The Cysteine-rich Domain of the Macrophage Mannose Receptor Is a Multispecific Lectin That Recognizes Chondroitin Sulfates A and B and Sulfated Oligosaccharides of Blood Group Lewisα and Lewisβ Types in Addition to the Sulfated N-Glycans of Lutropin

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Abstract

The mannose receptor (MR) is an endocytic protein on macrophages and dendritic cells, as well as on hepatic endothelial, kidney mesangial, tracheal smooth muscle, and retinal pigment epithelial cells. The extracellular portion contains two types of carbohydrate-recognition domain (CRD): eight membrane-proximal C-type CRDs and a membrane-distal cysteine-rich domain (Cys-MR). The former bind mannose-, N-acetylglucosamine-, and fucose-terminating oligosaccharides, and may be important in innate immunity towards microbial pathogens, and in antigen trapping for processing and presentation in adaptive immunity. Cys-MR binds to the sulfated carbohydrate chains of pituitary hormones and may have a role in hormonal clearance. A second feature of Cys-MR is binding to macrophages in marginal zones of the spleen, and to B cell areas in germinal centers which may help direct MR-bearing cells toward germinal centers during the immune response. Here we describe two novel classes of carbohydrate ligand for Cys-MR: chondroitin-4-sulfate chains of the type found on proteoglycans produced by cells of the immune system, and sulfated blood group chains. We further demonstrate that Cys-MR interacts with cells in the spleen via the binding site for sulfated carbohydrates. Our data suggest that the three classes of sulfated carbohydrate ligands may variously regulate the trafficking and function of MR-bearing cells.

Key words: chondroitin sulfate • cysteine-rich domain • lutropin (luteinizing hormone) • macrophage receptor • sulfo-Lewisα/β

Introduction

The mannose receptor (MR) is a type I transmembrane protein with an extracellular portion consisting of eight membrane-proximal C-type carbohydrate-recognition domains (CRDs), followed by a domain containing a fi-
blonectin type II repeat, and a membrane-distal cysteine-rich domain (Cys-MR; references 1, 2). M. R. was originally identified on macrophages, but it was also expressed on dendritic cells, hepatic endothelial cells, tracheal smooth muscle cells, retinal pigment epithelium, kidney mesangial cells, and Kaposi sarcoma cells (3). M. R. is a carbohydrate-recognition receptor that binds to mannose, fucose, and N-acetylgalactosamine via the CRDs (4). The CRDs mediate binding to a variety of polysaccharides such as those at the surface of pathogenic microorganisms, and the receptor has endocytic and phagocytic properties conferred by the cytoplasmic domain. These features render M. R. s important for macrophage uptake of bacteria, yeasts, and parasites, and thereby may contribute to innate immunity toward these pathogens (3). The endocytic function of M. R. is also important in adaptive immunity, namely, in the uptake of antigens by immature dendritic cells and their delivery to MHC class II compartments for antigen processing and presentation (5). The CRDs are not the only lectin domains on M. R. The Cys-MR has been shown to bind to the sulfated carbohydrate chains of the pituitary hormones, lutropin, and thyroid-stimulating hormones (6–9). The recognition motif is on the 4-sulfated N-acetylgalactosamine-terminating sequence GalNAc(4S)β1-4GlcNAc β1-2Man, which occurs on the N-glycans of these glycoproteins (10). It has been proposed (11) that Cys-MR on hepatic endothelial cells has a role in the rapid clearance of lutropin from the serum, which is important for maintaining hormone responsiveness in vivo. There is recent evidence that additional binding of lutropin, and also thyrotropin, occurs via nonsulfated carbohydrate domains to the CRDs of M. R. (12). A recombinant, soluble form of Cys-MR has been shown to bind to metallophilic macrophages in the marginal zones of the spleen, and in the subcapsular sinuses of lymph nodes, to cells in B cell areas, some with dendritic morphology in nascent germinal centers. It has also been proposed that the Cys-MR may help to direct M. R.-bearing cells toward germinal centers during immune responses (13). Moreover, macrophages secrete a soluble form of M. R., which may have a role in binding to and directing mannose-bearing antigens to cells bound by Cys-MR at sites where humoral immune responses are orchestrated (13, 14).

In the course of performing carbohydrate-recognition studies on the cysteine-rich domain (Cys-DEC) of the dendritic cell receptor (DEC-205), a transmembrane protein with a modular architecture similar to M. R. (15), we carried out parallel carbohydrate-binding experiments with Cys-MR. We found a lack of Cys-DEC binding to lutropin, but identified two additional types of sulfated carbohydrate recognition elements for Cys-MR. Here we describe glycosaminoglycan sequences of chondroitin sulfate type, and also sulfated oligosaccharide sequences of the blood group Lewis’ (Le”) and Le” series, which are recognized by Cys-MR, and provide evidence for the existence of a wider range of counterreceptors than hitherto anticipated for the multifunctional M. R.

Materials and Methods

Recombinant Soluble Cysteine-Rich Domain of the Mouse Macrophage M. R. A soluble form of the cysteine-rich domain of the murine macrophage mannose receptor (Cys-MR) fused to the Fc domain of human IgG (Cys-MR-Fc) was expressed and purified as described (13). Cys-DEC-Fc is a fusion protein containing the cysteine-rich domain of DEC-205 (15) and the Fc domain of human IgG. A spacer consisting of EGS was placed between the GPY-H at the end of the Cys-DEC and Igh. Cys-DEC-Fc was expressed and purified exactly as described for Cys-MR-Fc.

Olygosaccharides. Highly purified porcine lutropin (pLH SD477, 2.0 × LH NIH S1) was prepared essentially as described (16). Human lutropin (hLH), chondroitin sulfate A (CS-A, from bovine trachea containing 30% of CS-C), CS-B (from porcine intestinal mucosa containing 10% of CS-A and -C), CS-C (from shark cartilage containing 10% of CS-A), and heparin (from porcine intestinal mucosa) were from Sigma Chemical Co.

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graphed on an APS-2 (Hypersil, Astmoor) HPLC column (5 μm, 4.6 mm × 250 mm; Phenomenex) with monitoring at 206 nm. Elution was carried out with a linear gradient of N aH₄PO₄ (solvent C, 5 mM N aH₄PO₄ and solvent D, 50 mM N aH₄PO₄, from 10 to 50% solvent D in 40 min) at a flow rate of 1 ml/min. The major peak designated F3-2 was pooled, freeze dried, and desalted on a Sephadex G-10 column (1.6 × 36 cm). This in turn gave exclusively the two [M-H]⁻ ions at m/z 1,541, and 1,395 noted above. The oligosaccharide sequence in F3-2 was corroborated by the molecular ions and fragment ions of the derived neoglycolipids (NGLs, Fig. 1). The molecular ions at m/z 2,188 and 2,042 together with their sodiated ions at m/z 2,210 and 2,064 are consistent with those of NGLs of a sulfated monoantennary N-glycan with and without a fucose residue. The fragment ion at m/z 2,108 corresponds to the loss of HSO₃⁻ following oligosaccharides were synthesized chemically: 3-sialyl-Lewis₉ pentasaccharide, 3Su-Lex (24); 3-sulfated-Lex pentasaccharide, 3S-Su-Lex (25); and 6'-sulfated-Le'₉ pentaaccharide, 6Su-Le'₉ (25). The 3'-sulfated-lacto-N-tetraose, 3S-LNT, and 3'-sulfated-LNNT, 3S-LNNT, were prepared by mild acid hydrolysis (27) of 3S-Lee and 3S-Le'e, respectively.

NGLs. NGLs, unless otherwise stated, were prepared by the conjugation of oligosaccharides to the aminophospholipid L-1,2-dihexadecyl-sn-glycero-3-phosphoethanolamine (DHPE; Fluka), followed by isolation from the reaction mixtures by TLC as described previously (28). A modified procedure was used for the preparation of NGLs from CS oligosaccharides. In brief, lyophilized CS oligosaccharides (100 nmol) were dissolved in 5 μl H₂O, and 100 μl of DHPE solution (5 mg/ml in CHCl₃/MeOH 1:3, vol/vol) was added, followed by 20 μl of freshly prepared tetrabutylammonium cyanoborohydride (20 μg/ml in MeOH). The reaction mixtures were heated at 60°C for 70 h, conjugation was monitored by high performance TLC, and NGLs were purified using silica minicolumns (500 mg silica, Sep-Pak™ Vac Cartridge; Waters Corp.) essentially as described previously (29).

Biologically labeled oligosaccharides. Oligosaccharides were conjugated to 6-(biotinyl)-aminocapryl-hydrazide (BACH; Sigma Chemical Co.) and characterized as described previously (30, 31). In brief, the oligosaccharides were reacted with BACH for 16 h in methanol/water 95:5, vol/vol, purified by HPLC, and its chemical structures were corroborated by mass spectrometry.

Liquid Secondary Ion Mass Spectrometry. Negative-ion liquid secondary ion mass spectrometry (LSIMS) was carried out on a VG Analytical ZAB-2E mass spectrometer (VG Analytical) equipped with a cesium ion gun operated at 25 keV with an emission current of 0.5 μA. For the native and biotinylated oligosaccharides, 1 μg of sample was used for analysis with thioglycerol as the liquid matrix. NGLs were analyzed by in situ TLC/LSIMS with a mixture of diethanolamine/tetramethylurea/thioglycerol as the liquid matrix. NGLs, unless otherwise stated, were prepared by the conjugation of oligosaccharides to the aminophospholipid L-1,2-dihexadecyl-sn-glycero-3-phosphoethanolamine (DHPE; Fluka), followed by isolation from the reaction mixtures by TLC as described previously (28). A modified procedure was used for the preparation of NGLs from CS oligosaccharides. In brief, lyophilized CS oligosaccharides (100 nmol) were dissolved in 5 μl H₂O, and 100 μl of DHPE solution (5 mg/ml in CHCl₃/MeOH 1:3, vol/vol) was added, followed by 20 μl of freshly prepared tetrabutylammonium cyanoborohydride (20 μg/ml in MeOH). The reaction mixtures were heated at 60°C for 70 h, conjugation was monitored by high performance TLC, and NGLs were purified using silica minicolumns (500 mg silica, Sep-Pak™ Vac Cartridge; Waters Corp.) essentially as described previously (29).

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Binding Experiments. Binding of Cys-MR-Fc or of Cys-DEC-Fc, 0.5 μg/well (preincubated with anti-human IgG, at a chimeric to anti-IgG ratio of 1:3 [wt/wt]) to glycoproteins or to NGLs (reaction volumes 50 μl), was assayed essentially as described previously for L-selectin (33). For inhibition of Cys-MR binding to hLH (0.5 μg hLH added/well), the Cys-MR-Fc was used at 0.25 μg/well, which gave an absorbance in the range of 0.75–0.79 corresponding to 50% of maximum absorbance. Cys-MR binding was also examined to serglycin immobilized on microwells (1.5 μg added/well) and treated at 30°C for 4 h with either chondroitinase ABC (50 μU in 50 μl of 10 mM phosphate buffer, 150 mM NaCl, pH 7.5 [PBS]), with the heat-denatured enzyme, with heparinase I (2.5 U), or with PBS alone. After washing four times with PBS, binding of Cys-MR-Fc, 0.5 μg added/well, was assayed as above.

Figure 1. Mass spectrum of the NGLs derived from the sulfated N-glycan, pLH-F3-2, isolated from lutropin. The molecular ions and fragment ions correspond to those of the monoantennary N-glycan sequence illustrated, with and without a core fucose residue, which was described previously as one of the major oligosaccharide species in human lutropin (reference 10).
Binding of Cys-MR-Fc to biotinylated oligosaccharides was assayed essentially as described previously for plant lectins (30) and selectins (31). In brief, biotinylated oligosaccharides were immobilized in high capacity streptavidin-coated microtiter wells (Boehringer Mannheim) in TBS (10 mM Tris-HCl buffer, pH 8.0, 150 mM NaCl). Cys-MR-Fc chimera was at 5 μg/well (preincubated for 1 h with rabbit anti-human IgG, at a chimeric to anti-IgG ratio of 1:3 [wt/vol]). The diluted was TBS containing 0.5% (wt/vol) BSA, and reaction volumes were 200 μl. Binding was detected with peroxidase-labeled protein A (10 μg/ml) using ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-sulfonic acid) as substrate, and absorbance was read at 405 nm.

**Results**

Histochemical Experiments. Histochemical staining of spleens from nonimmunized C57 mice and mice 9-10 d after intraperitoneal immunization with 100 μg of chicken gammaglobulin precipitated in alum (Jackson ImmunoResearch Laboratories) was performed essentially as described (13). In brief, 8-μm cryostat sections were fixed for 12 min in acetone at 4°C or in 2% formaldehyde in PBS at 18°C. Fixed sections were washed in PBS, and Fc receptors were blocked for 10 min at 18°C using rat anti-mouse C16/CD32 (Becton Dickinson) at 10 μg/ml in 5% (vol/vol) donkey serum-PBS. Sections were incubated for 30 min at 18°C with the Cys-MR-Fc chimera at 10 μg/ml in donkey serum-PBS. Binding was detected using fluorescein-labeled donkey anti-human IgG (Jackson ImmunoResearch Laboratories) for 30 min at 18°C, and sections were viewed with a Zeiss ultraviolet epifluorescence microscope. Specificity of staining was corroborated by the lack of staining of sections similarly incubated with a recombinant human Fc dimer (gift of Dr. E. Hofer, University of Vienna, Vienna, Austria). B cell areas in the splenic white pulp were identified by staining with rat anti–mouse complement receptor type 1 (CD35; Becton Dickinson) at 10 μg/ml. Antibody binding was detected using rhodamine-labeled donkey anti–rat IgG (Jackson ImmunoResearch Laboratories).

In certain experiments, the fixed spleen sections were treated for 30 min at 37°C with chondroitinase ABC (500 μU/ml) or with heparinase I (100 μU/ml) diluted in PBS containing 0.05% (vol/vol) Tween 20 and 1 mM PMSF (Sigma Chemical Co.). Enzyme-treated sections were stained in both single and dual immunolabeling experiments with a rabbit antisera (1:100 dilution) specific for the core regions of cleaved CS chains (Biogenes.s Ltd.) and with the Cys-MR-Fc. Binding of the CS antibody was detected using rhodamine-labeled donkey anti–rabbit IgG (Jackson ImmunoResearch Laboratories). The staining patterns for the Cys-MR-Fc and the CS antibody were viewed simultaneously with a Zeiss LSM 510 confocal microscope, and apparent colocalization in selected areas was confirmed by Z sectioning.

For inhibition studies, Cys-MR-Fc was incubated for 1 h at 18°C with the monosaccharides GalNAc, 4-sulfated GalNAc, or 6-sulfated GalNAc (Sigma Chemical Co.) at 5 mg/ml before application onto spleen sections.

**Figure 2.** Binding experiments with Cys-MR and Cys-DEC. NGLs (A-C), lutropin (A, inset), and biotinylated oligosaccharides (D) were immobilized on microwells, and the binding of Cys-MR-Fc or Cys-DEC-Fc, 5 μg/well, was assayed as described in Materials and Methods.

Results

In accord with previous data, the Cys-MR-Fc gave a binding signal with lutropin. In contrast, no binding was detected with Cys-DEC (Fig. 2 A, inset). The NGL derived from the sulfated N-glycan, F3-2, isolated from lutropin was also strongly bound by Cys-MR-Fc (Fig. 2 A). As the Cys-MR binding was inhabitable with 4-sulfated N-acetylgalactosamine (concentration giving 50% inhibition of binding [IC50] 0.18 mM), and less strongly with 6-sulfated and 4,6-disulfated N-acetylgalactosamine (IC50 values 1.34 and 0.85 μM, respectively; Fig 3 A), we examined the protein for recognition of glycans with various sulfation patterns as described below, and see Table I.

Cys-MR R recognition of Chondroitin 4-Sulfate Sequences. Initial inhibition of binding experiments with the commercially available chondroitin sulfates, CS-A, CS-B and CS-C, and heparin (Fig. 3 B) revealed an inhibitory activity in the sample of CS-B. This inhibitor consists predominantly of the repeating disaccharide sequence of 4-sulfated N-acetylgalactosamine [GalNAc(4S)] joined by β1-4 linkage to iduronic acid (IdoA) as follows -[3GalNAc(4S)1-4IdoAβ1-]. There was negligible inhibition by the CS-C polysaccharide, which contained predominantly 6-sulfated N-acetylgalactosamine [GalNAc(6S)] joined by β1-4 linkage to glucuronic acid (GlcA); -[3GalNAc(6S)1-4GlcAβ1-], and by heparin, which contain predominantly N- and 6-sulfa-
fated glucosamine [GlcNS(6S)] joined by β1-4 linkage to 2-sulfated iduronic acid: [-4GlcNS(6S)β1-4IdoA(2S)]₃. The CS-A polysaccharide also gave negligible inhibition despite the fact that it contains predominantly GalNAc(4S), joined by β1-4 linkage to glucuronic acid: [-3GalNAc(4S)β1-4GlcAβ1-]. Knowing the heterogeneity of sequences, and of the terminal monosaccharides in glycoconjugates, we prepared structurally defined oligosaccharides (Table I) from the three CS polysaccharides, and evaluated their NGLs for binding by Cys-MR.

The NGLs of the CS-B and CS-A pentasaccharides, CS-B5 and CS-A5, were bound by Cys-MR-Fc (Fig. 2, A and B). These pentasaccharides have a common terminal GalNAc(4S) in contrast to the hexasaccharide, the CS-A hexasaccharide, CS-A6, which terminates with the unsaturated uronic acid, DUAβ1-3, did not give a detectable binding signal with Cys-MR-Fc despite the presence of three GalNAc(4S) residues along the oligosaccharide chain (Table I). First, these results show that Cys-MR recognizes GalNAc(4S) in a terminal (nonreducing end), rather than an internal position. Therefore, the lack of inhibitory activity in the original sample of CS-A polysaccharide suggests that the amount of terminal GalNAc(4S) is likely to be lower than in the CS-B polymer. Second, the carbohydrate-binding site of Cys-MR can accommodate...
oligosaccharides terminating with GalNAc(4S) joined by β1-4 linkage to glucuronic acid or iduronic acid, as well as to N-acetylgalactosamine, as found on the N-glycans of lutropin. The CS-C pentasaccharide, CS-C5, containing predominantly the terminal GalNAc(6S)β1-4GlcAβ1-3 sequence (Table I), gave a negligible binding signal with Cys-MR-Fc, in accordance with the weak inhibitory activity of the monosaccharide GalNAc(6S) relative to GalNAc(4S).

Cys-MR Recognition of Sulfated Blood Group Chains. We evaluated Cys-MR binding to the NGLs and biotinylated forms of sulfated oligosaccharides of the blood group series, based on the type 1 and type 2 backbone sequences with galactose joined by β1-3 and β1-4 linkage to N-acetylgalactosamine: Galβ1-3GlcNAc and Galβ1-4GlcNAc, respectively (Fig. 2, A, C, and D). Clearly, there is recognition of sulfated forms of both types of blood group chains when there is sulfate at position 3 of the terminal galactose. Moreover, there is binding to the corresponding sulfated Leα and Leβ sequences, which contain fucose joined by α1-4 and α1-3 linkage to the N-acetylgalactosamine of the type 1 and type 2 backbones, respectively. Overall, there is a preference for the type 2 analogues over the type 1, and for the nonfucosylated chains over the fucosylated forms. There was no detectable binding to the 6-sulfated-Leβ sequence, or to the 3S-Leα and 3S-Leβ sequences.

Inhibition by Sulfated N-Acetylgalactosamine of the Cys-MR Binding to Cells in the Spleen. Cys-MR-Fc showed strong staining of large cells with granular cytoplasm in the marginal zone of spleen from naive and immunized mice, as described previously by Martínez-Pomares et al. (13; Fig. 4 A). There were weaker punctate staining of groups of cells in the splenic white pulp within B cell areas (Fig. 5 B), which were identified by their strong staining with anti-CD35. Staining by Cys-MR-Fc of cells in this region was greater in immunized mice. There was no staining of spleen sections from naive or immunized mice when similarly incubated with the recombinant human Fc dimer (results not shown).

The Cys-MR-Fc staining of naive and immunized mouse spleen was completely inhibited in the presence of 4-sulfated N-acetylgalactosamine (Fig. 4 B), markedly diminished in the presence of the 6-sulfated analogue (Fig. 4 C), but unaffected in the presence of the nonsulfated N-acetylgalactosamine (Fig. 4 D).

Distribution of CS Immunostaining and Cys-MR Binding in the White Pulp of Spleen, and Binding of Cys-MR to Proteoglycans Secreted by Monocytes. As cells of the immune system are known to produce cell-associated and secreted forms of CS proteoglycans, the amounts and proportions of which may change after various stimuli (34–36), we compared the immunostaining pattern for CS chains with that of Cys-

Figure 5. Confocal images of a dual immunolabeling experiment showing the similar distribution and limited colocalization of the binding of Cys-MR and an antibody to the core regions of CS chains within the B cell areas of the splenic white pulp. Before immunolabeling, spleen sections from immunized mice were treated with chondroitinase ABC as described in Materials and Methods. The sections were thereafter overlaid with a rabbit antiserum, directed at the core region of cleaved CS chains, and Cys-MR-Fc, followed by rhodamine-labeled anti-rabbit IgG (anti-CS binding, A) and fluorescein-labeled anti-human IgG (Cys-MR binding, B). The two staining patterns are shown superimposed in C. The area within the white square in C is shown enlarged in D. Original magnifications (A–C) × 2,000; (D) × 10,000.
Discussion

Here we establish that the ligands of Cys-MR encompass two classes of oligosaccharide chains additional to the described previously (10) N-glycans on pituitary hormones, which terminate in 4-sulfated N-acetylgalactosamine β1-4 linked to N-acetylgalactosamine. The additional classes of ligands include sulfated glycosaminoglycans and sulfated blood group chains. The glycosaminoglycans strongly bound are of chondroitin 4-sulfate type having in common a terminal 4-sulfated N-acetylgalactosamine that is joined by β1-4 linkage to iduronic acid (CS-B) or to glucuronic acid (CS-A). In the sulfated blood group chains that are bound by Cys-MR, there is a terminal 3-sulfated galactose β1-4 or β1-3 linked to N-acetylgalactosamine.

The mechanism whereby the Cys-MR can accommodate the three classes of oligosaccharide ligand is elucidated by the structural study of the Cys-MR complexed with 4-sulfated N-acetylgalactosamine (38). The crystal structure shows that the sulfate group is involved in no less than six hydrogen bonds with the protein; the N-acetyl group has no contact, however, with the binding site of Cys-MR. This explains our finding that a sulfated galactose can be bound as well as a sulfated N-acetylgalactosamine. A structural explanation is also provided for the weak inhibitory activity we have observed in the present investigation for the 6-sulfated N-acetylgalactosamine (IC_{50} 1.3 mM) compared with that for the 4-sulfated form (IC_{50} 0.18 mM).

Molecular modeling by Liu et al. (38) based on the crystal structure shows that for optimum binding, the sulfate group must be at C-3 or C-4 of the terminal monosaccharide. When GalNAc is sulfated at C-6, it does not make favorable van der Waals contacts with Trp117 of the Cys-MR binding site. The modeling studies also show that the addition of a monosaccharide at C-3 of the 4-sulfated N-acetylgalactosamine would hinder the binding of the sulfate. This accounts for our observation that the hexosaminoglycans CS-A6 with a terminal uronic acid linked to C-3 of N-acetylgalactosamine was not bound by Cys-MR. We can now also explain the preferential binding of Cys-MR to the 3Su-Le^a over the 3Su-Le^b analogue. The modeling, based on the crystal structure, shows that the sulfated galactose and the fucose of Lex can be stacked readily against Trp117, whereas in the Le^b analogue, there is steric hindrance between the N-acetyl group and Asn102 in the Cys-MR binding site.

In contrast to Cys-MR, the cysteine-rich domain of DEC-205, another member of the MR family, did not give a binding signal with lutropin in the present investigation. The structural basis for this finding is apparent from the x-ray crystallographic study of the Cys-MR-ligand complex by Liu et al. (38) which defines the amino acid residues used to bind the sulfated carbohydrates. The amino acids in the analogous region are lacking in the Cys-DEC sequence.

A new finding in the present investigation is that the binding described for Cys-MR with cells both in the marginal zones and in the B cell areas of the naive and the immune spleen (13) is mediated by the binding site for sulfated carbohydrates. We further provide evidence that Cys-MR binds secreted proteoglycans of cells of the immune system, the major component of which is serglycin (36). Binding to serglycin is via the CS chains, which are known to be predominantly of chondroitin 4-sulfate type (37).

Serglycin accumulates in intracellular vesicles of cells of the immune system, and is secreted by the activated cells (36). The amounts of such proteoglycans that are retained in cells, or are secreted, differ after various stimuli (39). Thus, in lymphoid tissues in vivo, the serglycin has the potential to form a complex with Cys-MR and modulate the availability of the saccharide-binding site of both the transmembrane form and the secreted form of MR. It has been

Figure 6. Binding of Cys-MR to serglycin and abolition of binding after treatment of the proteoglycan with chondroitinase ABC but not heparinase. We conclude that Cys-MR interacts with these soluble proteoglycans via CS chains.

In both naive and immunized mice, there was similar distribution of the staining for CS chains and Cys-MR binding within the B cell area of the white pulp (Fig. 5, A and B), but they differed in the marginal zone (not shown). There was no fluorescein staining observed after incubation of sections with a recombinant human Fc dimer (not shown); in addition, there was no rhodamine staining observed in these areas before and after treatment with chondroitinase ABC (results not shown). Thus, although similar, the two staining patterns for MR staining. In both naive and immunized mice, there are two classes of oligosaccharide chains additional to the described previously (10) N-glycans on pituitary hormones, which terminate in 4-sulfated N-acetylgalactosamine β1-4 linked to N-acetylgalactosamine. The additional classes of ligands include sulfated glycosaminoglycans and sulfated blood group chains. The glycosaminoglycans strongly bound are of chondroitin 4-sulfate type having in common a terminal 4-sulfated N-acetylgalactosamine that is joined by β1-4 linkage to iduronic acid (CS-B) or to glucuronic acid (CS-A). In the sulfated blood group chains that are bound by Cys-MR, there is a terminal 3-sulfated galactose β1-4 or β1-3 linked to N-acetylgalactosamine. The mechanism whereby the Cys-MR can accommodate the three classes of oligosaccharide ligand is elucidated by the structural study of the Cys-MR complexed with 4-sulfated N-acetylgalactosamine (38). The crystal structure shows that the sulfate group is involved in no less than six hydrogen bonds with the protein; the N-acetyl group has no contact, however, with the binding site of Cys-MR. This explains our finding that a sulfated galactose can be bound as well as a sulfated N-acetylgalactosamine. A structural explanation is also provided for the weak inhibitory activity we have observed in the present investigation for the 6-sulfated N-acetylgalactosamine (IC_{50} 1.3 mM) compared with that for the 4-sulfated form (IC_{50} 0.18 mM).

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Serglycin accumulates in intracellular vesicles of cells of the immune system, and is secreted by the activated cells (36). The amounts of such proteoglycans that are retained in cells, or are secreted, differ after various stimuli (39). Thus, in lymphoid tissues in vivo, the serglycin has the potential to form a complex with Cys-MR and modulate the availability of the saccharide-binding site of both the transmembrane form and the secreted form of MR. It has been...
shown recently (17) that serglycin in the serum is rapidly taken up by hepatic endothelial cells (also referred to as sinusoidal scavenger endothelial cells), and that a hyaluronan receptor plays a major role in the uptake. We propose that interactions of Cys-MR with serglycin in lymphatic tissue fluid, or with serglycin in homogenates of hepatic cells, may underlie the reported lack of demonstrable binding activity of certain tissue isolates of M R toward sulfated oligosaccharides (8).

Chondroitin 4-sulfate chains occur also on cell membrane-associated proteoglycans of lymphocytes, monocytes, macrophages, and other cells of the hematopoietic system (34). Changes in their carbohydrate chains have been documented during cellular activation (36). Notable among chondroitin 4-sulfate-containing proteoglycans at the cell surface are several variants of the signal-transducing molecule CD44 (40). It will be interesting to investigate if such glycosylation variants of CD44 are among counter-receptors for the Cys-MR.

An intriguing candidate counterreceptor for Cys-MR is the CS-modified form of the class II invariant chain (II) referred to as II-CS. While II-CS amounts to only a small proportion (2–5%) of Ii that associates with newly synthesized class II, it appears nevertheless to have an important role in antigen presentation functions (41). Evidence has been presented that expression of II-CS in antigen-presenting cells enhances antigen presentation and thereby enhances the triggering of primary antigen responses by T cells (41). Knowing that an abundant amount of M R is co-distributed with class II molecules in the specialized antigen-loading MHC II compartment (42), there is the potential for II-CS interactions with Cys-MR, and we raise the possibility that such interactions may occur, and thus facilitate, the class II–Ii dissociation, simultaneously facilitating class II–peptide antigen binding.

Chondroitin sulfate chains occur, par excellence, on proteoglycans in extracellular matrices (34, 43). The presence and the accessibility of Cys-MR ligands in extracellular matrices deserve investigation. Here may lie determinants of the trafficking of macrophages and their localization in pathological tissues, and also clues to the interactions of M R-bearing kidney mesangial, tracheal smooth muscle, and retinal pigment cells.

Carbohydrate sequences of the blood group family occur widely at the surface of cells. They occur on glycoproteins and glycolipids, and constitute a class of differentiation antigens, a reflection of the pronounced differentiation-associated changes they undergo in the branching patterns of their backbone regions and in the capping residues that they bear in their peripheral regions (44). The sialyl-Leα and -Leα sequences occur variously as markers of granulocytes, monocytes, or subsets of lymphocytes. The sulfogalactosyl-Leα and -Leα sequences are differentially expressed on various normal and malignant epithelial cells (45), and they may constitute ligands that mediate interactions of cancer cells with the selectins. In its ability to bind to the sulfogalactosyl-Leα and -Leα sequences, Cys-MR overlaps with the selectins (46), but it differs in its preference for the nonfucosylated analogues. The distribution of sulfated blood group chains on cells of the immune system needs to be determined. It will be interesting to investigate whether they occur on the acidic glycoforms of glycoproteins, such as sialoahesin or CD 45 of lymphoid tissue and the spleen, that are bound by Cys-MR (47, 48).

So far, we have considered endogenous ligands for Cys-MR. The repertoire of sequences recognized by this domain raises the possibility that certain acidic microbial polysaccharides may be bound. Thus, it will be interesting to determine whether, in an analogous way to the mannos-binding C-type lectin domains on M R, the Cys-MR contributes to trapping of ligand-positive microbial pathogens, and to the delivery of ligand-positive antigens for processing and presentation.

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