# Two galactose-specific receptors in the liver with different function

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In the rat liver both hepatocytes and macrophages have been shown to express on the surface lectins with similar binding specificity for galactose residues. Functionally the two lectins differ in the uptake of ligands. Whereas the hepatocytes ingest molecules and small particles (< 10 nm), the macrophages take up particles only. Antisera raised against hepatic galactose-specific receptor failed to react with the macrophage lectin but blocked ligand binding to the hepatocyte lectin. Accordingly, labeling with these antisera and fluorescent protein A occurs with hepatocytes only, indicating either a different antigenic structure or membrane localization of the two lectins.

*Hepatocyte* *Liver macrophage* *Asialoglycoprotein receptor* *Antibody* *Electron microscopy* *Ligand uptake*

## 1. INTRODUCTION

D-Galactose-specific lectins are expressed at the surface of the various types of liver cells; i.e., hepatocytes, liver macrophages and endothelial cells [1–3]. Best characterized of the three liver lectins is that on hepatocytes, first described by Ashwell and Morell [1]. In recent years a similar lectin was discovered on liver macrophages [2], and further characterized [4–7]. Comparison of the hepatocyte and liver macrophage lectin revealed similar binding specificity [4,6] but also some marked differences in the uptake of ligands of various sizes [3]. The aim of this paper is to describe in detail differences between the corresponding lectins on hepatocytes and liver macrophages. These differences provide an explanation why several groups failed to demonstrate a galactose-specific receptor on liver macrophages by classical ligand-uptake tests.

*Abbreviations:* ASF, asialofetuin; FITC, fluorescein-isothiocyanate; Gal, D-galactose; GalNAc, *N*-acetyl-D-galactosamine; Lac-BSA, lactosylated bovine serum albumin

## 2. MATERIALS AND METHODS

### 2.1. *Isolation of liver cells*

Livers of male Wistar rats (100–200 g) were perfused with 0.6% collagenase (Boehringer, Mannheim) for about 10 min as described in [6]. Hepatocytes and liver macrophages were enriched by differential centrifugation as in [6]. The final cell suspensions were adjusted to $2 \times 10^6$ cells/ml.

### 2.2. *Erythrocytes*

Freshly drawn rat blood mixed with sodium citrate was washed 3 times with Eagle's medium supplemented with 10 mM sodium phosphate buf-

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fer (pH 7.4). Erythrocytes ($10^9$ cells/ml) were incubated with 0.1 units/ml of neuraminidase (from *Vibrio cholerae*, Behringwerke, Marburg), washed 3 times with modified Eagle's medium and suspended to give a final density of $1 \times 10^8$ erythrocytes/ml.

### 2.3. *Cell binding assay*

Hepatocytes or liver macrophages (50 $\mu$l, $2 \times 10^6$/ml) were incubated for 10 min with 10 $\mu$l inhibitor (carbohydrate or antisera) or buffer as control. Then 50 $\mu$l $1 \times 10^8$ erythrocytes/ml (either untreated or neuraminidase-treated) were added, the suspension spun down at $80 \times g$ for 5 min at 4°C followed by incubation for 1 h at 4°C. For evaluation of binding, cells were resuspended by gentle shaking of tubes and stained with crystal violet. The percentage of cell contact forming liver cells was counted in a haemocytometer.

### 2.4. *Fluorescence test*

Hepatocytes or liver macrophages (10 $\mu$l, $2 \times 10^6$ or $4 \times 10^6$/ml, respectively) were incubated for 20 min at 4°C with 2.5 $\mu$l antiserum and subsequently for 20 min at 4°C with the fluorescent label (F-Lac–BSA or FITC–protein A). The suspension was diluted with 1 ml Hanks' solution (with 20 mM Hepes and 2 mM $CaCl_2$, pH 7.4) and centrifuged for 4 min at $20 \times g$ (hepatocytes) or $80 \times g$ (liver macrophages). After resuspension in 20 $\mu$l buffer containing 0.25% glutaraldehyde cells were observed by fluorescence microscopy.

### 2.5. *Electron microscopy*

Electron microscopy and the preparation of electron-dense ligand were performed as in [7].

### 2.6. *Carbohydrates and antisera*

Saccharides were purchased from Serva (Heidelberg), FITC–protein A was from Pharmacia (Freiburg). Fluorescence-labeled lactosylated bovine serum albumin (FITC–Lac–BSA) was prepared as in [8]. Three different antisera to the isolated hepatic galactose-specific lectin were used. Antiserum 1 was prepared in a goat by immunization with galactose-receptors from whole rat liver. It contains antibodies against the lectin binding site [9]. Antisera 2 and 3 were prepared in goats in a similar way [10]. Antiserum 2 contains antibodies against the binding site, antiserum 3 binds to structures not involved in ligand binding.

## 3. RESULTS

### 3.1. *Binding studies*

The presence of receptors for D-galactose/*N*-acetyl-D-galactosamine on the surface of both hepatocytes and liver macrophages was demonstrated in two different ways. When using a large particulate ligand, i.e., neuraminidase-treated rat erythrocytes, binding in both liver cell types is seen. By this method 60–95% of hepatocytes and 80–98% of macrophages show galactose–lectin activity. Comparative inhibition studies show that the carbohydrate specificity of the two receptor types is highly similar (table 1). When liver cells are incubated with fluorescence-labeled Lac–BSA, surface fluorescence is observed with both cell types. By this method >50% of hepatocytes and liver macrophages show galactose–lectin activity.

### 3.2. *Uptake studies*

Another type of ligand (visible by electron microscopy) are gold particles coated with Gal/GalNAc exposing glycoproteins (e.g., asialofetuin, ASF). Experiments with gold particles of various sizes revealed remarkable differences in their uptake by either hepatocytes or liver macrophages (table 2).

Table 1

Saccharide inhibition of rosette formation of hepatocytes or liver macrophages with neuraminidase-treated rat erythrocytes

| | Hepatocytes | Liver macrophages |
|---|---|---|
| *N*-Acetyl-D-galactosamine | 0.5 ± 0.2 | 0.6 ± 0.3 |
| D-Galactose | 1.2 ± 0.4 | 1.4 ± 0.4 |
| Methyl-$\beta$-D-galactoside | 1.4 ± 1.0 | 3.8 ± 1.8 |
| $\alpha$-Lactose | 2.0 ± 1.8 | 5.0 ± 1.0 |
| Melibiose | 3.4 ± 1.2 | 5.0 ± 2.6 |
| Methyl-$\alpha$-D-galactoside | 7.6 ± 3.4 | 13 ± 1.4 |
| D-Mannose | >50 | >50 |
| *N*-Acetyl-D-glucosamine | >50 | >50 |
| D-Glucose | >50 | >50 |

The values give the concentration (mM) ± standard deviation for 50% inhibition and are from 3 expt

Table 2

Uptake of ASF-coated gold-particles of different size (5, 17 and 50 nm) by hepatocytes and liver macrophages, respectively, as determined by electron microscopy

| | $ASF\text{-}Au_5$ | $ASF\text{-}Au_{17}$ | $ASF\text{-}Au_{50}$ |
|---|---|---|---|
| Hepatocytes | + | − | − |
| Liver macrophages | + | + | + |

### 3.3. *Immunological studies*

We tested the immunological cross-reactivity between hepatocyte and liver macrophage lectin with antisera raised against hepatic asialoglycoprotein receptor in two different ways:

(1) In the fluorescence test labeling with the 3 antisera and FITC-protein A only occurred with hepatocytes but not with liver macrophages. In detail, liver cells were incubated as in section 2 with goat antisera 1–3 to rat liver galactose-specific receptor. Rim-shaped fluorescence was observed for each serum with >80% of freshly isolated hepatocytes but not with liver macrophages.

(2) Rosette formation of liver cells is obtained with neuraminidase-treated rat erythrocytes (see table 1). The cell adhesion can be blocked by the 3 antisera again only in hepatocytes (fig.1).

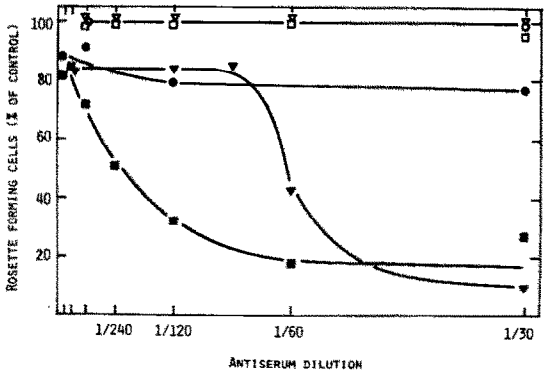


Fig.1. Effect of anti-receptor antibodies on the binding of neuraminidase-treated rat erythrocytes by hepatocytes and liver macrophages. Hepatocytes (closed symbols) and liver macrophages (open symbols) are preincubated with antiserum 1 (■, □), antiserum 3 (▼, ▽) or a control hyperimmune serum (●, ○) for 10 min at 4°C prior to addition of neuraminidase-treated erythrocytes. Rosette formation in controls: Hepatocytes 95% (inhibition by 25 mM GalNAc: 81%); liver macrophages 90% (inhibition by 25 mM GalNAc: 96%).

## 4. DISCUSSION

These results support our previous observation that not only hepatocytes but also liver macrophages express on the cell surface a lectin-like receptor for terminal Gal/GalNAc residues [4–7]. The two receptors appear to have highly similar binding specificity. Nevertheless we describe here marked differences between these lectins with respect to function (i.e., ligand uptake) and an apparent lack of immunological cross-reactivity.

The Gal/GalNAc-receptor of the hepatocyte mediates the internalization of molecular asialoglycoproteins and small particles only whereas the corresponding receptor on macrophages mediates endocytosis (pinocytosis) of particulate ligands without size restriction. Furthermore, the macrophage lectin also mediates phagocytosis of desialylated cells (Funke, Schlepper-Schäfer, Kolb-Bachofen, Kolb, in preparation). Non-particulate, molecular asialoglycoproteins, however, are apparently not taken up by macrophages [3]. This lack of ingestion of molecular ligands had led to the conclusion that liver macrophages are devoid of galactose-specific receptors [1,11–16]. The liver macrophage lectin therefore has been named galactose-particle receptor [3]. Considering their relative localization to the blood stream this differential function of hepatocyte and liver macrophage lectins appears plausible. The restriction of macrophages to galactose-particle uptake may find its explanation in the preclustered arrangement of galactose-receptors on the membrane of these cells [7] which appears suitable for the binding of large multivalent particles. The functional differences between hepatocyte and liver macrophage lectins are supported by our observation of different reactivity of the two receptors with antibodies. Three different antisera raised against Gal/GalNAc-specific lectins isolated from total liver tissue were tested. All of them bound to the hepatocyte receptor but none of them bound to or blocked the activity of the liver macrophage lectin. It is not clear at present whether this is due to antigenic differences or to positioning of the liver macrophage

receptor in such a way that it cannot be reached by the antibodies.

These experiments describe significant differences between the Gal/GalNAc-specific receptor of hepatocytes and the corresponding lectin on liver macrophages. It therefore seems probable that the two receptors have different physiological functions in vivo. Experiments to isolate the Gal/GalNAc-specific lectin from pure liver macrophages preparations and to raise antibodies are in progress.

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