Characterization of the interaction of galactose-exposing particles with rat Kupffer cells

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The characteristics of the recognition system involved in the binding of galactose-exposing particles to freshly isolated rat Kupffer cells were determined. For this purpose we used iodinated lactosylated low-density lipoprotein (^{125}I-Lac-LDL) as a ligand for the galactose receptor on Kupffer cells. The affinity of the binding of ^{125}I-Lac-LDL to Kupffer cells was saturable (23 500 galactose-specific binding sites per cell) and of high affinity (2.4 ± 0.3 nM). The order of potency of various carbohydrates in inhibiting the association of ^{125}I-Lac-LDL with Kupffer cells was as follows: *N*-acetylgalactosamine > L-fucose ≫ *N*-acetylglucosamine/mannan. Association of ^{125}I-Lac-LDL with Kupffer cells in the absence of Ca^{2+} was at the same level as in the presence of 50 mM *N*-acetylgalactosamine. A polyclonal antibody raised against the rat asialoglycoprotein receptor inhibited the binding of ^{125}I-Lac-LDL to Kupffer cells and reacted in a Western blot with two proteins (molecular mass 88 and 77 kDa), which correspond to the molecular mass of the fucose receptor [Lehrman, Haltiwanger and Hill (1986) J. Biol. Chem. **261**, 7426–7432]. Furthermore, the ability of fucosylated neoglycoproteins to displace ^{125}I-Lac-LDL from Kupffer cells was equally dependent on the extent of fucosylation as previously reported for the fucose receptor. We conclude that the fucose receptor and not the C-reactive protein, as recently proposed [Kempka, Roos and Kolb-Bachofen (1990) J. Immunol. **144**, 1004–1009], functions as the galactose-particle receptor on the Kupffer cell. The binding of galactose-exposing particles to the fucose receptor is a previously unknown property of this receptor.

INTRODUCTION

The liver contains several independent receptor systems for the elimination of carbohydrate-terminated substances from the circulation. Receptors have been described in the liver that are specific for galactose [1–6], mannose [7], *N*-acetylglucosamine [8] and fucose groups [9–15]. Galactose-specific uptake mechanisms have been characterized on both Kupffer [5,6] and parenchymal liver [1–4] cells.

The galactose-specific receptor on parenchymal liver cells, the established asialoglycoprotein receptor (ASGPr), has been characterized in detail at a physiological and molecular level [1–4]. The ASGPr specifically recognizes galactose groups of desialylated glycoproteins such as asialo-orosomucoid and asialofetuin. Binding of these proteins to the ASGPr is followed by internalization and degradation of the glycoproteins in the lysosomal compartment [1–4].

The galactose-specific receptor on Kupffer cells mediates the uptake of galactose-exposing particles and is therefore called the galactose-particle receptor (GPr) [5]. Particles of size 12 nm or more, such as lactosylated low-density lipoprotein (Lac-LDL; 22 nm) and desialylated rat erythrocytes, are cleared from the circulation by the GPr on Kupffer cells [5,6,16]. Smaller particles such as lactosylated high-density lipoprotein (12 nm) are, however, cleared from the circulation by the ASGPr on parenchymal liver cells [16]. In analogy with the ASGPr on parenchymal liver cells, binding of ligands to GPr on the Kupffer cell leads to internalization and degradation in the lysosomal compartment [6]. Recently, a galactose-binding protein has been isolated from the liver by perfusion of the liver with 20 mM EDTA. This lectin, with a molecular mass of 30 kDa, was claimed to be the GPr, as treatment of isolated Kupffer cells with 20 mM EDTA abolished the galactose-specific binding of desialylated rat erythrocytes [17,18]. Kolb-Bachofen and associates [19,20] have identified this lectin to be a subunit of C-reactive protein (CRP). It was suggested that membrane-associated CRP, which has galactose-binding properties, acts as the GPr on Kupffer cells.

Besides the GPr, Kupffer cells display various other carbohydrate-specific receptors which are involved in the uptake of carbohydrate-terminated proteins, i.e. relatively low amounts of the mannose receptor [7] and the fucose receptor [9–15]. The fucose receptor has been purified and cloned. It specifically recognizes proteins with high densities of fucosyl groups [9,14,15]. The fucose receptor interacts not only with fucose residues, but also with *N*-acetylgalactosamine and galactose-exposing neoglycoproteins [9].

The aim of the present study was to identify the recognition system on Kupffer cells involved in the uptake and degradation of galactose-exposing particles. The GPr in the rat Kupffer cell was screened using Lac-LDL as radioligand. This ligand is taken up by a galactose-specific mechanism in the Kupffer cell [21].

MATERIALS AND METHODS

Materials

Sodium cyanoborohydride was purchased from Aldrich Chemical Co. (Brussels, Belgium). Na^{125}I (carrier-free) in NaOH was obtained from Amersham International (Amersham, Bucks., U.K.). Collagenase type D and H were obtained from Boehringer-Mannheim (Mannheim, Germany). BSA (fraction V) and agarose-bound neuraminidase (from *Clostridium perfringens*, type IV-A) were purchased from Sigma (St. Louis, MO, U.S.A.). Dulbecco's modified essential medium was obtained from Flow Laboratories (Irvine, Scotland, U.K.). All other chemicals were reagent grade.

Polyclonal rabbit anti-(rat CRP) IgG and control non-immune

Abbreviations used: Lac-LDL, lactosylated low-density lipoprotein; GPr, galactose-particle receptor; ASGPr, asialoglycoprotein receptor; Fuc-BSA, fucosylated BSA; CRP, C-reactive protein.

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IgG were generous gifts from Dr. M. B. Pepys, Department of Medicine, Hammersmith Hospital, London, U.K. Polyclonal goat anti-[rat ASGPr (affinity-column-purified)] IgG and goat non-immune IgG were kindly provided by Dr. G. Ashwell (NIH, Bethesda, MD, U.S.A.). Anti-(rat CRP) antibody specifically reacted with rat CRP (isolated from rat serum) and anti-(rat ASGPr) antibody did not show any reactivity against CRP in an Ouchterlony test.

Isolation and radioiodination of asialo-orosomucoid and LDL

Human orosomucoid was isolated and subsequently desialylated enzymically as described [22]. Human LDL ($1.024 < d < 1.063$) was isolated by differential ultracentrifugation, as described by Redgrave et al. [23]. The (lipo)proteins were radiolabelled with carrier-free $Na^{125}I$ by the ICl method of McFarlane [24] as modified by Bilheimer et al. [25].

Modification of (lipo)proteins

Lactosylation of LDL was performed by reductive amination, and 390 lactose residues were incorporated per LDL particle [26,27].

Fucosylation of BSA was performed exactly as described [28,29].

Isolation of Kupffer cells and parenchymal cells

Parenchymal liver and Kupffer cells used for *in vitro* binding studies were isolated from male Wistar rats. Rats were anaesthetized with sodium pentobarbital (20 mg, intraperitoneal injection) and subsequently the liver was perfused for 10 min with collagenase [0.05% (w/v), type D] at 37 °C following essentially the method of Seglen [30,31]. Parenchymal cells were isolated by differential centrifugation (30 s at 50 *g*), and Kupffer cells were isolated by density gradient centrifugation and centrifugal elutriation (3250 rev./min at 70 ml/min). The purity of types of cell was more than 95%, and the viability (checked by ATP content and Trypan-Blue exclusion) was more than 95% throughout the experiments.

Association and degradation of ^{125}I-Lac-LDL by Kupffer cells

For binding studies, suspensions of Kupffer and parenchymal cells were incubated at a density of 1×10^6 cells/ml in Dulbecco's modified essential medium containing 2% (w/v) BSA and 25 mM Hepes (pH 7.4) [32]. Cell incubations were performed in Kartell plastic tubes in a circulating laboratory shaker (150 rev./min; Adolf Kuhner) at either 4 °C or 37 °C. At the end of the incubations, cells were washed (30 s at 50 *g* for parenchymal liver cells and 5 min at 500 *g* for Kupffer cells) twice with 10 mM Tris/HCl buffer, pH 7.4, containing 150 mM NaCl, 2.5 mM $CaCl_2$ and 0.2% (w/v) BSA and once with the same buffer without BSA. Finally, cells were resuspended in this buffer (without BSA), and cell-associated radioactivity was counted in a γ counter, and cell protein was determined by the method of Lowry et al. [33] with BSA as standard. Degradation of iodinated ligands was determined in the first cell-free supernatant after incubation of the cells for the indicated times at 37 °C, exactly as described [34].

The dissociation constant (K_d) and maximal specific binding ($B_{max.}$) were determined from displacement and binding curves according to a single-site displacement model using a computerized non-linear fitting program (Graph-Pad: H. Motulsky, ISI-Software) [32,34].

Rosette-formation assay

Desialylated rat erythrocytes were prepared by neuraminidase treatment as described previously [18]. Desialylated erythrocyte suspensions were mixed with an equal volume of rat Kupffer cells and centrifuged for 5 min at 80 *g* and subsequently incubated for 60 min at 4 °C [18]. The amount of Kupffer cells binding at least three desialylated rat erythrocytes was determined under the microscope in the presence or absence of competitors.

Liver cell membrane isolation and Western blotting

Kupffer and parenchymal liver cell membranes were prepared from freshly isolated cells. Briefly, cells were homogenized in buffer A (50 mM Tris, 150 mM NaCl, 0.2 mM EDTA, 1 mM phenylmethanesulphonyl fluoride and 100 kallikrein inhibitory units of trasylol/ml, pH 7.5) by ultrasonication. Intact cells were removed by centrifugation (30 s at 50 *g* for parenchymal liver cells and 5 min at 500 *g* for Kupffer cells). The resulting supernatant was centrifuged at 29000 *g* for 90 min. The (membrane) pellets were solubilized in buffer A containing 40 mM *N*-octylglucoside by incubation for 45 min at 4 °C. The solubilized membrane proteins were separated by SDS/PAGE (12% gel) as described by Laemmli [35] and blotted on to a nitrocellulose filter. Blots were incubated for 60 min at 37 °C with 5% skimmed milk in buffer B (10 mM Tris, 150 mM NaCl, 0.5 mM $CaCl_2$). Subsequently, the blot was washed four times with buffer B, incubated for 60 min at 37 °C with goat–anti-(rat ASGPr) antibody, washed four times with buffer B, and the bound antibody was visualized with rabbit–anti-goat IgG coupled to peroxidase followed by peroxidase staining.

RESULTS

The binding of Lac-LDL to freshly isolated rat Kupffer cells is shown in Figure 1. Galactose-specific binding of ^{125}I-Lac-LDL reached a maximum at concentrations of more than 10–15 nM. This typical binding curve is indicative of the presence of a single galactose-specific high-affinity binding site with an apparent affinity of 2.4 nM (1.3 μg of Lac-LDL/ml) and a maximum binding of 127 ng/mg of cell protein. The affinity of

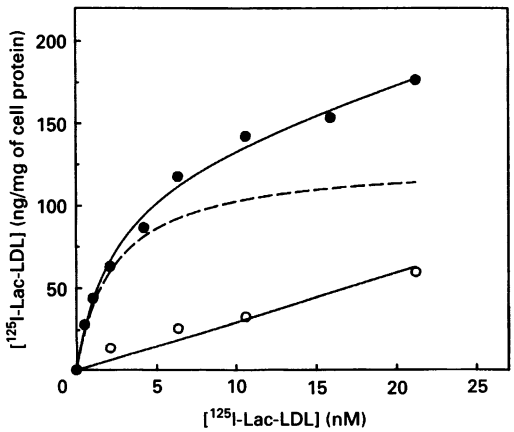

Figure 1 Binding of ^{125}I-Lac-LDL to rat Kupffer cells

The binding of increasing amounts of ^{125}I-Lac-LDL to rat Kupffer cells was determined in the presence (○, non-specific binding) or absence (●, total binding) of 100 mM *N*-acetylgalactosamine after incubation at 4 °C for 120 min. The broken line indicates the specific binding, defined as the difference between total and non-specific binding. Data are from one typical binding curve out of nine.

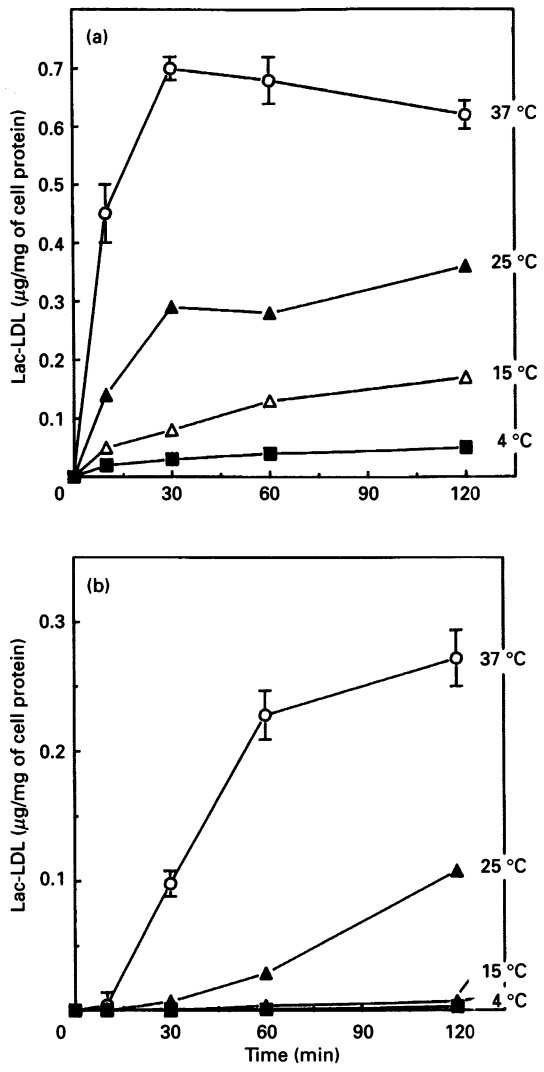

Figure 2 Association and degradation of ^{125}I-Lac-LDL by rat Kupffer cells at various temperatures

The association of ^{125}I-Lac-LDL (2 μg/ml) with (**a**) and degradation by (**b**) rat Kupffer cells was determined after incubation for various times at the indicated temperatures. Data are from one typical binding curve out of three.

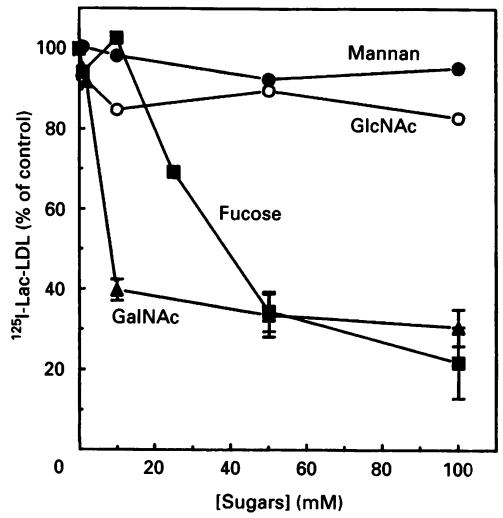

Figure 3 Effect of various carbohydrates on the association of ^{125}I-Lac-LDL with isolated Kupffer cells

The effect of increasing amounts of various carbohydrates on the association of ^{125}I-Lac-LDL (5 μg/ml) with isolated rat Kupffer cells was determined after 30 min of incubation at 37 °C. The following sugars were tested: *N*-acetylgalactosamine (▲), *N*-acetylglucosamine (○), mannan (●) and L-fucose (■). Data [means ± S.D. ($n = 4$)] are expressed as percentages of the association of ^{125}I-Lac-LDL with Kupffer cells in the absence of competitors.

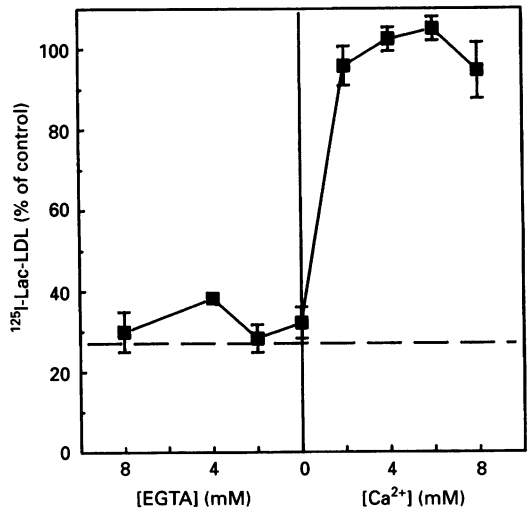

Figure 4 Effect of Ca^{2+} and EGTA on the association of ^{125}I-Lac-LDL with isolated Kupffer cells

The effects of various concentrations of EGTA or Ca^{2+} were determined on the association of Lac-LDL with rat Kupffer cells after incubation for 30 min at 37 °C in the presence of ^{125}I-Lac-LDL (5 μg/ml). Data [means ± S.D. ($n = 4$)] are expressed as percentages of the binding of ^{125}I-Lac-LDL in the presence of 2 mM Ca^{2+}. The broken line indicates the binding of ^{125}I-Lac-LDL in the presence of 50 mM *N*-acetylgalactosamine and represents non-specific binding.

Lac-LDL binding was constant from isolation to isolation [K_d 2.4 ± 0.3 nM (mean ± S.D., $n = 9$)], but the level of galactose-specific binding showed more variation from isolation to isolation: 142 ± 42 ng/mg of cell protein (mean ± S.D., $n = 9$) (approximately 23500 galactose-specific binding sites/Kupffer cell).

The effect of temperature on the association and degradation of ^{125}I-Lac-LDL by freshly isolated Kupffer cells is shown in Figure 2. The association increased markedly with increasing temperatures, indicating that the association of ^{125}I-Lac-LDL with rat Kupffer cells is apparently temperature sensitive. As could be expected, degradation was not observed below 20 °C. Degradation at 37 °C was detected after a lag phase of at least 10 min and proceeded linearly for 60 min. The kinetics are indicative of internalization of ^{125}I-Lac-LDL after binding followed by (lysosomal) degradation.

The carbohydrate specificity of the association of ^{125}I-Lac-LDL with Kupffer cells is shown in Figure 3. *N*-acetylgalactosamine was the most effective inhibitor. L-Fucose was also able to inhibit the association of ^{125}I-Lac-LDL, but its inhibitory capacity (IC_{50} = 30 mM) was much lower than that of *N*-acetylgalactosamine (IC_{50} = 5 mM). *N*-Acetylglucosamine and mannan were unable to compete for the binding of ^{125}I-Lac-LDL to Kupffer cells.

Figure 4 shows the Ca^{2+}-dependence of the association of ^{125}I-Lac-LDL with isolated rat Kupffer cells. Binding in the absence

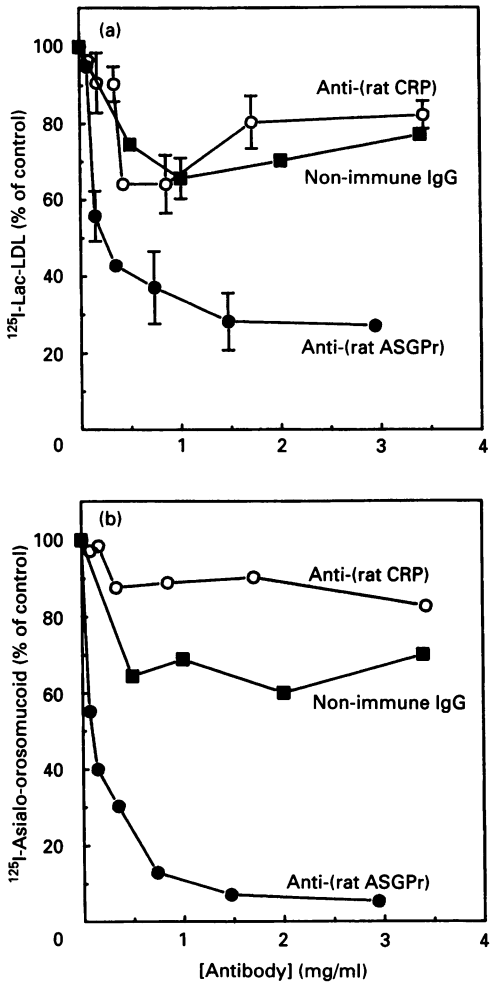

Figure 5 Effect of various antibodies on the association of ^{125}I-Lac-LDL with isolated rat Kupffer cells and ^{125}I-asialo-orosomucoid with parenchymal liver cells

The effects of antibodies against CRP (○) and ASGPr (●) were determined on the binding of ^{125}I-Lac-LDL (5 μg/ml) to rat Kupffer cells after incubation for 30 min at 37 °C (**a**). ■, Non-immune IgG. The effects of the same antibodies were tested on the binding of ^{125}I-asialo-orosomucoid to parenchymal liver cells after incubation for 10 min at 37 °C (**b**). Data [means ± S.D. ($n = 4$)] are expressed as percentages of the binding of ^{125}I-Lac-LDL or ^{125}I-asialo-orosomucoid to Kupffer cells and parenchymal liver cells in the absence of competitors.

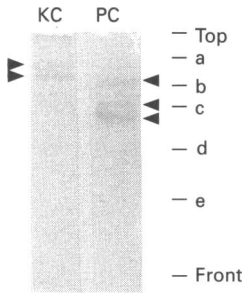

Figure 6 Western blot of membrane proteins from Kupffer cells and parenchymal liver cells with anti-(rat ASGPr) antibody

Solubilized membrane proteins from Kupffer (KC) and parenchymal liver (PC) cells were separated by electrophoresis and blotted on to nitrocellulose membranes. The blots were incubated with anti-(rat ASGPr) antibody, and the binding of anti-ASGPr antibody was subsequently visualized (see the Materials and methods section). Marker proteins are indicated by **a** (97 kDa), **b** (67 kDa), **c** (43 kDa), **d** (30 kDa) and **e** (21 kDa). The top and front of the gel are indicated.

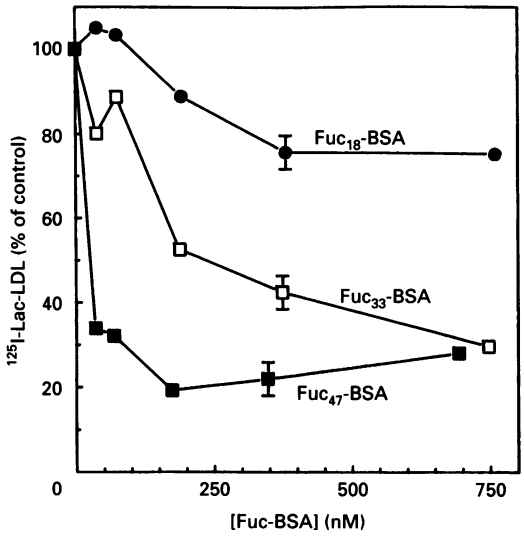

Figure 7 Effect of fucosylated neoglycoproteins on the binding of ^{125}I-Lac-LDL to isolated rat Kupffer cells

The effect of Fuc-BSA [carrying 18 (●), 33 (□) or 47 (■) fucosyl residues] on the association of ^{125}I-Lac-LDL (5 μg/ml) with rat Kupffer cells was determined after incubation for 30 min at 37 °C. Data [means ± S.D. ($n = 4$)] are expressed as percentages of the binding of ^{125}I-Lac-LDL in the absence of competitors.

of Ca^{2+} was 80% lower than in the presence of Ca^{2+} and comparable with the level of binding in the presence of 50 mM *N*-acetylgalactosamine.

As the GPr on rat Kupffer cells has been suggested to be a membrane-associated version of CRP, we tested polyclonal antibodies against rat CRP for their capacity to prevent the binding of ^{125}I-Lac-LDL to the GPr. As a control, we also determined the effect of polyclonal antibodies raised in goat against rat ASGPr on the binding of ^{125}I-Lac-LDL to Kupffer cells. As shown in Figure 5(a), the effect of anti-(rat CRP) antibody on the association of ^{125}I-Lac-LDL with Kupffer cells was not significantly different from the effect of non-immune IgG. Anti-(rat ASGPr) antibodies, however, significantly inhibited the association of ^{125}I-Lac-LDL with Kupffer cells by 80%. As a control the effect of both antibodies was tested on the galactose-specific binding of asialo-orosomucoid to rat parenchymal liver cells (Figure 5b). Only the anti-(rat ASGPr) antibody was able to completely inhibit the galactose-specific binding of ^{125}I-asialo-orosomucoid to parenchymal liver cells, indicating that the antibody was indeed reactive with ASGPr.

To determine whether the anti-(rat ASGPr) antibody reacted with specific protein(s)/receptors on rat Kupffer cells, we performed Western blotting with both parenchymal and Kupffer cell membrane proteins (Figure 6). The anti-(rat ASGPr) antibody specifically bound to three protein bands on parenchymal liver cells. A band with a molecular mass of 44 kDa stained most intensely, but two minor bands of molecular masses 54 and 72 kDa were also detected. The molecular mass of the major band closely resembles that of rat hepatic lectin (RHL)-1 (43 kDa), which constitutes 70% of the ASGPr in rat

Table 1 Effect of various competitors on rosette formation by Kupffer cells and association of ^{125}I-lac-LDL with isolated rat Kupffer cells

The effects of antibodies against CRP, ASGPr and neoglycoproteins (350 nM) derivatized with 18, 33 or 47 fucosyl residues were determined on the binding of ^{125}I-Lac-LDL (5 μg/ml) to rat Kupffer cells after incubation for 30 min at 37°C or on rosette formation. Data [means ± S.D. ($n = 4$)] are expressed as percentages of the Lac-LDL association or rosette formation in the absence of competitors. In both, the amount of aspecific binding is indicated by the amount of binding in the presence of *N*-acetylgalactosamine (50 mM).

	Binding of ^{125}I-Lac-LDL	Formation of rosettes
Control	100	100
N-Acetylgalactosamine	33.5 ± 5.4	28.1 ± 5.1
anti-(rat ASGPR)	28.2 ± 10.6	33.5 ± 12.0
anti-(rat CRP)	80.3 ± 7.0	83.6 ± 5.0
Control IgG	67.7 ± 3.0	89.6 ± 5.0
Fuc_{18}-BSA	75.6 ± 2.6	89.0 ± 4.7
Fuc_{33}-BSA	42.4 ± 2.6	50.0 ± 5.1
Fuc_{47}-BSA	22.0 ± 5.0	29.1 ± 11.3

parenchymal liver cells [36]. Lee and Lee [36] have also described two minor bands of the rat ASGPr with molecular masses of 52 (RHL-2) and 60 kDa (RHL-3). The two minor protein bands visualized in our Western-blot studies have slightly higher molecular masses. The anti-(rat ASGPr) antibody reacted with two Kupffer-cell membrane proteins with molecular masses of 77 and 88 kDa. These molecular masses are identical with those published for the fucose receptor monomers [9–15]. Western blots of parenchymal- and Kupffer-cell membrane proteins stained with anti-(rat CRP) antibody did not identify CRP on these cells. Furthermore, we could not detect any reaction of anti-(rat ASGPr) antibody with CRP by Western blotting.

Next we compared several characteristics of ligand recognition by the fucose receptor with those of the ^{125}I-Lac-LDL-binding site on Kupffer cells. Figure 7 shows that only extensively fucosylated BSA (Fuc-BSA) with 33 or 47 fucosyl residues/molecule of albumin was able to displace the binding of ^{125}I-Lac-LDL to Kupffer cells. The ability of Fuc_{47}-BSA to inhibit ^{125}I-Lac-LDL binding to Kupffer cells was much greater than that of Fuc_{33}-BSA. Fuc_{18}-BSA on the other hand was unable to displace ^{125}I-Lac-LDL from Kupffer cells at concentrations of up to 750 nM.

Finally, we determined the binding profile of another galactose-exposing particle, desialylated erythrocytes [18,37], to Kupffer cells and compared this with the binding profile of ^{125}I-Lac-LDL. Table 1 shows that the effect of anti-(rat ASGPr) antibody and anti-(rat CRP) antibody on rosette formation of desialylated erythrocytes with Kupffer cells is almost identical with the effect of these antisera on the association of ^{125}I-Lac-LDL. The pattern of inhibition of rosette formation by the various fucosylated neoglycoproteins was identical with the inhibition of ^{125}I-Lac-LDL association (Table 1). Fuc_{18}-BSA barely affected rosette formation, whereas an increasing degree of fucosylation (from 33 to 47 fucosyl residues per molecule of albumin) led to an increasing inhibition of rosette formation.

DISCUSSION

Galactose-terminated particles are cleared from the circulation by a galactose-specific uptake system on Kupffer cells. This so-called GPr shows a preference for particulate ligands that expose galactose groups, such as (desialylated) erythrocytes [5,19,20,37], LDL particles carrying terminal galactose groups (tris-galactoside-terminated cholesterol-LDL [6] and Lac-LDL [21]), carcinoma autoantigens [38], gold particles coated with lactosylated albumin [39] and plasma fibrinogen [40]. Kolb-Bachofen and co-workers suggested that a 30 kDa galactose-specific lectin that could be isolated from the liver using high concentrations of EDTA (20 mM) was identical with the GPr, as treatment of Kupffer cells with 20 mM EDTA abolished the galactose-specific binding by these cells. This 30 kDa lectin was more recently identified as a subunit of CRP, and it was suggested that the GPr might be a membrane bound CRP subunit [19,20].

In the present study we used Lac-LDL as a ligand for the GPr on isolated Kupffer cells. *In vivo*, Lac-LDL is predominantly taken up by Kupffer cells via a galactose-specific mechanism and is subsequently degraded in the lysosomes [21].

In vitro, freshly isolated rat Kupffer cells bind ^{125}I-Lac-LDL with an apparent affinity of 2.4 ± 0.3 nM in a galactose-specific way. Maximal specific binding of ^{125}I-Lac-LDL to Kupffer cells was 142 ± 42 ng/mg of cell protein, which is equivalent to approximately 23 500 binding sites per Kupffer cell. The number of receptors may, however, be underestimated, as one molecule of Lac-LDL, which is a large ligand (20 nm) and carried many recognition sites, may bind to more than one Kupffer cell receptor. The number of galactose-specific binding sites for ^{125}I-Lac-LDL is somewhat lower than the number of binding sites for fibronectin-coated gold particles (35000 binding sites), which also bind in a galactose-specific manner to rat Kupffer cells [39]. The association of ^{125}I-Lac-LDL with rat Kupffer cells increased with increasing temperature. However, it should be realized that at temperatures above 20 °C, internalization of the ligand–receptor complex occurs; the receptor is subsequently recycled at an apparent turnover rate of approx. 7.8 min (37 °C) [29]. When corrected for the internalized ligand, cell association of ^{125}I-Lac-LDL at 37 °C is still twofold higher than binding at 4 °C. This may be because the affinity of the receptor for ^{125}I-Lac-LDL increases at higher temperatures, as has been observed for other receptors [41,42].

Carbohydrate specificity and absolute Ca^{2+}-dependence of ^{125}I-Lac-LDL binding to Kupffer cells were almost identical with that observed for galactose-specific rosette formation by Kupffer cells [19,20,36]. The carbohydrate specificity and Ca^{2+}-dependence, however, match equally well the binding profile of the isolated fucose receptor, as described by Hill and co-workers [9].

More insight into the nature of the galactose-specific binding came from the use of polyclonal antibodies raised against rat CRP and rat ASGPr. The antibody raised against ASGPr specifically inhibited ^{125}I-Lac-LDL binding to Kupffer cells, and, in a Western blot, two Kupffer cell membrane proteins (88 and 77 kDa) reacted with the anti-(rat ASGPr) antibody. As the above molecular masses are identical with those observed for the isolated fucose receptor [13,14], and, in addition, the binding specificity of ^{125}I-Lac-LDL to Kupffer cells corresponds to the binding specificity of the isolated fucose receptor, we conclude that the fucose receptor and not CRP was primarily involved in the binding of ^{125}I-Lac-LDL to Kupffer cells. The unexpected cross-reactivity of the anti-(rat ASGPr) antibody with the fucose receptor on Kupffer cells may be the result of an impurity in the antigen (affinity-column isolation of ASGPr may also yield some fucose receptor, as this receptor also binds galactosyl residues on glycoproteins). The cross-reactivity of this polyclonal antibody might, however, also result from the great homology between the fucose receptor and the ASGPr [14,15]. Ii et al. [43] have demonstrated a galactose/*N*-acetylgalactosamine-specific 42 kDa lectin on rat peritoneal macrophages, the so-called macrophage asialoglycoprotein-binding protein. This lectin displayed a high

immunochemical cross-reactivity with the ASGPr on the parenchymal liver cell. However, in our Western-blot studies, we did not observe any staining of a 42 kDa protein in the Kupffer-cell preparation, indicating that the Kupffer cell does not possess significant levels of the macrophage asialoglycoprotein-binding protein.

As established, the affinity of the fucose receptor for neoglycoproteins increases exponentially with increasing degree of fucosylation. Only those neoglycoproteins with a high degree of fucosylation (33 or 47 fucose residues per albumin) were able to compete with ^{125}I-Lac-LDL binding to Kupffer cells. The neoglycoprotein that contained only 18 fucose residues per albumin had almost no ability to inhibit ^{125}I-Lac-LDL binding to Kupffer cells, in agreement with the observation that more than 24 fucose residues per molecule of albumin are needed to induce binding to the isolated fucose receptor [9].

Finally we tested whether the binding of sialylated erythrocytes to Kupffer cells is also mediated by the fucose receptor. Both the effect of the anti-(rat ASGPr) antibody and the effect of the fucosylated neoglycoproteins on rosette formation by rat Kupffer cells indicated that the binding of other galactose-exposing particles, such as desialylated rat erythrocytes, is also mediated by the fucose receptor.

In conclusion, the so-called GPr is identical with the well-defined fucose receptor [9–15]. CRP is therefore not involved in the interaction of galactose-exposing particles with Kupffer cells. The fucose receptor displays the previously unknown characteristic of mediating the binding and degradation of galactose-exposing particles by rat Kupffer cells.

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