Identification of an M, 64,000 Plasma Membrane Glycoprotein Mediating Adhesion of Tumor H-59 Cells to Hepatocytes1

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ABSTRACT

Tumor H-59 is a variant of the Lewis lung carcinoma which is metastatic to the liver. In previous studies we have shown that liver metastasis in this tumor model correlates with adhesion in vitro to hepatocyte monolayers (Brodt, P., Clin. Exp. Metastasis, 7: 525-539, 1989). In an attempt to identify the adhesion molecule(s) involved, monoclonal antibodies were produced. One monoclonal antibody (MAb C-11) was highly specific to hepatocyte-adherent tumor cells. The antibody (an IgG1) and F(ab)2 fragments blocked tumor cell attachment to hepatocytes while having no effect on tumor cell adhesion to basement membrane proteins coated onto culture dishes. Western blot analysis of solubilized H-59 plasma membranes or cell lysates showed that the antibody recognizes an M, 64,000 protein. Treatment with N-glycosidase F prior to Western blot analysis revealed that N-linked carbohydrate residues constitute approximately 43% of the total weight of this molecule. This glycoprotein is only weakly expressed on tumor M-27, a lung-specific subline of the Lewis lung carcinoma (Brodt, P., Cancer Res., 46: 2442-2448, 1986), is undetectable in plasma membrane preparations obtained from spleen cells and thymocytes, but can be detected on cultured hepatocytes and in hepatocyte cell lysates. Pretreatment of the hepatocytes with MAb C-11 also resulted in inhibition of tumor cell adhesion. These results suggest that this glycoprotein mediates the attachment of H-59 cells to hepatocytes.

INTRODUCTION

The host-tumor interactions which regulate the site selectivity of metastasis have been the subject of numerous investigations and much debate in the past century (1, 2). Evidence derived mainly from experimental tumor models and supported by clinical observations indicates that multiple factors, both specific and nonspecific, determine the ultimate site of growth of disseminated tumor cells. Thus, while the lymphatic and vascular drainage of the primary site initially determine tumor cell access to secondary organs, subsequent specific interactions between tumor cells and the target organ vascular endothelium (3-5), extracellular matrix (6-9), and stromal and parenchymal cells (10-12) are thought to regulate tumor cell potential to form metastases (for review, see Refs. 2 and 13). These specific interactions may involve tumor cell adhesion molecules which recognize organ-specific determinants on host cells and matrix and/or soluble mediators present in the organ microenvironment (14-16).

The liver is the primary site of metastasis for several common human malignancies (e.g., carcinomas of the gastrointestinal tract) (17). Yet, the mechanisms regulating liver colonization by tumor cells are not fully understood. A paucity of information is particularly noted in regard to neoplasms of epithelial origin because few experimental models of liver-homing carcinomas are available for study.

In previous studies we found that the potential of tumors H-59 and M-27, two sublines of the Lewis lung carcinoma with different organ specificities to metastasize to the liver, correlated with their adhesion to primary cultures of hepatocytes (18, 19). H-59 cells were subsequently fractionated into two subpopulations with high and low potentials to colonize the liver on the basis of their differential ability to adhere to hepatocyte monolayers (19). In the present investigation an MAb specific to a highly adherent and metastatic subpopulation of tumor H-59 was produced and used to characterize the adhesion molecule involved.

MATERIALS AND METHODS

Mice

C57BL/6 female mice (7-12 weeks old) were used for immunization and (C57BL/6 x BALB/c)F1 were used for production and maintenance of hybridoma ascites. The animals were purchased from Charles River Canada (Montreal, Quebec, Canada).

Cell Lines

The origin and metastatic and adhesive properties of sublines H-59 and M-27 of the Lewis lung carcinoma have been described previously (6,18,19). The tumors were maintained in vivo by s.c. implantation of liver (H-59) and lung (M-27) metastases derived from tumor-bearing mice, into new recipient animals.

Single-cell suspensions of the tumors were obtained by enzymatic digestion of the solid tumors in a 0.02% trypsin solution in PBS-EDTA as we described previously (18). Where required, tumor cells were cultured in RPMI-1640 supplemented with 10% fetal calf serum (Gibco, Burlington, Ontario, Canada), 0.01 M N-2-hydroxyethylpiperazine-N-ethanesulfonic acid, and 2 x 10-5 M glutamine. Tumor cells were cultured for 1-2 weeks prior to use in the adhesion assays or prior to preparation of plasma membranes and cell lysates.

Fibroblast line 3T6 was obtained courtesy of Dr. W. Lapp (Dept. of Physiology, McGill University, Montreal, Quebec, Canada) and maintained in RPMI-1640 supplemented with 10% fetal calf serum, 0.01 M N-2-hydroxyethylpiperazine-N-ethanesulfonic acid, and 2 x 10-5 M glutamine. All cells were cultured at 37°C in a 5% CO2 incubator and fed once or twice weekly as required. Monolayers were dispersed by incubation with enzyme-free PBS-EDTA for 5-10 min at 37°C (18). Monolayers of splenic lymphocytes in microtiter well plates were prepared using poly-L-lysine as previously described (20).

Adhesion Assay

Primary mouse hepatocyte cultures were prepared by collagenase perfusion (21). The culture conditions and adhesion assay were described in detail elsewhere (19). Nonadherent cells (H-59NA) were harvested by repeated washing of the hepatocyte monolayer with PBS. Adherent cells (H-59AD) were eluted off the monolayers by incubation in PBS-EDTA for 10 min at 37°C. Treatment of the tumor cells with...
Identification of Adhesion Receptor in Liver Metastasis

1.0

Fig. 1. Antibody C-11 recognizes a subpopulation of tumor H-59 selected for adhesion to hepatocytes. Target cells were seeded into 96-well plates at a density of 5 x 10⁴ cells/well. ELISA tests were performed on cell monolayers 48 h later. Columns, means (bars, ±SD) of 4 assays. O.D., absorbance.

tunicamycin and various enzymes prior to the adhesion assay was carried out as previously described (22).

Adhesion to Matrigel-coated Wells

Matrigel (a kind gift of Dr. H. Kleinman, National Institutes of Health, Bethesda, MD) was added to 24-well plates at a concentration of 1 mg/ml and allowed to dry in a laminar flow hood overnight (23). To each well 5 x 10⁴ ⁵¹Cr-labeled tumor cells were added and then incubated at 37°C for the specified time intervals. Adhesion was quantitated as described above for tumor adhesion to hepatocytes.

Production of Monoclonal Antibodies

Immunization. The method used to generate MAbs specific for H-59AD cells has been described in detail elsewhere (24). Tolerance to cell surface determinants expressed on H-59NA cells was induced in syngeneic C57BL/6 mice by i.p. injection of 10⁵ γ-irradiated (10,000 rads) H-59NA cells, followed 3 days later by an injection of cyclophosphamide (Procytox; Horner, Montreal, Quebec, Canada) as described before (22). Immunization with H-59AD cells followed, using injections of 10⁵ γ-irradiated cells on alternate weeks. Three days following the third inoculation the animals were sacrificed and their spleens removed for fusion.

Fusion Procedure and Screening. Myeloma line SP2/0-Ag 14 (obtained courtesy of Dr. A. Sullivan, McGill Cancer Centre; see Ref. 25) was used for the fusions. The fusion procedure was that previously described (22). Hybridoma supernatants were screened by ELISA (25). Hybridomas which secreted antibodies of interest were cloned by the limiting dilution method.

Isotyping of the Antibody. Antibodies were isotyped using the ELISA. A mouse monoclonal subisotyping kit obtained from HyClone Laboratories (Logan, UT) was used according to the instructions of the manufacturer.

Immunofluorescence Analysis. Indirect immunofluorescence labeling was carried out as we described in detail elsewhere (22).

Control Antibodies. The following antibodies were also used: (a) MAb 12/50 (an IgG1) was produced in our laboratory (22). The antibody reacts with tumor H-59 but does not discriminate between adherent and nonadherent cells; (b) MAb CL9002 (an IgG3) was obtained from Cedarlane Laboratories (Hornby, Ontario, Canada). It is specific for the H-2Dβ determinant of leukocytes, obtained from Cedarlane Laboratories (Hornby, Ontario, Canada). Rabbit antiserum to BSA was from Cappel (Organon Teknika, Scarborough, Ontario, Canada).

Purification of Antibodies. MAbs C-11 and 12/50 were purified by affinity chromatography with Affi-Gel protein A Monoclonal Antibody Purification System (MAPS II; Bio-Rad Laboratories, Mississauga, Ontario, Canada) according to the instructions of the manufacturer.

Preparation of F(ab)₃ Fragments. F(ab)₃ fragments of antibody C-11 were prepared using immobilized pepsin (Pierce, Rockford, IL) according to the instructions of the manufacturer. The fragments were separated on a protein A-agarose affinity column (see above), and the concentration of the Fc fragments was quantitated using high performance liquid chromatography (Waters; Protein Pak 300 SW; column, 0.75 x 30 cm), using 0.1 M sodium phosphate buffer, pH 6.5, and a flow rate of 0.5 ml/min. The absorbance was monitored at 254 nm (absorbance detector model 441), and the peak fractions were pooled, dialyzed, and concentrated. Purity of the fragments was confirmed by SDS-PAGE as described below.

Preparation of Cell Lysates. Cultured cells were harvested and lysed in a solution of 1% Triton X-100 containing 20 mM N-2-hydroxyethyl (LFA-1) determinant of leukocytes was obtained from Cedarlane Laboratories. Rabbit antiserum to BSA was from Cappel (Organon Teknika, Scarborough, Ontario, Canada).

Fig. 2. Indirect immunofluorescence labeling of H-59 cells with MAb C-11. Viable H-59 cells (2 x 10⁶ in 0.1 ml medium) were incubated with a 1:20 dilution of C-11 ascites for 1 h at 4°C. Fluorescein isothiocyanate-conjugated goat antimouse IgG was used as the second antibody. (× 4500).
Fig. 3. Antibody C-11 blocks the adhesion of tumor H-59 to hepatocytes. The adhesion of nonfractionated H-59 cells (A) and of the adherent tumor cells H-59AD (B) was measured following tumor cell incubation with ascites fluid (1:10) of antibodies C-11 and 12/50. MAb CL9002 to the H-2Db determinant was partially purified ascites fluid diluted 1:100 (dilution determined by ELISA to result in a level of antibody binding comparable to that of C-11 ascites fluid at the dilution used). MAb 01MLEUK10 to LFA-1 was used at a dilution of 1:25, at which maximal specific inhibition was seen. Cells (10^6 in 0.2 ml) were incubated with the antibodies for 1 h at 4°C and then washed 4 times with RPMI 1640 to remove unbound antibodies. Adhesion to hepatocytes was measured as described in “Materials and Methods.” Columns, means (bars, SD) of 3 experiments.

Fig. 4. Pretreatment of hepatocytes or H-59 cells with MAb C-11 inhibits cell-cell adhesion. Prior to the adhesion assay, MAB C-11 ascites (•) or a control ascites produced with myeloma SP2/0-Ag 14 (◊) were added at the dilutions indicated (reciprocal of dilutions shown) to 48-h-old hepatocyte cultures and incubated for 1 h at 4°C. Unbound antibody was removed by washing. Hepatocytes incubated with PBS were used as controls. Pretreatment of H-59 cells with C-11 (◇) or with the control ascites (◊) was carried out under similar conditions. As an added control the effect of MAB C-11 on the adhesion of H-59 cells to culture dishes coated with 1 mg/ml Matrigel (Δ) or 10 mg/ml BSA (■) were also tested. Incubation was for 60 min at 37°C. Unbound cells were removed by washing. Adhesion of untreated cells to Matrigel-coated dishes (not shown) was 30%, while adhesion to BSA-coated dishes was 2.4%.

Fig. 5. Inhibition of tumor cell adhesion to hepatocytes by F(ab)2 fragments of antibody C-11. One million H-59 cells were incubated for 1 h at 4°C with 0.2 ml of RPMI containing the specified concentration of purified antibody. Cells incubated with F(ab)2 were added to hepatocytes monolayers either directly (◇) or following extensive washing to remove unbound antibody (◇). The adhesion assays were carried out as described. Cells treated with whole antibody C-11 (◇) or with MAB 12/50 (○) were also washed prior to the adhesion assay. Results are based on triplicate samples. SD, 2-8% of the means.

Fig. 6. Western blot analysis with MAB C-11 (A and B) and anti-BSA (C). A, results of analyses carried out under reducing condition. Lane 1, H-59 lysate; lane 2, H-59 solubilized plasma membrane; lane 3, hepatocyte lysate; lane 4, M-27 lysate; lane 5, M-27 plasma membrane; lane 6, thymocytes lysate; lane 7, spleen cells lysate. B, nonreducing conditions. Lane 8, H-59 lysate; lane 9, M-27 lysate; lane 10, H-59 lysate blotted with normal mouse IgG; lane 11, H-59 lysate blotted with SP2/0-Ag 14 ascites. C, purified BSA blotted with antibody to BSA (lane 12) and MAB C-11 (lane 13). Proteins were resolved on a 10% SDS-PAGE.
polyacrylamide gels as specified, using the method of King and Laemmli (27).

Western Blot Analysis. The electrophoretic transfer of proteins onto nitrocellulose paper (0.2 μm; Schleicher and Schuell, Montreal, Quebec, Canada) and the subsequent analyses with MAb C-11 were performed as described by Towbin et al. (28). After the transfer, the nitrocellulose strips were first incubated for 18 h at 4°C with a solution of 5% skim milk and 3% BSA to block nonspecific binding sites and then for 18 h with C-11 ascites diluted 1:25 in PBS. Alkaline phosphatase-conjugated goat anti-mouse immunoglobulin (Jackson Immuno Research Laboratories) at a dilution of 1:5000 was used as the second antibody and nitroblue tetrazolium/5-bromo-4-chloro-3-indolylphosphate (BCIP), (Bio-Can, Toronto, Ontario, Canada) was used as substrate.

Enzyme Treatment. In some experiments the cell lysate was subjected to enzyme digestion with either N-glycosidase F (N-glycanase; Genzyme, Boston, MA) or with pronase (from Streptomyces griseus; Boehringer-Mannheim, Penzberg, West Germany) prior to the Western blot analysis. Cell lysate samples were prepared according to the instructions of the manufacturer, and 25 μg of protein was treated with 0.2–1 unit of N-glycanase or 0.35–1.75 units of pronase for 18 h at 37°C (29).

Control samples were incubated without enzyme.

Immunoaffinity Chromatography. Five mg of purified antibody was coupled to Affi-Gel Hz hydrazide gel (Bio-Rad) according to the instructions of the manufacturer. To remove plasma membrane proteins which react nonspecifically with IgG, a second column was prepared using 8 mg of affinity-purified mouse IgG (Jackson Immuno Research Laboratories). PM preparations were solubilized with Triton X-100, and 2 mg protein in PBS, pH 7.4, containing 0.1% Triton X-100 was applied to the IgG column. The column was washed with 0.5–0.25 M NaCl containing 0.05% Triton X-100 and the unbound protein applied to the C-11 column at a rate of 0.15 ml/min. The C-11-bound protein was eluted at the same flow rate with 0.2 M glycine-HCl, pH 2.5. The pH was readjusted with Tris-HCl, pH 9.0, and the peak fractions (determined on the basis of absorbance at 280 nm) were pooled and concentrated in an Amicon ultrafiltration cell. The eluate was separated by SDS-PAGE, and the protein band was silver stained using Bio-Rad reagents according to the instructions of the manufacturer.

Table 1 Effect of enzyme or tunicamycin treatment on the adhesion of tumor H-59 to hepatocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adhesion (% of control)</th>
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<tr>
<td>β-galactosidase</td>
<td>100 ± 7</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>Neuraminidase + β-galactosidase</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>Exoglycosidases*</td>
<td>30 ± 1.9</td>
</tr>
<tr>
<td>Tunicamycin</td>
<td>30 ± 1.5</td>
</tr>
<tr>
<td>Pronase</td>
<td>6 ± 2.2</td>
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* A cocktail of C. lypas exoglycosidases was used containing the following enzymes: α-N-acetylgalactosaminidase, β-N-acetylhexosaminidase, α-L-fucosidase, β-galactosidase, and α-mannosidase in a buffer of 8 mm Na2HPO4, 1 mm KH2PO4, and 0.1 M sodium citrate (pH 6.2).
RESULTS

Hybridomas were screened by ELISA on monolayers of H-59AD, H-59NA, and M-27 cells (19, 22). Hybridoma C-11 was subsequently cloned and selected for further study because it was highly specific for the adherent subpopulation of tumor H-59 as shown in Fig. 1. The antibody reacted poorly with tumor M-27, 3T6 fibroblasts, and normal syngeneic splenocytes but gave a strong positive reaction with primary cultures of hepatocytes (Fig. 1). Immunofluorescence analysis with fluorescein isothiocyanate-conjugated goat anti-mouse IgG confirmed the specificity of the antibody for the adherent subpopulation as 60% of the adherent cells, but only 12% of the nonadherent cells were positively labeled. A tumor H-59 cell labeled with the antibody is shown in Fig. 2.

The ability of antibody C-11 to block tumor cell adhesion to hepatocyte monolayers in vitro was subsequently tested. Tumor cells were pretreated with antibody C-11 or with three other MABs with different antigenic specificities and their attachment to hepatocyte monolayers were compared to that of untreated cells. Results of these experiments, shown in Fig. 3, demonstrate that antibody C-11 inhibited tumor binding significantly better than any of the other antibodies tested (87% inhibition). MAB 12/50 and a MAB directed against the H-2D\(^b\) determinant failed to inhibit binding (Figs. 3 and 5), while a MAB specific for the LFA-1 molecule (30) had a minor inhibitory effect on the adhesion of H-59AD cells (maximal inhibition, 29%).

Adhesion could also be inhibited by pretreatment of the hepatocytes with MAB C-11 as shown in Fig. 4. Normal mouse IgG and a control ascites did not significantly block tumor cell adhesion to hepatocytes, while antibody C-11 failed to block adhesion of H-59 cells to Matrigel or BSA-coated culture dishes (Fig. 4). Adhesion to laminin and type IV collagen-coated dishes was also not affected by pretreatment with MAB C-11 (not shown).

F(ab\(_2\))\(_2\) fragments of antibody C-11 also inhibited tumor cell adhesion to the hepatocytes as shown in Fig. 5. Treatment with F(ab\(_2\))\(_2\) fragments was most effective when they were added to the hepatocyte cultures together with the tumor cells.

Western blot analysis as shown in Fig. 6 revealed that the antibody detected an \(M_1\), 64,000 protein also found in hepatocyte cell lysates. Under reducing conditions this protein migrated to the \(M_1\), 71,000 region. There was no detectable band in cell lysates obtained from spleen cells and thymocytes, while a weak reaction was detected with tumor M-27. The protein did not react with either normal mouse IgG or ascites fluid obtained with myeloma SP2/0-Ag 14 (Fig. 6B). MAB C-11 gave no reaction with purified BSA (Fig. 6C), while antibodies to BSA did not react with a cell lysate of tumor H-59 (not shown).

Treatment of the cell lysate with \(N\)-glycosidase F did not affect antibody binding and revealed that \(N\)-linked carbohydrate residues constitute approximately 43% of the total weight of this molecule (Fig. 7). Pretreatment with pronase abolished antibody binding.

Immunofluorescently, the antibody C-11 reacted with the M, 64,000 plasma membrane protein. To test whether carbohydrate residues played a role in tumor cell adhesion to the hepatocytes, the cells were treated with either tunicamycin or with several exoglycosidases prior to the adhesion assay. Results shown in Table 1 demonstrate that tumor cell adhesion to hepatocytes was significantly reduced following treatment with tunicamycin or Charonia lampas exoglycosidases and to a lesser extent by a combination of neuraminidase and \(\beta\)-galactosidase. Treatment with either \(\beta\)-galactosidase or neuraminidase alone had no effect.

DISCUSSION

CAMs are now known to play a central role in the process of cancer dissemination by mediating cell-cell and cell-substrate interactions which are essential for tumor cell invasion and proliferation (2). While the potential involvement of CAMs in the organ site specificity displayed by some metastatic cancers has been postulated (1, 2, 13), few tumor adhesion molecules which are involved in the homing of cancer cells, carcinomas in particular, have actually been identified (12, 31).

The present study was prompted by our earlier observation that a subpopulation of tumor H-59 which was highly metastatic to the liver in vivo had a significantly elevated level of adhesion to hepatocyte monolayers in vitro as compared to nonmetastatic cells. This was in agreement with other reports based on studies of lymphoma and lymphosarcoma lines which also linked hepatic metastases formation with tumor potential to adhere to hepatocytes (32) and/or invade hepatocyte monolayers in vitro (33).

In an attempt to identify the adhesion receptors involved in tumor-hepatocyte adhesion in this tumor model, a MAB specific for the liver-adherent fraction of tumor H-59 (H-59AD) was produced. This antibody (C-11) inhibited the adhesion to hepatocytes in a specific manner and had little effect on the attachment of H-59 cells to BSA or extracellular matrix protein-coated dishes or primary cultures of liver sinusoidal endothelial cells. Moreover, adhesion to hepatocytes could not be blocked by pretreatment of the tumor cells with either normal mouse IgG or two control IgG MABs. A third MAB to the LFA-1 determinant inhibited adhesion only minimally (maximal inhibition, 29%). These findings coupled with our observation that the antibody bound poorly to the lung-specific line M-27 or to H-59NA cells suggest that C-11 recognizes a cell surface molecule directly involved in the adhesion of H-59 cells to hepatocytes.

Western blot analysis revealed that C-11 recognizes an \(M_1\), 64,000 plasma membrane glycoprotein which under reducing conditions shifted to the \(M_1\), 71,000 region, indicating the presence of intrachain disulfide bonds. \(N\)-linked carbohydrate residues were found to constitute approximately 43% of the weight of this molecule. Our data suggest that the \(N\)-linked carbohydrate moieties are not essential for MAB C-11 recognition but may play a functional role in adhesion because the enzymatic removal of cell surface carbohydrates significantly reduced tumor adhesion to hepatocytes. These results, however, do not rule out the possible involvement of glycoconjugates present on cell surface molecules other than the C-11 antigen.

The C-11 determinant is also expressed on hepatocytes as shown by ELISA and Western blot analysis. Blocking experiments suggested that cell-cell adhesion in this model requires participation of the molecules expressed on both cell types. Whether this interaction involves a homophilic cadherin-like recognition process (34) or is mediated by different receptor and ligand structures expressed on both cell types (heterotypic...
adhesion) remains to be determined.

Several classes of adhesion receptors which mediate cell-cell adhesion in developmental processes, inflammation, host immune responses, and possibly metastasis have been described. Best characterized among them are (a) the integrin family of adhesion receptors, in particular the β2 subgroup which plays a critical role in leukocyte adhesion (for review, see Ref. 35), (b) the adhesion receptors belonging to the IgG superfamily, also implicated in blood cell interactions (36), (c) the Ca\(^{2+}\)-dependent cadherins which mediate homophilic adhesion and play a regulatory role during development (37), and (d) the LEC CAMS which are expressed on a variety of cell types and mediate lectin-like adhesive cell-cell interactions which can be blocked by specific sugars. This group includes several adhesion receptors expressed on leukocytes (LECAM 1) and endothelial cells (ELAM 1 or LECAM 2) which play a role in leukocyte traffic and homing (38, 39).

Our data suggest that the adhesion receptor recognized by C-11 does not belong to one of the leukocyte adhesion systems previously described (35) because MAb C-11 did not react with lymphocytes and thymocytes in ELISA and no reaction could be detected when cell lysates of spleen cells and thymocytes were blotted with the antibody. In recent preliminary studies (not shown), we found that a MAb directed to the mouse β2-integrin chain (antibody M18.2.a.8 obtained courtesy of Dr. T. Springer, Boston, MA) failed to block adhesion of H-59 cells to hepatocytes. Thus, it appears that the adhesion in the present model system is mediated by mechanisms different from the β2-integrin-dependent adhesion described recently for liver-metastasizing lymphoma lines (40). The mechanism responsible for the low level of inhibition observed when H-59AD cells were treated with antibodies to LFA-1 is unclear. One possible explanation may be that H-59 cell adhesion to hepatocytes increases expression of the LFA-1 receptor on the tumor cells and that once expressed these molecules play a secondary role in the adhesion.

The molecular weight range of the C-11 antigen and its relatively high degree of N-linked glycosylation exclude many of the above-mentioned CAM families including the integrin subunits, the cadherins, and some of the larger adhesion receptors of the IgG superfamily. Because our findings suggest that carbohydrates are involved in the present adhesion system, it is conceivable that a hepatocyte lectin which could be abnormally expressed on tumor H-59 cells may be involved in the adhesion. This is supported by our previous results of an in situ lectin-binding study. In this study it was found that liver metastases of tumor H-59 expressed increased levels of peanut agglutinin receptors (as compared to the primary s.c. tumor) and that high levels of these receptors could also be detected on hepatocytes (41).

Tumor cell surface oligosaccharides have previously been shown to play a role in metastasis (42, 43) and to mediate adhesion to hepatocytes (12). Endogenous tumor lectins have also been implicated in tumor cell adhesion and metastasis (44). One of the better characterized liver lectins, namely, the asialoglycoprotein receptor, has previously been shown to mediate cell-cell adhesion to hepatocytes as well as to Kupffer cells and liver sinusoidal endothelial cells (45). Although the participation of this lectin in the present adhesion system cannot be entirely ruled out, the results of the enzyme studies argue that it is probably not the major hepatocyte receptor involved, because treatment of the tumor cells with neuraminidase or β-galactosidase failed to modify adhesion, while treatment with both enzymes only reduced adhesion by 50%.

The role of tumor cell adhesion to hepatocytes in the process of liver colonization is still unknown. Sargent et al. (15) have recently shown that tumor-hepatocyte contact is required for a growth stimulatory effect exerted by a diffusible hepatocyte factor on a liver-metastasizing subline of melanoma B-16. Preliminary data in our laboratory also indicated that, under conditions which permit cell-cell contact, H-59 cells cocultured with hepatocytes show an increased uptake of \(^{3}H\)thymidine as compared to controls.\(^{6}\) The nature of the growth factor(s) and the relationship between adhesion and proliferation in the present tumor model are the subject of present investigations in our laboratory and should be facilitated by the availability of antibody C-11.

Recently, we reported the inhibition of liver colonization by administration in vivo of MAb C-11 (46). Similar findings were also reported by McGuire et al. (47). Although the mechanism of action of the C-11 antibody in vivo remains to be elucidated, our data and studies by other laboratories (48) suggest that inhibition of cellular adhesion in vivo by specific reagents, in conjunction with existing treatment protocols, will provide a promising new avenue for therapeutic intervention in the process of cancer metastasis.

**REFERENCES**


IDENTIFICATION OF ADHESION RECEPTOR IN LIVER METASTASIS


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