

# Different adhesion inhibiting activities of antisera against plasma membranes of liver and Morris hepatoma 7777

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Received 11 January 1984

Cell-substratum adhesion of rat hepatocytes was inhibited by antisera raised against plasma membranes of liver (anti-liver antiserum) and Morris hepatoma 7777 (anti-hepatoma antiserum). Similar concentrations of both antisera inhibited adhesion on collagen. Anti-liver antiserum also inhibited the adhesion of hepatocytes on plastic, whereas anti-hepatoma antiserum was only able to inhibit the adhesion on collagen completely. These results suggest the existence of at least two different adhesion-involved molecules. Cells adhere to plastic by means of both molecules, whereas adhesion on collagen is mediated by only one of them. The results further suggest that hepatoma cells lost the molecule involved in adhesion on plastic.

<i>Cell-substratum adhesion</i>	<i>Collagen</i>	<i>Plastic</i>	<i>Hepatocyte</i>	<i>Hepatocarcinoma</i>
				<i>Plasma membrane</i>

## 1. INTRODUCTION

Antisera have been applied to the study of cell-cell and cell-substratum interactions [1,2]. They provide useful tools to characterize the membrane components involved in such interactions [3,4]. The adhesion-inhibiting activity investigated here is assumed to be the result of the reaction of antibodies with the adhesion-involved molecules [2-4]. These molecules are thought to play important roles in the control of cell proliferation and morphogenesis [5,6]. Alterations in adhesive properties may be involved in metastasis [7]. It is of considerable interest to compare adhesion-involved molecules of normal and malignant cells by studying the effects of antisera raised against purified plasma membranes of rat liver and Morris hepatoma 7777. This study suggests that the adhesion capabilities are altered in tumor cells in comparison to liver.

## 2. MATERIALS AND METHODS

### 2.1. Tumors and tumor cells

Morris hepatoma 7777 was inoculated into both hind legs of Buffalo rats. A cell line from Morris hepatoma 7777 was cultured by removing 2 weeks after inoculation parts of the tumor under sterile conditions. Minced tissue pieces were treated with collagenase for 30 min at 37°C. Single cells and small aggregates obtained by gentle aspiration were filtered through a nylon mesh. After 3-4 days in culture all living cells had formic lucid round cell aggregates, which could be easily separated by centrifugation.

### 2.2. Isolation of hepatocytes and adhesion inhibiting assay

Isolated hepatocytes were prepared from the livers of Wistar rats by collagenase perfusion [8]. The cells were incubated at a density of  $3 \times 10^4$  cells/cm<sup>2</sup> in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. For

adhesion-inhibiting assays various concentrations of antisera were added 2 h after plating. Adhesion inhibition was measured after overnight incubation by comparing the average spreading of untreated cultures with that of antiserum-treated cultures. The following 4 adhesive states were discerned: Cells fully spread like untreated cells, (3+); slight reduction of spreading, (2+); strong reduction of spreading, (+); no spreading, (-). Coating of plates with collagen was carried out as in [9] using rat tendon as collagen source. Plasma fibronectin was isolated as in [10].

### 2.3. Plasma membranes

Plasma membranes from normal liver were prepared from Wistar rats (Ivanovas, Kisslegg, FRG) by a modification of the method in [11,12]. For the isolation of plasma membranes from Morris hepatoma 7777 a procedure yielding membrane fractions of comparable purity was used [13].

### 2.4. Production of antisera

Antisera against plasma membranes were produced in rabbits by 3 subcutaneous injections of 10 mg plasma membranes at 2-week intervals. Sera were obtained 10 days after the last boosting. Complement system was destroyed by heat inactivation at 56°C for 30 min.

## 3. RESULTS AND DISCUSSION

Antisera raised against plasma membranes of rat liver and Morris hepatoma 7777 (anti-liver antiserum, anti-hepatoma antiserum) were both able to inhibit cell-substratum adhesion of hepatocytes in a reversible and non-toxic manner. The adhesion inhibition is assumed to be the result of binding of antibodies to adhesion-involved molecules. The viability of the cells was not affected by the antisera as judged by the trypan blue exclusion assay. By removing antiserum-containing culture supernatants after different incubation times and determination of the remaining adhesion-inhibiting activity, it was found that approx. 70% of the adhesion-inhibiting activity was adsorbed by the cells within the first 2 h. Adhesion was restored when the cultures were incubated for more than 24 h in the presence of antiserum, probably by internalization of bound immunoglobulins and expression of new or recycled adhesion-involved

molecules. Washing of the cells within the first 2 h resulted in restored adhesion in less than 24 h. Adhesion-inhibiting activity was not due to an unspecific mechanism, e.g., saturation of the surface with immunoglobulins or binding to any surface component. This was shown by incubations with antisera raised against 6 purified glycoproteins from liver plasma membranes [14,15]. The antisera did not affect hepatocyte adhesion either alone or in a mixture. Indirect immunofluorescence studies were made with 5 of them. Two antisera (aGP160, aGP140) bound to fibrillar structures of the hepatocyte surface while the others (aGP120, aGP80, aGP60) revealed as yet uncharacterized spot-like distributions (aGP120, aGP80, aGP60) (unpublished). It is noteworthy that all of the antisera did not show any significant fluorescence staining of substratum adherent material (SAM) obtained by rinsing off adherent hepatocytes. This indicates that the glycoproteins do not occur in cell-substratum attachment sites. In contrast, the anti-liver antiserum and anti-hepatoma antiserum bound to SAM, which suggests that they contain antibodies directed against substances of the attachment sites.

Anti-liver antiserum and anti-hepatoma antiserum had a similar effect on the adhesion of hepatocytes to collagen (fig.1). Anti-liver an-

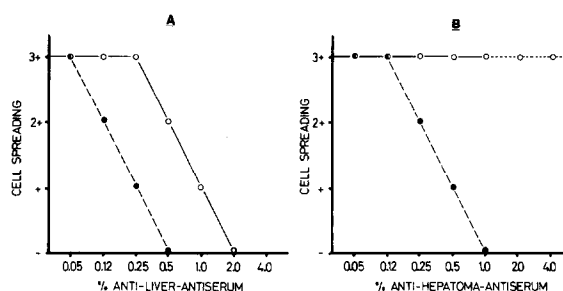


Fig.1. Effects of anti-liver antiserum (A) and anti-hepatoma antiserum (B) on hepatocytes plated on tissue culture plastic (○) and collagen (●). Coating with collagen was performed by drying 50  $\mu$ l of a collagen solution (10  $\mu$ g/ml) in microtest plates not treated for tissue culture use. Cell spreading of antiserum-treated cultures was compared with untreated cultures. Whereas the effect on cell spreading was mostly similar for all cells, anti-hepatoma antiserum at concentrations higher than 1% round up some cells and left most fully spread (see section 3). This contrary effect is marked by the dotted line.

tiserum was slightly more active than anti-hepatoma antiserum. In contrast, the antisera had quite different effects on the adhesion of hepatocytes to plastic. Whereas anti-liver antiserum inhibited spreading of all cells at concentrations greater than 2%, anti-hepatoma antiserum only detached 20–40% of the cells at concentrations greater than 2%. Even at 10% antiserum concentration the rest of the cells appeared fully spread. This observation suggests that the cells adhered to the substratum by means of a molecule which was not recognized by anti-hepatoma antiserum. Thus, at least two types of adhesion-involved molecules seem to exist on the surface of hepatocytes.

For further confirmation of this idea plastic dishes were coated with decreasing concentrations of collagen. The amount of anti-liver antiserum which was sufficient to inhibit adhesion to each substratum was determined (fig.2). With decreasing amounts of collagen on tissue culture plastic the concentration of anti-liver antiserum needed for adhesion inhibition increased to the level characteristic for uncoated plastic. This suggests that the adhesion-involved molecules used on collagen were stepwise substituted by those used for

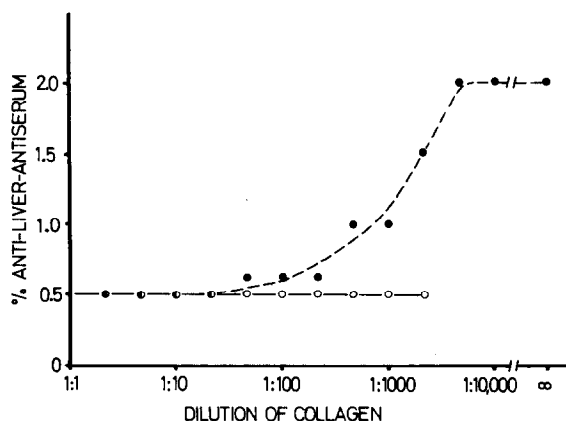


Fig.2. Effect of coating concentrations of collagen on the amount of anti-liver antiserum needed for adhesion inhibition. Coating of tissue culture plastic (●) and microbiological plates (○) was performed by drying 50  $\mu$ l of the diluted stock solution containing 100  $\mu$ g/ml collagen. For microbiological dishes dilutions of more than 1:2000 resulted in diminished adhesion, thus the amount of antiserum could not be determined for these collagen concentrations.

adhesion on plastic. As a control bacteriological dishes were coated similarly. No change of adhesion-inhibiting activity was observed, but adhesion diminished with decreasing collagen concentration. Thus, changes of adhesion-inhibiting activity were not due simply to a reduced collagen coating.

Authors in [4] showed by using a similar anti-liver antiserum that attachment of hepatocytes to collagen but not to fibronectin (cold-insoluble globulin) was inhibited. In our adhesion-inhibiting assay, however, which gives a measure of spreading rather than attachment, the adhesion to bovine fibronectin and collagen-coated dishes was inhibited by the same amount of antiserum. This suggests that adhesion on collagen and fibronectin is mediated by the same adhesion-involved molecule, possibly due to rapid adsorption of serum fibronectin to collagen. The role of fibronectin as an adhesion factor on plastic in serum-containing cultures is contested [16–18]. Since the results suggest that hepatocytes recognize different adhesion factors on plastic, at least one of them must be different from fibronectin.

Cells from Morris hepatoma 7777 were cultured and their adhesion properties investigated. Single cells adhered on collagen, however, also floating aggregates were soon formed. Adhesion was blocked by both antisera, again showing that both contain antibodies against molecules involved in adhesion on collagen. The adhesion on plastic was very rare. This result and the finding that anti-hepatoma antiserum did not inhibit hepatocyte adhesion to plastic completely suggest the loss of one type of adhesion-involved molecules in Morris hepatoma 7777. In vivo the cells of this carcinoma are able to detach from the original liver tissue matrix and invade other tissues. However, more detailed information is needed before claiming a connection between both findings. Using an antibody-blocking assay [1,3] we are now characterizing the adhesion-involved molecules, and have recently succeeded in separating two types from liver, but only one type from Morris hepatoma 7777 [19].

#### ACKNOWLEDGEMENTS

We like to thank Professor C. Bauer, A. Becker, Dr C. Hanski, Dr D. Josić and Dr R. Tauber for

helpful discussions. This research was supported by the Deutsche Forschungsgemeinschaft (SFB 29) and the Fonds der Chemischen Industrie.

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