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Short communication

HOMOLOGOUS LIVER PARENCHYMAL CELL-CELL ADHESION MEDIATED BY AN ENDOGENOUS LECTIN AND ITS RECEPTOR

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Abstract: Many studies have implicated cell-surface lectins in heterologous cell-cell adhesion, but little is known about the participation of lectins in cellular adhesion in homologous cells. Here, we show the development of a cell model for investigating the direct role of a cell-surface lectin in homologous cell-cell adhesion. Parenchymal cells were isolated from caprine liver using a perfusion buffer, and dispersed in a chemically defined modified Ringer's solution. These cells undergo autoagglutination in the presence of Ca^{2+}. The autoagglutinated cells can be dissociated specifically with D-galactose (50 mM), which also inhibits the liver cell autoagglutination event. The blood serum protein fetuin has no effect on liver cell autoagglutination, whereas desialylated fetuin (100 μM), with its terminal D-galactose residue, showed a high affinity for blocking the autoagglutination event. The data demonstrates the occurrence of a Ca^{2+}-dependent D-galactose-specific lectin and a lectin receptor on the parenchymal cells. Furthermore, it shows that the observed autoagglutination event is caused by the interaction of the cell-surface lectin with its receptor on the neighbouring homologous cells. The data supports the view that homologous cell-cell contact in mammalian tissues is triggered by such lectin-receptor interaction and that the previously reported cell-surface adhesive proteins serve as a secondary force to strengthen cell adhesion. This cell model could be extremely useful for investigating the direct role of cell-surface lectin and its receptor in homologous cell adhesion in a variety of tissues under normal and pathological conditions.

Key words: Autoagglutination, Homologous cell-cell adhesion, Lectin, Lectin receptor, Liver, Parenchymal cell

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Abbreviation used: CAMs – cell adhesion molecules

INTRODUCTION

Cellular adhesion is the binding of a cell to another cell or to a surface or matrix. The cells of multicellular organisms are capable of distinguishing between different cell types, and they adhere preferentially to cells of their own type. Cellular adhesion is regulated by specific cell adhesion molecules (CAMs) that interact with molecules on the apposing cell or surface [1-4]. Some of the major classes of CAM that have been identified in eukaryotes are fibronectins, cadherins, integrins, and Ig superfamily members. CAMs are large intrinsic membrane glycoproteins localized on the external cell surface [5, 6]. Carbohydrate-mediated cell-cell recognition plays a vital role in the orderly development and functioning of multicellular organisms [7, 8]. Cell-surface lectins have been implicated as vital in cell adhesion in mammalian tissues [9-11]. Lectins of one cell surface bind to specific complementary sugar residues of an apposing cell to induce cellular adhesion. Reported studies on the role of cell surface lectin in cell-cell and cell-substratum adhesions in mammalian tissues under normal and pathological conditions primarily center on the binding of one cell type with another cell type, i.e. heterologous cell-cell adhesion [8, 12-19]. Selectin, a member of the C-type lectin family that mediates heterologous cell-cell contacts, is a classic example. It is involved in attachment processes including those of leukocytes to the vascular endothelium; lymphocytes to the high endothelial venules of lymph nodes [12]; colon carcinoma cells to the lung endothelium [13], and colorectal tumor cells to endothelial cells [14]. D-galactose-specific lectin localized on liver parenchymal cell (hepatocyte) and Kupffer cell surfaces can bring about specific cell-cell contacts with appropriate non-homologous cells such as desialylated erythrocytes or lymphocytes [15-18], untreated thymocytes [16] or tumor cells [19]. However, the specific role of lectins in cell-cell adhesion in a population of homologous cells in mammalian organs is largely unknown. Here, we show for the first time the development of a mammalian cell model (using liver parenchymal cells) for investigating the direct role of a cell-surface lectin in homologous cell-cell adhesion.

MATERIALS AND METHODS

Chemicals

L-Fucose, D-mannose, D-glucose, sucrose and D-galactose were purchased from the British Drug House, Bombay. Fetuin was obtained from Sigma Chemical Co., St. Louis. Asialofetuin was obtained by removing the terminal sialic acid residue of fetuin, as described by Spiro [20]. Fresh liver tissues of adult goats were obtained from local slaughterhouses 2-3 h after the slaughter of the animals.

Isolation of liver cells

Parenchymal cells were isolated from caprine liver as per the method of Seglen [21] with some modification. The livers were cut into small pieces and incubated

for 30 min at 37ºC in a perfusion buffer (125 mM NaCl, 15 mM Na_2HPO_4, 2.47 mM KH_2PO_4, 5 mM glucose, 1 mM $CaCl_2$; pH 7.2). The pieces of liver were mildly homogenized in the perfusion buffer [21] in a loosely fitting glass homogenizer using two gentle strokes to make the uniform suspension needed for the separation of parenchymal and non-parenchymal cells. After filtration through cheesecloth, the suspension was kept at 4ºC for 1 h while the parenchymal cells sedimented. The supernatant fluid containing the non-parenchymal cells was removed with a Pasteur pipette. The sedimented parenchymal cells were re-suspended in the perfusion buffer and centrifuged at 50 g for 2 min. This process of washing was repeated three times to remove the contaminating tissue fluid. The isolated cells were then dispersed in a chemically defined RPS medium (modified Ringer's solution) containing 119 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM glucose, 16.3 mM K-phosphate, pH 6.9, and 50 units/ml penicillin. The cells were washed twice in the RPS medium and finally dispersed in the same medium. The washed cells were then counted in a haemocytometer using a phase contrast microscope. The yield of cells per gram of liver was approximately $100x10^6$.

Measurement of the autoagglutination of the liver cells

The isolated liver parenchymal cells dispersed in the RPS medium were tested for their autoagglutination efficacy. The standard assay medium contained $6x10^6$ parenchymal cells in a total volume of 0.5 ml of RPS medium, and the cell suspensions were incubated at 37ºC for 15-30 min. Cell agglutination was observed under a light source and was also inspected under a phase contrast microscope at 400x magnification. The cell agglutination data was expressed with '+' signs, with 4+ representing maximal cell agglutination when more than 90% of the liver cells were agglutinated, 1+ representing minimal detectable visible liver cell agglutination (approx 25% cell agglutination), and 3+ and 2+ respectively representing approximately 75% and 50% cellular agglutination.

Assay of anti-agglutination

The anti-agglutinin activities of different sugars were estimated using the liver cell as the model that undergoes autoagglutination when incubated *in vitro*. Intact parenchyma cells of the liver were incubated with and without the test substance in a total volume of 0.5 ml of RPS medium at 37ºC for 60 min. The degree of cell aggregation was then assessed as described above. The anti-agglutination potency of a sample was expressed as its efficacy to inhibit liver cell agglutination. Anti-agglutinin-mediated decreases in cell agglutination from the initial value of 4+ to final values of 2+, 1+ and 0+ were respectively assumed to represent inhibitions of cell agglutination to the extents of 50, 75 and 100%.

RESULTS AND DISCUSSION

Parenchymal cells are the main cell type in the liver. The isolated parenchymal cells were well defined and homogeneous (Fig. 1A). When incubated *in vitro* at

37ºC in a modified Ringer's solution, these highly purified liver cells adhered to each other forming cell clusters of varying sizes (Fig. 1B). This autoagglutination was time-dependent. It was detectable after 15 min of incubation of the liver cell suspension, and the maximal agglutination was noted after 60 min of incubation, when more than 75-80% of the cells had agglutinated. Studies with different bivalent metal ions showed that the agglutination of liver cells is dependent on Ca^{2+}, while Mg^{2+}, Zn^{2+} and Co^{2+} had no appreciable effect on liver cell agglutination. Ca^{2+} at a 1 mM concentration caused maximum cell autoagglutination (Tab. 1).

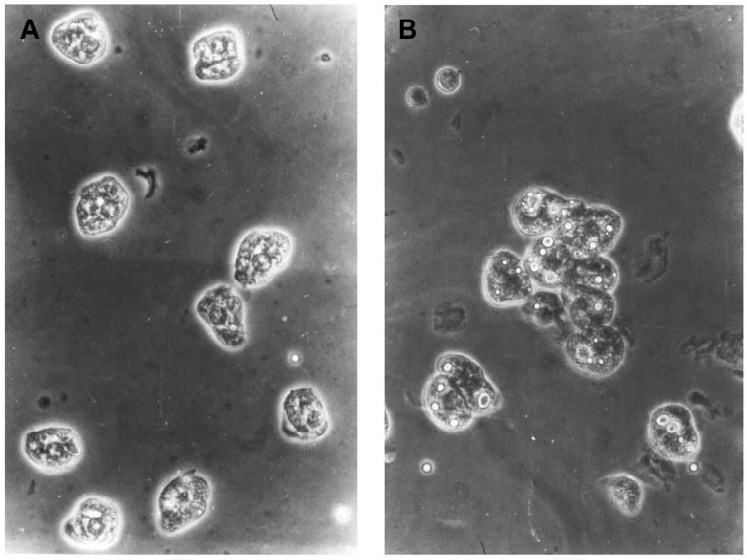

Fig. 1. Autoagglutination of liver parenchymal cells under the standard assay conditions. Micrographs (magnification of 400x) of liver cells before (A) and after (B) the autoagglutination event. The results are representative of three experiments.

Tab. 1. The effect of calcium on liver cell autoagglutination. Parenchymal cells from the livers of adult goats were isolated using the procedure described in the Materials and Methods section, except that the perfusion medium lacked Ca^{2+}. The effect of the various concentrations of Ca^{2+} on the agglutination of the liver cells was studied under the standard assay conditions. The results are representative of three experiments.

System	Agglutination (%)
Control	Nil
+ $CaCl_2$ (0.1 mM)	25
+ $CaCl_2$ (0.25 mM)	50
+ $CaCl_2$ (0.5 mM)	75
+ $CaCl_2$ (1 mM)	100

Tab. 2. The effects of different sugars on liver cell autoagglutination. Agglutination studies were performed under standard assay conditions except for the additions of various sugars at concentrations of 1 mM and 50 mM. The results are representative of three experiments.

Additions	Agglutination Concentration of sugar	
	1 mM	50 mM
Control (Nil)	4+	4+
D-Glucose	4+	3+
L-Fucose	4+	4+
D-Mannose	4+	4+
Sucrose	4+	4+
D-Galactose	2+	Nil

Several sugars were tested for their anti-agglutinin efficacy with a view to finding out if the adhesion process is dependent on a cell-surface sugar-binding protein (Tab. 2). L-Fucose, D-mannose and sucrose at concentrations of 1 mM and 50 mM had no appreciable effect on the autoagglutination event. Of all the sugars tested, D-galactose was the most potent inhibitor of liver cell adhesion. Glucose at 50 mM showed a low level of inhibition of the autoagglutination process. Galactose-mediated inhibition of liver cell agglutination was dose-dependent (Fig. 2), and maximum inhibition (100%) was noted at a sugar concentration of 50 mM. The data implies the localization on the liver cell outer surface of a Ca^{2+}-dependent D-galactose-specific lectin that binds to its specific receptor on the neighbouring homologous cells.

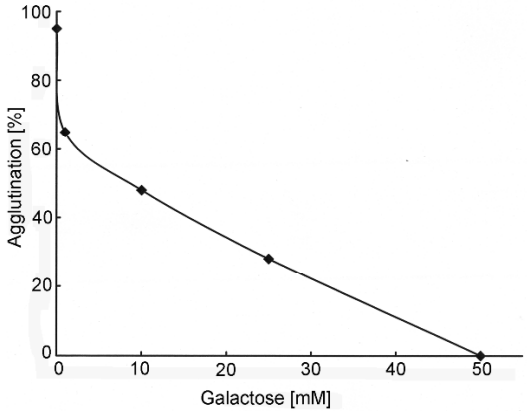

Fig. 2. The dose course of D-galactose for its inhibitory action on the autoagglutination of isolated goat liver cells under standard conditions. Each value is the mean of closely agreeing duplicate determinations and the data is representative of three experiments.

Commercially available purified proteins such as serum albumin, myoglobin and fetuin (2.4 mg/ml) did not show any appreciable anti-agglutinin activity in the liver cell autoagglutination model. Asialofetuin, generated by the removal of the terminal sialic acid residue from fetuin [20], contains a D-galactose residue at its terminal end. Asialofetuin strongly inhibited liver cell agglutination in a dose-dependent manner (Fig. 3). At a concentration of 10 μM, asialofetuin inhibited the liver cell autoagglutination process by approximately 60%, whereas at 100 μM, it nearly completely inhibited cellular agglutination. By contrast, fetuin, which has no D-galactose at the terminus of the sugar chain, did not show any appreciable anti-agglutinin action when tested at varying concentrations from 10 μM to 100 μM (data not shown). The data indicates that the D-galactose located at the sugar terminus of the polypeptide chain in asialofetuin confers anti-agglutinin potency to the protein molecule, but the protein loses this property if the hydroxyl group of the galactose at the C-4 position is blocked by sialic acid. The results also demonstrate that specific protein-bound D-galactose is much more potent as an anti-agglutinin than free galactose.

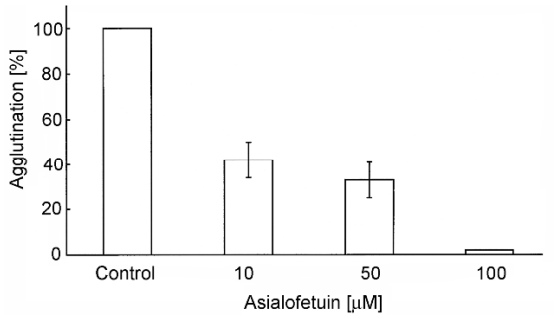

Fig. 3. Agglutination studies were done by microscopic analysis under the standard assay conditions with the addition of asialofetuin at concentrations of 10, 50 and 100 μM. Agglutination was 100% in the control system (without asialofetuin). The data is the means ± s.e.m. of three experiments ($p < 0.01$).

Membrane-bound D-galactose-specific lectin (asialoglycoprotein receptor) has been identified in the liver of several species of animals [22-24]. D-galactose-specific lectin localized on the liver parenchymal and the Kupffer cell surfaces can bring about specific cell-cell contacts with various appropriate non-homologous cells including lymphocytes, thymocytes and tumor cells [15-19]. The receptor for D-galactose-specific lectin has been identified in the small non-parenchymatous cells of the liver, but is absent from the large parenchymal cells, which are the main cells of the liver [25]. This study provides for the first time several lines of evidence supporting the occurrence of a D-galactose-specific lectin and its receptor on the outer surface of the same parenchymal cells. The observed liver cell autoagglutination (Fig. 1B) can thus be attributed to the interaction of the D-galactose-specific lectin of a parenchymal cell with the receptor of the neighbouring homologous cells and *vice versa*. To the best of our

knowledge, this is the first report on the localization of a lectin and its receptor on the external surface of homologous cells of a mammalian organ. Earlier studies from our laboratory revealed the existence of a synchronous modulation of cell-surface D-galactose-specific lectin and its receptor in the homologous goat epididymal spermatozoa as they reach maturity during epididymal transit [26]. The D-Galactose/asialofetuin-mediated inhibition of liver cell autoagglutination and the complete dissociation of the liver cell clusters with D-galactose or asialofetuin (Tab. 2, Figs 2, 3) show that cell-surface lectin-receptor interaction is the primary mechanism for the liver cell-cell adhesion. As the liver cells were obtained by applying a mild mechanical force [21] without any proteolytic/other enzymic modifications of the cell surface macromolecules, these cells were likely to have on their surface the adhesive proteins reported earlier [1-6]. As evident from the results of this investigation, the parenchymal cell-surface adhesive proteins did not manifest any appreciable activity in relation to the observed lectin receptor-mediated homologous cell adhesion *in vitro*. The precise biochemical role of the cell surface adhesive proteins [1-6] in the context of lectin-receptor interactions of the homologous cells is not clear. The cell surface adhesive proteins may serve as the secondary force to strengthen the cell-to-cell contact triggered by the lectin-receptor interactions. This cell model may be extremely useful for investigating the direct role of the cell-surface lectin and its receptor in cell-cell adhesion in a variety of tissues under normal and pathological conditions. It is well documented that cell-cell adhesion, especially cell detachment and attachment, play vital role in cancer metastasis. Our novel finding may thus have an important implication in tumor biology research.

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