A Monoclonal Antibody to Lewis Lung Carcinoma Variant H-59 Identifies a Plasma Membrane Protein with Apparent Relevance to Lymph Node Adhesion and Metastasis

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ABSTRACT

Tumors H-59 and M-27, two stable metastatic variants of the Lewis lung carcinoma, differ in their ability to disseminate lymphatically. Tumor H-59 metastasizes to the regional lymph nodes regardless of the local site of growth and gives rise to widespread lymphatic dissemination, whereas tumor M-27 disseminates hematogenously without involvement of the regional nodes (P. Brodt, Cancer Res., 46: 2442-2448, 1986). In a previous paper we reported that this divergent potential to disseminate lymphatically correlated well with adhesion to frozen sections of syngeneic lymph nodes and spleens (P. Brodt, Clin. Exp. Metastasis, 7: 343-352, 1989). A monoclonal antibody (12/50) specific for tumor H-59 was subsequently generated. This antibody (an IgG1) but not three control antibodies, which reacted with tumor H-59, significantly reduced tumor cell binding to the frozen sections. Western blot analysis revealed that it recognized a plasma membrane protein of M, 37,000 on tumor H-59 cells. No antibody binding was detected when solubilized plasma membrane preparations of tumor M-27 were used. Subsequent enzymatic assays indicated that the binding of monoclonal antibody 12/50 was insensitive to cell treatment with exoglycosidases but could be significantly reduced by pretreatment of the tumor cells with Pronase. Together these results suggest that monoclonal antibody 12/50 recognizes a cell surface adhesion protein relevant to lymphatic dissemination of this tumor.

INTRODUCTION

Rapid progress in recent years in our understanding of the cellular and molecular mechanisms which underly the process of tumor dissemination has made it clear that the metastatic process is driven by a series of specific adhesive interactions between the tumor cells and various host tissue components. Thus, numerous studies have shown that tumor cell potential to metastasize is linked to its ability to attach to vascular endothelial cells (1, 2), different constituents of the extracellular matrix (3), and parenchymal cells of the secondary organs (4) (see Ref. 5 for an extensive review). In several laboratories this has recently led to the production of reagents such as monoclonal antibodies (6, 7) and synthetic peptides (8, 9) which can block tumor cell attachment in vitro. Some of these reagents were subsequently shown to inhibit tumor metastasis in vivo (10-12).

Many of the common human carcinomas metastasize via the lymphatic route (13). The extent of lymphatic involvement is in fact a major factor in the staging and prognosis of primary malignancies (14). Yet despite progress in recent years in the characterization of adhesion molecules which play a role in hematogenous dissemination, little is presently known about the role (if any) of such molecules in lymphatic metastasis. One reason for this paucity of information may be the lack of appropriate experimental models (15).

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1 Supported by a grant from the National Cancer Institute of Canada.

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We have been studying host-tumor interactions in lymphatic metastasis using the Lewis lung carcinoma (3LL) model recently developed in our laboratory (16). Previous studies have shown that tumor cell potential to disseminate lymphatically in this model correlated well with adhesion to cryostat sections of syngeneic lymph nodes and spleens (17). Here we report on the identification and partial characterization by a MoAb3 of a cell surface protein which plays a role in this adhesion.

MATERIALS AND METHODS

Animals. Female C57BL/6 mice, 8-16 weeks old, were used for all the experiments, for maintenance of the tumor lines, and for the immunization procedures. They were supplied by Charles River of Canada (Montreal, Quebec). Ascites were generated and maintained in (C57BL/6 × BALB/c)F1 female mice bred in our animal facilities. The animals, routinely tested for the presence of murine infectious viruses, were negative throughout the period of these studies.

Cell Lines. The origin and metastatic properties of tumors M-27 and H-59, two highly metastatic sublines of the Lewis lung carcinoma, have been described in detail elsewhere (16). The tumors were maintained in vivo by s.c. implantation of lung (M-27) and liver (H-59) metastases derived from tumor-bearing mice into new recipient animals.

To obtain single-cell suspensions of the tumors, tumor fragments were trimmed of necrotic tissue and enzymatically digested in 0.02% trypsin solution, in Ca2+- and Mg2+-free PBS containing 0.02% EDTA. Tumor cells were cultured in RPMI 1640 medium supplemented with 10% FCS (GIBCO, Burlington, Ontario), 0.01 M N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid buffer, and 2 × 10-5 M glutamine (RPMI-FCS). Tumor cells were normally cultured for no longer than 4 weeks prior to use in the experiments.

Fibroblast line 3T6 was obtained courtesy of Dr. W. Lapp (McGill University). It was maintained in RPMI-FCS. MLg, a normal mouse lung cell line (18), was obtained from the American Type Culture Collection. It consists of a mixed population of fibroblast-like and epithelial cells. It was maintained in Dulbecco’s modified Eagle medium (GIBCO, Burlington, Ontario) supplemented as described above. 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 PBS-EDTA for 5-10 min at 37°C. Enzymatic digestion with 0.02% trypsin was applied only if required (16).

Splenic lymphocytes were obtained and layered in microtiter well plates using poly-L-lysine, as we described previously (17). In some of the experiments, the splenocytes were first fractionated into adherent and nonadherent cells, following a 4-h incubation of the cells at 37°C. Hepatocyte monolayers were prepared as we described elsewhere and used within 48-72 h (19).

Immunization Procedure. The selective immunization method used to generate tumor-specific MoAb was a modification of the method previously described by Kimura and Xiang (20). Namely, female C57BL/6 mice were first given i.p. injections of 107 γ-irradiated (10,000 rad) M-27 cells. Three days later, they received an injection of cyclophosphamide (CY.-Procystox; Horner, Montreal, Quebec) in order to induce tolerance to cell surface antigens expressed on this tumor. Two

3 The abbreviations used are: MoAb, monoclonal antibody; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; PM, plasma membrane; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; FCS, fetal calf serum.

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weeks later the animals were inoculated i.p. with 10^7 γ-irradiated H-59 cells. Injection with tumor H-59 was repeated once or twice at biweekly intervals. The animals were sacrificed 3 days after the last immunization and their spleens were removed for fusion.

**Fusion Procedure.** Myeloma line SP2/O-Ag 14 (obtained through the courtesy of Dr. A. Sullivan, McGill Cancer Centre) was used for fusion. The fusion protocol and selection medium were those described by Bazin et al. (21) and by Fong et al. (22), respectively.

Screening. Supernatants were screened for the presence of antibody by ELISA (23), 12 to 14 days after the fusion. Tumor cell monolayers plated into 96-well plates (Falcon Microtiter III plates) were used as targets. Nonspecific protein binding sites were blocked by incubation of the cells with 1% bovine serum albumin in PBS for 60 min. Cells were fixed prior to assay by incubation with 0.125% glutaraldehyde in PBS for 3 min. A peroxidase-conjugated goat anti-mouse immunoglobulin (Dimension Laboratories, Mississauga, Ontario) was used as second antibody and 2,2-azino-di-(3-ethyl-benzthiazolinsulfonate) (Boehringer-Mannheim, Montreal, Quebec) at a concentration of 0.005% in citrate buffer, pH 4.3, containing 0.003% H_2O_2 was used as substrate. Color intensity was measured at 410 nm using a Dynatech MiniReader II (Dynatech Laboratories, Inc., Alexandria, VA). Hybridoma cultures which secreted antibodies of interest were cloned by the limiting dilution method.

**Isotyping of the Antibodies.** The antibody was isotyped by ELISA using tumor H-59 cells, isotype-specific rabbit antisera to mouse immunoglobulins (Dimension Laboratories), and a peroxidase-conjugated goat anti-rabbit IgG antibody.

**Immunofluorescence.** Immunofluorescence assays were carried out as described elsewhere (24). Cells at a concentration of 5 x 10^5 in 0.1 ml of RPMI-FCS were incubated for 1 h at 4°C with an equal volume of antibody. The second antibody was a fluorescein isothiocyanate-conjugated AffiniPure goat anti-mouse IgG (H+L) (Jackson Immunoresearch Laboratories, Inc., West Grove, PA). Cytospots were prepared (10^4 cells/spot) in a Shandon Elliot Cytospin centrifuge. The spots were rapidly dried, fixed in an ice-cold solution of 95% ethanol-5% acetic acid for 15 min, dried again, and mounted in 90% (v/v) glycerol in PBS (pH 8.0) containing 0.1% p-phenylenediamine (w/v) (25). The proportion of fluorescein-labeled cells was determined with the aid of an Epifluorescence microscope (Carl Zeiss Canada, Don Mills, Ontario) equipped with a HBO-50 mercury lamp, phase contrast optics, and ×100 oil immersion phase contrast objective. For each slide, 300-500 cells were counted.

**Enzyme Treatment.** The effect of various enzymatic treatments on the binding of the MoAb to the tumor cells was assessed using the immunofluorescence technique. The concentrations and assay conditions used were those determined in preliminary tests to give a minimal loss in cell viability and, where indicated, an optimal reaction with a soluble substrate.

**Exoglycosidases.** Tumor cells at a concentration of 2 x 10^6/ml were incubated for 1 h at 37°C with 1 mg/ml of a cocktail of *Charonia lampas* exoglycosidases (Seikagaku Kogyo Co., Tokyo, Japan) containing the following enzymes: N-acetylglactosaminidase, β-N-acetylgalactosaminidase, α-1-fucosidase, β-galactosidase, and α-mannosidase, in a buffer containing 8 mM Na_2HPO_4, 1 mM KH_2PO_4, and 0.1 M sodium citrate, pH 6.2. The cells were washed and the treatment was repeated once with a fresh enzyme solution. Cell viability at the end of this treatment was >90%. Enzymatic activity was monitored using the substrate phenyl-N-acetyl-α-D-galactosaminide and assay conditions as per manufacturer instructions.

**Neuraminidase and β-Galactosidase.** Treatment with neuraminidase was as we described previously (17). Tumor cells at a concentration of 2 x 10^6/ml were incubated for 1 h at 37°C with 0.02 unit *Vibrio cholera* test neuraminidase (Hoechst-Behring, West Germany) in PBS, pH 6.2. This was followed by a second incubation for 30 min with a fresh enzyme solution. Cleavage of sialic acid was monitored by prelabeling cells with [6-^3H]-N-acetylmannosamine, as we described previously (17). Some of the neuraminidase-treated cells were then incubated twice (for 60 and 30 min) with 75 units of β-galactosidase (Boehringer-Mannheim, Dorval, Quebec) in PBS, pH 7.8. Trypsin and Pronase. Trypsin and pronase (Boehringer-Mannheim, Dorval, Quebec) were used at a final concentration of 100 units/ml for 5 x 10^6 cells in PBS, pH 7.8 (26). Cells were incubated with the enzymes twice for 60 and 30 min at 37°C.

**Controls for all enzyme treatments consisted of cells incubated under identical conditions without the enzyme solution.**

**Antibody Purification.** IgG antibodies were purified from ascites fluid by affinity chromatography on Protein A-SEPHAROSE (27). The column (Affi-Gel Protein A MADES II) was obtained commercially from Bio-Rad Laboratories (Mississauga, Ontario) and used according to manufacturer’s instructions.

**Tumor Cell Adhesion to Frozen Sections.** The adhesion of tumor cells to 5–7 μm cryostat sections of syngeneic lymph nodes was measured as described previously (17). To test the effect of MoAb on adhesion, 10^6 tumor cells were incubated with the desired concentration of the antibody diluted in a total volume of 0.5 ml of ice-cold RPMI-FCS. The incubation was for 1 h. The unbound antibody was removed by four washes with RPMI. Cell viability was assessed and the cells were readjusted to the desired concentration.

Control antibodies in this study included: (a) MoAb C-11, a H-59- specific IgG1 antibody which recognizes a M, 67,000 plasma membrane protein and blocks the in vitro adhesion of the tumor to hepatocytes (28); (b) MoAb 12/57, an IgG1 which binds to various mouse cell lines including tumors H-59 and M-27, 3T6 fibroblasts, and mouse lung cells; and (c) a commercially obtained MoAb (CL9902) specific for the H-2D^b determinant (Cedarlane Labs, Hornby, Ontario).

**Isolation of PM.** PM were prepared from cultured H-59 and M-27 cells by the method described by Monneron and d’Alayer (29), with some modifications. Tumor cells derived from enzymatically digested solid tumors were cultured for a period of 2–4 weeks, as previously described. A total of 5–8 x 10^6 cells, collected by a brief treatment of the cell monolayers with PBS-EDTA, were used per preparation. The cell pellet was resuspended in TKM buffer (50 mM Tris-HCl, pH 7.4, 35 mM KCl, 5 mM MgCl_2) and homogenized in ice using a Potter-Elvehjem Model K43 homogenizer at 50 rpm for 10–20 min. Cell disruption was monitored by trypan blue exclusion. The homogenate was adjusted to 40% sucrose in the TKM buffer and layered over a discontinuous 22.5–73% sucrose gradient in TKM buffer. The gradient was centrifuged for 2 h at 130,000 × g in an IEB International preparative ultracentrifuge at 4°C. The bands which formed at the two uppermost interfaces were collected, diluted in TKM buffer, and centrifuged again at 150,000 × g for 30 min in a Beckman L8-M ultracentrifuge. The pellet was suspended in a small volume of TKM buffer, centrifuged, and frozen at −70°C until used. In initial experiments it was confirmed that these bands were rich in PM by measuring 5'-nucleotidase activity (30). Prior to use, protein concentration in the PM preparations was determined using the Markwell-modified Lowry assay (31).

**Gel Electrophoresis.** Prior to analysis by SDS-PAGE, PM preparations were solubilized with 1% Triton X-100 and centrifuged for 4 min at 122,000 × g in a Beckman airfuge to remove aggregates. Samples were analyzed under reducing conditions using SDS-PAGE, as described by King and Laemmli (32). The Protean II vertical electrophoresis system (Bio-Rad) was used with 8% polyacrylamide gels. Protein bands on the gels were stained with Commassie blue.

**Western Blot Analysis.** The electrophoretic transfer of the membrane proteins onto nitrocellulose paper (0.2 μm; Schleicher & Schuell, Montreal, Quebec) and the subsequent analysis with MoAb 12/50 were performed as described by Towbin et al. (33), using the Blot-Transfer cell (Bio-Rad) according to manufacturer’s instructions. After the transfer, the nitrocellulose was cut into strips and incubated with 15 μg/ml MoAb for 18 h at 4°C. Peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dimension Laboratories, Mississauga, Ontario, Canada) was used as second antibody and 4-chloro-1-naphthol (3 mg/ml in methanol) as substrate.

**Statistics.** The Student t test was used to analyze differences in the levels of antibody binding determined by the ELISA.

**RESULTS**

In order to identify MoAb which recognize cell surface molecules expressed specifically on tumor H-59 (but not on M-27),

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hybridoma clones were initially screened by ELISA on cell monolayers of both tumor sublines. This resulted in the isolation of clone 12/50, which produced antibody that reacted strongly with tumor H-59 but failed to bind to tumor M-27. The antibody, an IgG1, was subsequently tested against other murine target cells. Results of these experiments using affinity-purified antibody are summarized in Fig. 1. Binding to tumor H-59 was significantly higher than to all other cell types tested. Binding to line M-27 and to freshly isolated murine hepatocytes was undetectable, even at the highest antibody concentration used (86 µg/ml), while binding to normal mouse splenocytes and 3T6 fibroblasts was negligible, as compared to the binding to tumor H-59. A low but consistent level of binding was seen with the mouse lung cell line (MLG) however, it was significantly lower than the levels seen with tumor H-59 (P < 0.001) at all antibody concentrations used.

Immunofluorescence analysis subsequently confirmed the ELISA findings indicating that, while approximately 80–90% of H-59 cells labeled with the antibody, only 1–3% of M-27 cells were positive. High intensity labeling was seen with the majority (>90%) of H-59 cells (see example in Fig. 2); however, because this method is only semiquantitative, minor differences in the intensity of labeling among different tumor cell subpopulations could not be assessed.

To determine whether the antibody identified a cell surface molecule relevant to the metastatic phenotype of the tumor, we tested its ability to block the preferential adhesion of tumor H-59 cells to frozen sections of lymph nodes (17). Tumor cells were treated with the antibody and their attachment to the frozen sections was measured as described previously (17). MoAb 12/57 and an anti-H-2D<sup>b</sup> antibody which bind to both tumors H-59 and M-27 were initially used as controls, while MoAb C-11 (an IgG1 antibody), which binds selectively to tumor H-59 and blocks its adhesion to hepatocytes (28), was used in later experiments. Results shown in Table 1 and in Fig. 3 demonstrate that antibody 12/50 reduced adhesion to the frozen sections by approximately 70%, while the control antibodies failed to block adhesion when used under similar experimental conditions. In other experiments (not shown), we found that MoAb 12/50 failed to inhibit the adhesion of H-59 cells to frozen sections of mouse liver and lung, as well as to isolated mouse hepatocytes.

To identify the antigenic molecule recognized by antibody 12/50, Western blot analysis was used on solubilized PM preparations obtained from tumors H-59 and M-27 and separated by SDS-PAGE.

Shown in Fig. 4 are Commassie blue-stained gels of the PM preparations. No marked differences were found between the protein profiles obtained from the two tumors. When the proteins were subsequently transferred electrophoretically onto nitrocellulose and incubated with MoAb 12/50, the antibody

**Table 1** Inhibition of tumor cell adhesion to lymph node sections by monoclonal antibodies

<table>
<thead>
<tr>
<th>Antibody added</th>
<th>Nil</th>
<th>MoAb 12/50</th>
<th>MoAb 12/57</th>
<th>Anti-H-2D&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of adherent</td>
<td>6 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2 ± 0.15</td>
<td>6.4 ± 0.15</td>
<td>5.4 ± 0.15</td>
</tr>
<tr>
<td>tumor cells (× 10&lt;sup&gt;4&lt;/sup&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Results are expressed as means ± SD of quadruplicate samples.

<sup>b</sup> One million <sup>3</sup>H-labeled tumor cells were incubated for 60 min at 4°C with 0.2 ml of a 1:1 dilution of ammonium sulfate-precipitated hybridoma culture supernatants (12/50 and 12/57), containing 5 mg protein/ml. Cells were washed, added to 5–7-µm cryostat sections of syngeneic lymph nodes, and incubated for 45 min at 5°C, as described (2). The anti-H-2D<sup>b</sup> antibody concentration (1:100 dilution of a partially purified ascites) was that determined by ELISA to give a colorimetric reaction comparable to antibodies 12/50 and 12/57 at the concentrations indicated.
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Fig. 3. Inhibition of H-59 adhesion to lymph node sections with purified MoAb 12/50 and C-11. Tumor cell adhesion to the sections was measured using $^{131}$I-labeled tumor cells and 5-7-μm cryostat sections. For pretreatment with the antibody, $10^6$ tumor cells were incubated for 60 min at 4°C with 0.2 ml affinity-purified antibodies (○, C-11; ■, 12/50). Experiments were carried out with quadruplicate samples and the results represent means and SD of three experiments. The mean number of adherent cells in the untreated controls in these experiments was $4.3 \pm 0.6 \times 10^4$, corresponding to $8.6 \pm 1.2\%$ of the total number of cells added to the sections.

Protein concentration (μg/ml)

![Graph showing protein concentration vs. inhibition percentage.]

Table 2 Effect of enzymatic treatment on the binding of MoAb 12/50

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Fluorescein-labeled H-59 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>94 ± 2</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>Neuraminidase + β-galactosidase</td>
<td>96 ± 1</td>
</tr>
<tr>
<td>Exoglycosidases</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>Exoglycosidases + neuraminidase</td>
<td>92 ± 1</td>
</tr>
<tr>
<td>Trypsin</td>
<td>78 ± 3</td>
</tr>
<tr>
<td>Pronase</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>

In previous studies we found that the preferential adhesion of tumor H-59 to cryostat sections of lymph nodes could be significantly reduced following the treatment of the tumor cells with tunicamycin or a combination of neuraminidase and β-galactosidase (17), as well as by treatment with Pronase (60% reduction), implicating glycoproteins in the attachment (17). To determine whether the glycoconjugates also played a role in antigen recognition by antibody 12/50, the effect of various enzymatic treatments on the binding of the antibody was assessed using H-59 and M-27 cells in combination with the immunofluorescence technique. The results of these experiments are shown in Table 2. No significant reductions in the number of labeled cells or in the intensity of labeling were observed following treatment of the cells with exoglycosidases, with neuraminidase alone, or with neuraminidase in combination with β-galactosidase and exoglycosidases. Treatment of the cells with trypsin decreased labeling by approximately 17%, while treatment with Pronase resulted in a complete inhibition of labeling. Pretreatment of M-27 cells failed to modify antibody binding, which remained low at <1% (results not shown).

DISCUSSION

Cellular adhesion molecules have now been identified as major mediators of cell attachment, growth, differentiation, and migration both during embryogenesis and in various postembryonic processes such as wound healing and lymphocyte recirculation (34, 35). In recent years it has become increasingly clear that cellular adhesion molecules also play a central role in the binding of MoAb 12/50 was specific.

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Fig. 4. SDS-PAGE protein profiles and Western blots of tumor cell membrane preparations. PM samples were separated on 8% SDS-polyacrylamide gels. The molecular weight standards (lane e) used were phosphorylase b (M, 92,500), bovine serum albumin (M, 66,200), ovalbumin (M, 45,000), carboñic anhydrase (M, 31,000), soybean trypsin inhibitor (M, 21,500), and lysozyme (M, 14,400), obtained from Bio-Rad. Shown are Coomassie-blue stained solubilized plasma membrane of tumor M-27 (lane c) and tumor H-59 (lane d), and peroxidase-labeled electrophoretically transferred PM of tumors H-59 (lane a) and M-27 (lane b).

The binding of MoAb 12/50 was specific.

In previous studies we found that the preferential adhesion of tumor H-59 to cryostat sections of lymph nodes could be significantly reduced following the treatment of the tumor cells with tunicamycin or a combination of neuraminidase and β-galactosidase (17), as well as by treatment with Pronase (60% reduction), implicating glycoproteins in the attachment (17). To determine whether the glycoconjugates also played a role in antigen recognition by antibody 12/50, the effect of various enzymatic treatments on the binding of the antibody was assessed using H-59 and M-27 cells in combination with the immunofluorescence technique. The results of these experiments are shown in Table 2. No significant reductions in the number of labeled cells or in the intensity of labeling were observed following treatment of the cells with exoglycosidases, with neuraminidase alone, or with neuraminidase in combination with β-galactosidase and exoglycosidases. Treatment of the cells with trypsin decreased labeling by approximately 17%, while treatment with Pronase resulted in a complete inhibition of labeling. Pretreatment of M-27 cells failed to modify antibody binding, which remained low at <1% (results not shown).

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role in the process of tumor dissemination (5). In several laboratories the identification of such molecules has in fact led to the successful inhibition of tumor metastasis by reagents which can block the attachment (7, 9, 12).

Studies to date have focused mainly on tumor adhesion molecules likely to play a role in hematogenous dissemination. For example, the cell surface receptors for laminin and fibronectin, two extracellular matrix glycoproteins which line blood vessel endothelial cells, have been extensively studied and well characterized (3, 8, 35). Little is known at present about the role of these or other cell adhesion molecules in lymphatic dissemination of tumor cells, mainly because fewer tumor models of lymphatic metastasis are available for study.

In the present study, the Lewis lung carcinoma model of lymphatic metastasis (16) has been utilized to determine the role of tumor adhesion in lymphatic metastasis formation. Using frozen lymph node sections of murine lymph nodes and spleens, it was shown that the potential for lymphatic metastasis in this model correlates with adhesion to the sections (17). Here this in vitro model was used to screen MoAb produced by syngeneic immunization against the metastasizing tumor H-59, resulting in the identification of MoAb 12/50, an antibody specific for tumor H-59, which significantly inhibited the adhesion of the tumor. This inhibition was specific to lymph node adhesion, because tumor cell adhesion to mouse lung and liver sections was unaffected.

The MoAb, an IgG1 molecule, bound poorly or not at all to several other murine cell lines tested, with the exception of a mouse lung epithelial line, which had a low but consistent level of binding. Because both lines were originally derived from lung epithelium, this may indicate that antibody 12/50 recognizes a cell surface molecule related to cellular differentiation in the lung. Western blot analysis subsequently revealed that the antibody recognized a plasma membrane molecule of Mr, 37,000. Enzymatic treatment of tumor H-59 to cleave terminal carbohydrates expressed on the cell surface, although it was effective in reducing adhesion, did not reduce the binding of antibody 12/50, whereas Pronase completely abolished binding, suggesting that the antibody recognized a polypeptide sequence.

It should be noted that a Mr, 37,000 protein band was also identified on SDS-PAGE protein profiles obtained with plasma membrane preparations of tumor M-27. This band failed, however, to bind antibody 12/50 in the Western blot analysis. It is presently unclear whether this protein represents an antigenic variant of the plasma membrane molecule detected on tumor H-59 or a second unrelated molecule. This question will be addressed in future experiments using two-dimensional gel electrophoresis and amino acid sequence analysis.

Cell adhesion to frozen organ sections has been shown by others to correlate with the metastatic phenotype of tumor cells (26). Butcher and co-workers (34) have shown that the adhesion of lymphocytes to cryostat sections of lymph nodes correlated with their homing patterns in vivo. In these studies the site of attachment on the sections was shown to be the high endothelial venules. Using a MoAb (MEL-14) which could inhibit lymphocyte adhesion in vivo, one of the adhesion molecules which mediates lymphocyte migration to the peripheral lymph nodes was subsequently identified as a Mr, 80,000–92,000 molecule. Pretreatment of lymphocytes with the MoAb was also shown to inhibit their migration to peripheral nodes in vivo (34). In a recent study by the same group (36), it was shown that the dissemination of malignant lymphoma lines is also regulated by the expression of high endothelial venule-binding molecules. Although the site of adhesion on the lymph node sections in

Presently we are analyzing the molecular structure of the 12/50 antigen, its relationship to adhesion molecules identified in other cellular adhesion systems, and its role in lymphatic metastasis in vivo.

* B. Zetter, unpublished observation.

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