Identification and Characterization of Murine SCARA5, a Novel Class A Scavenger Receptor That Is Expressed by Populations of Epithelial Cells*

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Epithelia are positioned at a critical interface to prevent invasion by microorganisms from the environment. Pattern recognition receptors are important components of innate immunity because of their ability to interact with specific microbe-associated structures and initiate immune responses. Several distinct groups of receptors have been recognized. One of these, the scavenger receptors, has been classified into at least eight separate classes. The class A scavenger receptors are characterized by the presence of a collagen-like domain and include macrophage scavenger receptor type A (SR-A1 I/II, SCARA1) and MARCO (SCARA2). These receptors are known to make important contributions to host defense. Here, we identify a novel murine scavenger receptor, SCARA5, which has a structure typical of this class. The cDNA encodes 491 amino acids, which predict a type II protein that contains C-terminal intracellular, transmembrane, extracellular spacer, collagenous, and N-terminal scavenger receptor cysteine rich domains. Expression in Chinese hamster ovary cells confirmed that the receptor assembles as a homotrimer and is expressed at the plasma membrane. SCARA5-transfected cells bound Escherichia coli and Staphylococcus aureus, but not zymosan, in a polyanionic-inhibitable manner. Unlike other class A scavenger receptors, the receptor was unable to endocytose acetylated or oxidized low density lipoprotein. Quantitative RT-PCR and in situ hybridization demonstrate SCARA5 has a tissue and cellular distribution unique among class A scavenger receptors. Because of the restriction of SCARA5 transcripts to populations of epithelial cells, we propose that this receptor may play important roles in the innate immune activities of these cells.

Mucosal surfaces are the predominant route of microbial entry into the host. Epithelial cells that line mucosa not only form a mechanical barrier that pathogens must overcome to invade the underlying tissues but also make specific contributions to the initiation of mucosal inflammation and associated immune responses (1). Although the immunological activities of epithelia have been under-investigated with comparison to those of hematopoietic cells, an increasing number of studies has given weight to the proposal that they are an active component of the innate immune system. The evidence is that epithelia such as those of the airway and urinary and intestinal tracts can sense microbes and signal their presence to cells of the underlying mucosa to activate appropriate anti-microbial and immunological responses (2). Recent demonstrations have shown that epithelial cells can themselves produce cytokines, chemokines, and other effector molecules following contact with bacteria and thus both influence and augment the immune response (3–5).

Cells of the innate immune system have evolved a number of germ line-encoded receptors, termed pattern recognition receptors (PRRs), which they employ to discriminate microbial cells from those of the host (6). PRRs are evolutionary ancient and have been highly conserved. PRRs function through their ability to interact with non-variable molecular structures of the surface of microbes, so-called pathogen-associated molecular patterns. The consequences of receptor-ligand engagement can promote phagocytic uptake of the microbe and triggering of pro-inflammatory cytokine production (7). Recently, there has been intense interest in the biological roles of the mammalian toll-like receptors (TLRs) (8) Investigations of the latter have demonstrated the essential contributions of these PRRs on epithelial cells as distinct from those of other immune cell types within the context of microbial infection. Hultgren and colleagues (9) showed that, in mice lacking TLR4, the principle receptor required for the recognition of lipopolysaccharide (10), uropathogenic bacteria persisted in the bladder epithelium in the absence of an inflammatory response. Secondly, by using a bone-marrow transplantation approach to create chimeric animals, the same workers confirmed that expression of the receptor on the epithelium and other stromal cells was essential to control early bacterial infection but not sufficient to activate an acute inflammatory response (11).

In addition to TLRs a number of other families of PRRs have been identified, which include C-type lectins and complement components (12). Knowledge of these receptors has come principally through analyses of antigen-presenting cells, such as macrophages and dendritic cells. In contrast, we have a much more limited understanding of the nature and diversity of PRRs that are expressed by epithelial cells.

Scavenger receptors (SRs), a structurally diverse group of membrane receptors, of which eight discrete classes have been now been recognized (13), were originally defined by their ability to recognize different forms of modified low density lipoprotein (14). It is this property and their association with the development of vascular disease that has been the main focus of research effort. However, the tissue distributions and the ability of specific SRs to bind microbe-associated molecules have inferred that some

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§ The abbreviations used are: PRR, pattern recognition receptor; SRs, scavenger receptors; SR-A, class A macrophage scavenger receptor; TLR4, toll-like receptor 4; CHO, Chinese hamster ovary cell; RT-PCR, reverse transcription-PCR; acLDL, acetylated low density lipoprotein; oxDL, oxidized low density lipoprotein; Dil, 1,1′dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; poly(G), polyguanylic acid; poly(I), polynosinic acid; poly(C), polycytidylic acid; SCARA5, class A scavenger receptor 5; FAC5, fluorescence-activated cell sorting; PBS, phosphate-buffered saline; GFP, green fluorescent protein; SLCR, scavenger receptor cysteine-rich domain; EGFP, enhanced GFP; MARCO, macrophage receptor with collagenous structure.

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behave as PRRs and contribute to innate immunity (15). The class A macrophage scavenger receptor (SR-AI I/II or SCARA1) is the prototypic SR. It is a trimeric membrane glycoprotein that exists in two major isoforms, type I and type II (16). It is composed of multiple protein domains of which one, the collagenous region, contains the binding site for modified lipoprotein (17). The receptor displays unusually broad, but specific ligand-binding properties, and its recognized ligands include lipopolysaccharide and lipoteichoic acid of Gram-negative and Gram-positive bacterial species. SR-AI I/II expression in the mouse is restricted to particular sub-populations of the myeloid lineage (18). Investigations of the biological activities of SR-AI I/II, facilitated by the creation of mice deficient in the receptor, have confirmed roles in host defense (19). SR-AI I/II can bind directly to *Staphylococcus aureus* and *Escherichia coli* (20), and SR-AI I/II knock-out mice have been shown to be more susceptible to infection-induced mortality by *Listeria monocytogenes* and *S. aureus* than wild-type animals (21, 22).

Recently other members of the class A group of SRs have been identified, all of which have helical collagenous domains of variable lengths. MARCO (macrophage receptor with collagenous structure) is closely related to SR-AI I/II, can bind Gram-positive and negative bacteria, but is more restricted in its tissue distribution (23). Ohtani et al. (24) isolated SRCL (SCARA4, also referred to as CL-P1), a class A transmembrane receptor that has a terminal carbohydrate recognition domain and is related to the soluble collectin group of molecules. Vascular endothelial cells predominantly express this receptor. It can also bind *E. coli* and *S. aureus* in vitro (25).

In the present study we report the characterization of a novel member of this group of SRs that is closely related in primary sequence to SR-AI I/II. It displays similar, but not identical binding properties, which include the recognition of specific microbes. However, unexpectedly and unique among the known class A SRs, its expression is apparently restricted to populations of epithelial cells associated with mucosal surfaces. These data suggest that this molecule is likely to be involved in the innate immune properties of these cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—CHO-K1 cells were cultured in Ham’s F-12 medium supplemented with 50 IU/ml penicillin G, 50 mg/ml streptomycin, 2 mM glutamine, and 10% fetal bovine serum (all from Invitrogen) and maintained at 37 °C with 5% CO2. The murine macrophage cell line RAW264.7 was maintained in RPMI1640 basal medium (Invitrogen) containing supplements as for CHO-K1 cells. The Sertoli-like cell line RAW264.7 was maintained in RPMI1640 basal medium (Invitrogen) containing supplements as for CHO-K1 cells. The Sertoli-like cell line RAW264.7 was maintained at 37 °C with 5% CO2.

**Cloning of Murine SCARA5**—Full-length Mouse SCARA5 was generated by PCR with the primers GGAAGCTTACATGGACAACAAA-GTCACCCCAGC (restriction enzyme sites underlined, Invitrogen), using Turbo (Stratagene) and employing as template National Institutes of Health Image clone ID 5321978 (GenBank accession number JC023907), obtained from MRC Geneservice, Hinxton, UK. The PCR product of the correct size was cloned into pEGFPc1 vector (Clontech) using standard molecular biological techniques and sequenced in its entirety.

**Generation of CHO-K1 Cells Stably Expressing SCARA5**—CHO-K1 cells were transfected with SCARA5 or pEGFPc1 alone using FuGENE 6 transfection reagent (Roche Applied Science) according to the manufacturer’s protocol. After 48 h cells were selected and maintained in complete medium containing 1 mg/ml G418 (Sigma). Expression was confirmed by FACS analysis (FacsScan, BD) and fluorescence microscopy (Zeiss Instruments). CHO-SR-A cells have been described previously (26).

**Western Blot Analysis of CHO-SCARA5**—Cells were lysed in protein lysis buffer (150 mM NaCl, 20 mM Tris, pH 8.0, 1% Nonidet P-40, containing protease inhibitor mixture) (Roche Applied Science) at 4 °C and centrifuged to remove insoluble debris. Lysates were separated by SDS-PAGE under either reducing or non-reducing conditions and transferred to polyvinylidene difluoride membrane (Amersham Biosciences). Blots were blocked for 2 h with PBS containing 0.05% Tween 20 and 5% nonfat milk, prior to incubation with rabbit anti-GFP antisera (Molecular Probes). Binding was detected with protein A-horseradish peroxidase (Bio-Rad) and visualized by chemiluminescence with ECL reagent (Amersham Biosciences).

**Surface Biotinylation and Immunoprecipitation**—CHO-SCARA5-transfected cells were resuspended in PBS containing 0.5 mg/ml EZ-link sulfo-NHS-LC biotin (Perbio) at 4 °C for 30 min. Cells were washed with PBS, lysed, and precleared with GammaBind™ Plus Sepharose (Amersham Biosciences) for 1 h at 4 °C. Antigen was precipitated with rabbit anti-GFP IgG by overnight incubation at 4 °C, followed by the addition of GammaBind™ Plus Sepharose for 1 h. Beads were washed, and specific protein was eluted with Laemmli sample buffer, separated on SDS-PAGE, and transferred to polyvinylidene difluoride membrane. Blots were probed with ExtraAvidin-peroxidase (Sigma) and developed with ECL chemiluminescence reagent.

**Analysis of Modified Lipoprotein Endocytosis**—CHO-K1, CHO-SCARA5, CHO-SR-A1, and RAW264.7 cells were incubated with 5 μg/ml Dil (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate)-labeled acetylated low density lipoprotein (Dil- acLDL, Molecular Probes) or Dil-oxidized LDL (Dil-oxLDL, Intracell) in complete medium at 37 °C for 4 h. Cells were washed three times with PBS and fixed with 4% paraformaldehyde before either FACS analysis or examination by a fluorescence confocal laser-scanning microscope equipped with appropriate filters (Zeiss Instruments).

**Analysis of Microorganism Binding**—Cells were cultured on glass coverslips and incubated at 37 °C for 1 h with tetramethylrhodamine-conjugated *E. coli* K12 strain bioparticles, *S. aureus* (Wood strain without protein A bioparticles or zymosan A (*S. cerevisiae*) bioparticles (all from Molecular Probes). These bioparticles are fluorescently labeled heat-killed bacteria or yeast. Cells were washed three times with PBS, fixed, stained with 4’,6-diamidino-2-phenylindole, and examined by fluorescence microscopy. Potential inhibitors of microbial binding (poly G, poly I, poly C, bovine serum albumin, and maleylated bovine serum albumin, all from Sigma) were added to a final concentration of 100 mg/ml 30 min prior to addition of the microorganism and maintained for the duration of the assay. To quantify the extent of inhibition of microbial binding by the polyamionic molecules, a minimum of 200 cells in random fields were examined for evidence of microbe recognition by fluorescence microscopy.

**Quantitative RT-PCR**—All equipment and reagents were obtained from Applied Biosystems. Primer and probe sequences specific to murine SCARA5 were selected using Primer Express software (Applied Biosystems). Forward primer, GTAGGCATGGAGCTTTGCA; reverse primer, CCCCCATGTTTCCCACTACCA; and probe, CCACCTCTCTCTCTCGGAGACTCTCGA. Selected sequences were compared with public databases to confirm specificities. Primer-probe sets to detect SR-A1 and CD68 (macrosialin) were as described previously (27). Primer pairs were tested using conventional RT-PCR to confirm generation of a single band of the expected amplicon size (not shown). cDNA was synthesized from 200 ng of total RNA (prepared using an RNeasy kit, Qiagen) in a 10-ml reaction volume using an Omniscript
FIGURE 1. A, the nucleotide (upper line) and predicted amino acid (lower line) sequence of murine SCARA5. The putative transmembrane region is underlined, the collagenous region is dash underlined, cysteine residues are shown in bold, and potential N-linked glycosylated asparagines residues are outlined. B, comparison of the predicted sequences of protein domains of murine SCARA5 (upper line) and murine SR-A1 type I (middle line). Sequences were aligned using ClustalW algorithm. Shaded boxes represent identical residues (consensus shown in the lower line). Dashes represent gaps in sequence to facilitate alignment. C, schematic of structural organization of members of class A scavenger receptors.
reverse transcription kit (Qiagen). PCR was performed using TaqMan universal master mix containing 300 nM primers and 100 nM probe and the equivalent of 20 ng of total RNA. Duplicate reactions were run on an Applied Biosystems Prism 7700 sequence detection system and quantified against a standard curve generated from reactions containing serial dilutions of plasmids encoding SCARA5, SR-A1, or CD68. The amount of RNA input into each reaction was determined by parallel measurement of glyceraldehyde-3-phosphate dehydrogenase levels. Data were

FIGURE 1—continued
calculated as relative SCARA5, SR-A1, or CD68 expression, normalized to glyceraldehyde-3-phosphate dehydrogenase.

RT-PCR—Conventional RT-PCR was performed using an Advantage PCR kit (Clontech) according to manufacturer’s instructions. cDNAs from tissues and MSC-1 cells were prepared as above. Full-length SCARA5 was amplified from cDNA for 30 cycles using forward primer ATGGACAA-CAAAGGCCATGTACCTG and reverse primer TCAGGGGACAGTACAAAGTCACC. Specific products were analyzed on 1% agarose gel.

In Situ Hybridization—A 389-bp fragment derived from the extracellular spacer region of SCARA5 was cloned into pcDNA3-TOPO (Invitrogen) and digoxigenin-labeled riboprobes synthesized using appropriate RNA polymerases (Roche Applied Science). Hybridizations and signal development were performed according to a previous study (28). Tissue sections were counterstained with methyl green (Vector Laboratories).

RESULTS

Identification and Predicted Domain Organization of the Class A Scavenger Receptor 5—We screened public murine and human DNA databases for sequences related to SR-A1 I/II and identified a number of clones that showed significant similarity. Further comparative sequence analysis revealed the existence of a full-length murine cDNA. Using these data we obtained a relevant Image clone that encodes this sequence, which was subcloned into appropriate expression vectors and sequenced in its entirety.

The sequence of the clone 5321978 (GenBank™ accession number BC023907) revealed an open reading frame of 1,473 bp, encoding an amino acid sequence of 491 acids with calculated protein molecular mass of 53,663 kDa (Fig. 1A). Analyses performed using the protein modular domain predictor SMART algorithm (29) indicated that the deduced amino acid sequence composed a multidomain type II transmembrane protein, with an organization characteristic of the class A SRs (Fig. 1, B and C). On the basis of our data and according to the agreed nomenclature for SRs, we have designated this receptor as SCARA5 or epithelial scavenger receptor, and the sequence has been submitted to GenBank™ under accession number DQ122126.

The receptor consists of the following predicted protein domains: a short, N-terminal cytoplasmic domain (Domain I; residues 1–59), a transmembrane domain (Domain II; residues 60–77), a spacer region (Domain III; residues 78–305), a collagenous domain (Domain IV; residues 306–378), and a scavenger receptor cysteine-rich domain (SRCR) (Domain V; residues 386–491). Comparison with existing members of class A SRs revealed the highest homology is with murine SR-A1 I/II; DQ122126 shares 34% identity with the murine SR-A1 type I isoform. Sequence identities are greatest for the SRCR domain (59%) and the collagen-like region (51%). DQ122126 has seven putative N-linked glycosylation sites, of which all except for Asn-16 are located within the extracellular spacer domain (Fig. 1A).

The predicted 59-amino acid domain I represents the N-terminal cytoplasmic region of the receptor. Sequence analyses indicate that it does not apparently contain any previously recognized endocytosis motifs and has only a single tyrosine residue at amino acid position 7, which does not comply with the surrounding sequence criteria required of a tyrosine phosphorylation site. However, consensus recognition sequences for protein kinase C isoforms (Thr-10), casein kinase II (Ser-18), and potential phosphorylation at five serine residues (Ser-18, Ser-28, Ser-30, Ser-54, and Ser-56) and a single threonine (Thr-10) were predicted. It may be relevant that a block of five amino acids beginning at Phe-24 is highly conserved with SR-A1 I/II, and mutagenesis of the bovine molecule has demonstrated that the residues Phe-24 and Asp-25 are important for efficient internalization and cell surface expression of the receptor (30).

The putative hydrophobic transmembrane domain (residues 60–82) is followed by a predicted extracellular 231-residue spacer domain (domain III). This domain displays 32% identity with the comparable region of mSR-A1 I/II and contains 9 heptad repeats (defined by the presence of hydrophilic residues, including Leu, Ile, or Val) that are also present in mSR-A1 I/II (Fig. 1B). Such repeats are characteristic of an α-helix, but analyses using Coils, Paircoil, or Multicoil programs (au.expasy.org/) did not predict formation of a coiled-coil structure.

The predicted collagenous triple helical domain of DQ122126 (domain IV) begins at position 306 with the triplet Gly-Pro-Pro, which is conserved absolutely in SR-A1 I/II across all species (31). This first triplet is followed by 24 additional Gly-Xaa-Yaa repeats, which is the same number as are present in human SR-A1/II and is only one greater than for murine SR-A1 I/II (31). In contrast, the collagenous domain of SRARA5 is significantly shorter than those of murine MARCO and SRCL, which contain 48 and 89 triplets, respectively (23, 25). Of the 25 Yaa residues in domain IV, 12 are Pro or Lys, (as compared with 14 in murine SR-A1 I/II), whereas 5 are Arg residues (4 in murine SR-A1 I/II).

Immediately after the collagenous region there is a stretch of eight amino acids that are located at the same relative position as the so-called hinge domain of SR-A1 type I (31). Adjacent to this is a predicted 102-residue domain with the features of an SRCR motif. This domain contains six cysteine residues that are spatially conserved with SR-A1 type I.
FIGURE 3. A, confocal microscopy of wild-type, CHO-K1 cells (a, f, k, and p), stable transfectants EGFP (b, g, l, and q), EGFP-SCARA5 (c, h, m, and r), and EGFP-SR-A1 (d, i, n, and s) or RAW264.7 cells (e, j, o, and t) incubated with either DiI-oxLDL or DiI-acLDL. Panels a–e and k–o represent nuclear staining of cells. Scale bars represent 5 μm. B, flow cytometry plots of CHO-K1 cells and CHO-K1 cells transiently transfected with EGFP, SCARA5, or SR-A1 and incubated with or without DiI-acLDL. Numbers in the upper right quadrants indicate percentage of transfected cells showing endocytosis of the fluorescent ligand.
This structural arrangement places the SRCR domain of BC023907 within the designated group A of the SRCR superfamily (32).

Microscopic and Biochemical Analyses of CHO Cells Overexpressing SCARA5—To investigate the biochemical and biological properties of SCARA5 we created N-terminal protein fusions with the GFP variant, EGFP. These plasmids were transfected into CHO-K1 cells, and stable integrants were selected with the appropriate antibiotic (Fig. 2A).

Confocal microscopy was performed on CHO-K1 cells transfected with EGFP-SCARA5 to analyze the sub-cellular distribution of the molecule. Diffuse fluorescence was seen throughout the cell, characteristic of the endoplasmic reticulum, together with a perinuclear halo typical of localization to the trans-Golgi network that was significantly different from that seen with the vector alone (Fig. 2). We acknowledge, however, that, because expression of the receptor was under the control of the cytomegalovirus immediate early promoter from within the plasmid construct, there is a possibility that the precise cellular distribution may be different when it is driven from the endogenous SCARA5 promoter.

To confirm the trimeric structure of SCARA5 predicted from the cDNA sequence we undertook Western blotting of transfected CHO cell lysates probed with anti-GFP antisera. We were able to identify two major protein bands of molecular sizes of ≈100 and >250 kDa that would correspond to monomeric and trimeric forms of the protein. Upon reduction with β-mercaptoethanol these collapsed to a single band of ≈100 kDa (Fig. 2B).

We employed cell-surface biotinylation to demonstrate that SCARA5 can localize to the plasma membrane. Viable cells (>95%) were labeled with membrane-impermeable biotin, then lysed, specific protein was immunoprecipitated with anti-GFP antisera, SDS-PAGE separated, and blotted, and the biotin-tagged receptor was identified with ExtraAvidin-horseradish peroxidase. We were able to detect specific protein of >250 kDa that corresponds to trimeric assembly of the polypeptides (Fig. 2C). We also observed a specific band at ≈100 kDa, the size of monomeric SCARA5, which may perhaps represent post-labeling dissociation of the trimeric receptor. It is also possible that the relative intensities of the two bands reflect a greater affinity of the anti-GFP antibody for the fusion protein in its monomeric form. Overall, these data are consistent with previous reports that all collagenous transmembrane proteins are trimers composed of identical polypeptides (33).

Binding Properties of SCARA5: Recognition of Modified Lipoproteins—SRs are defined by their ability to bind modified lipoproteins; however, the precise chemical modification that can be recognized is receptor-specific (14, 34). We therefore incubated SCARA5 transfected, mock transfected cells, wild-type CHO cells, and CHO cells overexpressing murine SR-A1 type I and RAW264.7 cells with fluorescently labeled modified low density lipoproteins and examined for evidence of binding and/or uptake by FACS and microscopy. Unlike either RAW 264.7 cells or CHO stably expressing mSR-A1 type I, we could not observe by fluorescent microscopy significant binding or endocytosis of acLDL or oxLDL, despite incubation periods of up to 6 h (Fig. 3A). FACS analyses of transiently transfected cells incubated with Dil-acLDL also confirmed the lack of significant binding or uptake by SCARA5 (Fig. 3B). We believe that the N-terminal GFP fusion was unlikely to have interfered with the activities of SCARA5, because a similarly tagged SR-A1 type I receptor transiently transfected into CHO cells retained the ability to mediate uptake of modified lipoproteins (Fig. 3B).

Binding of Microbes—We also investigated the capacity for SCARA5 CHO cells to bind microbes. Previous studies have shown that E. coli, S. aureus, and in some cases yeast can bind to other class A SRs (22–24,35). We observed that CHO-SCARA5 transfec transfectants avidly bound heat killed E. coli and S. aureus bioparticles (Fig. 4A, panels k and q) but not zymosan (Fig. 4A, panel v). Non-transfected or EGFP-expressing CHO cells did not bind any of the three particles. As expected, there was significant binding of E. coli and S. aureus bioparticles by CHO cells overexpressing SR-A (Fig. 4A, panels l and r) and uptake of zymosan particles by RAW264.7 cells (Fig. 4A, panel x).

The binding of E. coli and S. aureus bioparticles to SCARA5-transfected CHO cells was significantly inhibited by co-incubation with the polyanionic ligands poly(G), poly(I), and maleylated bovine serum albumin but not by the related polyribonucleotide poly(C) (Fig. 4B).

Tissue and Cell Type Distributions of SCARA5—We undertook real-time RT-PCR (TaqMan) analyses of total RNA purified from a number of mouse tissues to investigate the distribution of SCARA5 mRNA. We designed specific primer pairs and probes to detect SCARA5 that did not cross-react with the murine homolog of the human receptor (33).

FIGURE 4. A, confocal microscopy of bacterial binding by transfected CHO-K1 cells. Wild-type transfectants (CHO-K1 cells (a, e, and i), EGFP (b, f, and j), SCARA5 (c, g, k, m, o, q, and t), and SR-A1 (d, h, l, n, p, r, and u)) or RAW264.7 cells (s and v) were incubated with E. coli, S. aureus, or zymosan bioparticles. Upper sets of panels represent nuclear staining of cells, with EGFP fluorescence in rows beneath. Scale bars represent 5 μm. B, inhibition of SCARA5 binding of E. coli (open bars) and S. aureus (filled bars) bioparticles by specific polyanions. CHO-K1 cells stably expressing SCARA5 were co-incubated with bacteria and specific polyanion and frequency of binding determined by fluorescence microscopy. Error bars represent ± S.E.
not recognize any other sequence present in the public databases. These reagents produced a single DNA product of the expected size only from samples that had been reverse-transcribed and could not amplify from other class A SR templates (data not shown). We analyzed the same RNA samples for the presence of transcripts encoding SR-A1 I/II and macrosialin (CD68). The highest levels of SCARA5 mRNA were recovered from testis, bladder, trachea, adrenal, skin, lung, and ovary (Fig. 5). In contrast, SR-A1 I/II transcripts were more widespread and were greatest in testis, lymph node, spleen, bone marrow, and liver (Fig. 5). Macrosialin/CD68, a macrophage-specific gene (36), was most abundant in spleen, skin, lymph node testis, and liver (Fig. 5). We could not detect measurable levels of SCARA5 mRNA in the murine macrophage cell line RAW264.7 (data not shown).

In the absence of available antibodies we performed in situ hybridization on sections of selected tissues with a specific cRNA probe to determine the cell-type expression of SCARA5 (Fig. 6). We paid particular attention to those tissues in which we had detected significant levels of SCARA5 mRNA using TaqMan methodology (Fig. 5). We observed specific hybridization with the SCARA5 antisense probe, but little signal above background levels on all sections exposed to the sense reagent (Fig. 5). Hybridization of tissues taken from three independent animals gave comparable and consistent results (not shown).

In all tissues that were examined SCARA5 transcripts localized to specific epithelia. The pattern of specific hybridization signal in each of the tissues did not resemble the published distribution of myeloid cells (37, 38). Quantitative RT-PCR revealed the highest levels of SCARA5 transcripts were present in the testis (Fig. 5). In situ hybridization of sections from this organ confirmed the presence of specific mRNA, which localized to cells at the vascular-tubular boundary with the morphology of Sertoli cells (Fig. 6, b and c).

We examined particular tissues of the respiratory system, namely the trachea and lung, for evidence of SCARA5 expression. In sections of trachea, specific staining was confined to the epithelial surface layer and continuous with the pseudostriatified columnar epithelium that lines the mucosa of the tissue (Fig. 6, e and f). Little obvious signal was localized to the underlying connective tissue (lamina propria). Within lung tissues signal was detectable only on tracheobronchial epithelial cells of the upper airways and was apparently absent from both epithelia of the alveoli and alveolar macrophages (Fig. 6, h and i).

We selected the bladder and small intestine as examples of tissues within the urinary tract and gastrointestinal system, respectively, for examination. In general, staining of the bladder appeared less strong but more widespread than that observed in the other tissues that were studied (Fig. 6, k and l). Specific staining localized not only to cells within the transitional epithelium of the tissue, but also to individual cells that lay beneath the basal membrane. In sections of the small intestine hybridization for SCARA5 transcripts was restricted to the epithelial layer, was strongest at the bottom of villi, and was associated with crypts (Fig. 6, n and o). Staining was continuous for adjacent cells. The cellular origin of the hybridization signal was coincident with the location of enterocytes and Paneth cells, but the resolution of our hybridizations did not allow for absolute discrimination between the two cell types. Interestingly, we observed a gradient of staining across the basal-apical axis of each villus, with loss of hybridization signal toward the apical tip (Fig. 6o). Despite extensive analyses, on no occasion did we observe hybridization over lymphoid structures within sections of small intestine, which would suggest the absence of SCARA5 expression by M (microfold) cells.

SCARA5 Is Expressed by a Sertoli Cell-like Line—Although our in situ data indicated expression of SCARA5 was apparently restricted to epithelial cells, we wished to confirm that we could also detect transcript encoding the receptor in an immortalized epithelial cell line. MSC-1 is a transformed murine cell line derived from the testis that displays numerous features characteristic of Sertoli cells (39). RT-PCR using primers designed to generate full-length SCARA5 we were able to amplify a specific amplification product (1470 bp) from bladder, trachea, testis, and MSC-1 cells (Fig. 7). Sequencing of the specific PCR product confirmed its identity as SCARA5 (data not shown). These results are supportive of our real-time RT-PCR data (Fig. 5) and the in situ hybridization analyses (Fig. 6).

DISCUSSION

This study describes the identification, distribution, and some of the properties of the epithelial SR receptor SCARA5, a novel member of the
A class of SRs that is most closely related to murine SR-A1 I/II. Both molecules are, or, in the case of SCARA5, predicted to be, trimeric type II integral transmembrane glycoproteins (14). The molecular identities between the two proteins are distributed across all of the predicted structural domains but are greatest within the SRCR and collagen-like regions (Fig. 1B). Currently four class A SRs species have been identified, which are defined by the presence of a collagenous domain: SR-A1 I/II displays unusually broad ligand binding and is known to have roles in lipoprotein metabolism and the innate immune system (16); MARCO (SCARA2) is a macrophage-restricted membrane molecule, which has an extended collagen-like domain and also functions in host defense (23); CSR1 (SCARA3) has a leucine zipper domain as well as a collagenous region and can protect against cellular damage (40), whereas SRCL (SCARA4) is a molecule that is specifically on endothelia, is proposed to contribute to innate immunity, and contains a carbohydrate recognition domain (24, 25).

The identities of the protein domains that compose the predicted tertiary structure of SCARA5 revealed a very close relationship to those of murine SR-A1 type I (Fig. 1B). This is particularly true for the putative collagen-like and SRCR regions. In contrast, the predicted extracellular region of SCARA5 located between the putative transmembrane domain and the beginning of the predicted collagenous domain (residues 93–306) appears to differ significantly from the equivalent regions of SR-A1 I/II. This region of SR-A1 I/II is considered to compose of two structural domains. Domain III is the spacer domain, whereas the adjacent domain IV contains 17 heptad repeats and is predicted to form a long α-helical coiled-coil (31). Experimental evidence has demonstrated that it is required for trimeric assembly of the receptor (41). In contrast, the sequence of domain III of SCARA5 did not predict to assemble into an α-helical coiled-coil. It may, therefore, be more similar to MARCO, which has a 75-residue spacer domain that is ~2-fold longer than that of SR-A1 I/II and whose amino acid sequence also fails to predict an α-helical coiled-coil structure (23). Two cysteines in the spacer domain of murine MARCO participate in interchain disulfide bonding (23), but...
these are not present in either human MARCO (42) or murine SCARA5. It is likely therefore, that for these receptors the peptide chains are bound only through the triple helical collagenous domain. In the case of SCARA5, this will require confirmation by biosynthesis studies of mutant receptors.

Protein domain prediction from the primary sequence of SCARA5 indicated the presence of a C-terminal SRCR domain. Conservation of the SRCR motif was first recognized with the cloning of murine SR-A1 I/II (43), and sequences containing this structure have been identified subsequently from diverse animal phyla (44). There are mammalian examples of both membrane-bound and secreted molecules, many of which are found on cells associated with the immune system. Structure-activity investigations have revealed a significant difference in the binding activities of SR-A1 I/II and MARCO attributable to the SRCR domain. In the former, the ligand-binding properties of type 1 SR-A1 receptor, which contains a terminal SRCR domain and type II receptor that lacks it, are seemingly identical; the one or more ligand binding sites fall within the collagenous stem (31). In contrast, the binding site for bacteria and modified lipoprotein of MARCO has been assigned to the SRCR domain (45). Antibody blockade and mutagenesis manipulation have implicated an arginine-rich stretch of amino acids (45). Although two of the residues present in murine MARCO, Arg-424 and Arg-433, are identical in mSCARA5, the peptide sequence GRAVYY that is essential for bacteria binding by MARCO is poorly conserved in both SCARA5 and SR-A1 type I (42).

Despite the close similarities of the amino acid sequences of SR-A1 I/II and SCARA5 we were able to demonstrate significant differences in their binding activities. SCARA5 was unable to endocytose acLDL or oxLDL. Therefore, although structurally SCARA5 is a member of the class A SR family, it does not satisfy the definition of a SR as stated by Brown and Goldstein (46) because it cannot bind modified lipoprotein. This inability is shared with other receptors that contain collagen-like structures: human MARCO (47), human SRCL (24), and the complement component C1q (48), all of which are unable to bind acLDL, even though they can interact with other polyanionic molecules. There have been extensive experimental investigations of the structural requirements for binding of the modified lipoprotein by SR-A I/II. An original report confirmed that truncation of the collagen-like domain of the bovine receptor abolished binding (48). A second study of mutant bovine receptors with single amino acid substitutions demonstrated that activity required a cluster of lysine residues near the C-terminal of the domain (lysines 327, 334, 337, and 340) and was critically dependent upon residue Lys-337 (equivalent to Lys-338 in the mouse receptor) (41). A more recent investigation (49) concluded that SR-AI I/II recognition of the modified lipoprotein ligand is also affected by manipulation of the basic amino acid residues Lys-281, Arg-284, Lys-305, Arg-308, Arg-317, and Lys-325, which are located more proximally within the collagenous domain. Because of the inability of SCARA5 to recognize acLDL we compared details of the amino acid sequences of the collagen-like domains of the two receptors (Fig. 1B). Close examination of the amino acid sequences of the collagenous domains of SR-A1 I/II and SCARA5 suggests divergence of sequence that may underlie the different binding properties of the two molecules. SCARA5 does not contain an intact cluster of four lysine residues that are present in SR-A1 I/II (41). The conservation of specific basic amino acids between mSR-A1 I/II and SCARA5 is highest at the distal end of the domain, within which Lys-338 and Lys-335 are identical and Lys-341 is a conservative substitution to Arg-341. In contrast, toward the more proximal end Lys or Arg residues at positions 281, 284, 305, 308, 317, 325, and 328 of murine SR-A1 I/II, which have been shown also to influence lipoprotein binding (49), are replaced in SCARA5 with non-basic amino acids except for Arg-305. It would be of interest to examine whether appropriate substitution of one or multiple amino acids in SCARA5 would confer acetylated lipoprotein recognition.

The evidence that specific class A SRs are involved in host defense has accumulated from studies that have been made along three lines of investigation: the ability of the receptor to mediate binding or recognition of specific microbes or their products, expression of the receptor by populations of cells, such as macrophages, that are part of the innate immune system, and finally experimental depletion of the receptor, which results in enhanced sensitivity of the host to microbial challenge. In common with the known properties of SR-A1 I/II (20, 35), MARCO (23), and SRCL (24), we were able to demonstrate that transfection of SCARA5 alone into CHO cells was able to convey binding of particular Gram-positive and Gram-negative bacteria. This recognition is inhabitable with polyanionic molecules, indicating that the interaction is very likely dependent upon a charge-based mechanism. In light of the association of SCARA5 with specific epithelial surfaces, we suggest that it is likely to be worthwhile to test recognition of other microbial species that are known to invade the host via these sites. This may be predictive of a relevant infection model in which to examine the role(s) of the receptor.

We unexpectedly observed that expression of SCARA5 was restricted to certain populations of epithelia and thus is unique among class A SRs. Quantitative RT-PCR and in situ hybridization techniques demonstrated that the cellular distribution of SCARA5 did not overlap with those of other class A SRs. In addition, we were able to detect transcripts encoding SCARA5 in the Sertoli cell-like line MSC-1 (39). Immunocytochemistry has confirmed that expression of SR-AI I/II and MARCO are specific to myeloid-type cells, although their distributions among populations of tissue macrophages overlap, they are not identical, and the former is found additionally on sinusoidal epithelium of the liver (18, 23). On the other hand, Northern analysis has shown a widespread distribution of SRCL in human tissues, and the protein localized to vascular endothelium but not macrophages (50). There is no published evidence that epithelial populations express these three genes. The cellular distributions of SCARA5 and the other class A SRs are therefore not overlapping, although they may all be present within the same organ. For example, SR-AI I/II and MARCO are expressed by alveolar macrophages in the lung (51), whereas SCARA5 is specific to the airway epithelium. However, we cannot absolutely rule out the possibility of co-localization of SCARA5 with other class A SRs at other sites, because we examined its cellular distribution in only selected tissues. We speculate that, despite the broad similarity of their binding properties, the differential distribution of the three receptors may permit each to make comparable, but cell-restricted biological contributions. Our data would imply that one or more SCARA5-dependent activities on epithelial cells are unlikely to be duplicated by expression of the other known class A SRs. However, we cannot exclude the possibility that these epithelia express additional unidentified receptors that have similar recognition properties. There have been reports of the expression of members of other classes of SR by particular epithelial cells, notably the class B receptor SR-B1 in the gut (52), but to date there is no evidence of innate immune activities for this receptor.

Despite the acceptance that selected populations of epithelial cells display specific immune activities, the current understanding of how these cells detect and respond to microbes is incomplete. At present, the most detailed studies have focused on the contributions of certain TLRs, such as TLR4 (9) and TLR 11 (53), but these receptors appear to function predominantly in signal transduction, rather than binding or inter-
nalization of the pathogen. We detected the greatest expression of SCARA5 within the tests and transcripts localized to Sertoli cells (Fig. 6, b and c), which are able to produce inflammatory mediators such as interleukin-6 in response to appropriate stimuli (54) and are phagocytic for unwanted host cells (55).

We hypothesize that the tissue and cellular distributions, together with the in vitro bacteria binding activity, may implicate SCARA5 in immunity to infection. This would be consistent with the known contributions of structurally related SR-A1 I/II and MARCO (15). However, it is also accepted that specific PRRs are capable of interacting with and responding to environmentally derived non-infectious materials and/or endogenous host ligands and thus may function more broadly in tissue homeostasis (56). For example, ligands for SR-A1 I/II and MARCO include inert particles such as crocidolite asbestos and silica (14); mice that lack MARCO are more susceptible to inflammation of the lung provoked by the inhalation of titanium oxide (57). Therefore, SCARA5 may have non-microbial ligands that are biologically significant. Their identification will require a more extensive analysis of the binding properties of the receptor than was undertaken in this study. It may also be possible to directly identify and/or incorporate additional ligands would likely increase the number of biological contributions by this receptor.

The initial interaction between microbe and host recognition mechanisms defines the early phases of the infection. Although studies have emphasized the role of recognition receptors on professional phagocytes, especially macrophages and other types of antigen-presenting cells, in the normal route of invasion for many pathogens the first encounter is with the epithelium. Organisms that successfully attach and/or invade epithelial cells cause some of the most common infectious diseases (58). Therefore, greater understanding of the molecular basis of epithelial-microbe interactions and the consequences that result are likely to be beneficial for the development of treatments for these debilitating conditions. Here we report the identification of a new SR, with the capacity to bind bacteria and whose expression in the mouse is apparently restricted to specific epithelia. It may well be that SCARA5 is involved in the host defense properties of populations of human epithelial cells, because we have identified a probable orthologue in man (59).

Future studies will include those that clarify the nature of immunological and other activities that are dependent upon the receptor.

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