Specificity of Adhesion between Murine Tumor Cells and Capillary Endothelium: An in Vitro Correlate of Preferential Metastasis in Vivo

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ABSTRACT

We have compared the rate and extent of adhesion of various types of mouse tumor cells to endothelial cells derived from different organ sources. Our panel of tumors has included sarcoma, bladder carcinoma, glioma, teratoma, hepatoma, endothelioma, mammary adenocarcinoma, and lymphoma cells. Endothelial cell monolayers have included murine microvascular endothelial cells from ovary, brain, lung, and liver as well as large vessel endothelium from thoracic duct and dorsal aorta. Tumor cells differ both in their adhesive propensity and adhesive preference for different endothelial cells. Some, but not all, of the adhesive preferences correlate with the known in vivo metastatic behavior of these tumors. Our results support the hypothesis that endothelial cell surface-associated specificities may play a significant role in determining the pattern of metastasis.

INTRODUCTION

We have recently reported that glioma cells and teratoma cells differ in their relative adhesion to two endothelial cell targets (1). Teratoma cells adhered preferentially to ovary-derived endothelium compared to their adhesion to brain-derived endothelial cells, while conversely, glioma cells showed greater adhesion to brain microvascular endothelium than to capillary endothelial cells obtained from the ovary. Since the teratoma cells used in our study routinely seeded ovaries in vivo following i.p. or intracardiac injection, and since the glioma was of neural derivation, we suggested that our results were consistent with our working hypothesis that tumor/endothelial cell recognition may represent an important determinant of site-specific metastasis. The study, however, by being limited to two tumor lines and two endothelial targets, failed to provide the spectrum necessary to permit a secure conclusion concerning the relationship between in vitro cell adhesion to endothelium and in vivo metastatic behavior. The present study extends our observations by its use of a large panel of tumors assessed for adhesion to a broad spectrum of capillary and large vessel derived endothelial cell lines.

Central to our approach is the proposition that capillary endothelial cells from different organs are not alike, and that the differences they express are related to their developmental history (2, 3). The presence of readily identifiable organ-associated cell surface antigens on endothelial cells has further strengthened this hypothesis (3). To the extent that cell adhesion is a key component of histiotypic differentiation, the concept of organ-specific cell adhesion mediated by endothelial cells is a logical corollary of that proposition.

The idea of selective adhesion of tumor cells to specific organ-restricted target cells was already well formulated more than 30 years ago when tumor cells were shown to seed selective organ sites on injection into the embryonic circulation (4). In vitro correlates became available with the development of methods for quantitating cell adhesion and reaggregation (5); see Ref. 6 for references to the earlier literature. More recently Kahan (7), working with teratoma cells, and Nicolson and his associates (8, 9), using the melanoma lines developed by Fidler and his colleagues (10–12), have reported selective adhesion of tumors to organ-derived cell monolayers or to the subendothelial matrix, respectively. Schirrmacher (13) has recently prepared an excellent review of the field of cancer metastasis in which he outlines the many experimental approaches to metastasis: his review serves well to provide the literature background to the present study.

MATERIALS AND METHODS

Animals. BALB/cAu, C57BL/6Jau, and 129/J mice as well as various F1 hybrids were bred in our own animal colony.

Tumors. Teratoma cells were obtained from a spleen-seeking subline of OTT 6050. The line was developed in our laboratory by selection for spleen colonization, using a combination of in vitro culture and s.c. and i.p. retransplantation of cells from tumor-bearing spleens (14, 15). The OTT 6050 original tumor shows metastatic preference for ovaries and adrenal glands (16). S180 sarcoma cells were grown in vitro from tumor cells distributed by the NIH tumor bank. The C755 mammary carcinoma (C57BL/6 origin) was also obtained from this source. The endothelioma tumor line, derived from a spontaneous hemangioendothelioma of a strain 129 mouse, was obtained from G. Hoak and maintained since 1981 in our own culture facility. A second endothelioma line, CBr, arose by transformation from a long-term brain-derived BALB/c endothelial cell line. Two rat liver tumors, lines H7777 and H-4-11-II-E, were obtained from H. Pitot. A mouse bladder tumor (MBT-2) was provided by Y. A. Sidky. The BW 5147.3 T-lymphoma cell line was obtained from the American Type Culture Collection.

Endothelial Cells. Details of endothelial cell isolations have been presented elsewhere (1, 17, 18). In brief, MBE-cells were obtained by filtration of minced brain tissue through large-pore Nitex filters and retention on 45 μm filters, followed by collagenase treatment or direct explantation to obtain endothelial cell outgrowths. MHE cells were similarly obtained and sorted using a fluorescence-activated cell sorter (FACS-IV) to identify cells binding anti-ACE 3.1.1 antibody (17). MLE cells were obtained from thoracic duct fragment cultures (18). Thoracic ducts were visualized following feeding of evaporated milk, ligated, and removed as organ fragments. Endothelial cells were obtained either immediately, using collagenase treatment, or from outgrowth areas surrounding the explanted vessel fragments.

Cell Culture. Cells were maintained generally in Dulbecco's modified Eagle's minimum essential medium with 10–20% fetal bovine serum and antibiotics. Endothelial cell cultures were supplemented with tumor-conditioned (Sarcoma 180 or endothelioma) medium (20%) and, for some cells, with endothelial cell growth supplement (Collaborative Research). Heparin (5 units/ml) was added to some endothelial cell cultures.

Adhesion Assays. Endothelial cells were grown to confluence in 24-well culture plates (1). Tumor cells were labeled overnight with [3H]thymidine (10–50 μCi/10 ml medium, 1 mCi/ml, specific activity 2.0 Ci/mmol). Tumor cells were dispersed using EDTA followed by filtration

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2 Received 8/4/86; revised 11/24/86; accepted 12/1/86.
3 Summer medical student trainee supported by NIH training grants.
4 The abbreviations used are: MBE, mouse brain endothelium; MHE, mouse hepatic (liver) endothelium; MLE, mouse lymphatic (thoracic duct) endothelium.

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through 20-μm Nitex filters, and the cell concentration adjusted to 2–3 × 10^6 cells/ml in Dulbecco's modification of Eagle's minimum essential medium or L15 medium containing 10% fetal bovine serum. Aliquots were retained for later determination of total radioactivity to establish 100% labeling levels. In general, these aliquots yielded 150,000–300,000 dpm. Tumor cells were added to endothelial cell cultures which were then immediately placed on a rotating platform operating at room temperature at 100 rpm (1). At designated times cultures were removed from the shaker and rinsed 3x with medium to removed nonadherent cells. The retained cells were then lysed with 1 m NH4OH and assessed for radioactivity. Assignment of assay wells to different experimental groups was partially or completely randomized to minimize spurious differences associated with plate variables.

Data Presentation. Percentage of adhesion was determined according to the formula:

\[ \frac{\text{dpm sample} - \text{dpm blank}}{\text{dpm maximum} - \text{dpm blank}} \times 100 \]  

The minimum number of samples used was 3 for each tumor and each time point.

The specificity index (SI) was calculated on the basis of adhesion of two tumors \( t_1 \) to two endothelial cell monolayers \( e_1 \) and \( e_2 \) using the formula (1):

\[ \text{SI} = \frac{\% \text{ adhesion } e_1 t_1 \times e_2}{\% \text{ adhesion } e_1 t_2 \times e_2} \]  

where \( t_1 \) and \( t_2 \) represent two tumor types and \( e_1 \) and \( e_2 \) represent two different endothelial cell monolayers. The index was developed to minimize variables unrelated to specificity (cf. 1). This is of special importance when comparing, for example, two tumors which differ in tendency for adhesion (e.g., sarcomas versus lymphomas), or two monolayers with different amounts of presumably nonspecific cell adhesion molecules (e.g., fibrinogen). Log conversion of the ratios was carried out to obtain linear presentation of the data, and the SI values were transformed to +log or –log values for graphic presentation.

RESULTS

Adhesion to Large Vessel Endothelium. A comparison was made between the adhesion of different tumor cell lines to adult bovine aortic endothelium and MLE. The results for three tumors, the GL26 glioma, S180 sarcoma, and C755 mammary adenocarcinoma, are shown in Table 1. The experiments show (a) in contrast to the GL26 and C755 tumors, S180 adheres poorly to large vessel endothelium and (b) the GL26 glioma shows a marked preferential adherence to thoracic duct endothelium as compared to aortic endothelium, whereas no preference for one versus the other of these endothelial cells was expressed by the S180 or C755 tumors.

Additional studies have been carried out with the endothelioma and bladder tumors. The endothelioma pattern is similar to that of S180, while the MBT tumor appears similar to C755 (data not shown). Studies using mouse aortic endothelial cells are in progress.

Adhesion to Microvascular Endothelial Cells. Adhesion assays were carried out using microvascular endothelial cells derived from mouse liver, brain, lung, and ovary. The results are shown in Fig. 1–3. The experiments summarized in Fig. 1 show that (a) some tumors adhere more rapidly and more extensively to target endothelial cells than other tumors. For example, the S180 tumor is generally more adherent than the endothelioma; (b) the relative adhesion to different microvascular endothelial cells is not the same for different tumors. Each of the four tumor types shown in Fig. 1 has its own distinct pattern of relative adhesion to the test panel; (c) even for a single tumor type, adhesiveness will vary: the H7777 hepatoma, for example, is generally more adherent to cell monolayers than the H-4-II-E hepatoma (data not shown).

Fig. 2 represents one of seven experiments comparing H7777 and GL26 adhesion. Reproducibly, hepatoma cells adhered preferentially to liver endothelium as compared to brain endothelium, while glioma cells conversely adhered preferentially to brain endothelial cells. H-4-II-E cells, although less adherent than H7777 hepatoma cells, showed a similar preferential adhesion to the liver-derived endothelium (data not shown; three experiments).

The adhesion to MLE and 3T3 cells is also shown. Hepatoma cells adhere well to both MLE and 3T3 cells. In contrast, while the GL26 tumor adheres well to the thoracic duct endothelium (see also Fig. 1) it appears only weakly adherent to 3T3 fibroblasts. Additional studies of adhesion to 3T3 cells have been carried out with endothelioma cells. The original Hoak heman-gioendothelioma showed strong adhesive preference for 3T3 cells (Ref. 1; Fig. 6B); our current cell line, however, shows less adhesion to these cells (Fig. 1A); a third endothelioma line, CBR, isolated in our own laboratory, is only weakly (< 10% at 30') adherent to 3T3 cells.

Fig. 3 and Table 2 provide direct comparisons of specificity indexes obtained for GL26 versus H7777 adhesion data. Positive log transforms of the SI are given where \( t_1 = \) GL26; \( t_2 = \) H7777; \( e_1 = \) MBE; and \( e_2 = \) MLE, MHE, or 3T3. Negative log transforms of the SI are given where \( t_1 = \) H7777; \( t_2 = \) GL26; \( e_1 = \) MHE; and \( e_2 = \) MBE, MLE, or 3T3. The graphic display indicates preferential adhesion of GL26 to MBE cells by positive values, and preferential adhesion of H7777 to MHE cells by negative values. The shaded area represents the region of nonpreference. The experiments show that the glioma prefers MBE to MHE, MLE, or 3T3 cells, and that the hepatoma prefers MHE to MBE or MLE cells. The adhesion of the

Table 1 Relative adhesion of tumor cells to large vessel endothelial cells: mouse thoracic duct versus adult bovine aorta

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Exp. no.</th>
<th>Cell type</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL26 (Glioma)</td>
<td>6</td>
<td>MLE</td>
<td>11.4 ± 3.7**</td>
<td>24.2 ± 8.6</td>
<td>32.4 ± 13.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>ABAE*</td>
<td>6.3 ± 2.2</td>
<td>12.3 ± 4.9</td>
<td>19.9 ± 8.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MLE/ABAE</td>
<td>1.8</td>
<td>2.0</td>
<td>1.6</td>
</tr>
<tr>
<td>S180 (Sarcoma)</td>
<td>6</td>
<td>MLE</td>
<td>3.9 ± 1.1</td>
<td>8.2 ± 2.2</td>
<td>12.2 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>ABAE</td>
<td>4.4 ± 1.5</td>
<td>8.2 ± 2.3</td>
<td>14.2 ± 2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MLE/ABAE</td>
<td>0.9</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>C755 (Mammary carcinoma)</td>
<td>4</td>
<td>MLE</td>
<td>10.5 ± 2.2</td>
<td>14.5 ± 3.1</td>
<td>18.4 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>ABAE</td>
<td>10.0</td>
<td>14.1</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MLE/ABAE</td>
<td>1.1</td>
<td>1.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>

** Value represents the mean percentage of adhesion ± SE calculated using the mean values of triplicate assays from each experiment for each data point.
* ABAE, adult bovine aortic endothelium.
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**Fig. 1.** Adhesion of different mouse tumors (A, endothelioma; B, Sarcoma 180; C, teratoma; D, MBT-2 bladder tumor) to different murine confluent culture monolayers: (Δ) lung endothelium; (O) 3T3 fibroblasts; (□) liver; (□) thoracic duct lymphatic endothelium; (▲) brain endothelium.

**Fig. 2.** Adhesion of rat hepatoma H7777 (O) and mouse GL26 glioma (■) cells to mouse-derived cell cultures. A, liver endothelium; B, brain endothelium; C, thoracic duct lymphatic endothelium; and D, 3T3 fibroblasts.
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Fig. 3. Specificity index calculations for pooled data from six consecutive experiments using mouse glioma GL26 (open symbols) and rat Morris hepatoma H7777 (closed symbols). Positive log transforms were performed for GL26 specificity indexes comparing adhesion to mouse brain endothelium with adhesion to: mouse liver endothelium (A); mouse thoracic duct endothelium (O); and 3T3 cells (C). Negative log transforms were performed for H7777 specificity indexes comparing adhesion to mouse liver endothelium with adhesion to: brain endothelium (A); thoracic duct endothelium (O); and 3T3 cells (C). Values lying outside of the hatched region are significantly different (P < 0.05) from control values. Log ratios greater than +/−0.2 have a P value of < 0.01.

Table 2 Comparison of glioma and hepatoma adhesion to various target cell monolayers

<table>
<thead>
<tr>
<th>Cell type</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioma vs. hepatoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBE/MBE</td>
<td>1.20</td>
<td>2.07</td>
<td>2.61</td>
</tr>
<tr>
<td>MBE/MHE</td>
<td>3.07</td>
<td>3.84</td>
<td>5.04</td>
</tr>
<tr>
<td>MBE/3T3</td>
<td>15.78</td>
<td>10.56</td>
<td>19.11</td>
</tr>
<tr>
<td>Hepatoma vs. glioma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHE/MLE</td>
<td>1.48</td>
<td>1.86</td>
<td>1.93</td>
</tr>
<tr>
<td>MHE/MBE</td>
<td>3.07</td>
<td>3.84</td>
<td>5.04</td>
</tr>
<tr>
<td>MHE/3T3</td>
<td>0.19</td>
<td>0.36</td>
<td>0.26</td>
</tr>
</tbody>
</table>

* The specificity index is significantly different from random adhesion for all data points. Fig. 3 is derived from these data by +log transforms of glioma versus hepatoma ratios and −log transforms of the hepatoma versus glioma ratios.

DISCUSSION

We have previously shown that two different tumors, a glioma and an ovary-seeking teratoma, differed in their adhesive preference for endothelial cells (1). From a relative standpoint glioma cells adhered more readily to brain endothelium than to ovary endothelium, while the teratoma cells conversely showed preferential adhesion to the latter. The study provided our first clear experimental evidence for the hypothesis that the endothelium could represent an organ-specific selective mediator for metastasizing tumor cells (1). On the other hand, because we restricted our first analysis to two tumors and two endothelial cell targets only, we could not generalize from the results either in terms of the extent of organ-associated endothelial cell selectivity or in terms of relevance of the in vitro observations to the in vivo behavior of metastasizing tumor cells.

By extending our panel of tumor cells and the number of endothelial and nonendothelial target monolayers, we are now in a position to evaluate the extent and significance of the differential adhesion patterns seen in our in vitro assays. We conclude: (a) different tumors show differences in their adhesive preferences, such that relative adhesion to a panel of target monolayers provides an almost singular phenotypic description of each tumor; (b) for two of the tumor types, the hepatomas [both, i.e., hepatoma (H-4-II-E and H7777)], and glioma (GL26), there is distinct preferential adhesion to the endothelial cell monolayer derived from the corresponding organ from which it was isolated; (c) for two tumors (teratomas and hepatomas) preferential adhesion was seen to those endothelial cell monolayers which correspond to sites of metastasis when the tumors are grafted heterotypically (resp. ovary and liver derived endothelial cell cultures); (d) adhesion to lymphatic large vessel endothelium is greater for mammary adenocarcinoma cells than for sarcoma, teratoma, or bladder tumor cells, correlating with the presumed route of dissemination of the former tumors through the lymphatic system. (e) The weak adhesion of sarcoma cells to large vessel endothelium in general, contrasting with the high adhesion to fibroblasts, may be a reflection of the lack of metastatic competence of this tumor. Additional studies of adhesive preferences using other tumors with known metastatic preferences are currently under way.

We have now been able to obtain microvascular endothelial cells from murine placenta, urinary bladder, mammary gland, lymphatic capillaries, and adrenal gland (Ref. 18; and unpublished observation) and we are currently purifying the cultures by flow cytometric analysis and cell sorting. These cell lines can then be used with tumors such as C755 (mammary), MBT-2 (bladder), and OTT6050 (ovary and adrenal-seeking teratoma) for further tests of organ-specific selective adhesion.

Schirrmacher has emphasized the varied influences that mediate the final outcome of tumor cell dissemination (13). The role of the cell surface of cancer cells has long been a focal point for experimental analysis (e.g., 19, 20). Recent studies have focused on cell surface-associated carbohydrates (see Ref. 21), as shown by both lectin-binding properties of tumor cells and by the changes in adhesion induced by enzyme treatment. Antibodies directed against cell surface moieties, generally glycoproteins, moreover, were shown capable of interfering or altering metastatic behavior (22, 23).

The question of adhesive specificity in metastasis is a natural extension of the selective adhesion that has long been recognized as critical in the process of embryonic development (see Ref. 6). Specific adhesion-mediating molecules (24–28) have now been identified both by direct biochemical analysis and by inhibition studies using monoclonal antibodies, and it seems reasonable to propose that it is these or related molecules that are similarly responsible for the selective adhesion seen in our present experiments. It should be emphasized that our studies have focused on adhesion to endothelium since it is these cells that are accessible to tumor cells in the circulation. Adhesion to subendothelial matrix, fibroblasts or other cell types would certainly influence tumor development once extravasation has occurred.

Supporting this conclusion in principle is the fact that we have already been able to identify cell surface-associated, organ-specific antigens that are expressed on endothelial cells (3). We now need to examine whether antibodies against some of these antigens can interfere with the selective adhesion seen in our studies. In this connection the earlier finding that liver-metastasizing tumor cells interact selectively with liver parenchymal cells (29, 30) should be cited: these investigators suggested on the basis of selection experiments (cf. 31) that several surface glycoproteins served as specific ligands for tumor/hepatoocyte adhesion. The importance, specifically, of glycosyltransferases in modulating adhesive sites has been stressed by Lilien and his colleagues (32), by Schirrmacher (cf. 13), and by Shur (33).

Our studies on selected teratoma cell lines parallel the inves-
tigations reported by Kahan (7). In those investigations adhesion to organ monolayers was measured but without regard to the particular cell types involved. In the studies of Nicolson and his colleagues, emphasis was placed on the subendothelial matrix, leaving open the question of endothelial cell contributions to that matrix (22, 31). We do not know whether specificity seen in the support of tumor cell growth after traversing of the endothelial cell barrier is influenced by the same molecules as is the initial adhesion of tumor cells to the endothelium during their passage through the microvascular bed. Although the problem of tumor/host relationships in metastasis is a complex one, (see Refs. 13, 31, 34–36) it would seem that the initial arrest of cells by microvascular endothelium might, indeed, represent a significant primary event leading to metastatic specificity.

A critical question that we cannot yet answer is whether the preferential adhesion we have observed reflects a differential distribution of a few key molecules defining broad target subsets, or whether, in fact, it reflects unique organ-associated concentration in the lung may be responsible for lung-specific distribution of a few key molecules defining broad target sub.

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