thematic review

Thematic review series: The Immune System and Atherogenesis

Recent insights into the biology of macrophage scavenger receptors

David R. Greaves[1] and Siamon Gordon

Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, United Kingdom

Abstract Scavenger receptors were originally defined by their ability to bind and internalize modified lipoproteins. Macrophages express at least six structurally different cell surface receptors for modified forms of LDL that contribute to foam cell formation in atherosclerosis. In addition to their role in the pathology of atherosclerosis, macrophage scavenger receptors, especially SR-A, play critical roles in innate immunity, apoptotic cell clearance, and tissue homeostasis. In this review, we highlight recent advances in understanding the biology of macrophage scavenger receptors as pattern recognition receptors for both infectious nonself (pathogens) and modified self (apoptotic cells and modified LDL). We critically evaluate the potential of scavenger receptors and their ligands as targets for therapeutic intervention in human disease.—Greaves, D. R., and S. Gordon. **Recent insights into the biology of macrophage scavenger receptors. *J. Lipid Res.* 2005.** 46: **11–20.**

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MACROPHAGE-DERIVED FOAM CELLS

Fatty streaks, which are the earliest forms of atherosclerotic lesion visible in the artery wall, consist almost entirely of macrophage-derived foam cells (1–3). Monocytes that have been recruited to the subendothelial space at sites of endothelial cell activation differentiate into macrophages and express a range of scavenger receptors, which mediate the endocytic uptake of modified forms of LDL (4). Brown, Goldstein, and colleagues (5, 6) coined the term "scavenger receptor" to distinguish the uptake of modified LDL by macrophages from LDL uptake via the classical LDL receptor, which is feedback inhibited by the accumulation of cholesterol within the cell. Molecular cloning and biochemical characterization of the cellular receptors that mediate the uptake of modified forms of LDL have revealed an unexpectedly large number of endocytic receptors (7, 8). These membrane proteins have widely different structures, and all the scavenger receptors characterized to date bind a number of ligands of biological importance other than modified LDL. Even more puzzling is the recently described scavenger receptor SR-PSOX (scavenger receptor that binds phosphatidylserine and oxidized lipoprotein), which can act both as a receptor [for oxidized LDL (OxLDL)] and as a ligand (the membrane-bound CXC chemokine, CXCL16).

Their lack of feedback inhibition by intracellular cholesterol has made macrophage scavenger receptors scapegoats for the development of macrophage foam cell formation in the artery wall, but it is important to recognize that other metabolic pathways play an important role in the development of cholesteryl ester deposits in macrophage foam cells. Modified apolipoprotein B (apoB)-containing lipoproteins taken up via scavenger receptors are delivered to lysosomes and hydrolyzed to release free cholesterol and fatty acids. The conversion of free cholesterol into cholesteryl esters in macrophages is catalyzed by the enzyme ACAT, and free cholesterol can be converted by the mitochondrial enzyme Cyp27 into 27-hydroxycholesterol, one of several oxysterols that activate changes in macrophage foam cell transcription via the nuclear receptor liver X receptor (LXR) (9). The cloning and characterization of the gene defective in Tangier disease, a condition characterized by defects in HDL-mediated cholesterol efflux from cells, has shown that cholesterol efflux mechanisms in macrophages are important in macrophage foam cell formation and the development of atherosclerosis (reviewed in 10).

In recent years, we have come to appreciate that the macrophage foam cell is an important target for a series of ligands that exert their effects via binding to nuclear receptors. These include ligands of the LXR, the retinoic acid receptor-related orphan receptor (RORα) (NR1F1) receptor, and the peroxisome proliferator-activated recep-

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[1] To whom correspondence should be addressed.
e-mail: david.greaves@path.ox.ac.uk

tors (PPARs) PPARα, PPARγ, and PPARδ (11). The ligands for these receptors are either physiologically important mediators, such as free fatty acids (for PPARα), cholesterol, or cholesterol sulfate (for RORα), or oxysterols (for LXR). Synthetic ligands that bind this class of receptors have found application as drugs used in the treatment of cardiovascular disease [e.g., fibrates (PPARα)] and type II diabetes [e.g., thiazolidinediones (PPARγ)].

The intense interest in nuclear receptor ligands as mediators of macrophage foam cell biology has been spurred by three observations. First, Tontonoz and colleagues (12, 13) reported that changes in macrophage gene expression induced by OxLDL were attributable, in part, to the presence of the PPARγ ligands [9-hydroxyoctadecadienoic acid (HODE) and 13-HODE] present in OxLDL, thus implicating both nuclear and cell membrane receptors in the transcriptional response of macrophages to OxLDL. One of the first macrophage genes reported to be upregulated via PPARγ was the scavenger receptor CD36. Second, Ricote et al. (14) and Jiang, Ting, and Seed (15) independently reported that treatment of activated macrophages with PPARγ ligands significantly suppressed the production of inflammatory cytokines. Ricote et al. (14) also showed that macrophage scavenger receptor SR-A expression was decreased by treatment with natural and synthetic PPARγ ligands. These results have subsequently been confirmed and extended by gene array experiments with wild-type and PPARγ null macrophages (16, 17). Finally, a number of laboratories have shown that long-term administration of thiazolidinediones and LXR ligands significantly slows atherosclerotic lesion development in apoE$^{-/-}$ and LDL receptor knockout (LDLR$^{-/-}$) mice (18, 19). Although these nuclear receptor ligands have multiple cellular targets outside of the arterial wall (not least adipocytes, hepatocytes, and myocytes), all of them have the potential to significantly alter macrophage foam cell gene expression, including the therapeutic modulation of macrophage scavenger receptor activity.

VASCULAR SMOOTH MUSCLE CELL-DERIVED FOAM CELLS

More than 25 years ago, primary smooth muscle cells (SMCs) cultured from both human and cholesterol-fed rabbit arteries were shown to bind and endocytose labeled acetylated LDL (AcLDL) (20, 21). Rabbit SMC AcLDL receptor activity was shown to be upregulated by platelet secretion products and the activation of protein kinase C, and these treatments upregulated the expression of both SR-AI and SR-AII receptors at the cell surface (22). Further studies identified both transforming growth factor-β1 and human cytomegalovirus infection as major regulators of SMC scavenger receptor activity (23, 24). The diverse patterns of SMC gene expression observed both in vitro and in vivo have led to the idea that arteries contain at least two inherently different SMC subtypes, and a number of cell lines have been cloned from a single piece of human internal thoracic artery that display stable differences in cell phenotype in culture (25, 26). Recent experiments conducted with these cell lines suggest that SMC subsets differ in their response to atherogenic lipoproteins (27). Further evidence supporting the existence of different SMC populations may come from laser microdissection analysis of mRNA expression (28). A recent report has documented significant changes in the program of gene expression of cholesterol-loaded murine aortic SMCs, with decreased expression of SMC markers and the induction of macrophage markers, including CD68 and Mac-2 (29). Similar experiments have not yet been reported for human SMCs, but the possibility exists that transdifferentiation within the arterial wall may mask the cellular origin of foam cells in atherosclerotic plaques.

BRIEF BIOGRAPHIES OF THE "CLASSICAL" MACROPHAGE SCAVENGER RECEPTORS

In this section, we will briefly introduce the senior members of the macrophage scavenger receptor family, highlighting recent insights into their biology and newly identified ligands. For more comprehensive descriptions, the reader is referred to several previous reviews (8, 30–32).

CD36

Initially identified as the platelet integral membrane glycoprotein receptor for thrombospondin-1, CD36 has been shown to have multiple biologically relevant ligands, including modified LDL, *Plasmodium falciparum*-parasitized erythrocytes, sickle cell erythrocytes, collagen I and IV, anionic phospholipids, and long-chain fatty acids (33). CD36 is a heavily glycosylated 53 kDa protein expressed by microvascular endothelium, adipocytes, skeletal muscle, dendritic cells, erythroid precursors, and platelets as well as cells of the monocyte/macrophage lineage. Defined as a class B scavenger receptor having N- and C-terminal transmembrane regions, CD36 family homologs have been identified in *Drosophila melanogaster* and *Caenorhabditis elegans*. The availability of CD36 null mice and humans with platelets and monocytes deficient in CD36 expression (Nak^a-negative individuals) has allowed for an unambiguous assignment of CD36 ligands and an assessment of the contribution of CD36 to the binding of ligands recognized by multiple scavenger receptors.

In 1993, CD36 was shown to bind and internalize minimally modified forms of LDL (34), and peritoneal macrophages of CD36 knockout mice exhibit a 60–80% decrease in both OxLDL binding and OxLDL uptake (35). The importance of CD36 as a receptor for modified LDL in vivo was confirmed when CD36$^{-/-}$ mice were crossed with apoE$^{-/-}$ mice, resulting in a 70% reduction in atherosclerotic lesion size in animals fed a high-fat diet (36). This work has been confirmed and extended by the analysis of apoE$^{-/-}$ mice reconstituted with macrophages deficient in CD36 expression (37).

The role of CD36 in atherosclerosis was further strengthened by the analysis of CD36 ligands. The antibody E06, which recognizes the phosphorylcholine head

group of oxidized phospholipids, was shown to block OxLDL uptake by macrophages (38), and subsequent studies confirmed the nature of the ligand and identified the macrophage scavenger receptor as CD36 (39). Interestingly, the same phosphorylcholine head group is recognized by the scavenger receptor class B type I (SR-BI) receptor (40). Podrez et al. identified a series of oxidized choline glycerophospholipids as ligands of the CD36 receptor and showed that these $oxPC_{CD36}$ ligands are enriched in atherosclerotic lesions of WHHL rabbits (41, 42). Given the role of CD36 in apoptotic cell clearance, antigen "cross-priming" by dendritic cells (43), long-chain fatty acid metabolism, binding of advanced glycation end products (AGE)-modified proteins, and the potential involvement of CD36 in the development of insulin resistance, it will be important to determine if the same regions of the CD36 protein are responsible for the recognition of its diverse set of physiologically important ligands. Generation of mice defective in CD36-mediated OxLDL uptake but still competent in CD36-mediated apoptotic cell uptake would be very useful for the study of the role of CD36 in innate and adaptive immunity and pathologies such as atherosclerosis and Alzheimer's disease.

SR-BI

SR-BI, like CD36 and lysosomal integral membrane protein II, has been classified as a member of the class B scavenger receptor family. SR-BI was initially identified as a scavenger receptor activity of CHO cells and subsequently shown to be a heavily *N*-glycosylated 82 kDa protein of 504 amino acids (44). An alternatively spliced form of the gene (SR-BII) that generates a protein with an altered cytoplasmic tail has been reported (45, 46). SR-BI can bind typical scavenger receptor ligands, including AcLDL and OxLDL, advanced glycation end products, apoptotic cells, and anionic phospholipids, but SR-BI can also bind native forms of HDL (47). The ability of SR-BI to facilitate cholesterol transfer from macrophages to HDL and its ability to mediate selective cholesterol delivery to the liver and steroidogenic tissues without HDL particle degradation marks SR-BI as an important player in reverse cholesterol transport.

In the liver, SR-BI expression and activity at the plasma membrane is regulated by the binding of a protein called PDZK1 to the cytoplasmic terminus of SR-BI. Decreased liver expression of SR-BI in PDZK1 knockout mice leads to a significant increase in plasma HDL levels, whereas gene transfer using an adenovirus expressing SR-BI reduces plasma HDL levels, showing that hepatic SR-BI expression is a potential target for therapeutic regulation of HDL levels (48–50).

The importance of SR-BI in atherosclerosis has been amply demonstrated using transgenic and knockout mouse studies. Hepatic overexpression of SR-BI achieved via transgenic expression in $LDLR^{-/-}$ mice significantly protects against the development of atherosclerosis (80% decrease) under conditions of mild hypercholesterolemia (51), and similar results were obtained using adenovirus-mediated SR-BI gene transfer (52). Remarkably, $apoE^{-/-}$ mice that are defective in SR-BI expression develop occlusive coronary artery lesions on a normal chow diet that result in myocardial infarctions followed by cardiac dysfunction, myocardial scarring, and/or sudden death at a young age (53). Such apoE/SR-BI double knockout animals may provide a useful model to study the development of coronary artery disease, and it is noteworthy that treatment of apoE/SR-BI knockout animals with probucol can prevent the early onset of atherosclerosis and cardiac problems in these animals (54). The importance of macrophage expression of SR-BI for the atheroprotective function of SR-BI in murine models of atherogenesis has been demonstrated by using $SR\text{-}BI^{-/-}$ bone marrow transplantation into $apoE^{-/-}$ mice (55, 56, reviewed in 57).

The SR-A family

The molecular cloning of the bovine macrophage scavenger receptor in 1990 marked the start of an era of functional dissection of macrophage scavenger receptor function and allowed for the identification of a wide range of polyanionic scavenger receptor ligands (58). Careful analysis of macrophage scavenger receptor knockout mice, originally developed by Kodama and coworkers (59), has revealed multiple, unexpected roles for this family of cellular receptors in areas as diverse as antimicrobial immunity, cell adhesion, apoptotic cell clearance, antigen processing, and atherogenesis.

The macrophage scavenger receptor gene was shown to produce two distinct forms of macrophage scavenger receptor protein through alternative mRNA splicing; the larger type I receptor (SR-AI) differs from the type II receptor (SR-AII) in having a cysteine-linked C-terminal extension of 110 amino acids (60). Scavenger receptor cysteine-rich (SRCR) domains have been found as a protein motif in a number of different proteins, including CD5, CD6, CD163, and Spα (61, 62). A third naturally occurring splice variant of the human SR-A gene has been described that encodes a protein with an altered C- terminal amino acid sequence, human SR-AIII, which is trapped within the endoplasmic reticulum. This novel splice variant can act as a dominant negative mutant of SR-A activity in transfected cells (63).

The in vivo expression profile of human SR-A has been analyzed using anti-SR-A-specific antisera and monoclonal antibodies (64, 65). In addition to its role in the endocytosis of modified LDL, the SR-A receptor has been shown to mediate macrophage adhesion in vitro, and a role for SR-A in the phagocytosis of apoptotic thymocytes has been inferred from the in vitro analysis of SR-A gene knockout animals.

The initial evaluation of modified LDL uptake by SR-A-deficient macrophages suggested that SR-A was responsible for the majority (~80%) of macrophage uptake of AcLDL but only ~50% of OxLDL uptake (66). Notwithstanding this less than complete block to macrophage uptake of atherogenic OxLDL, SR-A knockout mice show a significant decrease in atherosclerotic lesions in the $apoE^{-/-}$ and $LDLR^{-/-}$ mouse models (66, 67).

The generation of transgenic mice lacking both SR-AI/SR-AII and CD36 scavenger receptors allowed Kunjathoor

et al. (68) to show that together SR-A and CD36 accounted for up to 90% of total macrophage uptake of both AcLDL and OxLDL. At first sight, this result seems to suggest that other scavenger receptors are unlikely to contribute to macrophage foam cell formation in the arterial wall. Nakagawa-Toyama et al. (69) reported a comparative analysis of CD36 and SR-A expression in human atherosclerotic lesions. A nonoverlapping pattern of SR-A and CD36 expression was observed in atherosclerotic plaques taken from 48 different aortic samples at autopsy. The authors' histological data suggested that SR-A is expressed in cells closer to the lumen of the artery and CD36 expression is highest within the macrophage foam cells found in the center of the atheroma. A second note of caution in interpreting in vitro uptake experiments relates to the nature of the OxLDL used in different experiments, with suggestions that different scavenger receptors have different affinities for different LDL modifications.

The original description of the macrophage scavenger receptor null mice by Kodama and coworkers (66) showed that mice lacking SR-AI and SR-AII expression were unexpectedly more susceptible to infection in vivo by the Gram-positive pathogen *Listeria monocytogenes* and the viral pathogen HSV-1. The demonstration that the SR-A receptor can recognize the lipid A moiety of lipopolysaccharide (LPS) (70) and that SR-A null mice are protected from endotoxic shock in a primary bacterial infection-LPS challenge model (71) led to the idea that SR-A is an important pattern recognition receptor of the innate immune system. This idea has been strengthened by the observation that SR-A can mediate the nonopsonic uptake of the Gram-positive pathogen *Staphylococcus aureus* (72) and the Gram-negative bacterial pathogen *Neisseria meningitidis* (73, 74). In recent work, we have compared the pattern of macrophage activation after stimulation via SR-A using *N. meningitidis* as a ligand and stimulation via the T-helper cell-1 cytokine IFN-γ. Macrophage activation via SR-A leads to a different pattern of cell surface receptor expression, some of which is dependent on LPS signaling via Toll-like receptor (TLR)4 (74).

There have been a number of reports implicating SR-A in adaptive immunity, most recently including a report that SR-A may be involved in the capture of antigen from live cells by a process of "antigen nibbling" (75). Although yet to be critically evaluated for their role in antigen capture and processing, the expression of scavenger receptors by dendritic cell populations hints at scavenger receptor functions in adaptive immunity as well as their role in the innate immune system and the arterial wall.

A structurally related molecule expressed by murine macrophages was cloned in 1995 and termed MARCO (macrophage receptor with collagenous structure) (76). MARCO shows the same overall domain structure as the SR-A type I receptor but differs in having a longer extracellular domain and completely lacking an α-helical coiled-coil domain. MARCO has been demonstrated to bind bacteria, but its ability to bind modified forms of LDL is less well studied. Interestingly, the binding site for bacteria has been mapped to an arginine-rich segment of the C-terminal SRCR domain (77), a site distant from and distinct from the ligand binding site of SR-A, which lies within the collagenous domain of the receptor (78, 79). MARCO$^{-/-}$ mice are susceptible to *Streptococcus pneumoniae* infection as a result of a defect in alveolar macrophage phagocytosis of unopsonized bacteria (80). The availability of MARCO null mice and MARCO/SR-A double knockout mice will allow for a more complete evaluation of the SR-A family of scavenger receptors in macrophage function in the arterial wall and beyond. Already, it is clear that ligand binding to the closely related SR-A and MARCO receptors results in markedly different patterns of macrophage activation (74, 80).

CD68

The human CD68 gene and its murine homolog, macrosialin, were cloned from expression libraries using anti-CD68 monoclonal antibodies and shown to be heavily glycosylated transmembrane proteins expressed in the endosomal compartments of tissue-resident macrophages (81, 82). Ligand-blotting experiments with ^{125}I-labeled OxLDL identified both human CD68 and murine macrosialin as macrophage proteins able to bind OxLDL in vitro (83–85), but the lack of a CD68 knockout mouse has prevented an unambiguous demonstration that the OxLDL binding activity of CD68 is important in macrophage foam cell formation in vivo.

LOX-1

The lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) was originally characterized as a receptor for OxLDL but not AcLDL expressed by bovine aortic endothelial cells. Molecular cloning showed that LOX-1 is a type II glycoprotein consisting of a short intracellular domain, a transmembrane-spanning region, and an extracellular C-type lectin domain (86). LOX-1 is heavily modified by *N*-linked carbohydrate, and experiments with tunicamycin-treated cells have suggested that this *N*-linked glycosylation may contribute to OxLDL binding (87). Interestingly, Xie et al. (88) recently reported that human LOX-1 functions as a disulfide-linked homodimer at the cell surface. In addition to acting as a membrane receptor for OxLDL, soluble forms of LOX-1 have been identified that are secreted by tumor necrosis factor-α-stimulated endothelial cells (89).

Cells transfected with a LOX-1 expression vector support the binding and internalization of OxLDL (90), and LOX-1 will bind a range of other ligands, including activated platelets, Gram-positive and Gram-negative bacteria, and apoptotic cells (reviewed in 91, 92). Although originally described as an endothelial cell scavenger receptor, LOX-1 is expressed by macrophages and vascular SMCs (93). An immunohistochemical analysis of human atherosclerotic lesions has shown that endothelial cells associated with early atherosclerotic lesions express LOX-1 but in more advanced lesions vascular SMCs and lipid-laden macrophages express readily detectable levels of LOX-1 (94). A definitive assessment of the role of LOX-1 in atherogenesis awaits the development of LOX-1 knockout mice.

RECENT ADDITIONS TO THE MACROPHAGE SCAVENGER RECEPTOR FAMILY

Despite the identification and detailed characterization of CD36, SR-A, LOX-1, and SR-BI, the examination of non-macrophage cell type uptake of modified LDL combined with expression cloning and genomic approaches have yielded several new cell surface receptors capable of specifically binding modified LDL. We have singled out some of the more recent interesting additions to the macrophage scavenger receptor family for further discussion.

SR-PSOX (CXCL16)

The macrophage scavenger receptor SR-PSOX was identified by an expression cloning strategy using a cDNA library generated from mRNA of THP-1 cells transfected into COS-7 cells and screened using 1,1′-dioctadecyl-1-3,3,3′,3′-tetramethylindocarbocyanine perchlorate DiI-labeled OxLDL (95). Human SR-PSOX is a type I membrane protein of 254 amino acids that shows no homology with other scavenger receptors. The transfected human SR-PSOX gene directs expression of a 30 kDa protein that can bind ^{125}I-OxLDL with a similar affinity to that of other scavenger receptors. Two years after the original identification of SR-PSOX, it was shown to be identical to an unusual membrane-bound CXC chemokine called CXCL16 (96, 97). Recent reports have shown that SR-PSOX/CXCL16 mediates the binding and phagocytosis of Gram-negative and Gram-positive bacteria and that this binding activity maps to the chemokine domain, which is also responsible for the recognition of OxLDL (98, 99). Immunohistochemistry and quantitative RT-PCR have shown that SR-PSOX is expressed by macrophage foam cells present in atherosclerotic plaques of human carotid and coronary arteries (100) as well as in atherosclerotic lesions of apoE$^{-/-}$ mice (101). A quantitative assessment of the contribution that SR-PSOX makes to activated macrophage OxLDL uptake and foam cell formation in vivo awaits the generation of SR-PSOX knockout mice.

FEEL-1 (stabilin-1/CLEVER-1)

The FEEL [fasciclin epidermal growth factor (EGF)-like, laminin-type EGF-like, and link domain]-containing scavenger receptor-1 is a large (2,570 amino acid) multidomain protein independently cloned by several groups (102–105). FEEL-1 contains 7 fasciclin-like, 16 EGF-like, 2 laminin-type EGF-like, and 1 link domain next to the single transmembrane region. Originally identified in a screen for AcLDL binding activities in HUVECs, FEEL-1 and the related FEEL-2 protein have been shown to be endocytic receptors for AGE-modified proteins (106). The in vivo expression pattern and cell biology of these recently identified scavenger receptors need to be better characterized before we can speculate on the role of FEEL-1 and FEEL-2 in human vascular disease.

SREC

The scavenger receptor expressed by endothelial cells (SREC) encodes a protein of 830 amino acids, and SREC-I has been shown to mediate the binding and internalization of AcLDL (102). SREC-I and the closely related SREC-II are type I membrane proteins with seven epidermal growth factor receptor (EGFR)-like cysteine pattern domains, a transmembrane-spanning region, and a long intracellular C terminus. Tamura et al. (107) have shown that SREC-I is expressed by LPS-treated macrophages, and the generation of SREC-I knockout mice has allowed a comparison of the contribution of SR-AI/SR-AII and SREC-I with macrophage AcLDL binding, with SR-A accounting for 90% of total ^{125}I-AcLDL degradation and SREC-1 only 6% of total macrophage AcLDL degradation. The availability of SREC-I knockout mice will allow for a direct assessment of the role of SREC-I in murine models of atherogenesis and will aid in the search for specific SREC-I and SREC-II ligands (108) as well as a reexamination of the role of SREC-I in cell adhesion and cell morphology (109).

CD163

Tissue macrophages remove hemoglobin produced by the lysis of erythrocytes in a complex with haptoglobin via a cell surface receptor containing nine tandem SRCR domains. This macrophage receptor was identified as the CD163 differentiation antigen, which is expressed at a high level by tissue-resident macrophages, and its expression level can be further augmented by glucocorticoids and the cytokines interleukin-10 and interleukin-6 (which also increases haptoglobin synthesis) (110). Although there are no reports of CD163 binding modified LDL, the macrophage scavenger for hemoglobin CD163 serves to remind us that tissue-resident macrophages have evolved a wide range of endocytic receptors designed to scavenge or clear potentially harmful products of host origin, including but not limited to modified LDL. Increased serum levels of soluble CD163 shed from activated macrophages have been observed in patients with rheumatoid arthritis (111), and CD163-mediated clearance of modified forms of hemoglobin may be important in atherogenesis in diabetic subjects (112).

The phosphatidylserine receptor—a note of caution

The exposure of phosphatidylserine on the outer side of the plasma membrane of apoptotic cells is important for the uptake of apoptotic cells by macrophages. The specific macrophage receptors that mediate the uptake of apoptotic cells by recognition of phosphatidylserine are not fully elucidated, but until recently the best candidate was the phosphatidylserine receptor Ptdsr (113). Many of the functional studies showing a role for Ptdsr in apoptotic cell uptake and expression cloning of the Ptdsr cDNA were performed using the monoclonal antibody 217G8E9. A detailed characterization of fetal liver-derived macrophages of Ptdsr$^{-/-}$ animals showed normal levels of apoptotic cell engulfment in vitro and in vivo, although the authors did observe some changes in macrophage cytokine production after apoptotic cell phagocytosis (114). An explanation for this puzzling result lies in the observation of Bose et al. (114) that there is no difference in staining of Ptdsr$^{-/-}$ and Ptdsr$^{+/+}$ with the anti-Ptdsr monoclonal an-

tibody 217G8E9. This result strongly suggests that the anti-Ptdsr monoclonal antibody recognizes at least two different proteins, one of which is critically important for apoptotic cell recognition and remains to be identified.

TOWARD A NEW DEFINITION OF MACROPHAGE SCAVENGER RECEPTORS?

With so many cell surface receptors displaying some aspects of scavenger receptor activity, do we need to tighten our definition of macrophage scavenger receptors? Specifically, should we retain the title of scavenger receptor only for those receptors that can bind and internalize modified forms of LDL (thus excluding CD163), or should we embrace these related molecules under the umbrella of macrophage clearance receptors, recognizing the importance of macrophages in tissue homeostasis? Given the fact that several macrophage scavenger receptors can bind bacteria in vitro (e.g., SR-A, MARCO, LOX-1, and CXCL16), should we think of macrophage scavenger receptors as fully paid-up nonopsonic pattern recognition receptors? Medzhitov and Janeway's (115) original model of self-versus-nonself recognition included the need for the immune system to recognize altered self (e.g., modified LDL) as well as pathogenic nonself, although why these two activities should so often find themselves encoded in the same molecule (e.g., SR-A and anti-streptococcal T15 IgM antibodies that recognize modified LDL) is not clear (116).

WHY SO MANY RECEPTORS? WHY SO MANY LIGANDS?

None of the macrophage scavenger receptors we have described (except CD163) bind a unique ligand. However, wherever scavenger receptor gene knockout mice have been generated, they have all exhibited a specific phenotype. Just because there are multiple receptors for the same ligand does not mean that there is redundancy in the macrophage scavenger receptor family. What is driving the evolution of scavenger receptors? Is it their role as pattern recognition receptors or is it their role in the recognition and clearance of effete cells and molecules? What are the true physiological ligands of macrophage scavenger receptors, and how will we recognize them when we find them?

All scavenger receptors (except CD163) are relatively low-affinity receptors that recognize large polyanionic ligands via extended ligand binding domains, so acquisition of novel ligand binding activities through amino acid substitutions may be easier than for other cell surface receptors (e.g., GPCRs) in which gene duplication followed by divergence has preceded the emergence of new ligand binding activities. Another macrophage cell surface receptor that has acquired multiple, relatively low-affinity ligand binding activities is the CR3 complement receptor, which recognizes a range of ligands, including opsonized bacteria and viruses, *Leishmania* surface antigens, intracellular adhesion molecule-1, and fibrinogen, and can also mediate macrophage adhesion (117, 118).

SCAVENGER RECEPTORS AS THERAPEUTIC TARGETS?

In contrast to G protein-coupled receptors, which generally have only a single high-affinity ligand that binds to a relatively small binding site, scavenger receptors have multiple ligands with diverse structures that tend to bind to extended regions of the receptor. These properties of scavenger receptors suggest that they will not be the most attractive targets for drug design. However, as discussed above, scavenger receptor expression by macrophage foam cells can be modified by treatment of cells with PPARγ- and LXR-specific ligands, and there are good reasons to think that modifying scavenger receptor activity is an important outcome after treatment with these drugs. The statins, which inhibit the key enzyme in cholesterol biosynthesis, have also been reported to downregulate the scavenger receptors SR-A and LOX-1 (119, 120). Through their ability to upregulate hepatic LDL receptor expression, statins dramatically reduce plasma LDL cholesterol and presumably reduce the amount of modified LDL in the arterial wall for macrophage scavenger receptors to accumulate.

Rather than looking at scavenger receptors as potential drug targets, perhaps scavenger receptor ligands represent a better target for therapeutic intervention in human disease. LDL oxidation and modification have fallen from favor as rational targets in the treatment of cardiovascular disease after the limited success of antioxidant therapy in placebo-controlled clinical trials in patient groups with established cardiovascular disease (121–123). LDL modification in vivo is mediated, in part, by specific enzymes, including 15-lipoxygenase-1. Recently, genes of the 5-lipoxygenase (LO), 5-LO-activating protein pathway have been identified as genes associated with increased risk of cardiovascular disease, notably in studies in Icelandic populations (124). Specific enzymes might represent a better target for the development of drugs. Scavenger receptors and lipoxygenases are already of interest to medicinal chemists because of their role in the pathology of asthma.

UNANSWERED QUESTIONS

• Are there more macrophage scavenger receptors waiting to be discovered? There are probably a few more members of the scavenger receptor family still to be identified. One obvious candidate is the receptor recognized by the "phosphatidylserine receptor" monoclonal antibody that blocks the macrophage uptake of apoptotic cells. There are probably several new scavenger receptor ligands to be characterized, including several encoded by microbial pathogens and the cellular and extracellular substrates for macrophage adhesion.

• Are there anatomical sites other than the arterial wall where we should be looking for new biological activities of macrophage scavenger receptors? Potentially interesting sites to look for macrophage scavenger receptor function would include adipose tissue (125), lymphatic endothelium (126), and sites of microglia activation and macrophage recruitment in neuropathologies including Alzheimer's disease (127).

• What role do macrophage scavenger receptors play in antigen presentation and the elaboration of an adaptive immune response? A potential role for scavenger receptors in adaptive immunity is hinted at by the role of apoptotic cell phagocytosis in antigen cross-priming (43) and the expression of SR-A on dendritic cells (75).

• Is heterogeneity in scavenger receptor expression in vivo important in the biology of tissue-resident macrophages and dendritic cells (DC)s? A better picture of macrophage heterogeneity with respect to scavenger receptor expression and function will require the development of better antibodies for use in histochemistry and more detailed phenotyping of receptor knockout mice. Recent studies by Geissman, Jung, and Littman (128) have suggested that monocytes in blood are heterogeneous with regard to their surface phenotype (CX3CR1 and CCR2 expression as well as CD14 and CD16 antigens), which can be correlated with their ability to be recruited by inflammatory stimuli or constitutively to peripheral tissues. It is not known whether recruitment to the arterial wall selects for a similar subpopulation of monocytes as infectious or physiologic stimuli. Normal circulating monocytes do not express SR-A, but experimental models with fluorescent markers for different receptors now make it possible to approach this question experimentally (129).

FUTURE PROSPECTS

Despite their central role in atherogenesis, we have not yet been able to exploit our knowledge of scavenger receptor biology to develop better treatments for cardiovascular disease. Such a goal may seem unrealistic given the multiplicity of scavenger receptors and their ligands, but the emerging worldwide epidemic of type II diabetes and the continuing burden of cardiovascular disease argue strongly that we need to improve our knowledge of these receptors if we are to develop a better understanding of the pathological processes that drive the development of atherosclerotic plaques and diabetic vasculopathy.

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