PRESENCE OF A LECTIN-LIKE RECEPTOR FOR D-GALACTOSE ON RAT PERITONEAL MACROPHAGES

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1. Introduction

Neuraminidase-treated erythrocytes adhere to macrophages in vitro [1,2] as well as in vivo [3-5]. In vivo desialylated erythrocytes are taken up by macrophages of the liver (Kupffer cells) when injected intravenously [5]. We have found that adhesion of neuraminidase-treated erythrocytes to Kupffer cells is due to a galactose-specific lectin on the macrophage [6].

We now report the presence of a similar galactose-specific receptor on rat peritoneal macrophages.

2. Materials and methods

2.1. *Animals*

Osborn-Mendel rats (200–250 g) were used. The rats were kindly provided by Dr L. Herberg.

2.2. *Peritoneal cells*

Rats were injected intraperitoneally with 0.2 ml drawing ink (Gunther Wagner, Pelikan fount India), dialysed against physiological saline. Rat peritoneal cells were obtained 45 min later by washing the peritoneal cavity with 15 ml Eagle's medium (Flow, Bonn). Cells were washed twice by centrifugation at 100 × *g* for 3 min. Blood was withdrawn from the tail, mixed with sodium citrate, and washed 3 times with Eagle's medium. Erythrocytes (10^9 cells/ml) were incubated with 0.1 units/ml of neuraminidase (from Vibrio cholerae, Behringwerke, Marburg) at 37°C for 30 min and washed 3 times.

2.3. *Cell adhesion assay*

Peritoneal cells (100 μl) containing 5×10^6 macrophages were mixed with an equal volume of erythrocytes (10^8 cells/ml) in 3 ml plastic test tubes. After 1 h at 5°C cells were resuspended by gentle shaking of tubes by hand and counted in a hemocytometer. Cells which had phagocytosed carbon particles were regarded as macrophages. Cell adhesion was regarded as positive when ⩾3 erythrocytes adhered to 1 macrophage (rosette).

Inhibition studies were performed by preincubating peritoneal cells with saccharides (Sigma, Munich) or glycoproteins at 5°C for 5 min, or with sodium azide, cytochalasin B, colchicine (Serva, Heidelberg) or antirat IgG at 37°C for 30 min. Gamma globulin fractions of goat and rabbit anti-rat immunoglobulin antisera (Nordic, Tilburg) were prepared by precipitation with sodium sulfate and absorbed twice with a mixture of untreated and desialylated rat erythrocytes [7]. Antisera reacted with H-chains of IgG and L-chains of all Ig classes.

3. Results

When peritoneal macrophages were mixed with neuraminidase-treated erythrocytes spontaneous rosette formation was observed (table 1). Cell contacts could be specifically inhibited by preincubation of macrophages with mono- or oligosaccharides related in structure to D-galactose with the exception of D-galactosamine (table 1).

The specificity of the inhibition reaction is further demonstrated in fig.1. The concentration leading to 50% inhibition of cell contacts were 16 mM for D-galactose and 9 mM for *N*-acetyl-galactosamine. Even at 100 mM, inhibition by D-glucose and D-galactosamine was not significant.

Adhesion between macrophages and desialylated erythrocytes could also be blocked by the addition of

Table 1
Recognition of desialylated erythrocytes by peritoneal macrophages

Erythrocytes	Saccharides[a]	Rosette-forming peritoneal macrophages (% ± SD)	Inhibition (%) of rosette formation
Untreated	None	8.9 ± 3.6	
Desialylated	None	61.2 ± 13.0	
Desialylated	D-Glucose	60.2 ± 11.3	1.6
Desialylated	*N*-Acetyl-D-glucosamine	60.0 ± 10.0	2.0
Desialylated	D-Mannose	58.4 ± 11.6	4.6
Desialylated	D-Galactose	17.2 ± 3.7	71.8
Desialylated	D-Galactosamine	58.0 ± 11.6	5.2
Desialylated	*N*-Acetyl-D-galactosamine	14.8 ± 3.2	75.8
Desialylated	D-Fructose	58.0 ± 10.8	5.2
Desialylated	L-Fucose	22.0 ± 5.2	64.1
Desialylated	D-Fucose	19.1 ± 4.5	68.8
Desialylated	Lactose	21.9 ± 4.9	64.2
Desialylated	Lactulose	17.4 ± 4.3	71.6
Desialylated	Melibiose	30.9 ± 6.3	49.5

[a] Peritoneal cells were preincubated with the saccharide (25 mM) for 5 min at 5°C

glycoproteins with terminal galactosyl residues (table 2). Glucosylated bovine serum albumin also impaired rosette formation but was 10-times less effective than galactosylated BSA.

Peritoneal macrophage receptor function was found to depend on the presence of Ca^{2+} (table 3). Similarly, pretreatment of cells with an inhibitor of ATP-formation (NaN_3) impaired rosette formation. No effect was seen after disturbing microfilament or microtubulus function by cytochalasin B or colchicine, respectively (table 3). Also receptor function could not be blocked by the addition of polyvalent anti-rat immunoglobulin antisera.

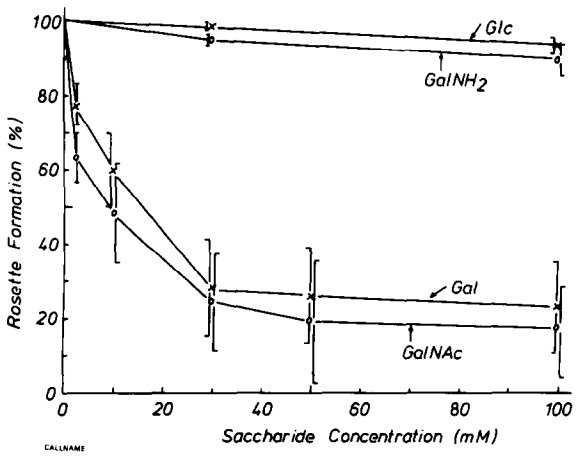

Fig.1. Inhibition of peritoneal macrophage receptor function by monosaccharides. Peritoneal cells were preincubated with the saccharide for 5 min at 5°C. Bars indicate standard deviation.

4. Discussion

The experiments reported here demonstrate the presence of a receptor on the cell surface of peritoneal macrophages with specificity for galactosyl and glucosyl residues. The receptor is able to mediate cell contacts between macrophages and erythrocytes. The receptor reacts with monosaccharides sharing with D-galactose the *stereo* configuration of the C4 hydroxyl group, the only exception being D-galactosamine. Also oligosaccharides with D-galactose at the non-reducing end are recognized. At the level of glycoproteins not only D-galactose but also D-glucose is recognized. The avidity of the receptor for glucosylated BSA, however, was found to be 10-times lower than for galactosylated BSA.

The peritoneal macrophage receptor is not an immunoglobulin for the following reasons. The assay system is devoid of serum, i.e., serum antibodies are not present. Blocking of residual membrane bound immunoglobulin on macrophages with anti-immunoglobulin sera is of no effect. In contrast to immunoglobulins receptor function is dependent on the presence of Ca^{2+}. Finally, macrophage subpopulations

Table 2
Recognition of glycoproteins by the peritoneal macrophage receptor

Glycoproteins[a]	Conc. (μM)	Rosette-forming peritoneal macrophages (% ± SD)	Inhibition (%) of rosette formation
None		66.7 ± 10.2	
Orosomucoid[b]	50	63.3 ± 10.7	5.1
Asialo-orosomucoid[b]	50	17.7 ± 3.2	73.5
D-Galactosyl-BSA[c]	1	15.0 ± 3.1	77.5
D-Glucosyl-BSA[c]	10	16.1 ± 4.0	75.9
BSA	10	61.6 ± 7.7	7.6

[a] Peritoneal cells were preincubated with glycoproteins for 5 min at 5°C
[b] Orosomucoid and asialo-orosomucoid were a gift from Dr G. Ashwell (National Institutes of Health, Bethesda, MD)
[c] D-Galactosyl bovine serum albumin (37 mol D-galactose/mol BSA) and D-glucosyl-BSA (35 mol D-glucose/mol BSA) were a gift from Dr Y. C. Lee (The Johns Hopkins University, Baltimore, MD)

Table 3
Characteristics of peritoneal macrophage receptor function

Inhibitor[a]	Conc.	Rosette-forming peritoneal macrophages (% ± SD)	Inhibition (%) of rosette formation
None		63.5 ± 8.3	
EDTA	2 mM	7.4 ± 1.4	88.3
EGTA	2 mM	6.1 ± 1.4	90.4
NaN_3	10 mM	20.9 ± 5.5	67.1
Cytochalasin B	40 μM	63.2 ± 8.2	0.5
Colchicine	250 μM	59.9 ± 8.0	5.7
Anti-rat IgG (Rabbit)	20 μl	60.4 ± 7.3	4.8
Anti-rat IgG (Goat)	20 μl	59.1 ± 7.7	6.9

[a] Peritoneal cells were preincubated with inhibitors for 30 min at 37°C

exist in the rat, which do not express the receptor although they carry immunoglobulin on the surface (Y. N., H. K., in preparation).

The peritoneal macrophage receptor closely resembles the hepatic lectin first described in [8,9] and a lectin found on Kupffer cells [6,7]. In each case the receptor recognizes both galactosyl and glucosyl residues [7,9] and its activity is calcium-dependent. Inhibition of ATP production, however, only affected receptor activity of peritoneal macrophages but not of hepatocytes [10] or liver macrophages [7].

The function of D-galactose/D-glucose-specific receptors on macrophages is not known. Terminal galactosyl residues, however, are known to be expressed on a number of virus infected cells [11], tumor cells [12] and bacteria [13]. We therefore have suggested that macrophage lectins may function as receptor for 'altered self' and 'non-self' [14,15].

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