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A LECTIN-LIKE RECEPTOR ON MAMMALIAN MACROPHAGES

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SUMMARY: Rat Kupffer cells in vitro strongly bind neuraminidase-treated rat erythrocytes but not untreated erythrocytes. Binding between cells is inhibited by preincubation of macrophages with D-galactose and related sugars, but not with unrelated saccharides. We therefore suggest that cell adherence is mediated by a galactose-specific receptor on the Kupffer cell membrane.

In multicellular organisms phagocytes are able to distinguish between self and nonself and also between young and old cells (1). The recognition mechanism involved in phagocyte action remains unclear, though many hypotheses have been brought forward (see 2,3,4). Recently the adhesion of neuraminidase-treated erythrocytes to macrophages of the liver and mononuclear spleen cells in vitro has been described as a model for phagocytic recognition of senescent cells (5). We have now further analysed this type of cell interaction and found that carbohydrate specific receptors on the phagocyte mediate adhesion of asialo-erythrocytes.

MATERIALS AND METHODS

A single cell suspension from the rat liver was prepared by the collagenase perfusion method as described previously (6). Kupffer cells were separated from hepatocytes by centrifuging twice at 20g for 3 min. The supernatant contained between 30 and 60% Kupffer cells. Kupffer cells were identified by their ingestion of ink particles which had been injected into the rat i.v. 2 hours prior to sacrifice.

Rat erythrocytes (10^9 cells/ml) were incubated with 50 units of Vibrio cholerae neuraminidase per ml (Behring-

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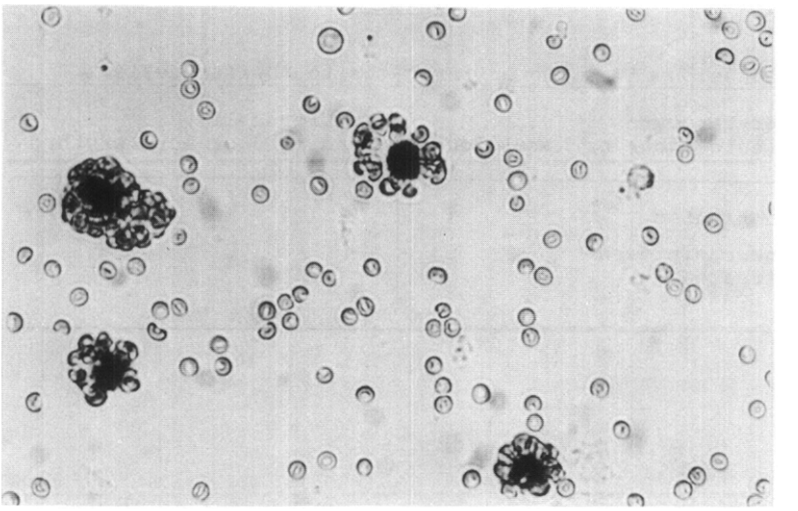

Figure 1: Cell contacts between Kupffer cells and asialo-erythrocytes. Kupffer cells appear dark due to the ingestion of ink particles.

werke, Marburg, W. Germany) for 30 min at 37°C and washed three times in Hank's buffer. 100 µl of Kupffer cells (10^6 cells/ml) were mixed with 100 µl of asialo-erythrocytes (10^8 cells/ml) in round bottomed tubes, centrifuged for 5 min at 120g and incubated on ice for 60 min. Cells were then resuspended by shaking up by hand.

RESULTS

When Kupffer cells and neuraminidase-treated rat erythrocytes were mixed, adhesion between the two cell types occured (Fig. 1). Almost 100% of Kupffer cells were covered with a single layer of asialo-erythrocytes whereas non-treated rat erythrocytes adhered only to a small degree (Tab. 1).

In order to test whether β-D-galactosyl residues, which are revealed on erythrocytes after neuraminidase-treatment, were involved in binding we performed hapten inhibition experiments with various mono- and oligosaccharides.

As shown in Table 2 the formation of cell contacts between Kupffer cells and asialo-erythrocytes could be preven-

TABLE 1

Adhesion of asialo-erythrocytes to Kupffer cells

Erythrocytes (autologous or homologous)	Kupffer cells binding 3 or more erythrocytes
untreated	5 - 10%
neuraminidase-treated	95%

TABLE 2

Specificity of contacts between Kupffer cells and asialo-erythrocytes

Saccharide *	Concentration necessary for 50% inhibition (mM)
D-Glucose	>100
N-Acetyl-D-Glucosamine	>100
D-Mannose	>100
L-Fucose	49
D-Fucose	28
Lactose	22
D-Galactose	16
N-Acetyl-Galactosamine	5

* Kupffer cells were preincubated with the saccharide for 5 min at 0°C.

ted by the addition of galactose related sugars. Since unrelated saccharides were not inhibitory in even 10 times higher concentrations an unspecific effect on cell adhesion can be ruled out. Binding of asialo-erythrocytes is not dependent on the metabolic state of Kupffer cells since clusters were formed at 0°C as well as at 37°C, both living and dead cells, were involved. Electron microscopic studies showed that cell

contacts lead to total surrounding of erythrocytes by Kupffer cell microvilli with subsequent phagocytosis. (Kolb-Bachofen and Kolb, to be published).

DISCUSSION

The results reported here indicate that Kupffer cells bear a membrane receptor with specificity to D-galactosyl residues. We have reported previously that hepatocytes are also able to bind asialo-erythrocytes (7) and asialo-lymphocytes (8) via a similar receptor. The hepatic receptor involved is probably the same as that described by Ashwell and co-workers (9) for the binding of asialo-glycoproteins to the liver cell membrane. The fine specificity of hepatocyte recognition of asialo-erythrocytes is almost identical to binding properties of the Kupffer cell receptor. In both cases N-acetyl-D-galactosamine is the most inhibitory saccharide. The relative inhibitory activity of D-galactose, D-fucose, L-fucose and lactose is also comparable with the hepatocyte receptor. This would indicate close similarities of D-galactose specific receptors on both Kupffer cells and hepatocytes. In vivo, binding of asialo-glycoproteins could not be observed with rabbit Kupffer cells in autoradiographic studies but only with parenchymal liver cells (10). In a more recent study, however, binding of aislao-glycoproteins to Kupffer cells in mice in vivo was observed (Newman, personal communication). The experiments reported here also suggest that (in the rat) Kupffer cells bind both desialylated proteins and cells but they may take up only particles by endocytosis whereas glycoproteins could be metabolized by hepatocytes exclusively. It is known that only particles and not soluble proteins are taken up by primitive phagocytic cells (11).

At present it cannot be decided whether the Kupffer cell receptor is identical to the hepatocyte receptor or not. The finding of similar fine specifity in both cases is not sufficient to prove identity since lectins with similar binding properties are also found in primitive organisms like slime molds (12).

The physiological function of the receptor has not yet been determined. We suggest that the Kupffer cell receptor is involved in the entrapment of asialo-erythrocytes, asialo-lymphocytes and senescent blood cells in the liver; in addition the hepatic receptor may also be involved (7,8 and Kolb and Kolb-Bachofen, to be published). The finding of a D-galactose-specific protein on the Kupffer cell surface demonstrates that macrophages are able to differentiate between self and nonself (old) not only by unspecific but also by specific recognition mechanisms.

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