DIFFERENT CELL SURFACE GLYCOPROTEINS ARE INVOLVED IN CELL–CELL AND CELL–COLLAGEN ADHESION OF RAT HEPATOCYTES

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1. Introduction

Two fundamental characteristics of many vertebrate tissues are that the cells in the tissues (a) adhere to each other [1] and (b) are anchored to extracellular matrix components such as collagen and fibronectin [2,3]. Both of these features are important in the formation and maintenance of the tissues. Cell–cell and cell–matrix interactions are also essential for other basic cell biological phenomena, e.g., regulation of motility and of proliferation [1]. Adhesion of actively metabolizing cells to other cells or to matrix components is mediated by specific cell surface molecules [4–8]. However, our knowledge about such adhesion molecules is still limited. It is not known if various adhesion phenomena in the same cell, such as cell–cell and cell–matrix adhesion, are mediated by the same or by different surface molecules. Nevertheless cell–substrate adhesion has been considered to be essentially the same phenomenon as cell–cell adhesion [9]. Here we show that different cell surface molecules seem to be involved in the initial reactions of cell–cell and cell–collagen adhesion of rat hepatocytes.

2. Materials and methods

2.1. *Cell adhesion assays*

Rat hepatocytes were isolated by a collagenase perfusion procedure as in [10]. Procedures have also been published for the determination of: (a) intercellular adhesion measured as cell aggregation [7]; (b) cell attachment to collagen-coated culture dishes [3]; (c) inhibition of intercellular adhesion [7] or cell-collagen attachment [11] by antibodies directed against hepatocyte plasma membranes; and (d) neutralization by solubilized membrane components of the antibody-inhibition of hepatocyte adhesion [7].

2.2. *Membrane preparation*

Hepatocytes are polarized in vivo, their surfaces having three different regions which face the sinusoids, neighbouring hepatocytes and the bile canaliculi, respectively [12]. Plasma membranes representing each of these 3 regions can be prepared [12]. Here two such preparations were used. Sinusoidal membranes were prepared as in [12] (fraction M-L). Membranes enriched in the contiguous faces of neighbouring hepatocytes were prepared as in [13]. The latter type of membranes contain junctional complexes. In addition microsomes, prepared by classical differential centrifugation after homogenization in 0.25 M sucrose [12], were used. Antisera against JM and SM, denoted anti-JM [7] and anti-SM [11], respectively, were prepared as in [7,11].

2.3. *Membrane solubilization and fractionation*

In order to produce specific anti-sera, effective in inhibiting cell–cell and cell–collagen adhesion of hepatocytes, solubilized membrane components were fractionated and were then used for immunization. The details of the purification procedures and of the characterization of the antisera will be published elsewhere. Briefly, the fractionations were carried out as follows.

Membrane components involved in hepatocyte

Abbreviations: SDS, sodium dodecyl sulfate; JM, junction-containing membranes; SM, sinusoidal membranes; CAM cell adhesion molecules; PBS, phosfate-buffered saline; app. M_r, apparent relative molecular mass

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intercellular adhesion were partially purified after solubilization of JM with papain [7]. The ability of these components to neutralize anti-JM antibody-mediated inhibition of hepatocyte aggregation was used to follow their purification [7]. The papain-solubilized components were applied to affinity-chromatography on immobilized *Lens culinaris* hemagglutinin. The material which was adsorbed to the lectin and desorbed with α-methyl mannoside was chromatographed on Sephadex G-200. Components having K_{av} 0.17–0.33 were pooled and subjected to chromatography on DEAE-Sephadex at pH 6.0 Material eluting with 0.225–0.275 M NaCl was pooled and was finally subjected to preparative polyacrylamide electrophoresis in SDS. Components with neutralizing activity had app. M_r of 60 000–100 000 according to their migration in the polyacrylamide gel electrophoresis. These components, denoted cellCAM were used to immunize a rabbit and the resultant antiserum was denoted anti-cellCAM.

Membrane components involved in hepatocyte-collagen attachment were partially purified after solubilization of microsomes with Triton X-100. Microsomes were chosen since the yield of SM, which are prepared from microsomes, is too low to make further fractionation meaningful. The ability of these components to neutralize anti-SM antibody-mediated inhibition of hepatocyte attachment to collagen was used to follow their purification. However, in this assay the membrane components were first adsorbed to a hydrophobic matrix (Biobeads SM-2, Biorad) and washed to remove the detergent which otherwise would destroy the cells. The detergent-solubilized components were applied to affinity chromatography on immobilized *Lens culinaris* hemagglutinin. The material which was adsorbed to the lectin and desorbed with α-methyl mannoside was subjected to affinity chromatography on collagen–Sepharose. The collagen–Sepharose column was developed with NaCl gradient. The fractions with neutralizing activity were pooled and these components, denoted collagenCAM, were used to immunize a rabbit. The resultant antiserum was denoted anti-collagenCAM.

2.4. *Antibody production*

Antisera were produced by immunizing rabbits with membranes or isolated membrane components as in [7,11]. The IgG fraction, and Fab fragments thereof, of the antisera were prepared as in [7,11].

2.5. *Immune precipitation*

Rat liver plasma membranes (JM) were labelled with ^{125}I by the lactoperoxidase technique [14]. Iodinated membranes (1.3 mg protein/ml) were solubilized with 1% Triton X-100 in PBS in the presence of 2 mM phenylmethyl sulfonyl fluoride for 60 min at room temperature. The suspension was centrifuged at 100 000 × *g* for 60 min and to 400 μl supernatant, 50 μl respective anti-serum were added. After 15 h at 4°C formaldehyde-fixed bacteria (200 μl 15% *Staphylococcus aureus*, strain Cowan I) were added. The mixtures were incubated at room temperature for 30 min and were then centrifuged at 2000 × *g* for 20 min. The pellets were washed 3 times in 0.1% Triton X-100 in PBS. The washed pellets were treated with 100 μl 4% SDS, 6 M urea, 0.068 M Tris–HCl (pH 6.8) and were boiled for 2 min. The suspensions were centrifuged at 2000 × *g* for 30 min and the supernatants were reduced with 5% β-mercaptoethanol. The reduced supernatants were analyzed by SDS–polyacrylamide gel electrophoresis on 10% gels according to [15]. The gels were dried and exposed to Kodak No-screen X-Ray films.

3. Results and discussion

The strategy used in investigating if identical or different cell surface molecules are involved in cell–cell and cell–collagen adhesion, respectively, was to prepare and compare the effects of various antisera with abilities to inhibit these adhesion reactions. In the first approach we tested antisera against SM and JM, respectively. If different surface molecules are involved in cell–cell and cell–collagen adhesion, antibodies prepared against the sinusoidal sides of the hepatocytes, where the collagen-containing reticular fibres are found, should preferentially inhibit attachment to collagen. Analogously, antibodies directed against the contiguous intercellular junction-containing faces should preferentially inhibit cell–cell adhesion. Indeed these antibodies had the expected relative effects, anti-JM being more effective than anti-SM in preventing cell aggregation and anti-SM being a more potent inhibitor of cell–collagen attachment than anti-JM (not shown). However, this specificity was not absolute and at higher concentrations, and after further booster injections, both types of antibodies effectively inhibited both types of hepatocyte adhesion. This behaviour could be explained

by the reasonable assumption that the various plasma membrane fractions are not exclusively derived from the sinusoidal or the contiguous faces, but are cross-contaminated.

Thus more specific antisera were required, and with this in mind anticellCAM and anti-collagenCAM were produced as in section 2. Anti-cellCAM and anti-collagenCAM antibodies were found to have a remarkable specificity in the hepatocyte adhesion assays. Anti-cellCAM only inhibited intercellular adhesion and had no effect on cell–collagen attachment, whereas anti-collagenCAM only inhibited cell–collagen attachment and had no effect on cell–cell aggregation (fig.1). These results clearly demonstrate that the hepatocyte surface molecules involved in the initial reactions of cell–cell and cell–collagen adhesion, respectively, are immunologically different. These molecules seem to be of glycoprotein nature since (a) they can be released from hepatocyte membranes by papain or trypsin indicating that they are proteins; and (b) they bind to the *Lens culinaris* hemagglutinin lectin, the binding being abolished by α-methyl mannoside, which indicates that they contain carbohydrate. Immune precipitation of detergent-solubilized ^{125}I-labelled plasma membrane components also demonstrated that the anti-cellCAM and anti-collagenCAM antisera were both more specific than the anti-JM or anti-SM antisera (fig.2). The most specific antiserum was anti-cellCAM which essentially reacted with two proteins only, having app. M_r of 110 000 and 150 000, respectively. Thus cell surface glycoproteins, that are involved in intercellular adhesion of rat hepatocytes, should be found among these high M_r antigens.

Earlier work from our laboratory has indicated that the initial attachment of hepatocytes to fibronectin substrates is mediated by other surface molecules than those involved in the initial attachment to collagen [11] and that fibronectin is neither involved

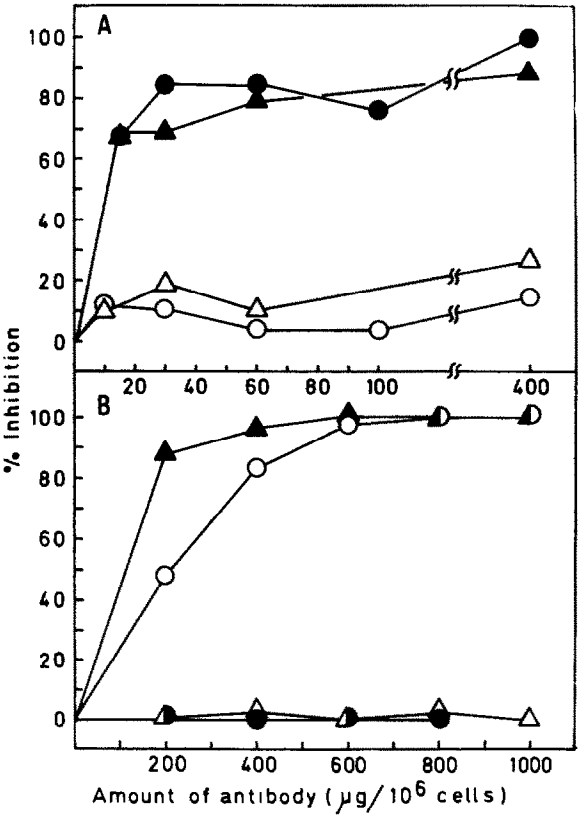

Fig.1. Effects of various antibodies on intercellular (A) and cell–collagen (B) adhesion of rat hepatocytes. All experiments were performed in a calcium and magnesium-containing medium (medium A in [3]). The cells were preincubated for 30 min at 4°C in the presence of the indicated amounts of antibodies. Intercellular adhesion was determined as the decrease in single cells due to aggregation. Cells (1×10^6) in 0.5 ml medium, with or without antibodies, were gyrated at 80 rev./min in flat-bottomed plastic dishes (16 mm diam.) at 37°C for 40 min. The no. single cells was determined with a Celloscope 302. Cell attachment to collagen substrates was determined by seeding 1×10^6 cells in 1 ml medium, with or without antibodies, into Petri dishes (35 mm diam.) coated with 150 μg rat skin collagen. After incubation at 37°C for 30 min the dishes were washed and the no. cells attached was determined from the activity of lactate dehydrogenase in a Triton X-100 lysate of the attached cells. Inhibition of 0% of intercellular adhesion was set to equal the no. remaining single cells in the absence of any antibodies. In the absence of antibodies 20–40% of the cells remained as single cells after 40 min incubation. Inhibition of 100% of intercellular adhesion was set to equal the no. single cells in the original cell suspension prior to incubation at 37°C. Inhibition of 0% of cell–collagen attachment was set to equal the number of cells attached in the absence of any antibodies. Inhibition of 100% of cell–collagen attachment means that no cells had attached. (A) Aggregation was determined in the presence of the indicated amounts of Fab fragments of anti-JM (▲), anti-cellCAM (●), anti-collagenCAM (○) and pre-immune serum (△), respectively. Fab fragments of anti-SM inhibited aggregation essentially in the same way as Fab fragments of anti-JM. (B) Attachment to collagen was determined in the presence of the indicated amounts of IgG molecules of anti-SM (▲), anti-cellCAM (●), anti-collagenCAM (○) and pre-immune serum (△), respectively. IgG molecules of anti-JM inhibited attachment essentially in the same way as IgG molecules of anti-SM.

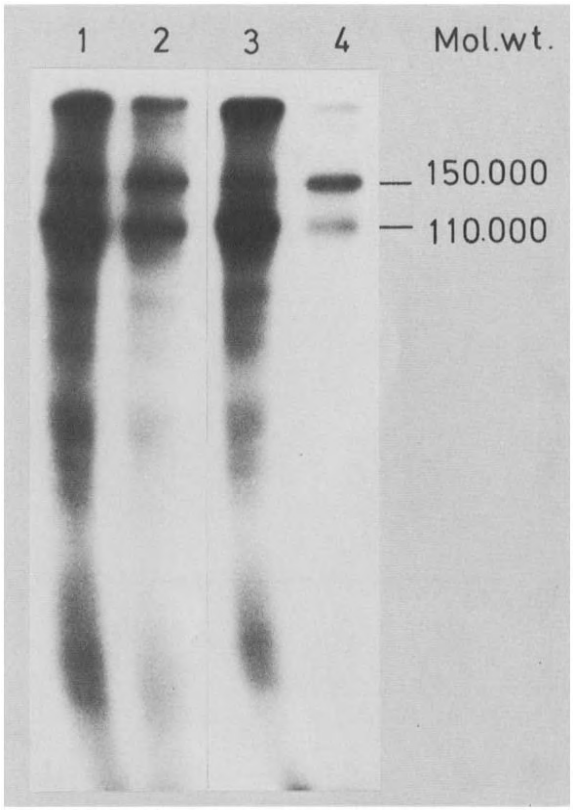

Fig.2. Autoradiogram of polyacrylamide gels after electrophoresis of immunoprecipitated ^{125}I-labelled plasma membrane components: (1) immune precipitate obtained with anti-SM; (2) immune precipitate obtained with anti-collagen-CAM; (3) immune precipitate obtained with anti-JM. (4) immune precipitate obtained with anti-cellCAM. The app. M_r indicated in the figure were obtained from comparison with the electrophoretic mobilities of standard proteins. The radioactive material at the top of the gels represent aggregated material which did not enter the gels; the amount of this material varied from one experiment to another.

in attachment to collagen [3,11] nor in intercellular adhesion [7] of rat hepatocytes. Thus we conclude that 3 different and probably physiologically important adhesion reactions of rat hepatocytes involve different cell-surface molecules. However, there seems to be more than one mechanism for initial cell–cell adhesion in one single cell type [8,16,17]. The existence of different types of intercellular junctions also points in the same direction. Thus it is possible that hepatocytes might adhere to each other, or to collagen, in more than one way and it might accordingly be possible that some cell–cell and cell–matrix adhesion phenomena could exhibit molecular similarities. Our results only demonstrate that on cell type, rat hepatocytes, possesses different molecular mechanisms for a few various adhesion phenomena.

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