Differential Expression of a Lamininlike Substance by High- and Low-Metastatic Tumor Cells

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High-metastatic murine fibrosarcoma cells readily attached to Type IV (basement membrane) collagen, whereas low-metastatic cells isolated from the same tumor did not. The addition of laminin—a glycoprotein that facilitates the adherence of epithelial cells to their basement membranes—enhanced the attachment of the low-metastatic cells, but not the high-metastatic cells. Using anti-laminin antibodies and a laminin-binding lectin as probes, the authors were able to identify by immunofluorescence a moiety associated with the high-metastatic cells, but not the low-metastatic cells, which cross-reacted with murine laminin purified from the EHS sarcoma. When extracts from the high-metastatic cells were separated by affinity chromatography, with the laminin-binding lectin as the affinity substrate, a substance was isolated that had an apparent molecular weight of 56,000 daltons. The affinity-purified material reacted strongly with anti-laminin antibodies by enzyme-linked immunosorbent assay. (Am J Pathol 1983, 111:27–34)

IN PREVIOUS WORK we isolated from a murine fibrosarcoma tumor cell lines that differed greatly in their tumorigenicity and spontaneous metastatic ability.[1,2] Numerous studies have been conducted in an effort to delineate the biologic basis for the differences in *in vivo* behavior. A number of functional properties, both *in vitro* and *in vivo*, have been found to distinguish the high- and low-metastatic cells,[1-8] but the molecular basis for these differences is unknown.

In this paper we report that the high-metastatic cells (but not the low-metastatic cells) express a moiety that cross-reacts immunologically with murine laminin prepared from the EHS sarcoma. Since it has been suggested that laminin plays a role in tumor metastasis,[9] the expression of this substance by the high-metastatic cells may contribute to their enhanced ability to metastasize spontaneously from primary tumors and to form lung tumors after intravenous injection.[1-3]

Materials and Methods

Cells

The procedures used to isolate the high- and low-metastatic cells have been described previously.[1,2] Briefly, cells from a 3-methylcholanthrene-induced tumor in a C57 bl/6 mouse were established in culture on medium 199 supplemented with 10% fetal bovine serum. To obtain the high-metastatic tumor cells, a syngeneic mouse was given 1×10^5 cells injected in the right rear footpad. A primary tumor developed at the site of injection, and cells from the tumor spontaneously metastasized to the lungs. Cells from the metastatic lung tumor were subsequently reestablished in culture. After reestablishment in culture, these cells would form tumors in virtually 100% of the syngeneic mice given 1×10^5 cells injected in the footpad. Tumor-bearing mice would subsequently develop pulmonary metastases.

The low-metastatic cells were isolated from the same tumor by culturing the cells *in vitro* over a 4-month period in Medium 199 containing 10% normal

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human serum in place of the fetal bovine serum. Originally, the number of cells that survived and grew in human serum-supplemented medium was small. However, by continually selecting for those cells that did grow in medium with human serum, we were eventually able to establish a human-serum-adapted cell line. After isolation, these cells were maintained under the same conditions as the high-metastatic cells (i.e, in medium supplemented with 10% fetal bovine serum). The human-serum-adapted cells would routinely form tumors in only 20–25% of the animals given 1×10^5 cells injected in the footpad. Spontaneous metastasis formation was extremely rare. Cloned lines were established from both populations and have proven to be phenotypically stable when maintained *in vitro* over a 2-year period or when passed through syngeneic mice and reestablished in culture.[10]

In the present study, the two cell lines were maintained under identical conditions. These included growth on RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. The cell lines were grown at 37 C in a humidified atmosphere containing 5% CO_2. They were subcultured on a 3–4-day schedule at a split ration of 1:4. Both cell lines were tested for *Mycoplasma* contamination by growth in *Mycoplasma* broth and growth on *Mycoplasma* agar and found to be free of contamination.

Reagents

Murine laminin was isolated from the EHS sarcoma according to the method of Timpl et al.[11] The tumor was a gift from Dr. L. Liotta (Laboratory of Pathophysiology, National Institutes of Health).

Antiserum to the purified murine laminin was prepared by repeated intradermal injections of the laminin emulsified in Freunds' adjuvant into the backs of New Zealand White rabbits. Blood was obtained by periodic venipuncture, and the serum was separated after the blood had been allowed to clot overnight.

An Isolectin I mixture from *Griffonia simplicifolia* (GS I) was prepared in our laboratory by the method of Hayes and Goldstein.[12] Fluorescein-isothiocyanate–labeled GS I-B_4 (FITC-GS I-B_4) was obtained from Sigma Chemical Company (St. Louis, Mo).

Immunofluorescence

Live cells were processed for immunofluorescence as previously described.[13] We used FITC-GS I-B_4 to study lectin binding. An indirect immunofluorescence method was used with the rabbit anti-laminin antiserum. The second label for this was rhodamine-conjugated goat anti-rabbit IgG (Cappel Laboratories, Cochranville, Pa). In one study the cells were sequentially labeled with both the antiserum and the lectin. In this study, the cells were labeled first with the anti-laminin antiserum, followed by the commercial rhodamine-conjugated anti-rabbit IgG and finally the FITC-GS I-B_4. As a control for the lectin studies, binding was attempted both in the presence and the absence of the competing haptenic sugar—10 mM methyl-α-D-galactopyranoside (Pfanstiehl laboratories, Inc., Waukegan, Ill). Preimmune rabbit serum was used as the control for the rabbit anti-laminin antiserum.

Cytotoxicity Assay

The cells were harvested from culture and washed twice in serum-free medium. The washed cells were diluted to 1×10^5 cells in 0.9 ml of medium and added to the wells of a 24-well culture dish. Each well was then supplemented with 0.1 ml of the appropriate sera (rabbit anti-laminin antiserum or preimmune rabbit serum) and 0.1 ml of commercially prepared rabbit complement (Accurate Chemical and Scientific Corporation, Westbury, NY). The cells were incubated at 37 C in 5% of CO_2 for 1 hour. After incubation, the cells were examined microscopically, and the relative amount of cytotoxicity was evaluated. The supernatant fluids were harvested into tubes, and 1 ml of 0.25% trypsin (Difco, Detroit, Mich, 1:250), was added to each well. The attached cells were released by the trypsin, and these cells were added to the supernatant fluid from the same well. The total number of viable cells harvested from each well was then determined using a hemocytometer. Trypan blue was used as a vital stain but its use was really not necessary since the cytotoxic sera rapidly lysed the killed cells.

Affinity Chromatography

Extracts prepared from the high and low metastatic cells were separated by affinity chromatography using GS I as the affinity probe. The isolectin mixture was immobilized onto cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the manufacturer's directions. The affinity gel was packed into a 0.7×10 cm column and equilibrated with phosphate-buffered saline (PBS) containing 0.15 M NaCl, 0.1 M phosphate buffer, pH 7.2, 0.1 mM $CaCl_2$ and 0.04% NaN_3. To prepare cell extracts, 10 75-sq cm flasks of

each cell line (approximately 1–2 × 10^7 cells/flask) were labeled with 10 μCi of ^{14}C-leucine 24 hours prior to harvesting. The cells were in active logarithmic growth at this time. The cells were harvested either by trypsinization and resuspension in PBS or by washing in PBS and then scraping directly into the PBS. After disruption of the cells by sonication, the cell extracts were stirred for 48 hours at 4 C to insure complete solubilization and then centrifuged at 27,000*g* for 30 minutes to remove the particulate fraction. After dialysis against PBS, the supernatants were applied to the GS I affinity column. The column was washed with PBS at a flow rate of 5 ml/hr, and 4.4 ml fractions were collected. All column separations were performed at 4 C. Aliquots (200 μl) of each column fraction were analyzed for amount of ^{14}C present. When the ^{14}C counts per minute returned to baseline value, the column was eluted with 50 mM of methyl-α-D-galactopyranoside. The radioactive tracer was used to quantitate the amount of material from each cell line that was specifically bound to the lectin column and eluted with the haptenic sugar.

In addition to the ^{14}C-leucine–labeled cell extracts, we also separated nonlabeled extracts from the high-metastatic cells by the same procedure. The nonlabeled cell extract that was bound to the column and subsequently eluted was concentrated and separated into two pools. One of the two pools was examined by SDS-polyacrylamide gel electrophoresis, and the other was examined for immunologic cross-reactivity with the antibodies prepared to laminin.

An enzyme-linked immunosorbent assay (ELISA) was used to determine reactivity between the affinity-purified extract from the high-metastatic cells and the anti-laminin antibodies. The ELISA was performed with modifications of a method previously described by us.[14] Briefly, the affinity-purified extract was diluted in 0.05 M carbonate buffer, pH 9.6. Fifty microliters of the antigen solution was added to each well of a 96-well microtiter plate and allowed to incubate for 3 hours at 37 C. A control plate to which was added 50 μl/well of the purified laminin (10 μg/ml) was handled identically. After incubation for 3 hours, the plates were washed three times with phosphate-buffered saline, pH 7.2, containing 0.05% Tween 20 (PBS-T). Serums appropriately diluted in PBS-T were then added to the wells (50 μl/well), and the plates were incubated at 37 C for 30 minutes. The plates were again washed with PBS-T, and 50 μl of the appropriately diluted alkaline phosphatase-conjugated second antibody were added to each well. After 30 minutes' incubation at 37 C with the second antibody, the plates were washed three times with PBS-T. One hundred microliters of a 1 mg/ml solution of *p*-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, Mo) in 0.05 M carbonate buffer, pH 9.6, were added to each well and the absorbance (OD_{405}) of the wells was measured at various time intervals with a Titertek Multiskan (Flow Laboratories, McLean, Va).

Adherence Assays

The adherence of the high- and low-metastatic cells to Type I/III collagen and Type IV collagen was measured. The Type I/III collagen was prepared from rat tail tendon by acid extraction and contains nearly all Type I collagen.[15] However, it has been shown to contain a very small amount of Type III collagen and so will be referred to as Type I/III collagen in this article. The Type IV collagen was a generous gift from Dr. L. Liotta (Laboratory of Pathophysiology, National Institutes of Health). It was obtained from the EHS sarcoma by acid extraction and further purified as previously described.[16] Bacteriologic dishes (35 mm in diameter) were coated with 10 μg per dish of either collagen type in 1 ml of 0.1 M acetic acid and allowed to dry overnight. The dishes were then washed three times in cold PBS and were ready to use. To coat the collagen substrates with laminin, we incubated each dish with 10 μg of laminin in PBS for 30 minutes at 37 C prior to the addition of the cells. A single-cell suspension was prepared with each cell line, and the rate of attachment was determined as described previously.[4,5]

Results

Adherence to Collagen Substrates

Since laminin is thought to play a role in the adherence of normal epithelial cells[9] and metastatic tumor cells[17] to basement membrane (Type IV) collagen, we examined the attachment of the high- and low-metastatic cells to substrates prepared from Type I/III collagen and Type IV collagen. The collagen-coated plates were prepared as described in the Materials and Methods section. The high- and low-metastatic cells were then added to replicate dishes and the rate of attachment determined.[4,5] Figure 1 shows the rate of attachment of the two cell lines to Type IV collagen under serum-free conditions. It can be seen that in the absence of exogenous laminin, the high-metastatic cells (left-hand panel) attached much more rapidly than did the low-metastatic cells (right-hand panel). It can also be seen that when the Type IV collagen-coated dishes were pretreated with 10 μg of laminin per dish, the attachment of the low-metastat-

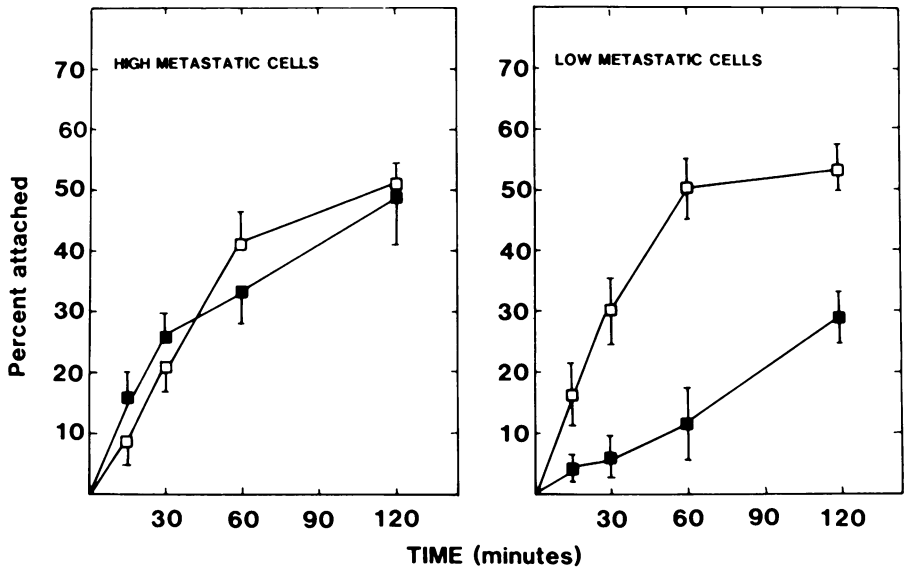

Figure 1—Attachment of the high- and low-metastatic cells to Type IV collagen. The dishes were prepared and the assay carried out as described in Materials and Methods. The high-metastatic cells are shown in the left-hand panel, and the low-metastatic cells are shown in the right-hand panel. Collagen-coated dishes treated with laminin are indicated by ■-■; collagen-coated dishes without laminin are indicated by □-□. The adherence assays were run three times (with duplicate plates per time point), and the data shown are the average number of cells attached ± SEM of all three experiments.

ic cells was dramatically increased. In contrast, we observed no significant increase in the high-metastatic cell attachment following pretreatment of the dishes with laminin. Although not shown we observed that both cell lines attached rapidly to Type I/III collagen-coated dishes and there were no significant differences between the cell lines. Furthermore, laminin pretreatment had no effect on the attachment of either cell line to the Type I/III collagen (data not shown).

Immunofluorescence Studies

The observations in the adherence assay were consistent with the suggestion that the high-metastatic cells (but not the low-metastatic cells) were expressing a substance that would mimic the effects of exogenous laminin. We therefore examined the cells by immunofluorescence to determine whether a substance that cross-reacted with laminin could be identified. For these studies the cells were grown on coverslips and examined by indirect immunfluorescence in the viable state. Anti-laminin antibodies prepared in rabbits against laminin purified from the EHS sarcoma were used as the primary antibodies, and rhodamine-conjugated goat anti-rabbit IgG was used as the secondary antibody. Nearly 100% of the high-metastatic cells stained brightly under these conditions, whereas virtually none of the low-metastatic cells did. The staining of the high-metastatic cells appeared to be confined to the cell surface, although it is difficult to accurately localize antibody binding on live cells by immunofluorescence. No staining was observed in either cell line treated with normal rabbit serum.

The cells were next examined for the ability to bind FITC-GS I-B_4. Similar results were obtained. Nearly all of the high-metastatic cells stained brightly with this lectin, whereas the low-metastatic cells showed no significant fluorescence. The specificity of lectin binding by the high-metastatic cells was confirmed by the fact that fluorescence was completely abrogated when the cells were treated with 10 mM of the competing haptenic sugar, methyl-α-D-galactopyranoside, along with the lectin.

Finally, the high- and low-metastatic cells were stained with both the anti-laminin antibodies and the GS I-B_4 lectin. For the double-labeled cells, the anti-laminin antibodies were added first. This was followed by the rhodamine-labeled anti-rabbit IgG and finally the FITC-GS I-B_4 lectin. The double-labeled cells are shown in Figure 2. The high-metastatic cells are shown in the top panel, and the low-metastatic cells are shown in the bottom panel. Rhodamine fluorescence is shown in the left-hand panels, and FITC fluorescence is shown in the middle. It can be seen that the same cells exhibiting rhodamine fluorescence also show FITC fluorescence. This indicates that it is the same cells within the population that bind both the anti-laminin antibodies and the GS I-B_4 lectin. Examination of the upper panels suggests that the fluorescence pattern obtained with the two probes is different. This could be due to the fact that the lectin is recognizing the carbohydrate structure primarily, whereas the anti-laminin antibodies may be directed toward the protein part of the receptor mole-

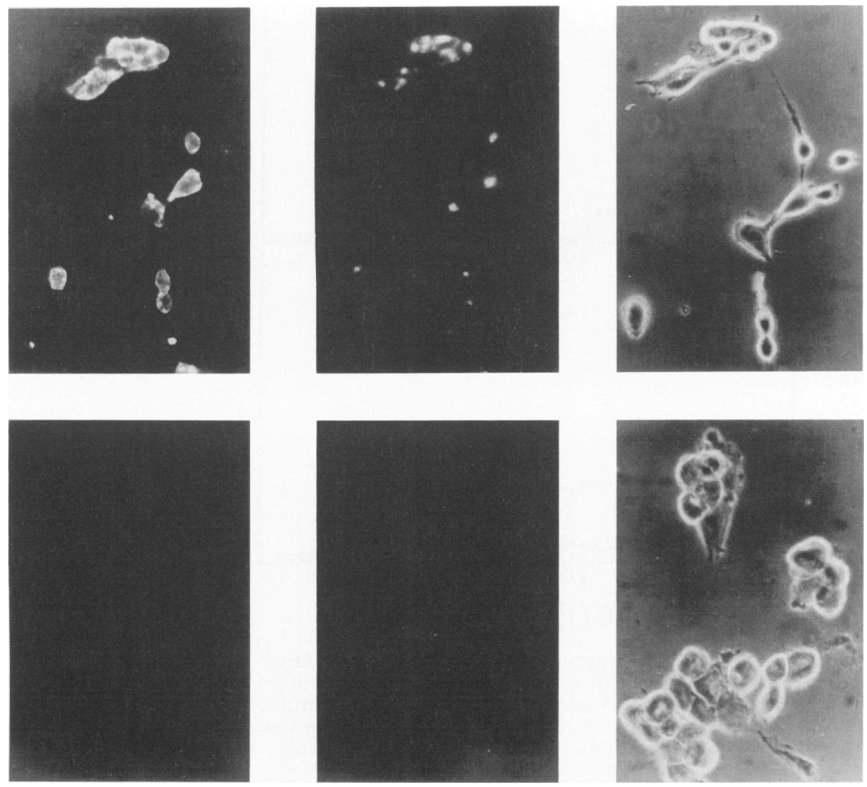

Figure 2—Binding of anti-laminin antibodies and the GS I-B_4 lectin by the high- and low-metastatic cells. Live cells were processed for immunofluorescence as described in Materials and Methods. They were first incubated with rabbit anti-laminin, followed by rhodamine-conjugated goat anti-rabbit IgG and finally with FITC-GS I-B_4. Incubation of the high-metastatic cells with 10 mM of the haptenic sugar, methyl *α-D*-galactopyranoside eliminated FITC-GS I-B_4 binding. Preimmune rabbit serum controls showed no rhodamine fluorescence. All exposures were for 1 minute at a magnification of ×400. The high-metastatic cells are shown in the upper panels; the low-metastatic cells are shown in the lower panels. Rhodamine (anti-laminin) fluorescence is shown in the left-hand panels; FITC (GS I-B_4) fluorescence is shown in the middle. Phase/contrast photomicrographs are shown in the right-hand panels.

cule. Alternatively, the patchy nature of the lectin fluorescence could be due to the fact that the lectin induces cap formation. It should be pointed out that when the cells were stained with the lectin after treatment with paraformaldehyde (unlike the cells shown in Figure 2, which were stained in the viable state), the fluorescence pattern was uniform around the cell periphery.

Cytotoxicity Studies

The high- and low-metastatic cells were examined for cytotoxicity following incubation with either normal rabbit serum or the rabbit anti-laminin antiserum. The assay was carried out in the presence of rabbit complement as described in the Materials and Methods section. The results shown in Table 1 indicate that the high-metastatic cells were very susceptible to the anti-laminin antiserum, whereas the low-malignant cells were much more resistant. The normal rabbit serum was not toxic to either cell line.

Separation of Cell Extracts by Affinity Chromatography

The immunofluorescence and cytotoxicity studies indicated a significant difference between the high- and low-metastatic cells in their ability to react with anti-laminin antibodies. The immunofluorescence studies also indicated a significant difference in the binding of GS I-B_4 lectin between these cell lines. In an effort to quantitate this difference, we labeled the high- and low-metastatic cells with ^{14}C-leucine, sonicated them, and separated the extracts on a GS I affinity column. The results are shown in Figure 3. It can be seen in the top panel that after application of the extract to the column, very little of the radioactive extract prepared from the low-metastatic cells was specifically eluted with the haptenic sugar (the arrow indicates when the hapten was added). In the

Table 1—Cytotoxicity of Rabbit Anti-Laminin Antiserum and Normal Rabbit Serum for Murine Tumor Lines*

	Percent killed (± SEM)†	
Cell line	Normal rabbit serum	Rabbit anti-laminin antiserum
High-metastatic line	7 ± 3	90 ± 8
Low-metastatic line	5 ± 3	22 ± 4

* The cytotoxicity assay was carried out in the presence of rabbit complement as described in Materials and Methods.

† The experiment was conducted twice; each experiment consisted of duplicate samples. The data shown is the average percent killed (± SEM) of the two experiments combined.

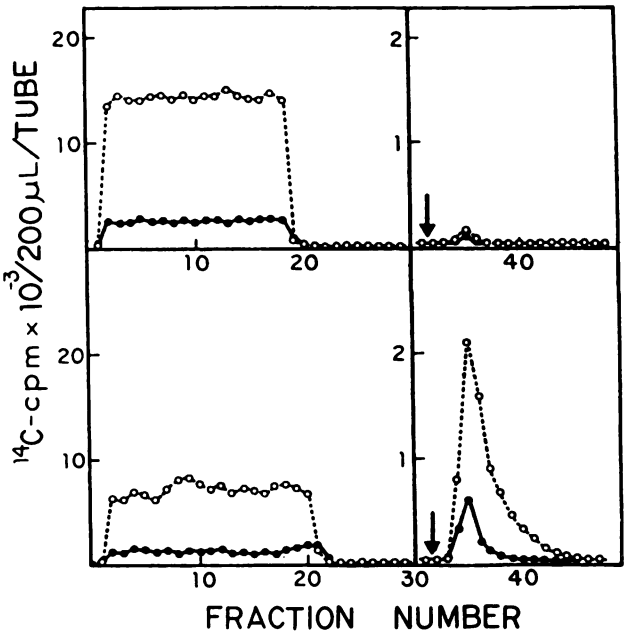

Figure 3—Separation of the high- and low-metastatic murine fibrosarcoma cell extracts on a GS I affinity column. The preparation of the affinity column and separation of the cell extracts was carried out as described in Materials and Methods. Shown is the elution profile of the low-metastatic cells (upper panel) and the high-metastatic cells (lower panel). The *arrow* indicates when the methyl α-*D*-galactopyranoside was added (extracts prepared by trypsinization, O——O; extracts prepared by scraping, ●——●).

bottom panel it can be seen that much more of the radioactive extract prepared from the high-metastatic cells was specifically released from the lectin column by the haptenic sugar. When the amount of material specifically released by the hapten was compared with the total amount added to the column, the value was between 20 and 50 times greater with the high-metastatic cells than with the low-metastatic cells.

Extracts prepared from nonlabeled high-metastatic cells were also separated on the affinity column. After the hapten-eluted material had been pooled and concentrated, it was divided into two portions. One portion was examined on a 5% SDS-polyacrylamide gel run under reducing conditions. Molecular weight standards run along with the sample included bovine serum albumin (~66,000 daltons), ovalbumin (~45,000 daltons), and rabbit muscle aldolase (~40,000 daltons). After electrophoresis and staining with Coomassie Brilliant Blue R-250, a single band with an apparent molecular weight of approximately 56,000 daltons was obtained from the high-metastatic cell extract. No other staining material was observed (Figure 4).

The second portion of the affinity-separated extract was used as the antigen in ELISA. The material was dried onto the wells of 96-well microtiter plates and the assay carried out as described in Materials and Methods. The rabbit anti-laminin antiserum was tested for reactivity against this material. Purified laminin from the EHS sarcoma was used as a control antigen, and normal rabbit serum was used as a control antibody preparation. In addition to these serums, we also used a second anti-laminin antibody prepared in sheep. The sheep anti-laminin was provided as a gift by Dr. L. Liotta, Laboratory of Pathophysiology, National Institutes of Health. The

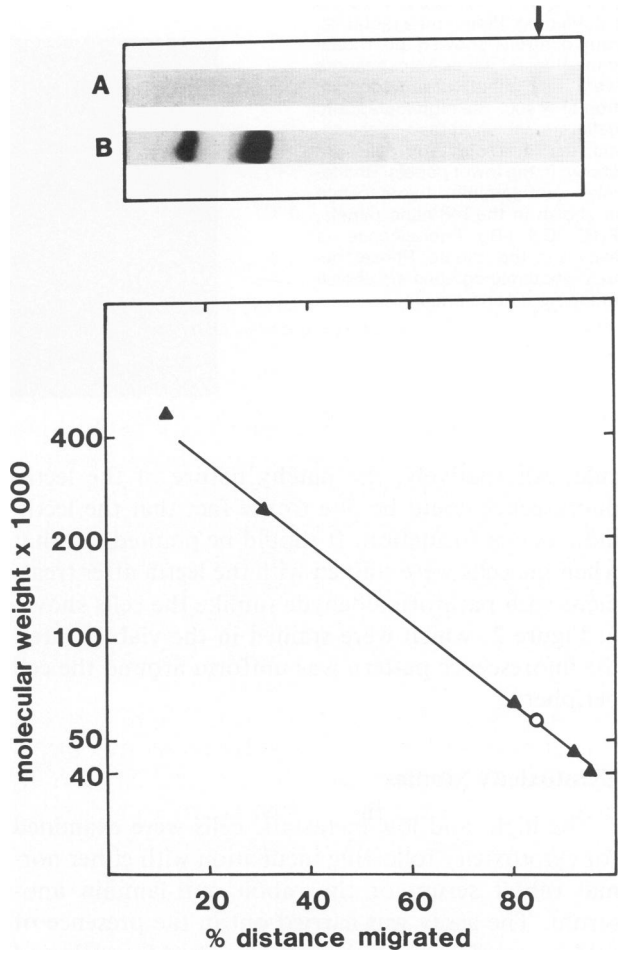

Figure 4—SDS-polyacrylamide gel electrophoresis of the affinity purified extract from the high metastatic cells. A 5% SDS-polyacrylamide gel was used, and the sample was electrophoresed in the presence of 2-mercaptoethanol. Molecular weight standards run along with the samples included bovine serum albuminin (~66,000 daltons), ovalbumin (~45,000 daltons), and rabbit muscle aldolase (~40,000 daltons). The gels were stained with Coomassie Brilliant Blue R-250. The electrophoretic pattern of the affinity-purified cell extract is shown in Channel A of the upper portion of the figure. A single Coomassie blue-staining band is seen (*arrow*). A laminin standard run under identical conditions is shown for comparison in Channel B. In the lower portion of this figure can be seen a plot of the molecular weight standards that we used to determine the molecular weight of the affinity-purified cell extract. The x-axis represents the distance migrated by each standard (as a percentage of the total gel length). The standards are represented by ▲. The affinity purified extract is indicated by O.

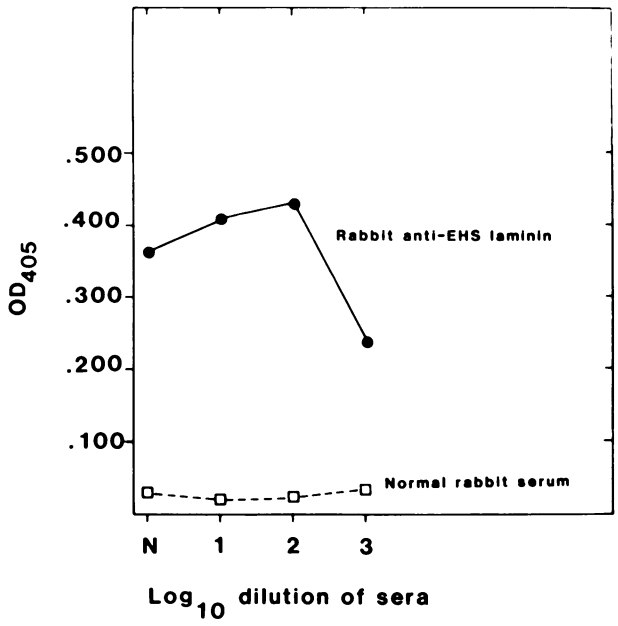

Figure 5—Reaction in ELISA of rabbit anti-laminin antiserum with the affinity-purified extract from the high-metastatic cells. Details of the procedures are described in Materials and Methods. The absorbance at 405 nm (OD_{405}) was measured after allowing the substrate to develop for 13 minutes.

ELISA results showed that the affinity-purified material from the high-metastatic cells did react with the anti-laminin antibodies. Significant reactivity with the rabbit anti-laminin antiserum was obtained at all concentrations between undilute and 1:1000 (Figure 5). When purified laminin was used as the antigen in ELISA, the same antiserum reacted at concentrations between undilute and 1:10,000. The sheep anti-laminin antiserum was examined againt the affinity-purified extract and was reactive at similar dilutions. In contrast, neither normal rabbit serum nor normal sheep serum reacted with either the affinity-purified material or the murine laminin at any concentration tested (undilute 1:10,000) (data not shown).

Discussion

Previous studies in our laboratory have led to the isolation and characterization of several tumor lines from a mouse fibrosarcoma that differ in their *in vivo* behavior (ie, tumorigenicity and metastatic activity)[1-3,18] Studies carried out over the past few years with prototype high and low-metastatic lines from this tumor have identified a number of functional properties that distinguish the lines.[1-8,10,18] For example, the high-metastatic cells are more adherent than the low-metastatic cells, as indicated by resistance to trypsin-mediated release from plastic culture dishes and monolayers of endothelial cells.[3-5,10,18] They are also more motile and chemotactic in response to the C5-derived tumor cell chemotactic factor.[1,2,67] Finally, the high-metastatic cells have lower cyclic adenosine monophosphate (cAMP) levels and are more sensitive to the inhibitory effects of dibutyryl cAMP.[8] The molecular basis for these differences is unknown. In the present study we demonstrate that the high-metastatic cells isolated from this murine tumor expressed a moiety that cross-reacts immunologically with laminin purified from the EHS (murine) sarcoma. This cross-reactive moiety could be detected by immunofluorescence on viable cells and by complement-mediated cytotoxicity with rabbit antiserum prepared against murine laminin. The high-metastatic cells also bind the GS I-B_4 lectin, as indicated by immunofluorescence. This lectin has been known for some time to react with basement membranes,[19] and very recent studies have shown that murine laminin binds this lectin.[20] It is likely that the anti-laminin antibodies and the GS I-B_4 lectin are recognizing the same substance on the high-metastatic cells, because when extracts of the high-metastatic cells are separated by affinity chromatography with GS I as the probe, the material specifically eluted by the haptenic sugar methyl-α-D-galactopyranoside reacts strongly with the anti-laminin antibodies by ELISA. In contrast to these results with the high-metastatic cells, no detectable reactivity between the low-metastatic cells and the anti-laminin antibodies or the GS I-B_4 lectin was observed by immunofluorescence. Furthermore, these cells were much more resistant to the cytotoxic effects of the anti-laminin antibodies than were the high-metastatic cells.

The nature of the laminin cross-reactive moiety on the high metastatic cells is not known at present. We examined the affinity-purified material on SDS-polyacrylamide gels and observed a single band with a molecular weight of approximately 56,000 daltons. There was no evidence of the high- molecular-weight bands (~200,000 and ~400,000 daltons) that characterize the electrophoretic pattern of laminin isolated from EHS tumor tissue.[11] There are a number of possibilities that could account for the differences in the electrophoretic patterns. Perhaps the 56,000-dalton substance is a cellular precurser of the laminin molecule. Alternatively, it may be a breakdown product of laminin. Laminin is known to be highly sensitive to proteolytic cleavage.[21] Further purification studies will have to be done before the relationship between laminin and the cross-reactive moiety is known.

A critical question to be answered regards the role of this moiety in the biologic behavior of the high-metastatic cells. Does the difference between the

high- and low-metastatic cells in the expression of this substance contribute to the *in vivo* and *in vitro* differences between these lines? While we cannot answer this question with certainty, we did observe a significant difference between the two lines in their ability to attach to Type IV collagen. The high-metastatic cells were found to attach to Type IV collagen in the absence of exogenous laminin, and the addition of laminin did not enhance the response. On the other hand, the low-metastatic cells did not readily attach to Type IV collagen in the absence of laminin but did when the collagen substrate was pretreated with laminin. These observations are compatible with the suggestion that the moiety on the high-metastatic cells which cross-reacts with murine laminin is serving a lamininlike function, ie, facilitating cell attachment to Type IV collagen. This, in turn, may contribute to the observed differences between the high- and low-metastatic cells in metastatic potential, since metastasizing tumor cells must adhere to and cross basement membranes during the process of secondary localization.

In summary, then, these observations show that high-metastatic cells derived from a murine fibrosarcoma express a moiety that reacts with anti-laminin antibodies and the GS I-B_4 lectin. Low-metastatic cells isolated from the same tumor do not express detectable quantities of this substance. Regardless of the nature of the moiety, the ability of these probes to distinguish the high- and low-metastatic tumor cells may be extremely important. If this finding turns out to be generally true, these probes may be very useful for identifying the most malignant cells in a heterogeneous tumor population. A recent report by Stanley and Chu[22] showed that FITC-GS I-B_4 bound to mouse L cells. A variant cell line was isolated from the parent L cells which did not bind to the lectin. Unlike the parent cell line, which formed tumors in mice, the lectin-negative line did not. Thus, in at least two unrelated models, the GS I-B_4 lectin was able to recognize the more tumorigenic or metastatic cell type. Further studies along this line are clearly warranted.

References

1. Varani J, Orr W, Ward PA: Comparison of subpopulations of tumor cells with altered migratory activity, attachment characteristics, enzyme levels and *in vivo* behavior. Eur J Cancer 1979, 15:585-592
2. Varani J, Orr W, Ward PA: Hydrolytic enzyme activities, migratory activity and *in vivo* growth and metastatic potential of recent tumor isolates. Cancer Res 1979, 39:2376-2380
3. Varani J, Lovett EJ, Elgebaly S, Lundy J, Ward PA: *In vitro* and *in vivo* adherence of tumor cell variants correlated with tumor formation. Am J Pathol 1980, 101:345-352
4. Varani J, Orr W, Ward PA: Adhesive characteristics of tumor cell variants of high and low tumorigenic potential. J Natl Cancer Inst 1980, 64:1173-1178
5. Varani J, Fantone JC: Phorbol myristate acetate-induced adherence of Walker 256 carcinosarcoma cells. Cancer Res 1982, 42:190-197
6. Orr FW, Varani J, Delikatny J, Jain N, Ward PA: Comparison of chemotactic responsiveness of two fibrosarcoma subpopulations of differing malignancy. Am J Pathol 1981, 102:160-167
7. Wass JA, Varani J, Piontek GE, Ward PA, Orr FW: Responses of normal and malignant cells to collagen, collagen-derived peptides and the C5-related tumor cell chemotactic peptide. Cell Differ 1981, 10:329-332
8. Elgebaly S, Kunkel S, Lovett EJ, Varani J: cAMP differences between clones of high and low malignant fibrosarcoma cells. Oncology 1982, 39:163-166
9. Terranova VP, Liotta LA, Russo RG, Martin GR: Role of laminin in the attachment and metastasis of murine tumor cells. Cancer Res 1982, 42:2265-2269
10. Varani J, Lovett EJ: Phenotypic stability of tumor cells *in vitro* and *in vivo*. J Natl Cancer Inst 1982, 68:957-962
11. Timpl R, Rohde H, Robey PG, Rennard SI, Foidart JM, Martin GR: Laminin—a glycoprotein from basement membranes. J Biol Chem 1979, 254:9933-9937
12. Hayes CE, Goldstein IJ: An α-D-galactosyl-binding lectin from Bandeiraea simplicifolia seeds. J Biol Chem 1974, 249:1904-1914
13. Johnson GD, Holborow EJ, Dorling J: Immunofluorescence and immunoenzyme techniques, Handbook of Experimental Immunology. Edited by D M Weir. Oxford, Blackwell Scientific Publications, 1978, pp 15.1-15.21
14. Michaelson JH, McCoy JP, Bigazzi PE: Antibodies to glomerular basement membrane: Detection by an enzyme-linked immunosorbent assay in the sera of Brown Norway rats. Kidney Int 1981, 20:285-288
15. Klebe RJ: Isolation of a collagen-dependent cell attachment factor. Nature 1974, 250:248-251
16. Timpl R, Martin GR, Bruckner P: Structure of basement membrane collagen obtained from a mouse tumor. Front Matrix Biol 1979, 7:130-141
17. Terranova VP, Rohrbach DH, Martin GR: Role of laminin in the attachment of PAM 212 (epithelial cells) to basement membrane collagen. Cell 1980, 22:719-726
18. Varani J, Lovett EJ, Elgebaly S, Lundy J: Characteristics of tumor cells with varying malignant potential, Metastasis: Clinical and Experimental Aspects. Edited by K Hellman, P Hillard, S Eccles. The Hague, Martinus Nijhoff, 1980, pp 189-193
19. Peters BP, Goldstein IJ: The use of fluorescein-conjugated *Bandeiraea simplicifolia* B_4-isolectin as a histochemical reagent for the detection of α-D-galactopyranosyl groups: Their occurrence in basement membranes. Exp Cell Res 1979, 120:321-334
20. Shibata S, Peters BP, Roberts DD, Goldstein IJ, Liotta LA: Isolation of laminin by affinity chromatography on immobilized *Griffonia simplicifolia* I lectin. FEBS Lett 1982, 142:195-198
21. Liotta LA, Goldfarb RH, Brundage R, Siegal GP, Terranova V, Garbisa S: Effect of plasminogen activator (urokinase), plasmin, and thrombin on glycoprotein and collagenous components of basement membrane. Cancer Res 1981, 41:4629-4636
22. Stanley WS, Chu EHY: BS I-B_4 isolectin as a probe for an investigation of membrane alterations and transformation phenotypes of mouse L cells. J Cell Sci 1981, 50:79-88