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 Lewis^a and Lewis^x Types in Addition to the Sulfated *N*-Glycans of Lutropin

By Christine Leteux,* Wengang Chai,* R. Wendy Loveless,* Chun-Ting Yuen,‡ Lars Uhlin-Hansen,§ Yves Combarnous,‖ Mila Jankovic,¶ Svetlana C. Maric,¶ Ziva Misulovin,¶** Michel C. Nussenzweig,¶** and Ten Feizi*

*From *The Glycosciences Laboratory, Imperial College School of Medicine, Northwick Park Hospital, Harrow HA1 3UJ, United Kingdom; the ‡Laboratory of Molecular Structure, National Institute for Biological Standards and Control, Hertfordshire EN6 3QG, United Kingdom; the §Department of Biochemistry, Institute of Medical Biology, University of Tromsø, N-9037 Tromsø, Norway; the ‖Institut National de la Recherche Agronomique, Unit 1291, Station Physiologie de la Reproduction des Mammifères Domestiques, 37380 Nouzilly, France; and the ¶Department of Molecular Immunology, and **The Howard Hughes Medical Institute, The Rockefeller University, New York, New York 10021-6399*

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-Lewis$^{a/x}$

Introduction

The mannose receptor (MR)[1] 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-

Address correspondence to Ten Feizi, The Glycosciences Laboratory, Imperial College School of Medicine, Northwick Park Hospital, Watford Road, Harrow, Middlesex HA1 3UJ, UK. Phone: 44-20-8869-3460; Fax: 44-20-8869-3455; E-mail: t.feizi@ic.ac.uk

[1]*Abbreviations used in this paper:* CRD, carbohydrate-recognition domain; CS, chondroitin sulfate; Cys-MR, cysteine-rich domain of the MR; ΔUA, 4,5-unsaturated uronic acid; ECM, extracellular matrix; Hex, hexose; HexNAc, *N*-acetylhexosamine; IC_{50}, half-maximal inhibitory concentration; Ii, invariant chain; LNNT, lacto-*N*-neotetraose; LSIMS, liquid secondary ion mass spectrometry; MR, mannose receptor; NGL, neoglycolipid; pLH, porcine lutropin (luteinizing hormone); 3S-Le^a, 3′-sialyl-Lewis^a; 3S-Le^x, 3′-sialyl-Lewis^x; 3Su-Le^a, 3′-sulfated-Lewis^a; 3Su-Le^x, 3′-sulfated-Lewis^x; 6Su-Le^x, 6′-sulfated-Lewis^x; 3Su-LNNT, 3′-sulfated-LNNT; TBS, Tris-buffered saline.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/2000/04/1117/10 $5.00
Volume 191, Number 7, April 3, 2000 1117–1126
http://www.jem.org/cgi/current/full/191/7/1117

bronectin type II repeat, and a membrane-distal cysteine-rich domain (Cys-MR; references 1, 2). MR 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). MR is a carbohydrate-recognizing receptor that binds to mannose, fucose, and *N*-acetylglucosamine 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 MRs important for macrophage uptake of bacteria, yeasts, and parasites, and thereby may contribute to innate immunity toward these pathogens (3). The endocytic function of MR 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 MR. 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 MR (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 MR-bearing cells toward germinal centers during immune responses (13). Moreover, macrophages secrete a soluble form of MR, 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 MR (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^x (Le^x) and Le^a 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 MR.

Materials and Methods

Recombinant Soluble Cysteine-rich Domain of the Macrophage MR. 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 ESG was placed between the GPYHEI at the end of the Cys-DEC and IgG. Cys-DEC-Fc was expressed and purified exactly as described for Cys-MR-Fc.

Glycoproteins, Proteoglycans, and Polysaccharides. 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.

CS proteoglycans were isolated from the conditioned, serum-free medium of the cultured monocytic cell line, THP-1 (17). In brief, the conditioned medium was subjected twice to Q-Sepharose anion-exchange chromatography. After extensive washing, the bound molecules were eluted with a gradient of 0.35–1.5 M NaCl in 0.5 M NaOAc, 6 M urea, pH 6.0. The proteoglycan-containing fractions, detected by safranin O staining (18), were pooled and desalted on a Sephadex G-50 (fine) column. After labeling with ^{125}I, the purified material was treated with chondroitinase ABC (Sigma Chemical Co.), and subjected to SDS-PAGE. A major band of ~14 kD appeared, consistent with the core protein of serglycin. The identity of the protein was confirmed by NH_2-terminal amino acid sequencing.

Oligosaccharides. The sequences of the oligosaccharides investigated in this study are shown in Table I.

Sulfated N-Glycan from pLH. *N*-glycans from 100 mg pLH were released by hydrazinolysis followed by re–*N*-acetylation (19). The solution was then desalted with 25 ml AG50W-X12 resin (H^+ form; Bio-Rad Laboratories) and freeze dried. Peptides were removed on a TSK Amide-80 (TosoHaas) HPLC column (4.6 × 250 mm) using a gradient of 80–20% acetonitrile in 2.5 mM trifluoroacetic acid and 10 mM NH_4OAc, pH 4.5 (solvent A and B, respectively) at a flow rate of 1 ml/min. Aliquots containing ~200 μg hexose were dissolved in and run for 3 min in 65% solvent A and 35% solvent B. A linear gradient rising to 75% solvent B was applied for 3 min, and the gradient was maintained for 2 min before decreasing to 35% solvent B. Under these conditions, oligosaccharides eluted between 6 and 11 min. Oligosaccharides containing ~2 mg hexose were recovered from 100 mg of pLH. These were fractionated by gel filtration chromatography on a Bio-Gel P-6 column (1.6 × 90 cm), eluted with 0.2 M ammonium acetate at 15 ml/h, and monitored using a refractive index detector. A peak designated F3 (834 μg hexose) contained mainly monosulfated monoantennary *N*-glycans as judged by liquid secondary ion mass spectrometry. The $[M-H]^-$ ion at m/z 1,541 corresponds to a composition of $HSO_3.Hex_3.HexNAc_4.dHex_1$ (where Hex is a hexose, HexNAc is an *N*-acetylhexosamine, and dHex is deoxyhexose [fucose]), and m/z 1,395 corresponds to $HSO_3.Hex_3.HexNAc_4$. These are consistent with the sequence HSO_3-GalNAc-GlcNAc-Man-(Man)Man-GlcNAc-GlcNAc with or without fucose (10). Small amounts of biantennary sulfated *N*-glycans were also present, as indicated by the $[M-H]^-$ ions at m/z 1,801 ($HSO_3.Hex_3.HexNAc_6$) and 1,881 ($[HSO_3]_2.Hex_3.HexNAc_6$). Fraction F3 was further chromato-

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 NaH_2PO_4 (solvent C, 5 mM NaH_2PO_4 and solvent D, 50 mM NaH_2PO_4, 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 HSO_3. In part, m/z 2,042 represents a fragment ion resulting from loss of the dHex. The fragment ion at m/z 1,905 corresponds to the loss of HSO_3.HexNAc; m/z 1,702, loss of HSO_3.HexNAc.HexNAc; m/z 1,540, loss of HSO_3.HexNAc.HexNAc.Hex; m/z 1,216, loss of HSO_3.HexNAc.HexNAc.Hex_3; and m/z 1,013, loss of HSO_3.HexNAc.HexNAc.Hex_3.HexNAc. This spectrum is consistent with the monoantennary *N*-glycan with and without a core fucose residue described as one of the major oligosaccharides on LH (10).

CS Oligosaccharides. Pentasaccharide fragments from CS-A, CS-B, and CS-C were prepared and purified essentially as described previously (20). In brief, the CS chains were partially depolymerized by limited digestion with chondroitinase ABC (EC 4.2.2.4, from *Proteus vulgaris*; Sigma Chemical Co.) to generate oligosaccharides consisting of even-numbered monosaccharide residues with a terminal unsaturated uronic acid (ΔUA) residue. These were chromatographed on a Bio-Gel P-6 column. The hexasaccharide fractions were subjected to oxymercuration treatment (20, 21) to remove the ΔUA residues, and the resulting pentasaccharides were isolated by gel filtration on a Sephadex G-10 column. Quantitation was carried out by the carbazole assay (22) using d-glucurono-6,3-lactone as a standard. The predominant sequences in the three pentasaccharides designated CS-A5, CS-B5, and CS-C5 (shown in Table I) were deduced by HPLC disaccharide-composition analysis after chondroitinase ABC digestion of the parent hexasaccharides (23).

Monosaccharides and Blood Group Oligosaccharides. The monosaccharides, 4-sulfated GalNAc, 6-sulfated GalNAc, and 4,6-disulfated GalNAc were from Sigma Chemical Co. Lacto-*N*-neotetraose (LNNT) was from Dextra. 3′-sialyl-Lewis^a pentasaccharide, 3S-Le^a, was from the Institute of Food Substances (Moscow). The following oligosaccharides were synthesized chemically: 3′-sialyl-Lewis^x pentasaccharide, 3S-Le^x (24); 3′-sulfated-Le^x pentasaccharide, 3Su-Le^x (25); 3′-sulfated-Le^a pentasaccharide, 3Su-Le^a (26); and 6′-sulfated-Le^x pentasaccharide, 6Su-Le^x (25). The 3′-sulfated-lacto-*N*-tetraose, 3Su-LNT, and 3′-sulfated-LNNT, 3Su-LNNT, were prepared by mild acid hydrolysis (27) of 3Su-Le^a and 3Su-Le^x, 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 H_2O, and 100 μl of DHPE solution (5 mg/ml in $CHCl_3$/MeOH 1:3, vol/vol) was added, followed by 20 μl of freshly prepared tetrabutylammonium cyanoborohydride (20 μg/μl 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).

Biotinylated Oligosaccharides. Oligosaccharides were conjugated to 6-(biotinyl)-aminocaproyl-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/*m*-nitrobenzyl alcohol (2:2:1, by volume) as the matrix as described previously (32).

Binding Experiments. Binding of Cys-MR-Fc or of Cys-DEC-Fc, 0.5 μg/well (preincubated with anti–human IgG, at a chimera 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 mU 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.

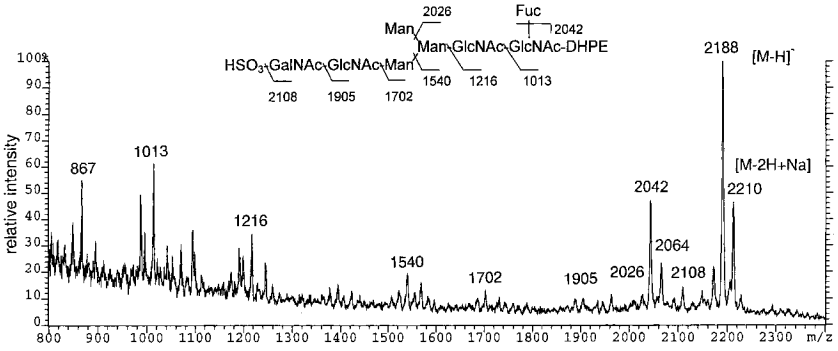

Figure 1. Mass spectrum of the NGLs derived from the sulfated *N*-glycan, pLH-F3-2, isolated from lutropin. The molecular ions and fragment ions correspond to those of the monoantennary *N*-glycan sequence illustrated, with and without a core fucose residue, which was described previously as one of the 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 chimera to anti-IgG ratio of 1:3 [wt/wt]). The diluent used 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′-azinobis[3-ethylbenzthiazoline-sulfonic acid]) as substrate, and absorbance was read at 405 nm.

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 CD16/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 mU/ml) or with heparinase I (100 mU/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 antiserum (1:100 dilution) specific for the core regions of cleaved CS chains (Biogenesis 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.

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 inhibitable with 4-sulfated *N*-acetylgalactosamine (concentration giving 50% inhibition of binding [IC_{50}] 0.18 mM), and less strongly with

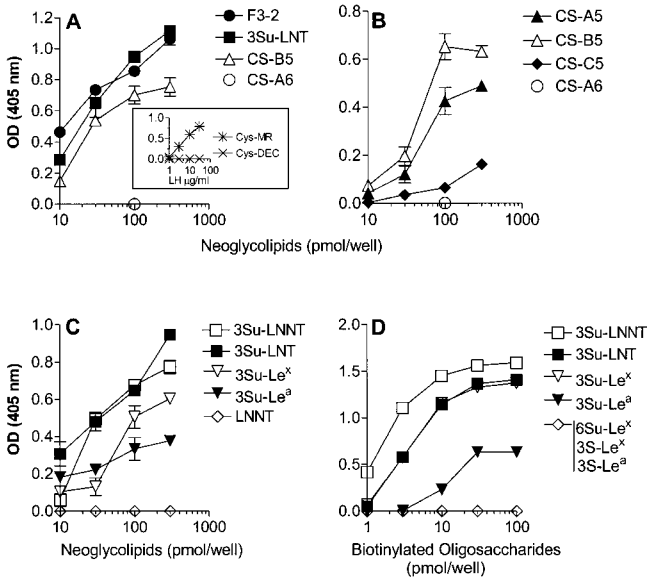

Figure 2. Binding experiments with Cys-MR and Cys-DEC. NGLs (A–C), lutropin (A, inset), and biotinylated oligosaccharides (D) were immobilized on microwells, and the binding of Cys-MR-Fc or Cys-DEC-Fc, 5 μg/well, was assayed as described in Materials and Methods.

6-sulfated and 4,6-disulfated *N*-acetylgalactosamine (IC_{50} 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 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)β1-4IdoAβ1-]$_n$. 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)β1-4GlcAβ1-]$_n$, and by heparin, which contain predominantly *N*- and 6-disul-

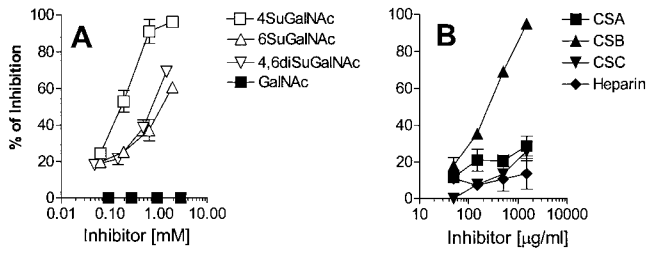

Figure 3. Inhibition of the binding of Cys-MR to lutropin by sulfated monosaccharides and glycosaminoglycans. hLH, 0.5 μg/well, was coated onto microwells, and the binding of Cys-MR-Fc, 0.25 μg/well, in the presence of the sulfated monosaccharides (A) or glycosaminoglycans (B), was assayed as described in Materials and Methods.

fated glucosamine [GlcNS(6S)] joined by β1-4 linkage to 2-sulfated iduronic acid: [-4GlcNS(6S)β1-4IdoA(2S)α1-]$_n$. 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-]$_n$. 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) (Table I). In contrast to the pentasaccharide, the CS-A hexasaccharide, CS-A6, which terminates with the unsaturated uronic acid, ΔUAβ1-3, 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

Table I. *Oligosaccharides Investigated and Their Cys-MR Binding Strengths*

Designation	*Sequence*	*Binding**
F3-2	Manα1-6 Manβ1-4GlcNAcβ1-4GlcNAc GalNAcβ1-4GlcNAcβ1-2Manα1-3/ |1,6 |4 +/-Fucα HSO_3	+++
CS-A6	ΔUAβ1-3GalNAcβ1-4GlcAβ1-3GalNAcβ1-4GlcAβ1-3GalNAc‡ |4 |4 |4 HSO_3 HSO_3 HSO_3	-
CS-A5	GalNAcβ1-4GlcAβ1-3GalNAcβ1-4GlcAβ1-3GalNAc‡ |4 |4 |4 HSO_3 HSO_3 HSO_3	+++
CS-B5	GalNAcβ1-4IdoAβ1-3GalNAcβ1-4IdoAβ1-3GalNAc‡ |4 |4 |4 HSO_3 HSO_3 HSO_3	+++
CS-C5	GalNAcβ1-4GlcAβ1-3GalNAcβ1-4GlcAβ1-3GalNAc‡ |6 |6 |6 HSO_3 HSO_3 HSO_3	+
3Su-Le^x	Galβ1-4GlcNAcβ1-3Galβ1-4Glc |3 |1,3 HSO_3 Fucα	+++
3Su-Le^a	Galβ1-3GlcNAcβ1-3Galβ1-4Glc |3 |1,4 HSO_3 Fucα	++
6Su-Le^x	Galβ1-4GlcNAcβ1-3Galβ1-4Glc |6 |1,3 HSO_3 Fucα	-
3Su-LNNT	Galβ1-4GlcNAcβ1-3Galβ1-4Glc |3 HSO_3	+++
3Su-LNT	Galβ1-3GlcNAcβ1-3Galβ1-4Glc |3 HSO_3	+++
3S-Le^x	Galβ1-4GlcNAcβ1-3Galβ1-4Glc |2,3 |1,3 NeuAcα Fucα	-
3S-Le^a	Galβ1-3GlcNAcβ1-3Galβ1-4Glc |2,3 |1,4 NeuAcα Fucα	-
LNNT	Galβ1-4GlcNAcβ1-3Galβ1-4Glc	-

*Binding strengths in the microwell assays, in which both the Cys-MR and the immobilized saccharides were in an oligometric state, are arbitrarily scored here as +++; ++; +; and −. These symbols denote binding signals (ODs at 405 nm) of 0.5 or more; 0.2–0.4; <0.2; and 0, respectively, using the NGLs at 100 pmol or the biotinylated oligosaccharides at 10 pmol/well.

‡The predominant sequences are shown. From the disaccharide composition analysis of the parent hexasaccharides, it is deduced that CS-A5 contains GalNAc(4S), 60%; CS-B5 contains GalNAc(4S), 90%; and CS-C5 contains GalNAc(6S), 85%.

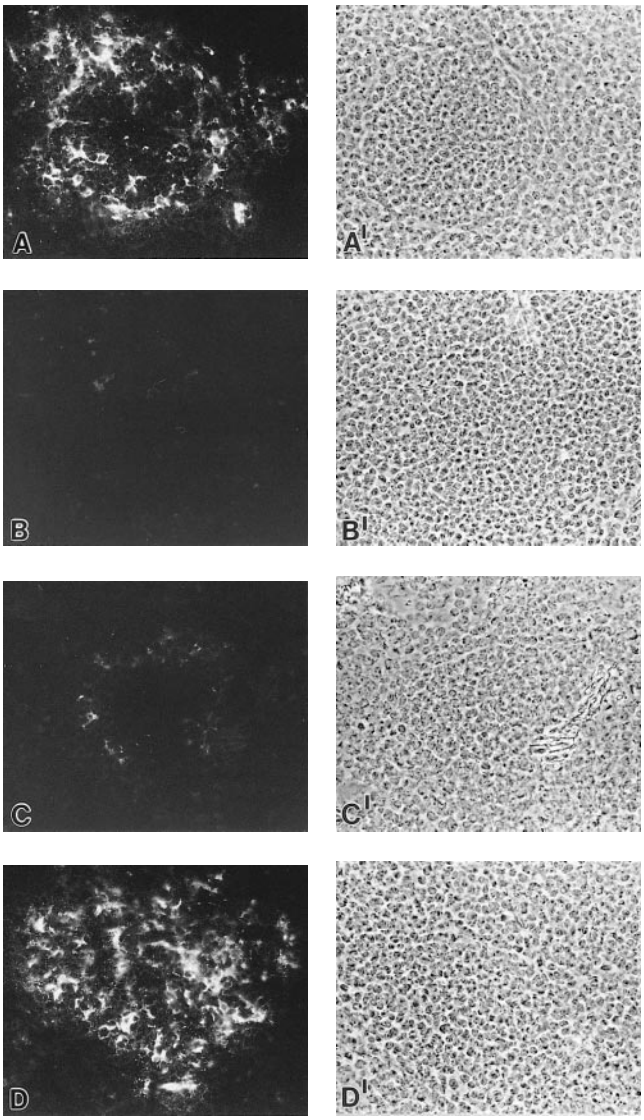

Figure 4. Immunofluorescence micrographs of sections of naive mouse spleen showing inhibition of Cys-MR binding in the presence of sulfated monosaccharides. Sections were overlaid with Cys-MR-Fc, 10 μg/ml, in the presence of PBS (A), 4-sulfated *N*-acetylgalactosamine (B), 6-sulfated *N*-acetylgalactosamine (C), or *N*-acetylgalactosamine (D), 5 mg/ml, and binding was detected with fluorescein-labeled donkey anti–human IgG as described in Materials and Methods. A′–D′ are the corresponding phase–contrast micrographs. Similar results were obtained using spleen sections from immunized mice (not shown). Original magnifications: ×650.

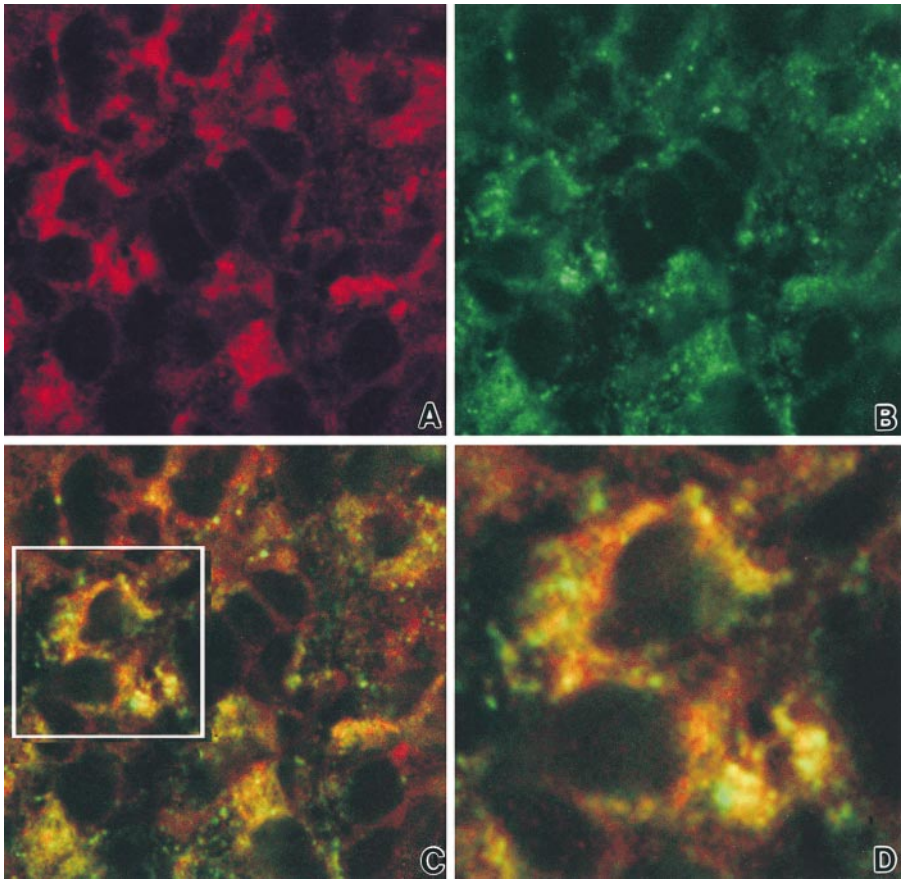

Figure 5. Confocal images of a dual immunolabeling experiment showing the similar distribution and limited colocalization of the binding of Cys-MR and an antibody to the core regions of CS chains within the B cell areas of the splenic white pulp. Before immunolabeling, spleen sections from immunized 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.

oligosaccharides terminating with GalNAc(4S) joined by β1-4 linkage to glucuronic acid or iduronic acid, as well as to *N*-acetylglucosamine, 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*-acetylglucosamine: 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 Le^a and Le^x sequences, which contain fucose joined by α1-4 and α1-3 linkage to the *N*-acetylglucosamine 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-Le^x sequence, or to the 3S-Le^a and 3S-Le^x 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-

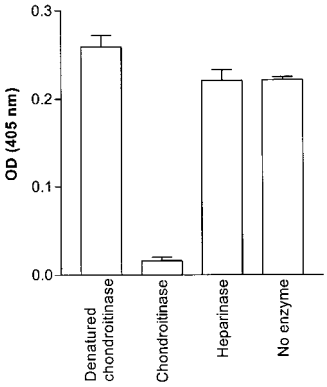

Figure 6. Binding of Cys-MR to serglycin and abolition of binding after treatment of the proteoglycan with chondroitinase ABC but not heparinase. Serglycin was coated onto microwells, 1.5 μg added/well, and incubated with chondroitinase ABC (50 mU), the heat-denatured chondroitinase ABC, heparinase I (2.5 U), or PBS. The binding of Cys-MR-Fc, 0.5 μg/well, was assayed as described in Materials and Methods.

MR staining. 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 the recombinant human Fc dimer (not shown); in addition, there was no rhodamine staining observed in the absence of the antibody to CS (not shown). Although similar, the two staining patterns for Cys-MR-Fc and anti-CS in the B cell area overlapped only in limited areas (yellow areas in Fig. 5, C and D). Moreover, there was no perceptible difference in the Cys-MR staining in these areas before and after treatment with chondroitinase ABC (results not shown). Thus, although substantial amounts of CS chains are present, it is likely that the Cys-MR binding forms are relatively minor components.

To directly evaluate the ability of Cys-MR to interact with chondroitin 4-sulfate proteoglycans produced by cells of the hematopoietic and immune systems (34–37), we examined its binding to a preparation of proteoglycans secreted by a monocytic cell line and known to be rich in serglycin. There was specific binding to the proteoglycan immobilized on microwells (Fig. 6), and treatment with chondroitinase ABC, but not heparinase, abolished the binding. We conclude that Cys-MR interacts with these soluble proteoglycans via CS chains.

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*-acetylglucosamine. 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*-acetylglucosamine.

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 hexasaccharide 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^x over the 3Su-Le^a analogue. The modeling, based on the crystal structure, shows that the sulfated galactose and the fucose of Le^x can be stacked readily against Trp117, whereas in the Le^a 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

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 MR 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 counterreceptors 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 MR is codistributed 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 MR-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 antigen, 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-Le^x and -Le^a sequences occur variously as markers of granulocytes, monocytes, or subsets of lymphocytes. The sulfo-Le^x and -Le^a 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 sulfo-Le^a and -Le^x 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 sialoadhesin or CD45 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 mannose-binding C-type lectin domains on MR, the Cys-MR contributes to trapping of ligand-positive microbial pathogens, and to the delivery of ligand-positive antigens for processing and presentation.

We are grateful to Dr. Suzanne Delpech (INRA, Nouzilly) for purification of the pLH, and to Dr. Christine Hale (Imperial College, South Kensington Campus) for confocal images. M.C. Nussenzweig is an associate investigator in the Howard Hughes Medical Institute.

This work was supported by grant 567 from the Human Frontier Science Program (Program grant G9601454 from the British Medical Research Council), and a project grant from the British Arthritis Research Council.

Submitted: 8 December 1999
Revised: 3 February 2000
Accepted: 7 February 2000

References

1. Taylor, M.E., J.T. Conary, M.R. Lennartz, P.D. Stahl, and K. Drickamer. 1990. Primary structure of the mannose receptor contains multiple motifs resembling carbohydrate-recognition domains. *J. Biol. Chem.* 265:12156–12162.
2. Ezekowitz, R.A., K. Sastry, P. Bailly, and A. Warner. 1990. Molecular characterization of the human macrophage mannose receptor: demonstration of multiple carbohydrate recognition-like domains and phagocytosis of yeast in Cos-1 cells. *J. Exp. Med.* 172:1785–1794.
3. Stahl, P.D., and R.A.B. Ezekowitz. 1998. The mannose receptor is a pattern recognition receptor involved in host defence. *Curr. Opin. Immunol.* 10:50–55.
4. Taylor, M.E., and K. Drickamer. 1993. Structural requirements for high affinity binding of complex ligands by the macrophage mannose receptor. *J. Biol. Chem.* 268:399–404.
5. Sallusto, F., M. Cella, C. Danieli, and A. Lanzavecchia. 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J. Exp. Med.* 182:389–400.
6. Fiete, D., V. Srivastava, O. Hindsgaul, and J.U. Baenziger. 1991. A hepatic reticuloendothelial cell receptor specific for SO_4-4-GalNAcβ1,4GlcNAcβ1,2Manα that mediates rapid clearance of lutropin. *Cell.* 67:1103–1110.
7. Fiete, D., M.C. Beranek, and J.U. Baenziger. 1997. The macrophage/endothelial cell mannose receptor cDNA encodes a protein that binds oligosaccharides terminating with SO_4-4-GalNAcβ1,4GlcNAcβ or Man at independent sites. *Proc. Natl. Acad. Sci. USA.* 94:11256–11261.
8. Fiete, D., and J.U. Baenziger. 1997. Isolation of the SO_4-

4-GalNAcβ1,4GlcNAcβ1,2Manα-specific receptor from rat liver. *J. Biol. Chem.* 272:14629–14637.
9. Fiete, D., M.C. Beranek, and J.U. Baenziger. 1998. A cysteine-rich domain of the "mannose" receptor mediates GalNAc-4-SO_4 binding. *Proc. Natl. Acad. Sci. USA*. 95:2089–2093.
10. Weisshaar, G., J. Hiyama, A.G.C. Renwick, and M. Nimtz. 1991. NMR investigations of the N-linked oligosaccharides at individual glycosylation sites of human lutropin. *Eur. J. Biochem.* 195:257–268.
11. Smith, P.L., G.R. Bousfield, S. Kumar, D. Fiete, and J.U. Baenziger. 1993. Equine lutropin and chorionic gonadotropin bear oligosaccharides terminating with SO_4-4-GalNAc and Siaα2,3Gal, respectively. *J. Biol. Chem.* 268:795–802.
12. Simpson, D.Z., P.G. Hitchen, E.L. Elmhirst, and M.E. Taylor. 1999. Multiple interactions between pituitary hormones and the mannose receptor. *Biochem. J.* 343:403–411.
13. Martínez-Pomares, L., M. Kosco-Vilbois, E. Darley, P. Tree, S. Herren, J.-Y. Bonnefoy, and S. Gordon. 1996. Fc chimeric protein containing the cysteine-rich domain of the murine mannose receptor binds to macrophages from splenic marginal zone and lymph node subcapsular sinus and to germinal centers. *J. Exp. Med.* 184:1927–1937.
14. Martínez-Pomares, L., J.A. Mahoney, R. Káposzta, S.A. Linehan, P.D. Stahl, and S. Gordon. 1998. A functional soluble form of the murine mannose receptor is produced by macrophages *in vitro* and is present in mouse serum. *J. Biol. Chem.* 273:23376–23380.
15. Jiang, W., W.J. Swiggard, C. Heufler, M. Peng, A. Mirza, R.M. Steinman, and M.C. Nussenzweig. 1995. The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature*. 375:151–155.
16. Hennen, G., Z. Pruzik, and G. Maghuin-Rogister. 1971. Porcine lutropin and its subunits. Isolation and characterization. *Eur. J. Biochem.* 18:376–383.
17. Øynebråten, I., B. Hansen, B. Smedsrød, and L. Uhlin-Hansen. 2000. Serglycin secreted by leukocytes is efficiently eliminated from the circulation by sinusoidal scavanger endothelial cells in the liver. *J. Leukoc. Biol.* 67:183–188.
18. Lammi, M., and M. Tammi. 1988. Densitometric assay of nanogram quantities of proteoglycans precipitated on nitrocellulose membrane with safranin O. *Anal. Biochem.* 168: 352–357.
19. Takasaki, S., T. Mizuochi, and A. Kobata. 1982. Hydrazinolysis of asparagine-linked sugar chains to produce free oligosaccharides. *Methods Enzymol.* 83:263–268.
20. Chai, W., H. Kogelberg, and A.M. Lawson. 1996. Generation and structural characterization of a range of unmodified chondroitin sulfate oligosaccharide fragments. *Anal. Biochem.* 237:88–102.
21. Ludwigs, U., A. Elgavish, J.D. Esko, E. Meezan, and L. Rodén. 1987. Reaction of unsaturated uronic acid residues with mercuric salts. Cleavage of the hyaluronic acid disaccharide 2-acetamido-2-deoxy-3-*O*-(β-d-gluco-4-enepyranosyluronic acid)-d-glucose. *Biochem. J.* 245:795–804.
22. Bitter, T., and H.M. Muir. 1962. A modified uronic acid carbazole reaction. *Anal. Biochem.* 4:330–334.
23. Chai, W., A.M. Lawson, M.J. Gradwell, and H. Kogelberg. 1998. Structural characterisation of two hexasaccharides and an octasaccharide from chondroitin sulfate C containing the unusual sequence (4-sulfo)-*N*-acetylgalactosamine-β1-4-(2-sulfo)-glucuronic acid-β-1-3-(6-sulfo)-*N*-acetylgalactosamine. *Eur. J. Biochem.* 251:114–121.
24. Hasegawa, A., T. Ando, A. Kameyama, and M. Kiso. 1992. Synthetic studies on sialoglycoconjugates. 40. Stereocontrolled synthesis of sialyl Lewis X epitope and its ceramide derivative. *J. Carbohydr. Chem.* 11:645–658.
25. Augé, C., F. Dagron, R. Lemoine, C. Le Narvor, and A. Lubineau. 1997. Syntheses of sulfated derivatives as sialyl Lewis^a and sialyl Lewis^x analogues. *In* Carbohydrate Mimics: Concepts and Methods. Y. Chapleur, editor. Verlag Chemie, Weinheim, Germany. 365–383.
26. Lubineau, A., J. Le Gallic, and R. Lemoine. 1994. First synthesis of the 3′-sulfated Lewis^a pentasaccharide, the most potent human E-selectin ligand so far. *Bioorg. Med. Chem.* 2:1143–1151.
27. Yuen, C.-T., A.M. Lawson, W. Chai, M. Larkin, M.S. Stoll, A.C. Stuart, F.X. Sullivan, T.J. Ahern, and T. Feizi. 1992. Novel sulfated ligands for the cell adhesion molecule E-selectin revealed by the neoglycolipid technology among O-linked oligosaccharides on an ovarian cystadenoma glycoprotein. *Biochemistry*. 31:9126–9131.
28. Feizi, T., M.S. Stoll, C.-T. Yuen, W. Chai, and A.M. Lawson. 1994. Neoglycolipids: probes of oligosaccharide structure, antigenicity, and function. *Methods Enzymol.* 230:484–519.
29. Stoll, M.S., and T. Feizi. 1997. Preparation of neoglycolipids for structure and function assignments of oligosaccharides. *In* A Laboratory Guide to Glycoconjugate Analysis. P. Jackson and J.T. Gallagher, editors. Birkhäuser Verlag, Basel. 329–348.
30. Leteux, C., R.A. Childs, W. Chai, M.S. Stoll, H. Kogelberg, and T. Feizi. 1998. Biotinyl-1-3-(2-naphthyl)-alanine hydrazide derivatives of *N*-glycans: versatile solid-phase probes for carbohydrate-recognition studies. *Glycobiology*. 8:227–236.
31. Leteux, C., M.S. Stoll, R.A. Childs, W. Chai, M. Vorozhaikina, and T. Feizi. 1999. Influence of oligosaccharide presentation on the interactions of carbohydrate sequence-specific antibodies and the selectins. Observations with biotinylated oligosaccharides. *J. Immunol. Methods*. 227: 109–119.
32. Chai, W., G.C. Cashmore, R.A. Carruthers, M.S. Stoll, and A.M. Lawson. 1991. Optimal procedure for combined high performance thin-layer chromatography/high sensitivity liquid secondary ion mass spectrometry. *Biol. Mass Spectrom.* 20: 169–178.
33. Galustian, C., R.A. Childs, C.-T. Yuen, A. Hasegawa, M. Kiso, A. Lubineau, G. Shaw, and T. Feizi. 1997. Valency dependent patterns of binding of human L-selectin towards sialyl and sulfated oligosaccharides of Le^a and Le^x types: relevance to anti-adhesion therapeutics. *Biochemistry*. 36:5260–5266.
34. Kolset, S.O., and J.T. Gallagher. 1990. Proteoglycans in haemopoietic cells. *Biochim. Biophys. Acta*. 1032:191–211.
35. Kresse, H., H. Hausser, and E. Schönherr. 1993. Small proteoglycans. *Experientia*. 49:403–416.
36. Kulseth, M.A., S.L. Mustorp, L. Uhlin-Hansen, F. Öberg, and S.O. Kolset. 1998. Serglycin expression during monocytic differentiation of U937-1 cells. *Glycobiology*. 8:747–753.
37. Toyama-Sorimachi, N., F. Kitamura, H. Habuchi, Y. Tobita, K. Kimata, and M. Miyasaka. 1997. Widespread expression of chondroitin sulfate-type serglycins with CD44 binding ability in hematopoietic cells. *J. Biol. Chem.* 272:26714–26719.
38. Liu, Y., A.J. Chirino, C. Leteux, T. Feizi, Z. Misulovin,

M.C. Nussenzweig, and P. Bjorkman. 2000. Crystal structure of the cysteine-rich domain of mannose receptor complexed with a sulfated carbohydrate ligand. J. Exp. Med. 191:1105–1115.

39. Kittlick, P.D., and D. Engelmann. 1992. The glycosaminoglycans in cultures of stimulated rat peritoneal macrophages. 1. Pattern and biosynthesis of glycosaminoglycans. *Exp. Toxicol. Pathol.* 44:407–413.
40. Piepkorn, M., P. Hovingh, K.L. Bennett, A. Aruffo, and A. Linker. 1997. Chondroitin sulphate composition and structure in alternatively spliced CD44 fusion proteins. *Biochem. J.* 327:499–506.
41. Naujokas, M.F., M. Morin, M.S. Anderson, M. Peterson, and J. Miller. 1993. The chondroitin sulfate form of invariant chain can enhance stimulation of T cell responses through interaction with CD44. *Cell.* 74:257–268.
42. Prigozy, T.I., P.A. Sterling, D. Clemens, P.L. Stewart, S.M. Behar, S.A. Porcelli, M.B. Brenner, R.L. Modlin, and M. Kronenberg. 1997. The mannose receptor delivers lipoglycan antigens to endosomes for presentation to T cells by CD1b molecules. *Immunity*. 6:187–197.
43. Ruoslahti, E. 1989. Proteoglycans in cell regulation. *J. Biol. Chem.* 264:13369–13372.
44. Feizi, T. 1985. Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are onco-developmental antigens. *Nature*. 314:53–57.
45. Loveless, R.W., C.-T. Yuen, H. Tsuiji, T. Irimura, and T. Feizi. 1998. Monoclonal antibody 91.9H raised against sulfated mucins is specific for the 3′-sulfated Lewis^a tetrasaccharide sequence. *Glycobiology*. 8:1237–1242.
46. Feizi, T. 1993. Oligosaccharides that mediate mammalian cell-cell adhesion. *Curr. Opin. Struct. Biol.* 3:701–710.
47. Martínez-Pomares, L., and S. Gordon. 1999. Potential role of the mannose receptor in antigen transport. *Immunol. Lett.* 65: 9–13.
48. Martínez-Pomares, L., P.R. Crocker, R. Da Silva, N. Holmes, C. Colominas, P. Rudd, R. Dwek, and S. Gordon. 1999. Cell-specific glycoforms of sialoadhesin and CD45 are counter-receptors for the cysteine-rich domain of the mannose receptor. *J. Biol. Chem.* 274:35211–35218.