

Selective Binding of the Scavenger Receptor C-type Lectin to Lewis^x Trisaccharide and Related Glycan Ligands*

Received for publication, April 18, 2005, and in revised form, April 21, 2005
Published, JBC Papers in Press, April 21, 2005, DOI 10.1074/jbc.M504197200

Peter J. Coombs[‡], Sarah A. Graham, Kurt Drickamer[‡], and Maureen E. Taylor^{‡§}

From the Glycobiology Institute, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, United Kingdom

The scavenger receptor C-type lectin (SRCL) is an endothelial receptor that is similar in organization to type A scavenger receptors for modified low density lipoproteins but contains a C-type carbohydrate-recognition domain (CRD). Fragments of the receptor consisting of the entire extracellular domain and the CRD have been expressed and characterized. The extracellular domain is a trimer held together by collagen-like and coiled-coil domains adjacent to the CRD. The amino acid sequence of the CRD is very similar to the CRD of the asialoglycoprotein receptor and other galactose-specific receptors, but SRCL binds selectively to asialo-orosomucoid rather than generally to asialoglycoproteins. Screening of a glycan array and further quantitative binding studies indicate that this selectivity results from high affinity binding to glycans bearing the Lewis^x trisaccharide. Thus, SRCL shares with the dendritic cell receptor DC-SIGN the ability to bind the Lewis^x epitope. However, it does so in a fundamentally different way, making a primary binding interaction with the galactose moiety of the glycan rather than the fucose residue. SRCL shares with the asialoglycoprotein receptor the ability to mediate endocytosis and degradation of glycoprotein ligands. These studies suggest that SRCL might be involved in selective clearance of specific desialylated glycoproteins from circulation and/or interaction of cells bearing Lewis^x-type structures with the vascular endothelium.

The scavenger receptor C-type lectin (SRCL)¹ is also known as collectin-placenta 1 (CL-P1). Although originally cloned from liver and placenta, SRCL is expressed in many tissues (1, 2). The receptor is found in endothelial cells from human umbilical vein and artery as well as in vascular endothelial cells of the heart, suggesting that it may be widely distributed in endothelia. The SRCL polypeptide contains an N-terminal intracellular domain, transmembrane anchor, and the extended extracellular domain (Fig. 1). The extracellular portion of the receptor is composed of a neck that contains coiled-coil and

collagen-like regions and a C-terminal carbohydrate-recognition domain (CRD).

The binding properties of SRCL have only been partially characterized. Binding to oxidized low density lipoprotein particles has been demonstrated and, by analogy to the type A scavenger receptors, this activity has been attributed to the collagen-like domain (1). Binding to Gram-negative bacteria has also been associated with the collagen-like domain, because a variant form of the protein lacking CRDs still shows binding activity for bacteria when expressed in Chinese hamster ovary cells (2). Although the cytoplasmic domain of SRCL contains a potential endocytosis signal motif, conflicting data have been presented regarding the uptake of particulate ligands into cells (1, 2).

C-type CRDs fall broadly into two subgroups, those that bind galactose and related ligands and those that bind mannose, *N*-acetylglucosamine, and fucose. Mutagenesis of galactose-binding C-type CRDs, combined with studies in which the specificity of a mannose-binding CRD was converted to galactose binding, have led to the identification of a small set of residues that are essential for high affinity binding to galactose (3, 4). Examination of the sequence of SRCL reveals that all of these key residues are present.² Thus, SRCL differs from DC-SIGN, the macrophage mannose receptor, serum mannose-binding protein, and other C-type lectins known to have roles in pathogen recognition, all of which fall into the second subgroup. Semiquantitative binding studies using cells expressing SRCL suggest that the receptor does bind sugar-containing ligands, with some preference for *N*-acetylgalactosamine (5).

The overall organization of SRCL shares features of both the type A scavenger receptors for modified low density lipoprotein and the collectins, which are soluble C-type lectins involved in pathogen recognition. The arrangement of domains in SRCL is most like the arrangement of the type A scavenger receptors, with the CRDs replacing cysteine-rich domains that are not related in sequence (6). Sequence comparisons reveal sequence similarities between the collagen-like and coiled-coil domains of SRCL and all three of the type A scavenger receptors, with particularly strong similarity to SR-A 3 (Fig. 1) (7). Although the collagen-like and coiled-coil domains would have similar elongated conformations in SRCL and the collectins, sequence comparisons provide no evidence for homology between SRCL and the collectins, and the domains are actually arranged in reverse order in the polypeptides. In addition, comparison of the CRD of SRCL with other C-type lectins shows that, in evolutionary terms, this domain falls within the family of type II transmembrane receptors that includes the asialoglycoprotein receptor, rather than forming part of the collectin cluster. Thus, it seems likely that the presence of similar domains in

* This work was supported by Grant 041845 from the Wellcome Trust, Studentship 10845 from the Biotechnology and Biological Sciences Research Council, Bursary 01194/G from the Nuffield Foundation, and National Institutes of Health Grant GM62116 to the Consortium for Functional Glycomics. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[‡] Present address: Division of Molecular Biosciences, Imperial College, London.

[§] To whom correspondence should be addressed: Division of Molecular Biosciences, Biochemistry Bldg., Imperial College, London SW7 2AZ, UK. Tel.: 44-20-7594-5281; Fax: 44-20-7594-5207; E-mail: maureen.taylor@imperial.ac.uk.

¹ The abbreviations used are: SRCL, scavenger receptor C-type lectin; SR, scavenger receptor; CRD, carbohydrate-recognition domain; BSA, bovine serum albumin; LNFP, lacto-*N*-fucopentaose.

² See www.imperial.ac.uk/research/animallectins for sequence alignments.

SRCL and the collectins represents independent gene shuffling events that have converged on a similar set of protein structural elements.

To assess the biological functions of SRCL, both the sugar-binding characteristics and intracellular trafficking properties of the receptor need to be better defined. To this end, the results of this study demonstrated that the CRD of SRCL binds with unusually high selectivity to glycans containing the Lewis^x epitope, that the CRDs are clustered in a trimer, and that the receptor is able to direct uptake and degradation of glycoprotein ligands.

EXPERIMENTAL PROCEDURES

Materials—Oligosaccharides and LNFP III-BSA were purchased from Dextra Ltd. (Reading, UK). Galactose-BSA was obtained from E-Y Laboratories (San Mateo, CA). Radioisotopes were from Amersham Biosciences. Rabbit polyclonal antibodies to the CRD from SRCL were generated by Eurogentec (Seraing, Belgium) following their standard protocol using 2 mg of bacterially expressed protein as antigen.

Cloning of SRCL cDNA—cDNA encoding human SRCL was amplified from human placental cDNA (Clontech) in two sections using the following pairs of primers: N-terminal section forward, 5'-AACCCACG-GTCACGGTCACCATGAAAGACGACTTCGCAG-3'; N-terminal section reverse, 5'-TTGAGCTGACCATGCTTGGAGTCTACTAGCTTC-3'; C-terminal section forward, 5'-AAGTGGTCATCATGAACCTCAACA-ACCTGAACCTG-3'; C-terminal section reverse, 5'-TTGCGGCCGCT-TATAATGCAGATGACAGTACTGTCTCCCTGTC-3'. Following denaturation at 95 °C for 1 min, 40 cycles of 95 °C for 30 s, and 68 °C for 1 min were carried out. PCR products were cloned into the vector pCRII-TOPO using the TOPO cloning kit (Invitrogen) and sequenced using an Applied Biosystems Prism 310 genetic analyzer. Segments of the cDNAs that were free of reverse transcription errors were combined using convenient restriction sites.

Expression of the CRD from SRCL in Bacteria—The region of the cDNA coding for the CRD only (from residue 603 to the C terminus) was amplified using the following primers: forward primer, 5'-AAGGCCG-CCGAGGACAATAGCTGCCGCTCACTGGAAG-3'; reverse primer, 5'-TTGCGGCCGCTTATAATGCAGATGACAGTACTGTCTCCCTGTC-3'. PCR products were digested at the FseI and NotI sites in the primers and inserted into a modified pNIIIompA2 expression vector containing restriction sites for FseI and NotI downstream of the ompA signal sequence (8). The correct reading frame was generated by digesting the vector with FseI, followed by trimming of the 3' extension with T4 polymerase and religation. The integrity of the final expression plasmid was verified by DNA sequencing. Luria-Bertani medium (1 liter), containing 50 µg/ml ampicillin, was inoculated with 30 ml of an overnight culture of *Escherichia coli* strain JA221 transformed with the expression plasmid. The culture was grown by shaking at 25–30 °C to an A₅₅₀ of ~0.8, and isopropyl-β-D-thiogalactoside and CaCl₂ were added to final concentrations of 50 µM and 100 mM, respectively. After growth, for a further 18 h at 25–30 °C, the cells were harvested by centrifugation at 4,000 rpm for 15 min in a Beckman JS-4.2 rotor. Bacterial pellets were resuspended in cold 10 mM Tris-Cl, pH 7.8, followed by centrifugation at 12,000 rpm for 15 min at 4 °C in a Beckman JA14 rotor. Bacteria were sonicated in 30 ml of 20 mM Tris-Cl, pH 7.8, 0.5 M NaCl, 20 mM CaCl₂ (loading buffer). Lysed bacteria were centrifuged at 10,000 × g for 15 min, and the supernatant was recentrifuged at 100,000 × g for 1 h at 4 °C. The supernatant was passed over a 10-ml column of galactose-Sepharose (9) equilibrated in loading buffer. The column was washed with 10 × 2 ml of loading buffer and eluted with 10 × 2 ml of elution buffer (20 mM Tris-Cl, pH 7.8, 0.5 M NaCl, 2 mM EDTA). Fractions were analyzed by SDS-polyacrylamide gel electrophoresis. The CRD was identified by N-terminal sequencing on a Beckman LF3000 protein sequencer following transfer to polyvinylidene difluoride membranes (10).

Expression of the Extracellular Domain of SRCL in Chinese Hamster Ovary Cells—DNA coding for the extracellular domain of SRCL, without the cytoplasmic tail and the transmembrane region, was produced by amplifying the region coding for the N-terminal section of the protein using the forward primer 5'-AAAGTTGTAGAGAAATGGACAATGT-CACAGGTGCC-3' with the reverse primer described above and combining this section with the region coding for the C-terminal section amplified as above. The resulting DNA, coding for the entire extracellular domain of SRCL (starting at residue 60), was fused to codons specifying the dog preproinsulin signal sequence. The preproinsulin signal sequence has previously been shown to direct efficient secretion of other truncated receptors (11, 12). The modified cDNA was inserted

into the vector pED between the XbaI and EcoRI sites following the region encoding the adenovirus major late promoter and preceding the region encoding the dihydrofolate reductase gene (13). The resulting plasmid was transfected into DXB11 Chinese hamster ovary cells (which are deficient in dihydrofolate reductase) using the calcium phosphate method (14). Transfectants were selected by growth in minimal essential α medium without nucleosides and supplemented with 10% (v/v) dialyzed fetal calf serum. Amplification of protein expression was achieved by passaging cells into medium containing increasing concentrations (up to 0.5 µM) of methotrexate over several weeks. For protein production, cells were grown in medium containing 0.5 µM methotrexate. Once the cells reached confluence, the medium was changed and batches of medium were harvested every 2 days for 8 days. Tris-Cl, pH 7.8, CaCl₂, and NaCl were added to the medium to give final concentrations of 20 and 20 mM and 0.5 M, respectively. Following centrifugation at 10,000 rpm in a Beckman JA14 rotor for 15 min, the medium was loaded onto a 10-ml column of galactose-Sepharose equilibrated in loading buffer. The column was washed with loading buffer before elution with elution buffer.

Expression of Full-length SRCL in Fibroblasts—DNA coding for full-length SRCL was inserted into the retroviral expression vector pVcos (15) at the EcoRI site. The neomycin resistance gene, under the control of the herpes virus thymidine kinase promoter, was inserted into the resulting vector at the unique ClaI site (16). The final plasmid was transfected into ΨCre packaging cells to produce a pseudovirus that was used to infect Rat-6 fibroblasts, and fibroblast lines stably expressing SRCL were selected using G418 (17). Cells were harvested by scraping, suspended in ~10 volumes of 125 mM NaCl, 10 mM Tris-Cl, pH 7.5, and 0.5% Triton X-100, sonicated briefly, incubated 1 h at 4 °C, and spun for 2 min at 18,000 × g. The supernatant was analyzed by SDS-polyacrylamide gel electrophoresis on a 12% gel, which was blotted onto nitrocellulose and probed with antibodies raised against the CRD (18).

Analytical Ultracentrifugation—Equilibrium sedimentation analysis was carried out in a Beckman Optima XL-A analytical Ultracentrifuge equipped with absorbance optics using an An60Ti rotor at 20 °C. Proteins were dialyzed extensively against 10 mM Tris-Cl, pH 7.8, 100 mM NaCl, 10 mM CaCl₂. The extracellular domain of SRCL was analyzed at 5,000 and 8,000 rpm. For the CRD, rotor speeds of 10,000 and 21,000 rpm were used. Equilibrium distributions from three different loading concentrations (0.5, 0.25, and 0.125 mg/ml for the extracellular domain and 0.2, 0.1, and 0.05 mg/ml for the CRD) were analyzed simultaneously using the Nonlin curve fit program supplied with the instrument. Partial specific volumes for the SRCL fragments were determined from their amino acid composition and estimates of the extent of glycosylation (19).

Labeling of the Extracellular Domain of SRCL—For radio-iodination, the extracellular domain of SRCL, ~100 µg of protein in 1.5 ml, was dialyzed against 150 mM NaCl, 25 mM HEPES, pH 7.8, 5 mM CaCl₂ and reacted with 50 µCi of ¹²⁵I-Bolton-Hunter reagent for 15 min at room temperature (20). Labeled protein was repurified by affinity chromatography on galactose-Sepharose. For fluorescent labeling, ~250 µg of protein was dialyzed against water, lyophilized, dissolved in 0.5 ml of 0.5 M NaCl, 20 mM Na-bicarbonate, 5 mM CaCl₂, and reacted with 75 µg of fluorescein isothiocyanate that was dissolved at 1 mg/ml in dimethyl sulfoxide and added as five aliquots of 15 µl, followed by reaction overnight at 4 °C. EDTA was added to a concentration of 10 mM, and the labeled protein was repurified by gel filtration on a 10 × 300-mm Superdex 200 column (Amersham Biosciences) eluted with 0.1 M NaCl, 10 mM Tris-Cl, pH 7.8, 2.5 mM EDTA at a flow rate of 0.5 ml/min.

Probing of Ligands—Glycoproteins were resolved on a 17.5% SDS-polyacrylamide gel and transferred to nitrocellulose. All of the glycoproteins were of bovine origin, except orosomucoid, which was from human serum. The blot was blocked at room temperature for 1 h with 2% bovine hemoglobin in wash buffer (125 mM NaCl, 25 mM Tris-Cl, pH 7.8, and 25 mM CaCl₂) and reacted for 1 h with the radio-iodinated extracellular domain of SRCL in the same solution at a concentration of ~1 µg/ml. The blot was washed four times for 5 min each with cold wash buffer, and radioactivity was detected using a phosphorimaging device from Molecular Dynamics. Neoglycolipids, prepared by coupling oligosaccharides to phosphatidylethanolamine dipalmitate, were resolved by thin layer chromatography and fixed with isobutylmethacrylate (21). The chromatograms were then blocked and probed as for the glycoprotein blots. Fluorescein-labeled extracellular domain was used to probe the glycan array following the standard procedure of Core H of the Consortium for Functional Glycomics.³

³ www.functionalglycomics.org.

Solid Phase Binding Assays—Polystyrene wells were coated overnight with either CRD or the extracellular domain of SRCL at a concentration of ~50 µg/ml in loading buffer. Neoglycoprotein reporter ligands were created by iodination (22). Binding competition experiments and determination of the pH dependence of binding were performed as previously described (17). Binding data were fitted using SigmaPlot software (Jandel Scientific).

Molecular Modeling—The crystal structure of the galactose-binding derivative of mannose-binding protein with bound galactose was used as a starting point (23). The structure of the Lewis^x trisaccharide abstracted from the crystal structures of this ligand bound to DC-SIGN was used to model the ligand (24), although similar results were obtained using coordinates derived from the crystal structure of sialyl-Lewis^x bound to E-selectin and from the NMR structure of the free oligosaccharide. The galactose moiety of Lewis^x was manually superimposed onto the galactose in the mannose-binding protein structure using Insight II modeling software.

Endocytosis Experiments—Analysis of uptake and degradation of ¹²⁵I-Gal-BSA or ¹²⁵I-LNFP III-BSA by fibroblasts expressing SRCL was performed as described previously (25).

RESULTS

Expression and Characterization of the SRCL Extracellular Domains—To initiate a study of the ligand-binding properties of SRCL, the C-terminal CRD was expressed in bacteria, whereas the entire extracellular domain was expressed in Chinese hamster ovary cells (Fig. 1). In the latter case, the insulin signal sequence was used to direct the protein to the endoplasmic reticulum so that it could be modified by hydroxylation and glycosylation before secretion into the medium. Both fragments of SRCL could be purified by affinity chromatography on galactose-Sepharose (Fig. 2). The CRD, on its own, binds weakly and washes off the affinity column even in the presence of Ca²⁺. The extracellular domain binds more effectively, and columns of sufficient capacity can be washed with Ca²⁺-containing buffer and eluted with EDTA. The extracellular domain fragment migrated as a broad band on SDS-polyacrylamide gels, suggesting that there is considerable heterogeneity in post-translational modification. The apparent molecular weight of 110–115 kDa deduced from the gels was consistent with the predicted presence of up to 13 N-linked glycans, 8 O-linked glycans, and mono- or disaccharide residues attached to hydroxylysine residues, all appended to a core polypeptide of 75 kDa.

Weak binding to highly substituted sugar resins is often characteristic of monomeric forms of C-type lectins. Therefore, the oligomeric states of the fragments were analyzed by equilibrium analytical ultracentrifugation (Fig. 3). The results demonstrated that the CRD is monomeric, but the entire extracellular domain formed a stable trimer. This result is consistent with the presence of the collagen-like domain, which would be expected to direct trimerization. In addition, the sequence of the N-terminal portion of the extracellular domain was predicted to form a 3-stranded coiled coil of α-helices, which would further stabilize the trimer. The relatively weak binding of both the CRD and the extracellular domain to galactose-Sepharose contrasts with other galactose-binding C-type lectins, such as the asialoglycoprotein receptor and the Kupffer cell receptor. Both monomeric and trimeric fragments of these receptors bind tightly to this resin and can only be eluted with EDTA, indicating that these receptors have inherently higher affinity for galactose. These results suggest that galactose on its own is not an optimal ligand for SRCL.

Binding of SRCL to Lewis^x and Related Structures—An initial screening for higher affinity ligands was undertaken by testing the binding of SRCL to a selection of glycoproteins. The glycoproteins were resolved by SDS-polyacrylamide gel electrophoresis, blotted onto nitrocellulose, and probed with the radiolabeled extracellular domain of SRCL (Fig. 4). Strong binding to asialo-orosomucoid is evident, but no binding to other

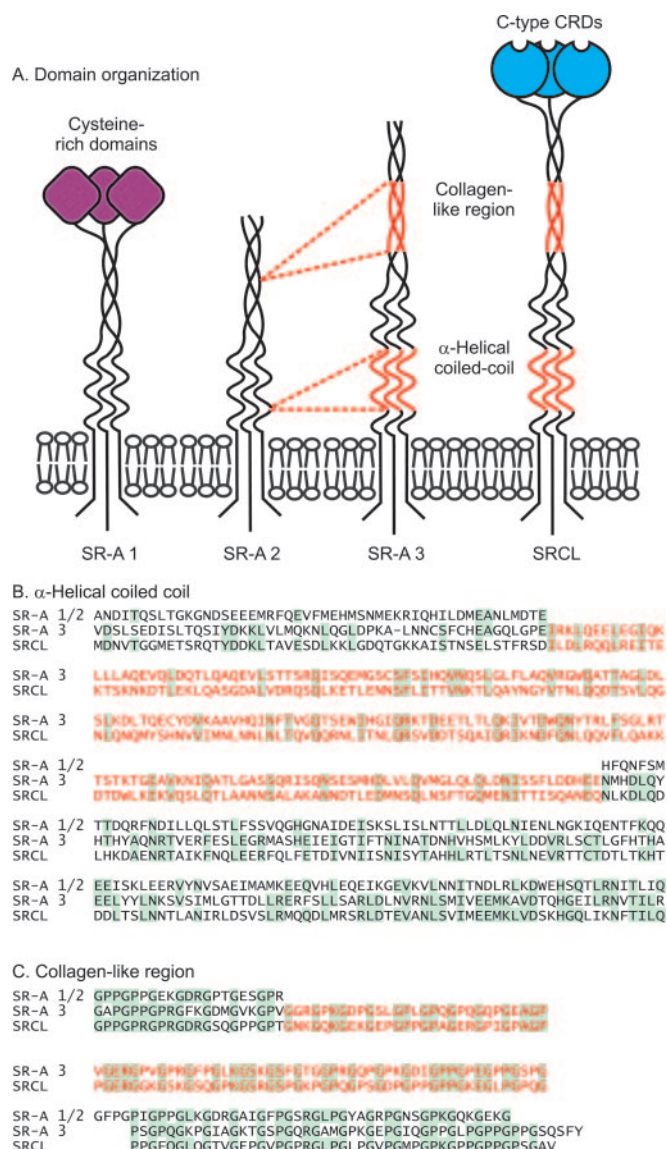


FIG. 1. Domain organization of SRCL and sequence comparisons with type A scavenger receptors. SR-A 1 and 2 are products of alternatively spliced mRNAs from a single gene, whereas SR-A 3 is the product of a distinct gene. The sequence comparisons suggest that SRCL is most similar to SR-A 3, as it has collagen-like and coiled-coil domains of exactly the same lengths. In SR-A 1 and 2, both regions are truncated.

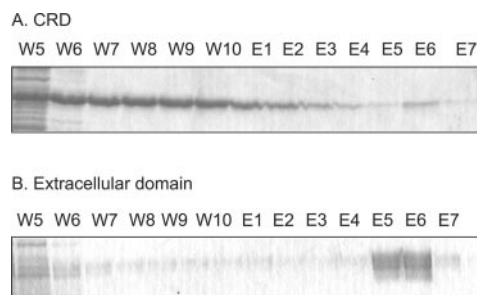


FIG. 2. Gel electrophoresis of fractions from affinity chromatography of fragments of SRCL on galactose-Sepharose. Fractions from washing in the presence of Ca²⁺ (W) and elution with EDTA (E) are shown. Gels were stained with Coomassie Blue.

desialylated glycoproteins was detected. Binding to glycoproteins bearing sialylated complex, high mannose, or sulfated N-linked glycans was also not detected.

There are two relatively unusual features to the N-linked

FIG. 3. Sedimentation equilibrium analytical ultracentrifugation of SRCL fragments. Data for three different loading concentrations, shown as symbols, were fitted globally, with the fitted curve shown as continuous lines. The deduced M_r of the CRD is 17,000 compared with the M_r of the CRD polypeptide calculated from the amino acid sequence, which is 16,441. The measured M_r of the extracellular domain is 317,000, which falls in the range of values calculated for a trimer with various possible degrees of glycosylation (313,000–322,000). Similar results were obtained at two different speeds for each sample. Results for 21,000 rpm for the CRD and 5,000 rpm for the extracellular domain are shown.

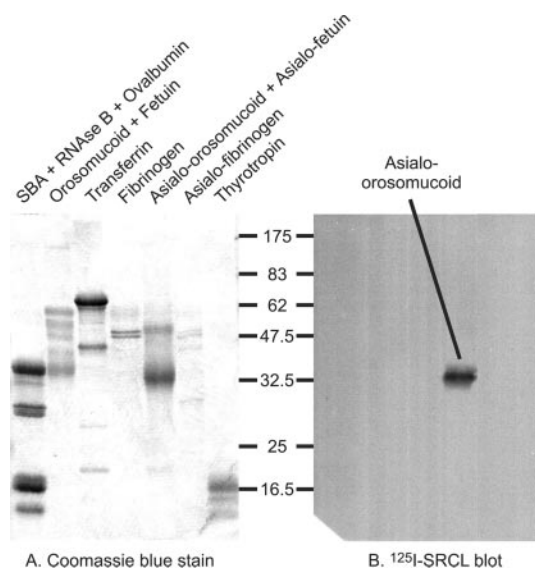
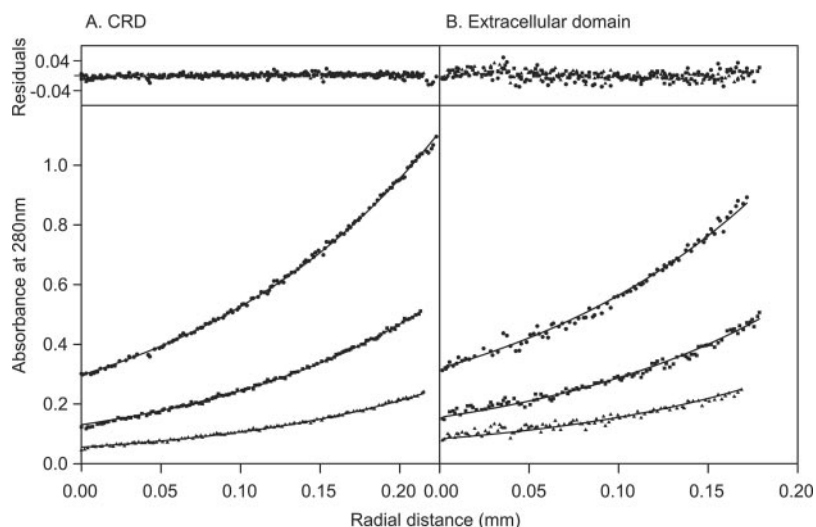


FIG. 4. Binding of ¹²⁵I-SRCL to glycoproteins. Aliquots of ~2 μg of each glycoprotein were resolved by SDS-polyacrylamide gel electrophoresis on a 17.5% gel. Portions of the gel were stained with Coomassie Blue and blotted onto nitrocellulose for probing with ¹²⁵I-SRCL. *SBA*, soybean agglutinin. *RNase*, ribonuclease. Samples in the first lane contain high mannose and hybrid glycans. Thyrotropin in the final lane contains sulfated glycans. Orosomucoid, fetuin, transferrin, and fibrinogen bear sialylated complex glycans.

glycans on asialo-orosomucoid. First, many of the glycans have highly branched tri- and tetra-antennary structures. Second, many of these branches bear outer-arm fucose residues (26). The contribution of these two features to the binding of SRCL to asialo-orosomucoid was assessed by testing the binding to highly branched glycans lacking fucose residues and glycans bearing single terminal fucose residues. The glycans were presented as neoglycolipids resolved by thin layer chromatography (Fig. 5). The radiolabeled extracellular domain of SRCL binds to the Lewis^x- and Lewis^a-containing ligands but not to the branched, unfucosylated oligosaccharides. Thus, SRCL shows selectivity for glycans bearing both terminal galactose and fucose residues.

A broader view of the binding selectivity of SRCL was obtained by probing a glycan array with fluorescently labeled extracellular domain. The array consists of over 150 different biotinylated oligosaccharides presented on streptavidin immobilized in polystyrene wells. The results reveal a striking selectivity for glycans containing terminal Lewis^x trisaccharides,

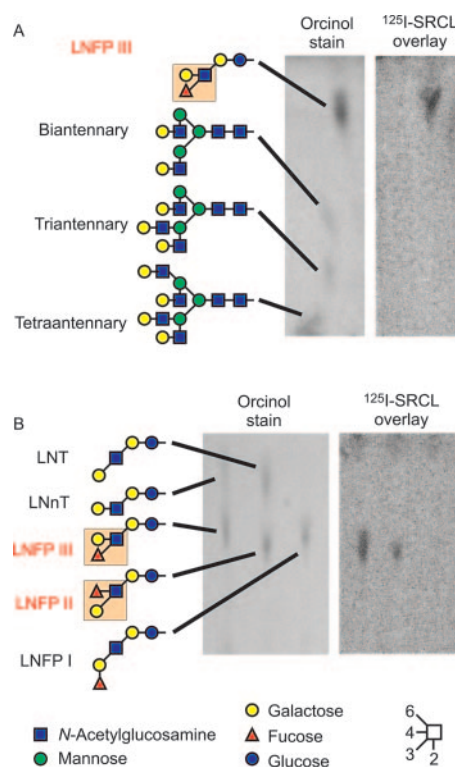
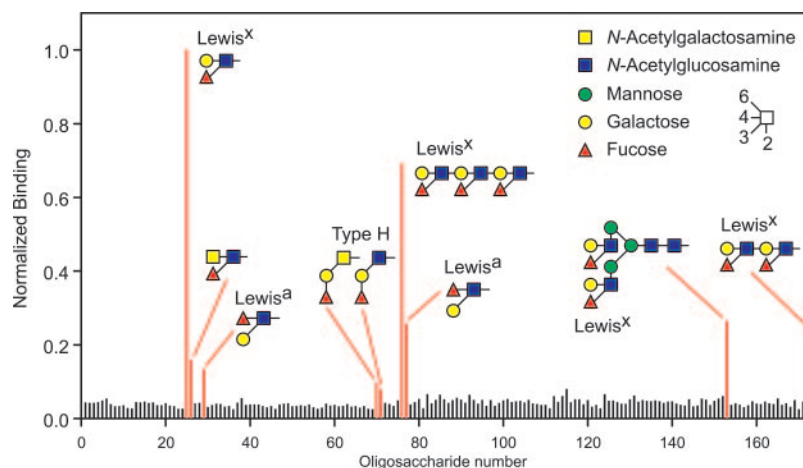


FIG. 5. Binding of ¹²⁵I-SRCL to neoglycolipids. A, chromatogram was developed with CHCl₃/methanol/H₂O 105:100:28. B, chromatogram was developed with CHCl₃/methanol/H₂O 11:9:2. Parallel chromatograms were stained with orcinol or fixed and probed with ¹²⁵I-SRCL. *LNT*, lacto-*N*-tetraose; *LNnT*, lacto-*N*-neotetraose. The boxes indicate Lewis^x and Lewis^a trisaccharide structures.

with no binding to sulfated or sialylated forms of these glycans (Fig. 6). Weaker binding was also observed for the isomeric Lewis^a trisaccharide and to Lewis^x, in which the galactose is substituted by *N*-acetylgalactosamine. In addition, very weak binding to blood group H substances was detected. The high degree of selectivity of SRCL is relatively unusual among C-type lectins.

Selectivity for Lewis^x and Mechanism of Binding—Previous studies with the dendritic cell receptor DC-SIGN have revealed that this receptor, similar to SRCL, binds Lewis^x and other glycans that bear terminal fucose and galactose residues, although it binds a broader range of such glycans. The primary binding site in the C-type CRD of DC-SIGN binds to the fucose moiety (24), whereas the primary binding site in SRCL has the

FIG. 6. **Binding of fluorescein-labeled SRCL to glycan array.** Biotin glycosides were immobilized in streptavidin-coated wells. Binding and washing were conducted in the presence of 150 mM NaCl, 25 mM Tris-Cl, pH 7.5, and 2 mM CaCl₂. The level of fluorescence was normalized to glycan 25 (Lewis^x).



hallmarks of a galactose-binding site. To clarify the nature of the primary monosaccharide-binding site, studies comparing the affinities of galactose, fucose, and Lewis^x trisaccharide were conducted (Fig. 7). The results, summarized in Table I, confirm that the affinity of SRCL for Lewis^x trisaccharide is much higher than the affinity for galactose. The affinity for fucose is roughly 4-fold lower than the affinity for galactose, suggesting that galactose is the primary site ligand. Comparison of α -methyl galactoside and α -methyl fucoside binding shows an even stronger preference for the galactose moiety, probably because the presence of the methyl group eliminates artifactual binding through the 1-hydroxyl group (27).

The availability of a crystal structure for a galactose-binding variant of the CRD from rat serum mannose-binding protein made it possible to model the binding site of SRCL. The conformation of the Lewis^x trisaccharide was derived from one of the several crystal structures that contain this oligosaccharide as part of a larger structure, and the galactose moiety was superimposed on the galactose residue in the CRD structure. Galactose occupies a primary binding site that involves coordination with Ca²⁺ and hydrophobic packing with a tryptophan residue (Fig. 8). The model shows that the fucose residue in the Lewis^x structure can be accommodated in the binding site with no steric clashes. The structure also suggests that the fucose might interact with the short segment-designated loop 5, consisting of the sequence Lys-Ala-Gly at residues 691–693. Mutants in which the sequence of the loop was modified to Arg-Pro-Gly, as found in the asialoglycoprotein receptor, or to Ala-Ala-Gly, to eliminate most potential side chain interactions, failed to change the affinity for Lewis^x compared with galactose (data not shown). These results suggest that interactions with loop 5 might be with backbone atoms rather than the side chains.

A weak binding interaction with the blood group H substances was also detected in the glycan array. Because these oligosaccharides do not contain terminal galactose residues, it was interesting to consider how they might be accommodated in the binding site. The fucose residue in these structures is attached to the 2 position of galactose, so the arrangement of coordination ligands with the 3- and 4-hydroxyl groups need not be perturbed. As no crystal structure with the H-type glycans has been reported, it is not possible to develop a detailed model of where the fucose residue is located. However, modeling of a fucose residue in linkage to the 2-hydroxyl group of galactose suggests that it can be accommodated and that it might make energetically favorable contacts with loop 5 or other residues on the surface of the CRD. Thus, the binding and modeling results provide a consistent picture of galactose- and fucose-containing oligosaccharides interacting with SRCL

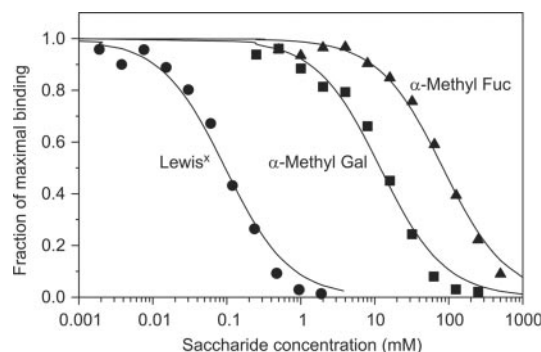


FIG. 7. **Binding competition assays for SRCL.** Competition assays for binding to SRCL-coated wells were performed using ¹²⁵I-LNFP III-BSA as reporter ligand. Assays were performed two or three times in duplicate, and representative results are shown.

TABLE I
Summary of binding competition assays for SRCL

The CRD of SRCL was immobilized on polystyrene wells and probed with ¹²⁵I-labeled LNFP III-BSA in the presence of competing sugars. Similar results were obtained using the trimeric extracellular domain and when ¹²⁵I-Gal-BSA was used as the reporter ligand. The K_I represents the concentration of free sugar needed to achieve 50% inhibition of reporter ligand binding. Assays were performed at least three times in duplicate for each competing sugar, except for Lewis^x, which was tested twice in duplicate. Values reported are means \pm S.D.

Competing sugar	K_I mM	$K_{I,sugar}/K_{I,galactose}$
Galactose	7.5 \pm 2.0	1
N-Acetylgalactosamine	1.9 \pm 0.5	0.28 \pm 0.05
α -Methyl galactoside	10.1 \pm 2.6	1.5 \pm 0.3
Fucose	24.5 \pm 4.3	3.8 \pm 0.4
α -Methyl fucoside	70 \pm 19	11.5 \pm 2.1
Lewis ^x	0.149 \pm 0.8	0.014 \pm 0.006

through a primary galactose-binding site and secondary contacts with fucose.

Endocytic Activity of SRCL—Although SRCL binds selectively to Lewis^x-type structures, its binding activity overlaps with the binding activity of the asialoglycoprotein receptor. The presence of SRCL on endothelial cells suggests that it might interact with a subset of circulating glycoproteins from which terminal sialic acid has been removed. It was, therefore, of interest to examine the ability of SRCL to mediate uptake of such glycoproteins.

The cytoplasmic domain of SRCL contains the sequence Tyr-Lys-Arg-Phe, which is a potential target for binding of adapter proteins that direct receptors to clathrin-coated pits for endocytosis (29). Studies with other receptors suggest that, in ad-

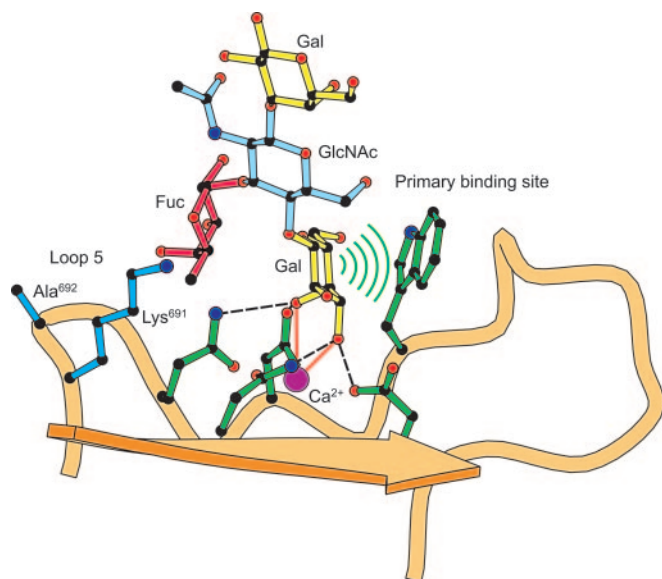


FIG. 8. **Model of SRCL-binding site.** Crystal structures of Lewis^x and a galactose-binding mutant of mannose-binding protein were combined to model SRCL. The primary binding site accommodates galactose through hydrogen bonding (*dashed black lines*), coordination bonds to Ca²⁺ (*solid red lines*), and hydrophobic packing of the B face against the side chain of a tryptophan residue (*green curves*). Possible secondary interactions of fucose would involve loop 5. This figure was generated using Molscript (28).

dition to such sorting signals, clearance activity requires the receptor be capable of ligand release at endosomal pH. Therefore, the ability of SRCL to release ligand in a pH-dependent manner was examined (Fig. 9). The results demonstrate that binding of carbohydrate ligands to SRCL is extremely dependent on pH, with a midpoint for release of ligand at a physiological Ca²⁺ concentration of approximately pH 6.5. This value is very similar to that observed for the asialoglycoprotein receptor, DC-SIGN, and other C-type lectins that function in endocytosis (24, 30). The steepness of the curve suggests that the transition from a binding to a nonbinding conformation involves titration of multiple groups in the CRD.

Based on these results, the endocytic activity of SRCL was investigated by expressing the full-length receptor in rat fibroblasts. Western blotting of cell extracts with antibodies raised to the CRD of SRCL was used to confirm that the protein was expressed, and endocytic activity was measured using ¹²⁵I-LNFP III-BSASY as a test ligand. Following an initial interaction with the cells, the ligand is gradually degraded and released back into the medium in acid-soluble form. The turnover calculated from an estimate of the number of receptors/cell is similar to that for other endocytic receptors in the C-type lectin family expressed in the same cell background. Similar results were obtained with ¹²⁵I-Gal-BSA. These results confirm that the receptor can mediate endocytosis and might be able to clear a selected set of asialoglycoproteins, including asialo-orosomucoid.

DISCUSSION

Similar to SRCL, serum mannose-binding protein and other collectins contain C-type CRDs, collagen-like domains and regions consisting of coiled coils of α helices (31). Because of the well established roles of several of the collectins in innate immunity, a similar role has been suggested for SRCL (1, 2). The results presented here, as well as the evolutionary considerations discussed in the Introduction, suggest that other possible functions for SRCL need to be considered.

The fact that bacteria and yeast can interact with SRCL is

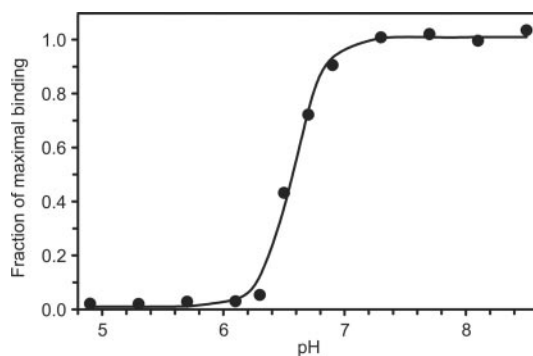


FIG. 9. **pH dependence of ligand binding to SRCL.** The curve was fitted to a binding equation in which both the pH of half-maximal binding and the order of the reaction were allowed to vary (30). Based on three separate assays, half-maximal binding is observed at pH 6.54 ± 0.04 , $n = 3.7 \pm 0.7$.

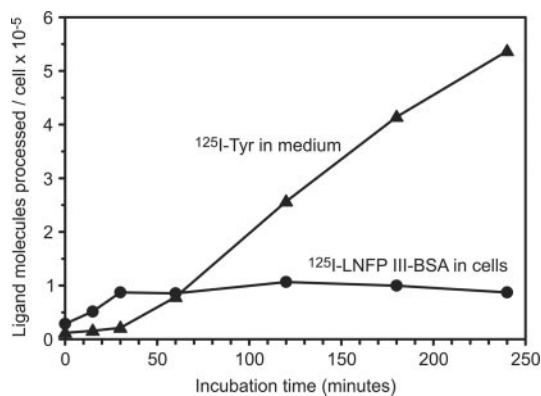


FIG. 10. **Uptake and degradation of ¹²⁵I-LNFP III-BSA by fibroblasts expressing SRCL.** Cells were preincubated for 30 min at 4 °C with ligand at a concentration of 2 μ g/ml. Following incubation at 37 °C for the times indicated, the cells were returned to 4 °C, the incubation medium collected, and the cells were washed three times with cold medium not containing ligand. Cell-associated radioactivity and acid-soluble degradation products released back into the medium were quantified.

consistent with a role in innate immunity (1, 2). However, it is important to note that SRCL is found on endothelial cells rather than cells such as macrophages or dendritic cells that are usually associated with the innate immune response. In addition, the way that SRCL interacts with microbes is very different from the way that the collectins bind to them. The CRDs of the collectins fall into the subcategory of C-type lectins that bind to mannose, GlcNAc, and related sugars (3). They bind weakly to individual sugars and achieve selective binding to the surfaces of bacteria and fungi by interacting with repeating arrays of sugars in the walls of these micro-organisms (32). The data on binding selectivity presented here indicate that the CRD of SRCL falls into the other subcategory of C-type CRDs, which bind galactose and related sugars in the primary binding site. This specificity is not typical of pathogen-binding molecules. Indeed, a truncated form of SRCL lacking the CRD seems to retain the ability to bind micro-organisms, leading to the suggestion that the binding site lies on the collagen-like domain (2). In contrast, the collagen-like domain of serum mannose-binding protein interacts with serine proteases that activate complement (33). Thus, SRCL differs from the collectins both in arrangement of domains and the functions of these domains.

The preferred ligands for SRCL have adjacent terminal galactose and fucose residues. Thus, SRCL shares with DC-SIGN the ability to bind Lewis^x-containing oligosaccharides (24). However, DC-SIGN binds a much broader range of fucose-

containing ligands, and the relative roles of fucose and galactose in the binding interaction are reversed. The different localizations of SRCL and DC-SIGN suggest that, despite their overlapping ability to bind the Lewis^x structure, they would probably target different glycoconjugate ligands.

The highly specific binding of SRCL to the Lewis^x trisaccharide suggests other possible roles for this receptor in the selective clearance of specific desialylated glycoproteins from circulation or selective interaction of cells bearing Lewis^x-type structures with the vascular endothelium. These roles would be more analogous to the functions of the asialoglycoprotein receptor (34) and the selectin cell adhesion molecules (35). Similar to the hepatic asialoglycoprotein receptor, SRCL has the ability to bind asialo-orosomucoid. Differences in the number of receptors may explain why the liver receptor normally provides the predominant route for clearance of this glycoprotein from circulation. SRCL might provide an alternative mechanism for clearance of some asialo-glycoprotein in asialoglycoprotein receptor-deficient mice (36, 37), although most asialoglycoproteins (which lack outer arm fucose residues) would not be taken up by this receptor.

The distribution of SRCL on vascular endothelial cells suggests parallels with the selectins, which bind to elaborated versions of the Lewis^x trisaccharide, bearing sialic acid and sulfate groups (35). The restricted binding to a specific glycan structure suggests possible roles in cell adhesion analogous to the leukocyte-endothelial interactions mediated by the selectins. E- and P-selectin are selectively expressed at sites of inflammation, whereas SRCL seems to be constitutively expressed on a wide variety of endothelial cells, although possible modulation of SRCL expression remains to be investigated. Knowledge of the glycan-binding selectivity of SRCL will also allow further study of possible glycoprotein and cellular target ligands.

Acknowledgments—We thank Richard Alvarez and Angela Lee of the Consortium for Functional Glycomics for screening the glycan array and Yuan Guo and Alex Powlesland for assistance with the sedimentation experiments. The University of Oxford Analytical Ultracentrifugation Facility is supported by the Wellcome Trust and the Biotechnology and Biological Sciences Research Council.

REFERENCES

1. Ohtani, K., Suzuki, Y., Eda, S., Kawai, T., Kase, T., Keshi, H., Sakai, Y., Fukuhara, A., Sakamoto, T., Itabe, H., Suzutani, T., Ogasawara, M., Yoshida,

- I., and Wakamiya, N. (2001) *J. Biol. Chem.* **276**, 44222–44228
2. Nakamura, K., Funakoshi, H., Miyamoto, K., Tokunaga, F., and Nakamura, T. (2001) *Biochem. Biophys. Res. Commun.* **280**, 1028–1035
3. Drickamer, K. (1992) *Nature* **360**, 183–186
4. Iobst, S. T., and Drickamer, K. (1994) *J. Biol. Chem.* **269**, 15512–15519
5. Yoshida, T., Tsuruta, Y., Iwasaki, M., Yamane, S., Ochi, T., and Suzuki, R. (2003) *J. Biochem. (Tokyo)* **133**, 271–277
6. Peiser, L., Mukhopadhyay, S., and Gordon, S. (2002) *Curr. Opin. Immunol.* **14**, 123–128
7. Han, H.-J., Tokino, T., and Nakamura, Y. (1998) *Hum. Mol. Genet.* **7**, 1039–1046
8. Grayeb, J., Kimura, H., Takahara, M., Hsiung, H., Masui, Y., and Inouye, M. (1984) *EMBO J.* **3**, 2437–2442
9. Fornstedt, N., and Porath, J. (1975) *FEBS Lett.* **57**, 187–191
10. Matsudaira, P. (1987) *J. Biol. Chem.* **262**, 10035–10038
11. Taylor, M. E., Bezouska, K., and Drickamer, K. (1992) *J. Biol. Chem.* **267**, 1719–1726
12. Taylor, M. E., and Drickamer, K. (1993) *J. Biol. Chem.* **268**, 399–404
13. Kaufman, R. J., Davies, M. V., Wasley, L. C., and Michnick, D. (1991) *Nuc. Acids Res.* **19**, 4485–4490
14. Graham, F. L., and van der Eb, A. J. (1973) *Virology* **52**, 456–467
15. Maddon, P. J., Littman, D. R., Godfrey, M., Maddon, D. E., Chess, L., and Axel, R. (1985) *Cell* **42**, 93–104
16. Southern, P. J., and Berg, P. (1982) *J. Mol. Appl. Genet.* **1**, 327–341
17. Stambach, N. S., and Taylor, M. E. (2003) *Glycobiology* **13**, 401–410
18. Burnette, W. N. (1981) *Anal. Biochem.* **112**, 195–203
19. Cohn, E. J., and Edsall, J. T. (1943) *Proteins, Amino Acids and Peptides as Ions and Dipolar Ions*, pp. 370–381, Reinhold, New York
20. Bolton, A. E., and Hunter, W. M. (1973) *Biochem. J.* **133**, 529–539
21. Feizi, T., Stoll, M. S., Yuen, C.-T., Chai, W., and Lawson, A. M. (1994) *Methods Enzymol.* **230**, 484–519
22. Greenwood, F. C., Hunter, W. M., and Glover, J. S. (1963) *Biochem. J.* **89**, 114–123
23. Kolatkar, A., and Weis, W. I. (1996) *J. Biol. Chem.* **271**, 6679–6685
24. Guo, Y., Feinberg, H., Conroy, E., Mitchell, D. A., Alvarez, R., Blixt, O., Taylor, M. E., Weis, W. I., and Drickamer, K. (2004) *Nat. Struct. Mol. Biol.* **11**, 591–598
25. Mellow, T. E., Halberg, D., and Drickamer, K. (1988) *J. Biol. Chem.* **263**, 5468–5473
26. Treuheit, M. J., Costello, C. E., and Halsall, H. B. (1992) *Biochem. J.* **283**, 105–112
27. Ng, K. K.-S., Drickamer, K., and Weis, W. I. (1996) *J. Biol. Chem.* **271**, 663–674
28. Kraulis, P. J. (1991) *J. Appl. Crystallogr.* **24**, 946–950
29. Traub, L. M. (2003) *J. Cell Biol.* **163**, 203–208
30. Wragg, S., and Drickamer, K. (1999) *J. Biol. Chem.* **274**, 35400–35406
31. Weis, W. I., and Drickamer, K. (1994) *Structure (Lond.)* **2**, 1227–1240
32. Weis, W. I., Taylor, M. E., and Drickamer, K. (1998) *Immunol. Rev.* **163**, 19–34
33. Wallis, R., Shaw, J. M., Uitdehaag, J., Chen, C.-B., Torgersen, D., and Drickamer, K. (2004) *J. Biol. Chem.* **279**, 14065–14073
34. Spiess, M. (1990) *Biochemistry* **29**, 10008–10019
35. Vestweber, D., and Blanks, J. E. (1999) *Physiol. Rev.* **79**, 181–213
36. Ishibashi, S., Hammer, R., and Herz, J. (1994) *J. Biol. Chem.* **269**, 27803–27806
37. Tozawa, R., Ishibashi, S., Osuga, J., Yamamoto, K., Yagyu, H., Ohashi, K., Tamura, Y., Yahagi, N., Iizuka, Y., Okazaki, H., Harada, K., Gotoda, T., Shimano, H., Kimura, S., Nagai, R., and Yamada, N. (2001) *J. Biol. Chem.* **276**, 12624–12628