGAGPPYRAGTACLPGPKGPPGIKGEAGDLOGLKGAPGKODAVGTPGROG 227					
Dog	PHLPGPLTGYILNSFSFPGPPGPRGPPGIKGEAG-ITGGKGRPGSAGNSGKKKVGD 203					
Cow	PHLPGPLTGYILNSFSFPGPPGPRGPPGIKGEAG-ITGGKGRPGSAGNSGKKKVGD 203 * **** ** * * * * * * * * * *					
Chimpanzee	EKGSKGDGGLIGPKGETGTKGEKGDLGLPGSKGDRGMKGDSGVMGPPGAQGSKGDSGRPG 286					
Human	EKGSKGDGGLIGPKGETGTKGEKGDLGLPGSKGDRGMKGDAGVMGPPGAQGSKGDFGRPG 286					
Orangutan Macague	EKGSKGDGGLIGPKGETGTKGDKGDLGLPGSKGDRGMKGDTGVMGPPGAQGSKGDSGRPG 282 EKGSKGDGGLIGPKGETGTKGDKGDIGLGPGEGPIGGVEGEOWVMGPPGAQGSKGDSGPPG 253					
TreeShrew	EKGSKGAGGLIGPKGEAGTKGDKGDIGLPGGKGDKGVKGDTGIIGPPGTPGSKGDPGKPG 290					
MouseLemur	EKGSKGDGGLVGPKGESGVKGDKGDLGLPGSKGDMGVKGDTGAMGPPGAQGGKGDSGKPG 285					
Cat	EKGSKGDGGLAGLKGEPGAKGDKGDLGLPGSKGDMGMKGDTGVMGPPGAQGSKGDSGKPG 287					
Horse	EKGSKGDGGLTGPKGEHGTKGDKGDLGLPGNKGDMGMKGDTGPMGSPGAQGGKGDAGKPG 285 EKGSKGDGGLTGPKGETGAKGEKGGLGLPGSKGDRGLKGDTGFMGLPGAPGSKGDSGRPG 287					
Dog	PSGARGGPGSGGETAGSEDKGADKFLGFSGRKGDMGMKGDMGPPGARGDKGDSGKPG 260					
Cow	PSGARGGPGSGGETAGSEDKGADKFLGFSGRKGDMGMKGDMGPPGARGDKGDSGKPG 260 .*::* * * ** . :*:.* *::*: :* ********					
Chimpanzee	PPGLAGFPGAKGDQGQ-PGLQGVPGPPGAVGHPGAKGEPGSAGSPGQAGLPGSPGSPGAT 345					
Human	PPGLAGFPGAKGDQQQ-PGLQGVPGPPGAVGHPGAKGEPGSAGSPGRAGLPGSPGSPGAT 345					
orangutan Macague	PFGLAGFPGAKGDQGQ=PGLQGVPGPPGAVGHPGAKGEPGSAGSPGKAGLPGSPGSPGAA 341 SPGLAGFPGAKGDPGO=PGLOGVPGPPGAVGOPGAKGEPGSAGSRGLTGLPGSPGSPGAA 312					
Trooghrow						

Fig. 3. Macrophage receptor with collagenous structure (MARCO). (A) CLUSTALW alignment of MARCO protein sequences of placental mammals (Eutheria). Sequences of 11 eutherian species are ordered on the basis of similarity. Amino acids are colored based on their properties: hydrophobic (red), acidic (blue), basic (magenta), hydroxyl, amine, and basic (green). An asterisk (\*) indicates an amino acid that is conserved in all sequences, a colon (:) indicates a conserved substitution between amino acids with similar properties (i.e. within the same color scheme described above), and period (.) indicates the presence of a semi-conserved substitution. Interestingly, conservation in the scavenger receptor cysteine-rich (SRCR) domain is much higher in MARCO than in scavenger receptor class A (SRA). (B) CLUSTALW phylogram of MARCO. All species with a complete MARCO sequence are included. Branch lengths are proportional to the amount of inferred evolutionary change. (C) Amino acid sequence identity and similarity of the SRCR, collagenous, and cytoplasmic domains of MARCO. Sequence identity is defined as the percentage of amino acids that are conserved among all aligned sequences (Eutheria = chimpanzee, human, orangutan, macaque, tree shrew, mouse lemur, cat, mouse, horse, dog, cow. All sequences include opossum, chicken, and zebrafish in addition to eutherian sequences). Sequence similarity is the highest in the SRCR domain and this is conserved not only in placental mammals but in more divergent species (i.e. chicken, opossum, and zebrafish). Sequences are most divergent in the cytoplasmic domain.

↓<sup>Dog</sup>



**Fig. 4. Promoter sequence analysis of MARCO.** (A) The promoter sequences of macrophage receptor with collagenous structure (MARCO) were analyzed using the database cisRED (76). This database contains conserved sequence motifs identified by genome-scale motif discovery, similarity, and co-expression analysis. Transcription factor-binding sites are generally 6-12 bp long, conserved among many species, and although they generally occur in the promoter region, they can occur as much as 1.5 kb upstream to 200 bp downstream of the transcription start site, within introns or even within coding exons. The promoter region of MARCO contains eight putative transcription factor-binding sites (P < 0.006 to P < 0.001), including those associated with NF-κB and the Ets family of transcription factors. (B) Alignments of the regulatory region of MARCO and high homology between multiple species were determined using evolutionary and sequence pattern extraction through reduced representations (ESPERR). The regulatory regions of human, chimpanzee, macaque, mouse, rat, dog, and cow are aligned and frequencies of short alignment patterns between known regulatory elements and neutral DNA are compared. A score above 0.1 indicates a potentially conserved regulatory element (77, 78).

this range of species, and thus a similar analysis cannot be performed at this time.

## Evidence for the existence of scavenger receptors or SR domains in lower species

In the jawed fishes (teleosts), SRs have been found to be expressed on subsets of non-specific cytotoxic cells (equivalent to natural killer cells), where they have been demonstrated to bind known scavenger receptor ligands including CpG-ODN, polyvinyl sulfate, and dextran sulfate (59). Macrophages derived from the head kidney of the rainbow trout (Oncorhynchus mykiss) phagocytose proteincoated beads in a scavenger receptor-dependent manner. This process was demonstrated by inhibiting phagocytosis with formaldehyde-treated bovine serum albumin, a scavenger receptor ligand. Indeed Fc and complement receptors did not appear to be involved, even when the beads were coated with complement (60). Although the identity of these scavenger receptors has not been identified, their expression on the plasma membrane of phagocytic cells and their specificity for known scavenger receptor ligands makes it highly likely that they are class A scavenger receptors.

In lower species, many proteins express individual domains of the scavenger receptors (e.g. SRCR domains), but insufficient analysis has been performed to determine the homology between these proteins and the class A scavenger receptors. For example, the sea urchin genome encodes approximately 150 genes consisting of one or more SRCR domains. The functions of these genes are not clear, as they do not appear to be regulated as part of the anti-pathogen response (i.e. they are not inducibly expressed in response to infectious stimuli) (61). An interesting and conserved feature of the scavenger receptor genes appears to be regulation by splicing. In mammals, both SRA and SRCL (17) have multiple splice variants of unknown function. In the sea urchin, many of the 150 proteins containing SRCR domains have multiple splice variants (61).

In species that do not have specialized immune cells (e.g. sponges), the scavenger receptors appear to be fairly evenly distributed, and indeed they may have entirely different roles such as adhesion and aggregation. A number of SRCR domain-containing proteins have been found in the sponge Geodia cydonium (46, 58, 62). It has been proposed that sponge SRCR domain-containing proteins may be involved in adhesion and aggregation, mediated through cell-cell interactions. Dissociated sponge cells re-aggregate through a process that requires plasma membrane-associated receptors and cell-cell contact. A receptor implicated in this process contains multiple SRCR domains (group A), a transmembrane domain, short conserved repeats, and a short cytoplasmic tail. This protein has alternative splice forms, although function has not been attributed to them (46). Another protein with an SRCR domain (group B), a fibronectin domain, and a short conserved region has been identified but no function has been attributed to it (62). Because of the presence of the fibronectin domain, it is possible that this protein is associated with adhesion, either to intracellular or extracellular components.

The recently sequenced genome of the unicellular green algae (Chlamydomonas reinhardtii) indicates that it contains a number of genes encoding proteins with either group A SRCR (22) or C-type lectin domains (4), or both (11, 63). As is the case in other lower organisms, many of these proteins consist of multiple repeats of SRCR domains. Interestingly, many of these proteins are not found in other closely related algae or in land plants, diatoms, marine algae, etc., indicating that they are not necessarily conserved in this class of life.

Whether the SRCR domain originated from a single ancestral gene early in evolutionary history or arose from multiple events over the course of millennia is not clear. Although it appears to be an integral component of the genome of lower organisms, such as sea sponges, and of higher organisms, such as mammals, there are organisms in between the evolutionary ladder that do not appear to have genes or proteins containing SRCR domains. For example, Caenorhabditis elegans does not have genes with identifiable SRCR domains, and, although it contains one protein that has homology to the collagenous domain of the class A scavenger receptors (WP:CE30854, J. Ewbank, personal communication), there are insufficient expression and functional data to determine whether this is a membrane expressed receptor with the properties of the scavenger receptors.

### Genetic variation within the class A scavenger receptors - role in health and disease

The focus of this review so far has been the evolution of the scavenger receptors and their domains through evolutionary history. However, access to an ever increasing amount of human genetic data demonstrates that the class A scavenger receptors are under continued selective pressure and that subtle genetic variability alters host responses to many diseases.

In mouse models of infection, MSR1/SRA has been shown to play a role in viral and bacterial infections (64, 65) in addition to models of septic or endotoxin-induced shock (13, 14). It has also been shown to play a role in mouse models of chronic disease including atherosclerosis (64) and Alzheimer's disease (66, 67). In general, murine models use mice that are completely deficient in the expression of the gene, an experimental situation that does not necessarily mimic the conditions found in humans. In humans, MSR1 is a polymorphic gene containing many regions of genetic variability. A scan of the database Ensembl for MSR1 (transcript identifier ENST00000262101) indicates that there are at least eight single-nucleotide polymorphisms (SNPs) that are synonymous in the coding region, five non-synonymous SNPs, one stop/frameshift mutation, two in known splice sites, approximately 50 in regulatory regions such as the 5'- and 3'-untranslated regions of the gene and many more in intronic regions. In addition, rare missense and nonsense mutations have been identified (68). These mutations and polymorphisms and genetic variations have been linked to chronic diseases such as prostate cancer (69). Although the evidence is less strong, genetic variation in humans has also been associated with breast cancer (70), heart disease (71), and chronic obstructive pulmonary disease (72). There have not been similar studies linking genetic variability in MSR1

Table 1. Selected frequencies of single-nucleotide polymorphisms in MSR1

Non-synonymous in coding region (approximately 8, e.g. rs3747531)				Splice site (at least 2, e.g. rs I 3306550)				
Frequencies*	Ancestral	Heterozygote	Variant	Frequencies	Ancestral	Heterozygote	Variant	
CEU (northern European)	0	0.08	0.92	CEU (northern European)		0	0	
HCB (Han Chinese)	0.02	0.38	0.6	HCB (Han Chinese)	0.8	0.2	0	
IPT (Japanese)	0.25	0.5	0.25	IPT (Japanese)	0.9	0.1	0	
YRI (West African)	0	0.14	0.86	YRI (West African)	1	0	0	

\*Populations as defined by the International HapMap Project (79).

to infectious disease. The frequency of these SNPs differs between different populations (Table 1). It has been proposed that evolution and selection of variants in different populations could explain why different populations have an increased or decreased risk of disease. Although insufficient data exist to determine whether genetic variability in MSR1 contributes to well-known differences in resistance/susceptibility among different populations to the above-mentioned chronic diseases, it could explain conflicting results in studies performed on different ethnic populations (73–75). There have been no studies on the genetic variability in humans of the other class A scavenger receptors.

#### Conclusion

It will not be possible to elucidate the precise appearance of the class A scavenger receptors or to understand the timing of the appearance of the domains of the class A scavenger receptors until more sequence data from a broader range of organisms are available. We can, however, hypothesize that certain scavenger receptor domains appeared early in evolutionary history (e.g. SRCR domain and collagenous domain) and that the functional plasticity of these domains resulted in their preservation and replication in multiple species over time. As an example, the SRCR domain is found in single-cell animals and even in algae but is not found in nematodes and then appears again in vertebrates. How organisms such as C. elegans compensate for the lack of class A scavenger receptors warrants further investigation. The combination of a collagenous and SRCR domain that defines the vertebrate class A scavenger receptors probably appeared before the vertebrate lineage, as it is found in lower vertebrates such as the zebrafish; however, there do not appear to be strong homologs in lower organisms such as the lamprey. Whether this is due to a dearth of complete sequence data or a genuine lack of homologs is not clear. As neutrophils lack SRA, it is likely that the vertebrate recombinant types of SR arose after their divergence from the macrophage lineage.

The fact that MARCO is more conserved across a broader range of species suggests that it could well be an ancestral gene of which duplication led to the evolution of SRA or it could imply that its presumed function in host defense is more highly conserved than that of the homeostatic functions of SRA. The role of SRA in the clearance of modified host proteins requires further analysis to elucidate the homeostatic needs of the individual host, whereas recognition of conserved pathogen-associated molecular patterns may be the primary function of MARCO.

The scavenger receptors (classes A–H) are diverse at the genetic and structural levels and, although they may share a conserved ability to bind selected polyanions, this fact does not appear to be due to conserved structural domains that are passed on by gene duplication. Thus, they are probably neither paralogs nor orthologs but have evolved for a variety of functions, including their ability to bind a range of different polyanionic molecules.

How does the evolution of the receptors of myeloid lineage cells compare with that of the better studied lymphoid lineage? Myeloid recognition receptors can function in roles that are independent of lymphoid receptors but in some cases have adapted to play a role in antigen presentation or as co-receptors. It is possible that their earliest roles were in cell–cell adhesion, phagocytic or pattern recognition but that over time these functions evolved in conjunction with receptors of the lymphoid lineage to participate in self versus non-self recognition, recognition of lipoproteins associated with pathogens, as well as homeostasis and clearance. Further studies of the roles of these receptors in lower organisms are required to determine if this is the case.

Myeloid lineage receptors such as the scavenger receptors are under selective pressure. Whether this is due to continued selection and counter-selection of pathogens, as might be the case for MARCO, or to changes in host requirements for homeostasis and lipid metabolism, as is likely for SRA, or due to as yet uncharacterized functions of these receptors, remains to be determined.

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