

COMMUNICATION

Two categories of mammalian galactose-binding receptors distinguished by glycan array profiling

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Profiling of the four known galactose-binding receptors in the C-type lectin family has been undertaken in parallel on a glycan array. The results are generally consistent with those of previous assays using various different formats, but they provide a direct comparison of the properties of the four receptors, revealing that they fall into two distinct groups. The major subunit of the rat asialoglycoprotein receptor and the rat Kupffer cell receptor show similar broad preferences for GalNAc-terminated glycans, while the rat macrophage galactose lectin and the human scavenger receptor C-type lectin (SRCL) bind more restricted sets of glycans. Both of these receptors bind to Lewis x-type structures, but the macrophage galactose lectin also interacts strongly with biantennary galactose- and GalNAc-terminated glycans. Although the similar glycan-binding profiles for the asialoglycoprotein receptor and the Kupffer cell receptor might suggest that these receptors are functionally redundant, analysis of fibroblasts transfected with full-length Kupffer cell receptor reveals that they fail to endocytose glycosylated ligand.

Key words: asialoglycoprotein receptor/glycan array/
Kupffer cell receptor/lectin/scavenger receptor

Introduction

The asialoglycoprotein receptor on hepatocytes is the prototype for the C-type lectin family. The receptor removes glycoproteins with terminal galactose or GalNAc residues from circulation (Ashwell and Harford, 1982). Three additional receptors in the C-type lectin family bind galactose- or GalNAc-containing ligands: the Kupffer cell receptor, the macrophage galactose lectin, and the scavenger receptor C-type lectin (SRCL) (Hoyle and Hill, 1988; Ii *et al.*, 1990; Nakamura *et al.*, 2001). The potentially overlapping functions of these receptors remain

poorly understood. It has been suggested that the Kupffer cell receptor might function in parallel with the asialoglycoprotein receptor, explaining failure of asialoglycoprotein receptor-deficient mice to accumulate uncleared asialoglycoproteins (Ishibashi *et al.*, 1994; Fadden *et al.*, 2003). Because the human macrophage galactose lectin binds strongly to the carcinoma-associated Tn antigen, GalNAc-Ser/Thr (Yamamoto *et al.*, 1994), this receptor may have a role in recognition of malignant cells by tumoricidal macrophages. Knockout studies suggest that one of the two mouse macrophage galactose lectins is involved in clearance of apoptotic cells (Yuita *et al.*, 2005).

The monosaccharide-binding profiles of the asialoglycoprotein receptor and Kupffer cell receptor are similar: both show a marked preference for GalNAc over galactose. In contrast, the rat macrophage galactose lectin exhibits no preference for GalNAc over galactose (Iobst and Drickamer, 1996). Binding of the asialoglycoprotein receptor (Lee *et al.*, 1983; Park *et al.*, 2003), the Kupffer cell receptor (Tiemeyer *et al.*, 1992), and the macrophage galactose lectin (Yamamoto *et al.*, 1994; Tsuiji *et al.*, 2002; van Vliet *et al.*, 2005) to distinct sets of oligosaccharide ligands has also been demonstrated. However, these results were obtained in a variety of different assay formats, so the results are not always directly comparable.

When the proteins are used in parallel to probe a recently developed glycan array, the results reveal that the receptors fall into two distinct subclasses that show selectivity for distinct classes of glycans. Further characterization of the intracellular trafficking capabilities of the Kupffer cell receptor indicates that it is unlikely to function in parallel with the asialoglycoprotein receptor in glycoprotein clearance.

Results

Two categories of galactose-binding receptors distinguished by glycan array profiling

In order to gain a broad view of the oligosaccharide-binding specificities of the galactose-binding receptors, fluorescently labeled trimeric extracellular domain fragments from each protein were used to probe a glycan array consisting of biotinylated oligosaccharides presented on immobilized streptavidin (Figure 1). Data from a previous screening of the array with SRCL (Coombs *et al.*, 2005) are shown for comparison. The results show that there is substantial overlap in binding specificities between the macrophage galactose lectin and SRCL. In contrast, the major subunit of the asialoglycoprotein

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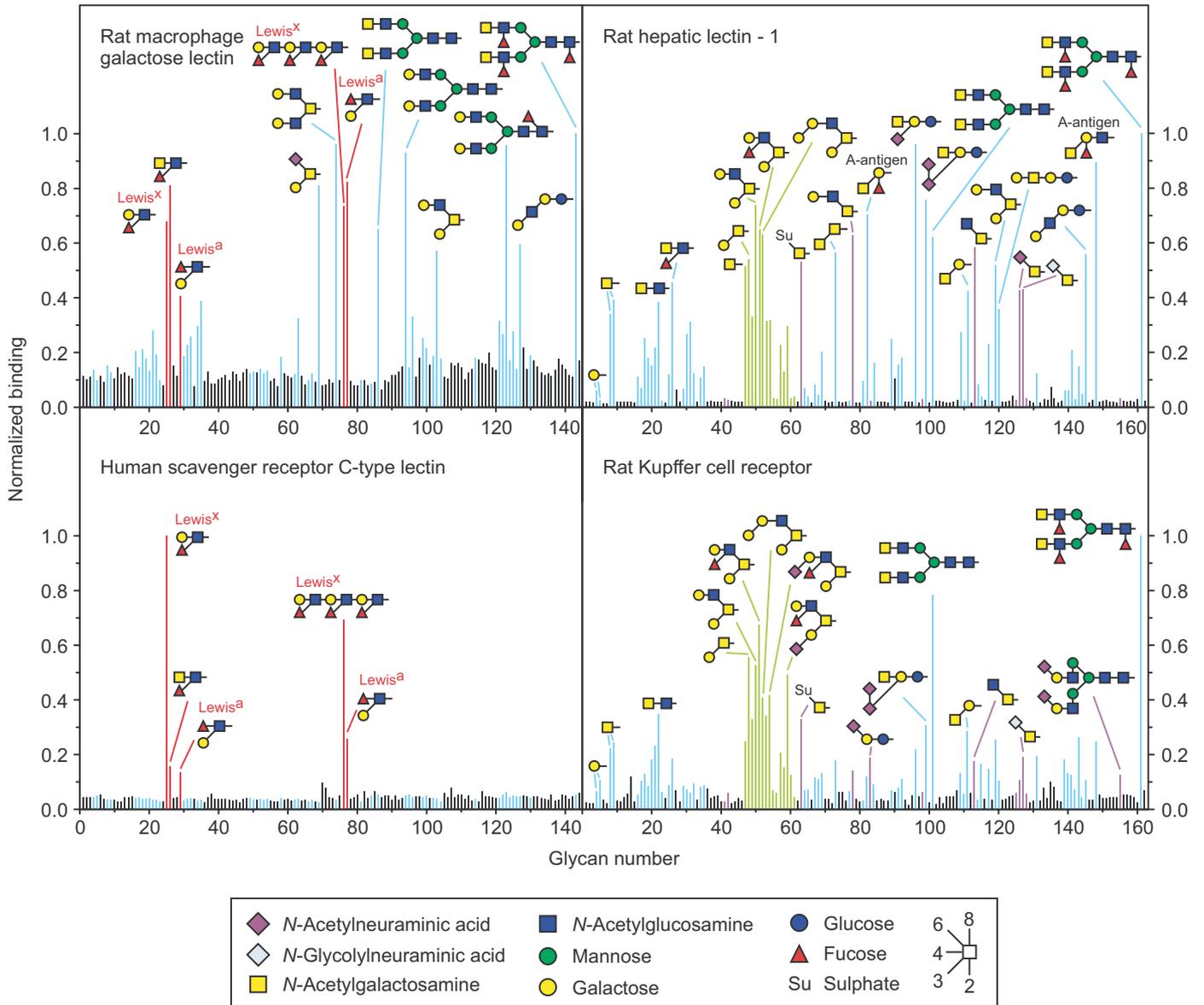


Fig. 1. Screening of a glycan array with galactose-binding receptors. Versions 3.3 and 2.3 of the glycan array were probed with SRCL (data from Coombs *et al.*, 2005) and macrophage galactose lectin, respectively. Results are shown for oligosaccharides common to both versions. Version 2.3 of the glycan array was used for RHL-1 and the Kupffer cell receptor. The level of fluorescence was normalized separately for each receptor, SRCL to glycan 25 (Lewis x), the macrophage galactose lectin to glycan 143 (biantennary GalNAc β 1-4(Fuc α 1-2)GlcNAc), and RHL-1 and the Kupffer cell receptor to glycan 161. However, the absolute affinity for an individual glycan cannot be compared between receptors. In the left-hand panels, galactose- and GalNAc-terminated glycans are indicated in blue and Lewis x, Lewis a, and GalNAc β 1-4(Fuc α 1-3)GlcNAc glycans are highlighted in red. In the right-hand panels, galactose- and GalNAc-terminated glycans are shown in blue, *O*-linked glycopeptides are indicated in green, and galactose- and GalNAc-terminated glycans with substituents at the 6-position are highlighted in purple. A complete listing of glycans is provided in *Supplementary Data*.

receptor, rat hepatic lectin-1 (RHL-1), and the Kupffer cell receptor both bind to a much broader range of oligosaccharides. Thus, the galactose-binding receptors can be divided into two specificity groups, the first group containing human SRCL and the rat macrophage galactose lectin and the second containing RHL-1 and the rat Kupffer cell receptor.

Comparison of macrophage galactose lectin and SRCL binding specificities

The glycan array profile for the macrophage galactose lectin reveals that this receptor binds two specific subsets of

glycans that terminate in galactose or GalNAc residues: branched glycans that bear two terminal galactose or GalNAc residues or smaller glycans in which fucose is adjacent to a terminal galactose or GalNAc residue. Amongst the fucose-containing ligands, Lewis x and Lewis a, as well as Lewis x in which the galactose is replaced with GalNAc, are all bound. The remaining highest-affinity ligands are all biantennary structures containing terminal galactose or GalNAc residues, except for glycan 69, which contains adjacent terminal galactose and NeuAc residues. On the basis of the known structures of C-type carbohydrate-recognition domains (CRDs) with bound ligands, these results suggest

that a galactose or GalNAc residue in the high-affinity ligands makes primary interactions with the macrophage galactose lectin, but binding is enhanced by secondary interactions with adjacent terminal galactose, GalNAc, fucose, or NeuAc residues. Binding of the macrophage galactose lectin is also observed to a few other galactose-terminated ligands including glycan 127 (Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc β). The extended structure of glycan 127 raises the possibility that binding may be due to the way the terminal galactose is presented on this glycan. Evidence that presentation can affect the binding of the receptor to glycans on the array is provided by the fact that Lewis a on a longer spacer arm (glycan 77) is bound more tightly than Lewis a on a short spacer arm (glycan 29).

SRCL exhibits an even narrower specificity than the macrophage galactose lectin, as it binds highly selectively to Lewis x, with weaker binding to Lewis a and Lewis x in which the galactose is replaced with GalNAc. SRCL has an absolute requirement for adjacent terminal galactose and fucose residues and does not bind galactose- or GalNAc-terminating glycans unless they form part of a Lewis x-type epitope.

Ligand-binding profile of RHL-1

Although the glycan array reveals that RHL-1 binds to a broad range of galactose- and GalNAc-terminated oligosaccharides over a range of affinities, some patterns in the type of ligand bound are evident.

First, the results for RHL-1 reflect the previously observed preferential binding of this receptor to GalNAc over galactose. Comparison of glycans 4 and 5 (α - β -linked galactose) versus glycans 8 and 9 (α - β -linked GalNAc) confirms the strong preference for GalNAc. RHL-1 also shows strong binding to blood group A antigen (GalNAc- α 1-3(Fuc α 1-2)Gal β) but almost no binding to the similar blood group B antigen (Gal α 1-3(Fuc α 1-2)Gal β) (glycans 23, 77, and 144), where the only difference is the presence of terminal galactose instead of GalNAc. However, preferential binding of GalNAc-terminating ligands by RHL-1 does not correlate with the ability of the receptor to bind a few monoantennary galactose-terminated ligands on the array, such as glycans 119, 120, and 145.

Second, modification of GalNAc by substitution on the 6-position is tolerated. These substituents include sulfate (glycan 63), Gal β 1-4GlcNAc (glycan 78), GlcNAc (glycan 113), and sialic acid (glycans 126 and 127). The orientation of the GalNAc in the CRD, where the sugar coordinates Ca²⁺ via its 3- and 4-hydroxyls, would project any substituent at the 6-position away from the primary binding site. In some cases, 6-substitution enhances binding, suggesting that 6-substituents make secondary contacts with the receptor. Binding to similarly substituted galactose residues is not observed. The ability to accommodate 6-substitution of galactose with GlcNAc or 6-substitution of GalNAc with sialic acid has been previously observed (Lee, 1982; Park *et al.*, 2003).

Third, binding to galactose- and GalNAc-terminated glycans is enhanced when there are multiple terminal residues. Previous studies on the asialoglycoprotein receptor show dramatic increases in binding in going from mono- to bi- to triantennary galactose-terminated structures (Rice *et al.*, 1990). However, RHL-1 shows much weaker binding to

biantennary glycans terminating in galactose (glycans 109 and 141) rather than GalNAc (glycans 101 and 161). The highest-affinity ligand on the array for RHL-1 is a complex biantennary glycan, in which both antennae terminate in a GalNAc β 1-4GlcNAc structure. This glycan also contains outer arm α 1-2-linked fucose on the subterminal GlcNAc and α 1-2-linked fucose on the asparagine-linked GlcNAc (glycan 161), but the nonfucosylated form (glycan 101) also binds well to RHL-1. Unfortunately, more branched tri- and tetra-antennary glycans that have been shown to compete particularly effectively for binding to the asialoglycoprotein receptor (Lee *et al.*, 1983) are not present on the array.

Finally, projection of glycans on a polypeptide core in the form of *O*-linked glycopeptides enhances binding. For example, binding of glycan 50, presented as an *O*-linked glycopeptide, is enhanced compared to the binding of glycan 119, the same oligosaccharide presented on a short spacer arm. The *O*-linked glycopeptides consist of oligosaccharides linked to an 18-amino acid peptide. The strong binding of galactose- and GalNAc-terminated *O*-linked glycopeptides suggests that *O*-linked glycans may be among the ligands bound by the receptor *in vivo*. Binding to a more limited set of *O*-linked glycans has been observed previously (Baenziger and Maynard, 1980).

Ligand-binding profile of the Kupffer cell receptor

The glycan array results indicate that the Kupffer cell receptor binds to essentially all of the same glycans as RHL-1, but the relative affinities of the two receptors differ in several respects. For example, compared to RHL-1, the Kupffer cell receptor shows less selectivity for GalNAc over galactose. Overall, the Kupffer cell receptor binds to very few glycans on the array substantially better than it binds to GalNAc. The two best ligands are biantennary GalNAc-terminated glycans, suggesting that for the Kupffer cell receptor the primary determinant of enhanced ligand binding is the formation of branched structures. In addition, the Kupffer cell receptor has enhanced affinity for the *O*-linked glycans on the array.

Like RHL-1, the Kupffer cell receptor binds to several GalNAc oligosaccharides modified at the 6-position with sulfate, sialic acid, or GlcNAc. However, the Kupffer cell receptor exhibits approximately equal or lower affinity for these glycans compared to those with terminal GalNAc presented alone, such as glycans 8, 9, and 22, implying the additional substituents are accommodated but do not contribute to binding affinity. In contrast to RHL-1, the Kupffer cell receptor does show weak binding to some 6-substituted galactose-containing glycans (glycans 83 and 155).

Testing for endocytic activity of the Kupffer cell receptor

The similarity between the ligand-binding profiles of the Kupffer cell receptor and RHL-1 on the glycan array is consistent with the possibility that the Kupffer cell receptor might function in parallel with the asialoglycoprotein receptor as a second clearance receptor for desialylated ligands. The Kupffer cell receptor releases ligand at endosomal pH (Lehrman, Haltiwanger, *et al.*, 1986; unpublished observations), as required for receptor-mediated endocytosis, but the ability of the receptor to mediate endocytosis has not

been demonstrated. To examine the endocytic capabilities of the Kupffer cell receptor, the full-length protein was expressed in rat fibroblasts. These cells show no internalization and degradation of ^{125}I -Gal-BSA, indicating that the receptor does not endocytose glycosylated ligands (Figure 2A). In a parallel assay, fibroblasts expressing SRCL exhibit ^{125}I -Gal-BSA uptake and degradation. Expression was confirmed by blotting of cell extracts with antibodies raised against the CRD of the receptor (Figure 2B). Two broad protein bands at ~70 and ~62 kDa represent the full-length receptor and the receptor after proteolytic cleavage, both with varying extents of glycosylation. Quantification of blots revealed that up to 250,000 molecules were expressed per cell, which is at least as high as the levels of other C-type lectins that reveal endocytic activity when expressed in these cells (Stambach and Taylor, 2003; Coombs *et al.*, 2005). When membrane-impermeable biotin was used to label cell-surface proteins, blotting of biotinylated proteins isolated from the cells on avidin confirmed the presence of Kupffer cell receptor at the cell surface.

It is possible that the Kupffer cell receptor requires the presence of accessory proteins not present in the fibroblasts to enable it to endocytose. However, this would be atypical of the C-type lectin family of receptors, many of which function as endocytic receptors when transfected into the cell line used in these experiments (Stambach and Taylor, 2003; Guo *et al.*, 2004; Coombs *et al.*, 2005). Unlike the other receptors investigated here, all of which are endocytic, the cytoplasmic tail of the Kupffer cell receptor lacks a tyrosine-based internalization motif, although it does contain a di-leucine motif (Figure 3).

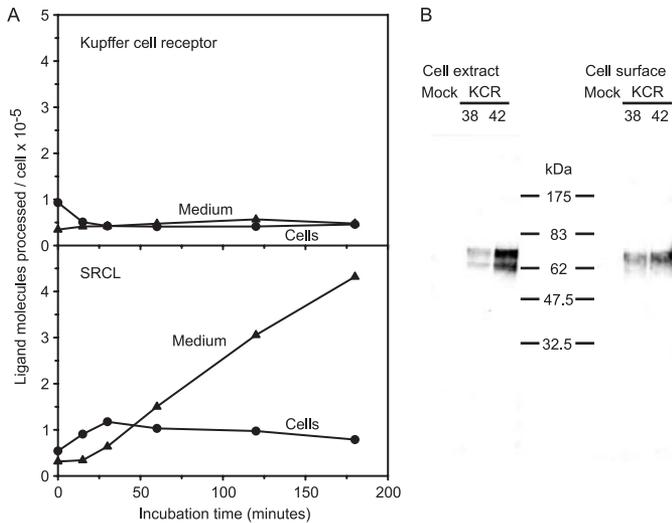


Fig. 2. Characterization of fibroblasts expressing Kupffer cell receptor. (A) Assay of uptake and degradation of ^{125}I -Gal-BSA. KCR-42 fibroblasts expressing full-length Kupffer cell receptor or SRCL were preincubated for 30 min at 4°C with 2 $\mu\text{g}/\text{mL}$ ^{125}I -Gal-BSA. Following incubation at 37°C for the times indicated, cell-associated radioactivity and acid-soluble degradation products released back into the medium were quantified. (B) Blots of Kupffer cell receptor from fibroblasts. Cell extracts and biotinylated cell-surface proteins from untransfected rat-6 fibroblasts (Mock) and two cell lines expressing Kupffer cell receptor (KCR-38 and KCR-42) were resolved by SDS-PAGE and probed with affinity-purified antibodies against the Kupffer cell receptor.

Hepatic lectins	
Human	MTKE ^Y QDLQHLDNEESDHHQLRKGGPPPPQ ^L LQRLCSGPR
Rat	MTKDYQDFQHLDNEN-DHHQLRGGPPAPRLLQRLCSGFR
Mouse	MTKD ^Y QDFQHLDNNDN-DHHQLRRGGPPPT ^R LLQRLCSGSR
Macrophage galactose lectins	
Human	MTRTY ^E NFQYLENKVKVQGFKNP-----LPLQS ^L LQRLCSGPCH
Rat	MTMAY ^E NFQNLGSEKNEAQEGK-----APPQSFLCN ^L LSWTH
Mouse 1	MIY ^E NLQNSRIEEKTQEPGK-----APSQSFLWRI ^L LSWTH
Mouse 2	MTMR ^Y ENFQNLEREKNEQMRNGDKKGGMESPKFALIPSQSFLWRI ^L LSWTH
Scavenger receptors	
Human	MKDDFAEEEEVQSF ^G YKRF ^G IQEGTQCTCKKNWALK
Rat	MKDDFAEEEEVQSF ^G YKRF ^G IQEGTQCTCKKNWALK
Mouse	MKDDFAEEEEVQSF ^G YKRF ^G IEGHTQCTCKINWALK
Kupffer cell receptors	
Rat	MKEAELNRDVAKFCTDNQCV ^L LQPPQLGPKSAAMPAPRTL ^R H
Mouse	MKEAELNRDMARYCTDNQCV ^L SLQPPQLGPKSAALMAPRTL ^R H

Fig. 3. Comparison of the cytoplasmic tail sequences of galactose-binding receptors. Potential tyrosine-based and di-leucine internalization motifs are boxed.

Discussion

The results presented here demonstrate that the glycan array provides a powerful tool for distinguishing the binding characteristics of superficially similar receptors. While previous studies have provided evidence that the galactose-binding C-type lectins differ in their relative affinities for monosaccharide ligands such as galactose and GalNAc, the full extent of their ligand-binding selectivity is only evident when larger oligosaccharide ligands are tested. The array provides an efficient way to make such comparisons. The major outcome of these studies is the demonstration that the galactose-binding receptors can be divided into two groups based on the distinct classes of ligands that they bind.

The binding profile of the Kupffer cell receptor presented here confirms that the receptor is primarily a galactose- and GalNAc-binding receptor. Although the receptor was originally referred to as the “fucose receptor,” it has low affinity for the α -linked fucose commonly found in naturally occurring glycans (Lehrman, Haltiwanger, *et al.*, 1986; Lehrman, Pizzo, *et al.*, 1986). The absence of fucose-binding activity, combined with the failure of the receptor to mediate endocytosis, makes it unlikely that it has a role in clearing fucose-containing glycoproteins from circulation. Results from the present studies combined with earlier work reveal that there are multiple C-type lectins that can endocytose fucose-containing ligands. Glycans bearing fucose in conjunction with another terminal sugar such as sialic acid or galactose, creating epitopes such as Lewis x, sialyl-Lewis x, or Lewis a, may be cleared by the asialoglycoprotein receptor and SRCL. Fucose is also bound by several receptors with mannose-type CRDs, including the mannose receptor, DC-SIGN, and langerin (Taylor, 2001; Stambach and Taylor, 2003; Guo *et al.*, 2004). This selection of C-type lectins that might be responsible for endocytosis of fucose-containing glycoproteins may explain earlier studies which suggested the presence of receptors for fucosylated ligands in addition to the asialoglycoprotein receptor and mannose receptor (Lehrman, Pizzo, *et al.*, 1986).

It has been suggested that the Kupffer cell receptor may function as a particle receptor, capable of internalizing particles greater than 12 nm in diameter coated with galactose or GalNAc (Biessen *et al.*, 1994). Such particles are much larger than Gal-BSA (~4 nm diameter). This type of phagocytic

activity might require additional specialized cellular machinery found in Kupffer cells but not in fibroblasts. Alternatively, the Kupffer cell receptor might function as an adhesion receptor. In any case, the function is not essential in mammalian biology, because no human ortholog of the Kupffer cell receptor has been identified (Fadden *et al.*, 2003).

Recent results for the human macrophage galactose lectin suggest that it differs significantly from the rat receptor studied here. An Fc-human macrophage galactose lectin chimera binds preferentially to terminal GalNAc (van Vliet *et al.*, 2005) and shows no enhanced affinity for fucosylated glycans such as GalNAc β 1-4(Fuc α 1-3)GlcNAc or for biantennary over monoantennary glycans. Other studies suggest a preference of mouse macrophage galactose lectin-1 for Lewis x and of mouse macrophage galactose lectin-2 for GalNAc-terminated sugars (Tsuiji *et al.*, 2002). It appears that rat macrophage galactose lectin is more closely related in ligand-binding characteristics to mouse macrophage galactose lectin-1, and the human receptor is more like mouse macrophage galactose lectin-2. Thus, rat and mouse both express macrophage galactose lectins capable of binding Lewis x, but this binding activity is absent in humans. Similarly, mouse and rat hepatic lectin-1 have been found to differ in their selectivity for Sia α 2-6GalNAc β -terminated glycans compared to GalNAc β -terminated glycans, even though they share very high amino acid sequence identity (Park and Baenziger, 2004). Thus, the results reported here, combined with the results of other recent studies of ligand-binding specificity of galactose-binding receptors, suggest the need for caution in making assumptions about the characteristics of receptors from one species based on results from another.

Both RHL-1 and the Kupffer cell receptor bind strongly to the GalNAc β 1-4GlcNAc epitope on the glycan array. This epitope is common on glycoproteins of invertebrates, such as helminth parasites (Cummings and Nyame, 1999), but is also found on some mammalian glycoproteins. For example, GalNAc β 1-4GlcNAc is present on glycoprotein hormones, where it is capped by 4-*O*-sulfate. Recognition of GalNAc β 1-4GlcNAc by RHL-1 raises the possibility that this receptor may be involved in interactions with parasites. Alternatively, RHL-1 might serve as a backup for the mannose receptor in clearance of glycoprotein hormones (Fiete *et al.*, 1991) on which GalNAc has become exposed due to loss of the 4-*O*-sulfate.

Materials and Methods

Expression, fluorescent labeling, and glycan array analysis

Trimeric extracellular domain fragments of the Kupffer cell receptor (KCR-B), RHL-1, and the macrophage galactose lectin (Iobst and Drickamer, 1996; Fadden *et al.*, 2003) were expressed and purified by affinity chromatography on galactose-Sepharose as previously described. The major subunit of the asialoglycoprotein receptor, RHL-1, is capable of forming homotrimers and binding glycosylated ligands (Braiterman *et al.*, 1989). The Kupffer cell receptor and the macrophage galactose lectin were dialyzed into 150 mM NaCl, 100 mM Na-HEPES (pH 7.8), and 10 mM CaCl₂, and RHL-1 was dialyzed into the same buffer but

containing 500 mM NaCl. Aliquots (~2 mL) containing 200–400 μ g of each protein were mixed with five aliquots of 10 μ L of fluorescein isothiocyanate dissolved at 1 mg/mL in dimethyl sulfoxide and left to react overnight at 4°C. Addition of an equal volume of loading buffer (1.25 M NaCl, 25 mM Tris-Cl [pH 7.8], and 25 mM CaCl₂) was followed by re-purification on a 1-mL column of galactose-Sepharose. Columns were washed with loading buffer, and proteins were eluted with 1.25 M NaCl, 25 mM Tris-Cl (pH 7.8), and 2.5 mM ethylene diamine tetraacetic acid (EDTA). Fractions containing the purified fluorescein-labeled extracellular domain fragments were used to probe the glycan array developed by the Consortium for Functional Glycomics (<http://www.functionalglycomics.org>) following their standard protocol.

Expression and analysis of full-length rat Kupffer cell receptor in fibroblasts

cDNA encoding the cytoplasmic tail and transmembrane region of the rat Kupffer cell receptor was amplified from rat liver cDNA (Marathon Ready cDNA, Clontech, Mountain View, CA) using the primers 5'-aggacagaccttagaatcgtggggcaagag-3' (forward) and 5'-gtgcagttgcttggggccatctggaggaa-3' (reverse). Following denaturation at 95°C for 1 min, 40 cycles at 95°C for 30 s and at 65°C for 1 min were carried out. Polymerase chain reaction (PCR) products were cloned into the vector pCRII-TOPO (Invitrogen, Glasgow, UK) and sequenced on an ABI prism 310 Genetic Analyzer. A fragment encoding the cytoplasmic tail and transmembrane domain was combined with the extracellular domain cDNA (Fadden *et al.*, 2003) to create the full-length receptor cDNA which was inserted into the retroviral expression vector pVcos along with the neomycin resistance gene (Stambach and Taylor, 2003). The plasmid was transfected into Ψ -Cre packaging cells, which produce a pseudovirus that was used to infect Rat-6 fibroblasts. Lines stably expressing Kupffer cell receptor were selected using G418. Analysis of the uptake and degradation of ¹²⁵I-Gal-BSA (Gal-BSA from E-Y Laboratories, San Mateo, CA) by Kupffer cell receptor and SRCL expressing fibroblasts was performed as previously described (Stambach and Taylor, 2003).

For blotting experiments, cells were harvested by scraping, suspended in 10 volumes of 125 mM NaCl, 10 mM Tris-Cl (pH 7.5), 0.5% Triton X-100, sonicated briefly, incubated for 1 h at 4°C, and spun for 2 min at 18,000 \times g. The supernatants were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 15% gel, which was blotted onto nitrocellulose. The membrane was blocked in 5% bovine serum albumin (BSA) in Tris-buffered saline and incubated with affinity-purified antibody at 1:100 dilution followed by protein A-alkaline phosphatase conjugate at 1:5000 dilution. For surface labeling (Davis *et al.*, 1998), cells at ~70% confluence were rinsed twice at 37°C with phosphate-buffered saline containing 0.1 mM CaCl₂ and 1 mM MgCl₂ followed by incubation with 2 mL of a 1 mg/mL solution of sulfo-*N*-hydroxysuccinimide-biotin (Perbio, Cramlington, Northumberland, UK) in the same buffer at 4°C for 20 min, with gentle shaking. The biotin solution was removed and the plates were washed thrice with phosphate-buffered saline containing 100 mM glycine and incubated for 45 min at 4°C in the

final wash. Plates were rinsed twice more and the cells were lysed by brief sonication in 1 mL of 100 mM Tris-Cl (pH 7.4), 150 mM NaCl, 1 mM EDTA, and 1% Triton X-100. Following incubation at 4°C for 10 min, lysates were centrifuged at 18,000 × *g* for 5 min and incubated with 100 μL of a 50% suspension of avidin-conjugated beads (Perbio) for 1 h at room temperature. Beads were recovered by centrifugation at 18,000 × *g* for 5 min and washed five times with 1 mL of lysis buffer. Proteins were eluted with 50 μL of 2× sample buffer containing 2% β-mercaptoethanol at 100°C for 5 min. Beads were removed by centrifugation, and aliquots of the supernatant were analyzed on a 15% SDS-PAGE that was blotted and stained as mentioned above.

Supplementary Data

Supplementary data are available at Glycobiology online (<http://glycob.oxfordjournals.org/>).

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Conflict of interest statement

None declared.

Abbreviations

BSA, bovine serum albumin; CRD, carbohydrate-recognition domain; RHL-1, rat hepatic lectin-1; SRCL, scavenger receptor C-type lectin.

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