Laminin and Fibronectin Promote the Haptotactic Migration of B16 Mouse Melanoma Cells In Vitro

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ABSTRACT The migration of tumor cells through basement membranes and extracellular matrices is an integral component of tumor invasion and metastasis. Laminin and fibronectin are two basement membrane- and extracellular matrix-associated noncollagenous glycoproteins that have been shown to promote both cell adhesion and motility. Purified preparations of laminin and fibronectin stimulated the directed migration of B16 murine metastatic melanoma cells in vitro as assessed in modified Boyden chambers. The stimulation of migration occurred over a concentration range of 1–100 μg/ml of laminin or fibronectin, with a peak response occurring between 12.5 and 25 μg/ml. The maximal response of these cells was 80–120-fold higher than control migration. Affinity-purified antibody preparations specifically abrogated the migration of these cells in response to the respective proteins. Tumor cells in suspension were preincubated in physiologic levels of plasma fibronectin prior to assay to partially mimic what occurs when a metastasizing cell is in the blood stream. This preincubation with plasma fibronectin had no effect on the subsequent migration of cells in response to either laminin or fibronectin. Furthermore, experiments using filters precoated with fibronectin or laminin indicated that these cells could migrate by haptotaxis to these two proteins. We conclude that tumor cell migration in response to such noncollagenous adhesive glycoproteins could be an important aspect in the invasion and metastasis of certain malignant cell types.

Metastasis is a process that is composed of a number of interrelated events that culminate in the successful translocation and growth of tumor cells within the host (1, 2). These events include penetration and entry into the vascular or lymphatic circulation from the primary site of tumor growth, followed by attachment to endothelium or subendothelial components, extravasation, and proliferation of cells at apparently selective sites that are distant from the original tumor site. The similarity between tumor metastasis and inflammation has been alluded to previously (3). Among a number of processes the two have in common is that both involve the recruitment and subsequent migration of cells through basement membrane and interstitial matrices. Numerous factors have been described that stimulate chemotaxis, the directed migration in response to concentration gradients of attractant, of inflammatory cells in vitro (4, 5). Several studies have also reported the existence of specific chemoattractants for tumor cells. These attractants include a proteolytic fragment of the complement fragment C5a (6), collagen and related peptides (7), and factors derived from resorbing bone (8) or tumor tissues (9). Injection of the complement-derived tumor cell attractant (10) as well as a tumor-derived attractant (11) have been reported to influence the formation of experimental metastases at the site of injection. These findings imply a role for tumor chemoattractants in the "recruitment" of metastasizing cells to sites distant from the primary tumor mass.

Cell adhesion proteins play an important role in the phenotypic behavior of diverse cell types ranging from normal to highly metastatic cells (12, 13). Fibronectin and laminin are two of the best characterized cell adhesion proteins. Both proteins apparently have discrete regions or domains that serve unique functions, such as binding to specific collagens and proteoglycans, as well as to cell surfaces (12, 13). Initially, it was believed that epithelial cells attach specifically to laminin and that mesenchymal cells utilize fibronectin for adhesion (14). However, recent evidence indicates that both cell types can synthesize and utilize either fibronectin or laminin for attachment (15, 16). One important function of fibronectin involves the ability to direct cell movement. Studies have shown that fibronectin will promote the directed movement in vitro of various cells, including fibroblasts (17) and neural crest cells (18). This activity may be important in embryolog-

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ical development or during inflammation and wound healing. For example, fibronectin is observed underneath the closing edge of epidermal wounds (19, 20) and fibronectin-coated coverslips have been recently reported to promote epithelial cell migration in healing wounds on newt limbs (21). Additionally, the incorporation of fibronectin into collagen gels has been shown to promote the invasion of metastatic melanoma cells into those gels (22). Less is known about the potential for laminin to stimulate cell movement, though recently laminin was shown to stimulate migration of Schwann cell-derived tumor cells and to promote outgrowth of embryonic chick and human fetal neurites (23–25).

Recent reports have indicated that laminin may be involved in the formation of tumor metastases. It has been demonstrated that cells that have been selected for attachment to laminin have enhanced metastatic potential (26). Also, laminin and laminin fragments can be located to the surface of certain metastatic tumor cells (27). It has been shown that laminin will bind to a specific receptor on tumor cell surfaces (28, 29).

The present study was performed to examine the role of laminin and fibronectin in promoting the migration of metastatic melanoma cells in vitro. The data demonstrate that laminin and fibronectin promote highly significant migration of B16 melanoma cells, and that this migration has both accelerated random and directional components. In addition, filters precoated with either attractant supported significant levels of tumor cell migration. Thus, these tumor cells can apparently migrate by haptotaxis in response to bound attractant proteins (23). These results indicate a likely role for interstitial and basement membrane noncollagenous adhesive glycoproteins in directly promoting the invasion of certain metastatic cell types in vivo.

MATERIALS AND METHODS

Cells: Murine melanomas $B16F_{10}$ and $B16F_1$ were provided by Drs. I. J. Fidler and I. R. Hart, Fredrick Cancer Research Center. Cells were maintained in vitro in Dulbecco's modified Eagle's medium (DME)[1] containing 5% fetal calf serum (heat inactivated; Gibo Laboratories, Inc., NY).

Migration Assay: The conditions used for the microchamber migration assay were similar to those described previously (23). Briefly, log-phase cultures were trypsinized, washed, and resuspended to a final concentration of 4×10^5/ml in DME with 0.015 M HEPES buffer, pH 7.2. Attractants were also diluted in this medium. The filter type used was a polyvinylpyrrolidone-free polycarbonate filter with 8.0-μm pore size. Incubation was for 4 h at 37°C, in a humid 5% CO_2 atmosphere.

Proteins and Antibodies: Fibronectin was purified from human plasma by gelatin affinity chromatography as previously described (30). Laminin was purified from the mouse EHS tumor by neutral salt extraction as described (31). The purity of both proteins was assessed by SDS-PAGE and enzyme-linked immunoadsorbant assay using affinity-purified antibodies. The concentration of both proteins was estimated by using previously determined weight extinction coefficients (23, 32).

Antibodies were generated against both proteins in New Zealand white rabbits as described previously (31). Antibodies were purified by affinity chromatography of appropriate antisera on the respective ligand immobilized to Affi-gel 15 (Bio-Rad Laboratories, Richmond, CA). Reactivity of antisera was verified using an enzyme-linked immunoadsorbant assay.

Migration of Melanoma Cells on Attractant-precoated Filters: Filters were precoated with the indicated concentrations of either fibronectin or laminin as previously described (22). Low ionic strength (0.05 M) carbonate buffer, pH 9.6, was used to precoate protein onto polyvinylpyrrolidone-free polycarbonate filters. Filters were coated for 14 h at 37°C in a humid, 5% CO_2 atmosphere. Filters were then washed extensively (four to five times) in PBS, once in water, and air dried before use in the migration assay. Control filters were treated similarly in carbonate buffer without attractant. Filters to be coated on both surfaces for the experiments were submerged in the solution for coating. Filters were also coated on only the lower side by floating the filter on the surface of the coating solution.

Diffusion of Attractant across Filter: Studies were performed to evaluate the diffusion of laminin across the filter in these assays. Laminin was tritium labeled by reductive methylation as described (33). Radioactive laminin was added at a concentration of 30 or 120 μg/ml to the lower wells of the migration chamber. Chambers were assembled and medium was dispensed into the upper wells. Samples were removed from the upper wells periodically during incubation at 37°C in a humidified atmosphere. Radioactivity diffusing to the upper well was solubilized in Aquasol II (New England Nuclear, Boston, MA) and counted in a Beckman LS 230 liquid scintillation counter (Beckman Instruments, Inc., Palo Alto, CA).

RESULTS

Migration of Melanomas

The data in Fig. 1 depict migration of the tumor cells to increasing concentrations of fibronectin and laminin. Fig. 1*A* shows that the metastatic murine melanomas $B16F_1$ and $B16F_{10}$ cells migrate in response to increasing concentrations of purified fibronectin. The increase in cellular migration occurs within a range of from 1.5 to 12.5 μg/ml of fibronectin, and plateaus at protein concentrations higher than this. The maximum level of migration to fibronectin was 80–95-fold greater than control migration for both cell types. A highly significant concentration-dependent increase in cell migration of $B16F_1$ and $B16F_{10}$ to laminin was also observed (Fig. 1*B*). Maximum migration of these cells occurred in response to 12.5 μg/ml of laminin. The highest level of laminin tested (200 μg/ml) led to lower B16 migration compared with the peak response, a phenomenon that occurs for other cells and chemoattractants. Neither cell type responded to BSA at any concentration tested (1.5–100 μg/ml) (data not shown).

Effect of Antibody on Melanoma Migration

The addition of affinity-purified antibody to fibronectin or laminin (Fig. 2) specifically inhibited melanoma migration in response to the respective attractants. Thus, the migration of B16 melanomas in response to low levels of laminin (3.1 μg/ml) was completely inhibited by the addition of 8 μg/ml affinity-purified antilaminin antibody (Fig. 2*A*). In contrast, the addition of 25 μg/ml affinity-purified antifibronectin had no effect on laminin-stimulated movement. A similar specific inhibition was observed for antifibronectin when fibronectin was used as the attractant with no effect of antilaminin in these circumstances (Fig. 2*B*).

Checkerboard Analyses

Checkerboard analyses (34) were performed to study the nature of cell migration in response to laminin and fibronectin (Fig. 3). The assay is constructed by examining the migration of cells in the presence of increasing levels of attractant both above and/or below the filter. A comparison of cell migration levels toward a positive gradient of fibronectin or laminin (Fig. 3, *A* and *B*, below the diagonal) with movement observed in a reversed gradient, when more attractant is on the near side of the filter (Fig. 3, *A* and *B*, above the diagonal) demonstrated that migration in response to both attractants was directional in nature. Additionally, random migration, or the migration in the absence of an established gradient, was increased in response to both proteins (Fig. 3, *A* and *B*, along

[1] *Abbreviation used in this paper:* DME, Dulbecco's modified Eagle's medium.

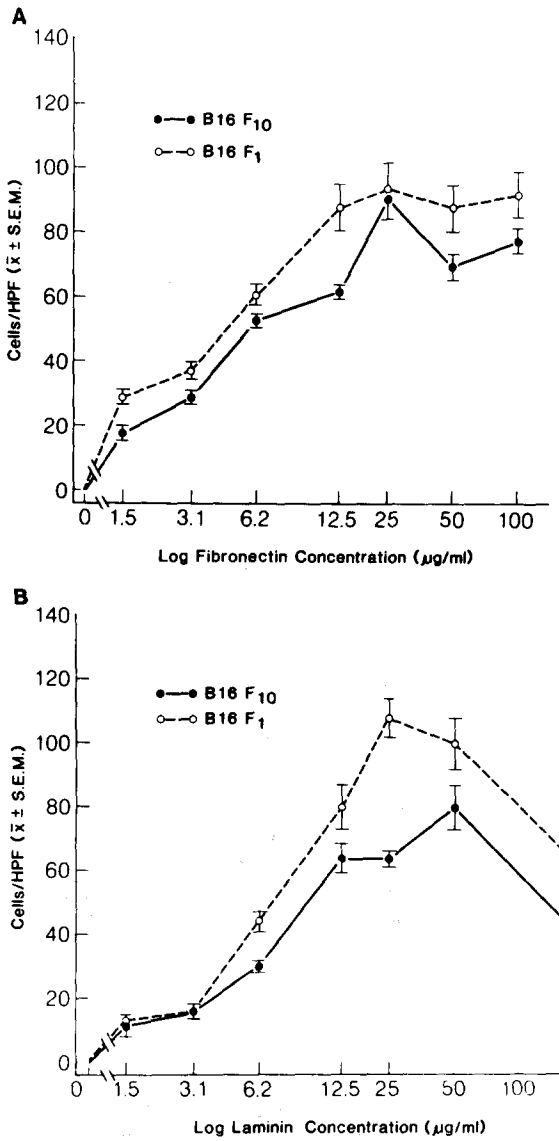

FIGURE 1 Stimulation of B16 melanoma migration by fibronectin and laminin. Indicated dilutions of either fibronectin (*A*) or laminin (*B*) were dispensed in 25-μl volumes into the blind well portion of microchambers. $B16F_1$ (O- - -O) or $B16F_{10}$ (●——●) cells were added to the upper well and chambers were incubated for 4 h at 37°C. Migrated cells were quantitated in 20 randomly selected high power fields (*HPF*, × 400 magnification). Data represent the mean of triplicate determinations plus or minus the standard error of the mean ($\bar{x}$ ± SEM).

the diagonal). Similar results for the checkerboard were observed using $B16F_1$ cells (not shown).

Preincubation of Melanoma Cells with Plasma Fibronectin

Since metastasizing cells in the blood stream come into contact with plasma fibronectin, experiments were performed to more closely parallel this circumstance. Suspensions of $B16\text{-}F_{10}$ melanoma cells were adjusted to a final concentration of 5×10^5/ml in DME/HEPES containing 2 mg/ml BSA. Cells were preincubated for 1 h at 37°C in the presence or absence or plasma levels (250 μg/ml) of fibronectin and washed twice to remove excess fibronectin. Studies with other chemoattractants have shown that preincubation may desensitize cells to further stimulation immediately after this incubation (35, 36). This preincubation step had no effect on the subsequent migration of cells towards laminin or fibronectin (Table I). This suggested that the fibronectin present in this soluble form did not interact with cells.

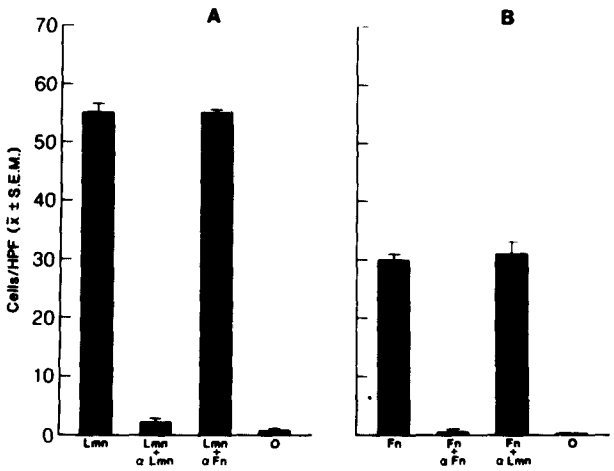

FIGURE 2 Effect of specific antibody on fibronectin- and laminin-stimulated $B16F_{10}$ migration. Dilutions of (*A*) laminin (3.1 μg/ml) or (*B*) fibronectin (3.1 μg/ml) were added to the lower well in the presence or absence of purified antilaminin (*αLMN*, 8.5 μg/ml) or antifibronectin (*αFN*, 25 μg/ml) antibodies. After incubation, migrated cells were fixed, stained, and quantitated on the lower filter surface. The data shown represent the mean number of migrated cells per high power field (*HPF*, × 400) plus or minus the standard error of the mean ($\bar{x}$ ± SEM).

Responsiveness of Melanoma Cells on Precoated Filters

Previous work has shown that a rat Schwann cell tumor line migrated over substratum-bound laminin in the absence of additional soluble attractant (23). The migration of cells due to substratum-bound attractant, termed haptotaxis, was more pronounced in the presence of a density gradient of substratum-bound laminin than in the absence of such a gradient (23). It was therefore of interest to determine if metastatic melanoma cells could respond similarly on precoated filters in the absence of additional soluble attractant. The data in Table II illustrate the results of this type of experiment. Low level random migration of these cells is observed on filters precoated on both sides in the absence of additional soluble attractant. Importantly, more cells accumulate on the lower surface when it is the only surface coated with the same concentrations of either fibronectin (a threefold difference) or laminin (a 15-fold difference), compared with "random" migration levels when both sides are uniformly coated with attractant proteins.

Effect of Soluble Attractant on Tumor Cell Migration over Attractant-precoated Filters

Preincubation studies suggested that fluid-phase fibronectin was not interacting with cells to promote migration in this system but instead was stimulating cells by first depositing on the filter surface. The precoating experiments further indicated that substratum-bound fibronectin (and laminin) could promote haptotaxis of these metastatic melanoma cells. To further elucidate the relationship between bound and soluble attractant, we next investigated melanoma migration using

A

Fibronectin Concentration (μg/ml)

Fibronectin Concentration (μg/ml) near / for	0	6.2	12.5	25	50	100
0	**0.4**	**0.4**	**0**	**2.8**	**0.2**	**7.4**
6.2	**36.9**	**28.2**	4.6	7.4	1.0	10.6
12.5	**53.2**	35.6	**14.0**	38.1	11.2	13.0
25	**44.0**	34.8	27.2	**19.2**	27.4	16.4
50	**32.8**	22.0	23.0	21.6	**19.8**	23.8
100	**39.2**	25.8	27.3	28.6	17.0	**20.7**

B16 (F_{10}) Melanoma Checkerboard

B

Laminin Concentration (μg/ml)

Laminin Concentration (μg/ml) near / for	0	6.2	12.5	25	50	200
0	**0**	**1.0**	**6.0**	**5.8**	**9.2**	**4.6**
6.2	**59.4**	**19.4**	14.4	11.2	9.8	4.8
12.5	**69.8**	30.2	**20.0**	14.6	12.6	3.4
25	**67.2**	27.3	26.8	**25.0**	16.6	4.8
50	**68.6**	37.2	45.0	16.0	**20.0**	4.0
200	**40.2**	34.4	34.4	25.0	12.8	**4.4**

B16 (F_{10}) Melanoma Checkerboard

FIGURE 3 Checkerboard analysis of $B16F_{10}$ response to fibronectin and laminin. Dilutions of either fibronectin (*A*) or laminin (*B*) were made as indicated in italics, and 25 μl of each concentration was dispensed into the blindwell portion of each chamber. Filters were overlayed and 50 μl of a cell suspension (4×10^5 cells/ml) containing the indicated attractant concentrations were dispensed into the upper wells. After incubation, filters were removed, fixed, and processed. Cells in 20 randomly selected high power fields were quantitated and the number shown indicates the mean of triplicate determinations of cells per high power field. Vertical boldfaced data indicate the migration observed to a maximal positive gradient. Horizontal boldfaced data represent migration observed to the maximum reversed gradient. Data along the diagonal represent accelerated random migration that occurs in the absence of an established gradient.

precoated filters in the presence or absence of additional soluble attractant. High levels of attractant (50 μg/ml of fibronectin and 100 μg/ml of laminin) were used to precoat filters on both the upper and lower surfaces. These concentrations of protein were chosen to saturate filter surfaces with bound attractant prior to use in the assay. The migration of tumor cells in response to additional soluble attractant on such uniformly coated filters was compared with migration levels observed on noncoated filters. The data in Fig. 4 illustrate the result of such experiments.

Melanoma cells were observed to migrate in a concentration-dependent manner, in response to increasing levels of soluble fibronectin using uncoated filters (Fig. 4*A*). In contrast, precoating the filter with fibronectin on both sides before the assay abrogated the response of these cells to soluble fibronectin in the lower well. We considered the possibility that this inhibition of the migration response to soluble fibronectin was due to the presence of potentially "deactivating" levels of fibronectin on the filter surface, which were created by the necessity of using high levels of fibronectin during coating to saturate the filter. However, this concentration of fibronectin when applied only to the lower surface of the filter, enhanced migration levels significantly compared with control (both sides coated, no soluble fibronectin), indicating that cells could effectively migrate over this density level of bound fibronectin.

The response of metastatic cells to challenge with soluble laminin on laminin-coated filters (Fig. 4*B*) differed from that of fibronectin in one respect: the addition of soluble laminin to the lower wells in chambers containing coated laminin filters did cause enhanced melanoma migration compared with the migration on coated filters in the absence of such soluble attractant. However, the increased response to soluble laminin on laminin-coated filters was less than that observed when noncoated filters were used. Again, results using filters on which only the lower surface was laminin coated indicated that cells could migrate effectively on this density level of

TABLE I

Lack of Effect of Preincubation of $B16_{10}$ Cells with Plasma Fibronectin on Subsequent Cellular Migration

	Preincubation	
Challenge	Medium	Fibronectin
μg/ml		
Laminin		
60	29.2	31.0
15	72.0	79.8
3.8	46.4	51.4
0.9	17.6	8.4
Fibronectin		
30	20.2	19.0
7.5	58.2	46.2
1.9	33.1	19.0
Medium	0	0

Cells were adjusted to a final concentration of 5×10^5/ml in DME with 2 mg/ml BSA. The suspensions were incubated for 1 h at 37°C in the presence or absence of plasma fibronectin (250 μg/ml). The cells were washed three times to remove excess plasma fibronectin and were then tested for responsiveness to laminin or fibronectin in the Boyden chambers. Data represent the mean number of migrated cells per high power (× 400) field. SEM <10% of the mean. Determinations were in triplicate.

TABLE II

Migration of B-16 Melanoma Cells on Precoated Filters

Protein coated	Surface coated	Migrated cells per HPF
μg/ml		
Fibronectin		
100	Both	11.4 ± 2.9
100	Distal	31.4 ± 4.4
Laminin		
10	Both	2.4 ± 1.0
10	Distal	30.0 ± 2.4
No coating		0.4 ± 0.2

Filters were precoated on both or only the lower (distal) side with either 100 μg/ml fibronectin or 10 μg/ml laminin. After washing and air drying, filters were used in the migration assay in the absence of further soluble attractant. Data represent the mean number of migrated cells per high power (× 400) field (*HPF*) ±SEM. Determinations were in triplicate.

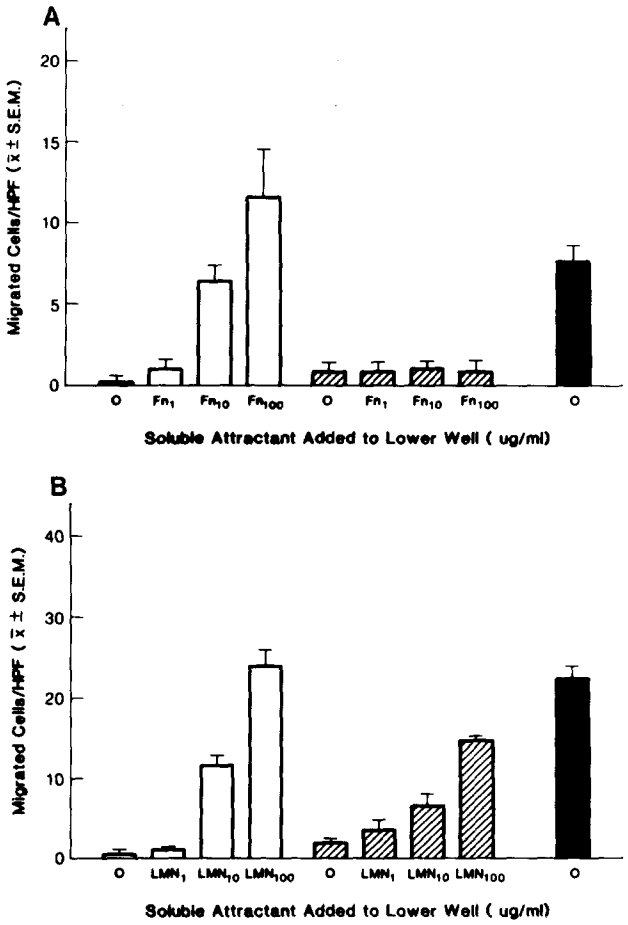

FIGURE 4 Migration of melanoma cells on attractant-precoated filters in the presence of gradients of additional soluble attractant. Filters were precoated on both sides as described with high concentrations of fibronectin (*A*) or laminin (*B*). Migration in response to positive gradients of fibronectin (*A*) or laminin (*B*) was then examined on attractant-precoated filters (hatched bars) or noncoated filters (open bars). Additionally, migration was assayed on filters precoated on the distal side only in the absence of additional soluble attractant (solid bar). Data represent the mean number of triplicate determinations of migrated cells per high power field plus or minus SEM.

laminin. Thus, we concluded that the partial inhibition of tumor cell movement toward soluble attractant on precoated filters compared with noncoated counterparts was probably not due to deactivating levels of laminin on the surface.

Diffusion of Laminin through Filter

Experiments were performed using [^{3}H]laminin labeled by reductive methylation (33) to determine if the filters used for migration maintain a concentration gradient of this attractant. These results, shown in Fig. 5, demonstrate that attractant added to the lower well begins to diffuse to the upper well within 30 min of incubation. Diffusion of the upper compartment increased until 2 h following the start of incubation, after which time a plateau appears. Even after 3 h of incubation, only 10% of the available [^{3}H]laminin was detected in the upper well.

DISCUSSION

These results indicate the potential for a direct role for fibronectin and laminin in tumor cell motility and invasion.

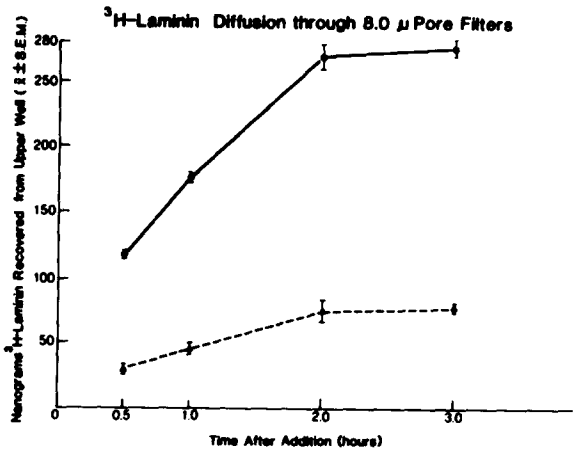

FIGURE 5 Diffusion of [^{3}H]laminin through 8.0-μm pore size filters. Aliquots of 25 μl of [^{3}H]laminin at 120 μg/ml (solid line) or 30 μg/ml (dashed line) were added to the lower well of a microchamber. The chamber was assembled with an 8.0-μm polycarbonate filter and medium was added to the upper well. Samples were removed from the upper well at the times indicated and analyzed for the presence of radioactivity. Data represent the mean of duplicate determinations plus or minus the standard error of the mean ($\bar{x}$ ± SEM).

Highly significant migration of melanoma cells in response to laminin and fibronectin was observed. The response was clearly due to specific protein effects, since the addition of BSA to the lower wells did not stimulate tumor cell migration. Both proteins stimulated maximal migration at concentrations between 12.5 and 25 μg/ml. Migration of tumor cells to laminin or fibronectin could be inhibited by the addition of the appropriate affinity-purified antibody.

Checkerboard analyses were performed to determine whether or not the migration was directional. These results indicate that maximal migration of these cells in response to laminin and fibronectin occurred in response to positive gradients of attractant (directed migration). Significant migration also occurred in response to challenge with increasing levels of attractant added simultaneously on both sides of the filter (increased random migration). Thus, migration of these metastatic melanoma cells to both attractants was due to accelerated random as well as increased directional components, as judged by this method.

The results of previous work in our laboratory on laminin-stimulated movement of a Schwann cell tumor line indicated that migration of these cells occurred primarily in response to substratum-bound attractant. This conclusion was supported by the observation that the filters bound laminin under the assay conditions used, and that filters precoated with laminin could support cell migration in the absence of additional soluble laminin. This type of migration due to substratum-bound laminin was operationally termed haptotaxis (based on Carter, reference 39) to distinguish it from chemotaxis, which involves the directed migration of cells in response to soluble concentration gradients of attractant. The distinction is important, since chemotactic mediators would be predicted to be operative over longer distances and as such may be important in the margination and active recruitment of metastasizing tumor cells at distant sites (10, 11). In contrast, haptotactic migration would be more involved with insolubilized constituents of the matrix or basement membrane directly promoting the invasion of metastatic tumor cells. Haptotaxis would be important for extravasation once the

tumor cell had come into contact with matrices following endothelial cell retraction or in regions of exposed basement membranes.

The results in this study indicate that metastatic melanoma cells can migrate in a haptotactic manner to both insolubilized fibronectin and laminin. This conclusion is supported by results obtained from measuring migration levels on attractant-precoated filters in the absence of a soluble stimulus. Precoating of only the lower side of the filter with either attractant promoted more cellular migration to the lower surface than that observed using filters coated on both surfaces with the same concentration of attractant. This finding was similar to that observed for the haptotactic migration of Schwannoma cells on laminin-precoated filters (23).

Metastasizing tumor cells in the blood stream would come into contact with high levels of soluble plasma fibronectin. It was therefore important to analyze the effect of preincubation with plasma levels of fibronectin on melanoma cell migration in response to fibronectin and laminin. It was necessary to wash away excess soluble fibronectin prior to the assay, in this case to eliminate the potential influence on cell migration created by binding of this level of excess soluble fibronectin onto the upper filter surface (see below). The results indicated that preincubation of melanoma cells with physiologic levels of plasma fibronectin had no appreciable effect on subsequent laminin- or fibronectin-promoted movement of these cells. This indicates that soluble plasma fibronectin, encountered by hematogenously metastasizing tumor cells, would likely not inhibit laminin-mediated extravasation. However, if fibronectin were deposited on a surface, or occurred naturally in a "solid phase," such as in basement membranes or within connective tissues, then it could effectively mediate tumor cell migration. The observation is consistent with previous reports that have demonstrated that plasma fibronectin in solution does not interact well with vertebrate cells in suspension (37, 38). Furthermore, it is suggestive that fibronectin-mediated melanoma migration in this system may be totally in response to substratum-bound attractant.

The preincubation experiments with plasma fibronectin suggested that cells in suspension did not bind soluble plasma fibronectin. It was not clear if this represented a property of the suspended cell or instead was due to differences (e.g., conformational) between bound and soluble fibronectin (40, 41). Thus, it was of interest to examine migration of melanoma cells on fibronectin-precoated filters in the presence of soluble fibronectin. These results indicated that cells promoted to adhere to a fibronectin-precoated surface did not respond to challenge with increasing levels of soluble fibronectin in the lower well. Furthermore, the high levels of melanoma cell migration, observed on filters coated on the lower surface only, indicated that precoated filter–mediated inhibition of the response to soluble fibronectin was not due to "deactivation" of the tumor cells. We therefore concluded that the interaction and subsequent migration of the tumor cells to fibronectin in this system is a result of deposition of density gradients of attractant on the filter surface, and as such represents a haptotactic response (22). It is quite possible that the haptotactic requirement for this migration is determined by conformational alterations of fibronectin that occur upon binding of the protein to the filter, thus allowing it to interact with the cell surface. This conformational alteration has previously been determined to regulate the cell attachment properties of fibronectin in promoting the cell attachment to collagen-coated (40) as well as to synthetic surfaces (41). These conclusions contrast with those of Seppä et al. (42) in which they indicated that a chymotryptic cell-binding fragment of fibronectin stimulated the chemotactic response of fibroblasts. These differences may indicate a difference in the nature of the migratory response to various cell types to fibronectin, or may reflect an altered configuration of the cell-binding fragment relative to intact fibronectin. Further work using proteolytic fragments in our system is necessary to distinguish between these two possibilities for fibronectin-induced tumor cell movement.

The same type of experiment using laminin-coated filters and soluble attractant (laminin) is more difficult to interpret. Results using filters coated on both sides with attractant indicate that B16 melanoma cells are partially responsive to challenge with soluble laminin in the presence of a high level of bound laminin. This partial responsiveness could be due to additional binding of laminin to the lower filter surface in the presence of excess soluble laminin, contributing to the formation of increased density gradients on the substratum. Alternatively, the effect of soluble laminin on melanoma migration may reflect both binding of soluble laminin to membrane receptors as well as substratum-mediated interactions of bound laminin with the cell surface. Experiments using [^{3}H]laminin indicated that a soluble concentration gradient was established and maintained by these filters for long periods of time. Only 10% of the available radioactive laminin diffused to the upper well after 3 h of incubation even with the highest concentration of laminin tested. Thus, it was concluded that cells are in the presence of a relatively steep (10-fold) concentration gradient of soluble laminin during the assay. At least one receptor for soluble laminin has been described for a number of tumor cell types (28, 29) and recently isolated (29, 43). Interestingly, the work of Brown et al. (44) would indicate that this receptor moiety, which they have termed connectin, can interact with actin filaments and cause bundling of these filaments. This observation allows the speculation that this cell surface receptor for soluble laminin is directly involved with stimulating the motility of the metastatic melanoma cells in the current study. Clearly, further work using different assay systems to assess tumor cell migration in the presence of bound and soluble attractants is necessary to accurately relate the nature of the cellular interactions of the matrix proteins with the subsequent attachment and motility responses of metastatic tumor cells.

It is clear that invasion of tissues by aggressive tumor cells must also be accompanied by the localized dissolution of matrices. Differences in the production and release of proteolytic enzymes that have been reported for cell types of varying metastatic potential are likely important in this regard (45–47). Of particular interest is a recent report indicating that migrating endothelial cells digest substratum-bound types IV and V collagen (48). An interruption of matrix-mediated tumor cell migration and associated enzymatic functions would greatly reduce, if not eliminate, the metastatic potential of a tumor population. A complete understanding of the involvement of extracellular matrix adhesive glycoproteins such as laminin and fibronectin in this regard may provide useful tools to ultimately control cancer metastasis, the major cause of death in cancer patients.

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Note Added in Proof: A recent study by Lacovara et al. (J. Lacovara, E. B. Cramer, and J. P. Quigley, *Cancer Res.*, 1984, in press) has reported similar conclusions concerning the haptotactic nature of fibronectin-mediated B16 melanoma migration through nitrocellulose filters in Boyden-type chambers (J. P. Quigley, personal communication).

REFERENCES

1. Poste, G., and I. J. Fidler. 1980. The pathogenesis of cancer metastasis. *Nature (Lond.)* 283:139–146.
2. Fidler, I. J., and I. R. Hart. 1982. Biological diversity in metastatic neoplasms: origins and implications. *Science (Wash. DC).* 217:998–1003.
3. Orr, F. W., J. Varani, J. Delikatny, N. Jain, and P. A. Ward. 1981. Comparison of the chematactic responsiveness of two fibrosarcoma subpopulations of differing malignancy. *Am. J. Pathol.* 102:160–167.
4. Schiffmann, E., and J. I. Gallin. 1979. Biochemistry of phagocyte chemotaxis. *Curr. Top. Cell. Regul.* 15:203–261.
5. Snyderman, R., and E. J. Goetzl. 1981. Molecular and cellular mechanism of leukocyte chemotaxis. *Science (Wash. DC).* 213:830–837.
6. Romualdez, A. G., and P. A. Ward. 1975. A unique complement derived chemotactic factor for tumor cells. *Proc. Natl. Acad. Sci. USA.* 72:4128–4132.
7. Mundy, G. R., S. DeMartino, and D. W. Rowe. 1981. Collagen and collagen-derived fragments are chemotactic for tumor cells. *J. Clin. Invest.* 68:1102–1105.
8. Orr, W., J. Varani, M. D. Gondek, P. A. Ward, and G. R. Mundy. 1979. Chemotactic responses of tumor cells to products of resorbing bone. *Science (Wash. DC).* 203:176–179.
9. Oshijama, K., H. Nishi, A. Ishikura, and H. Hayashi. 1976. Characterization of two different factors chemotactic for cancer cells from tumor tissue. *Vicrhows Arch. B Cell Pathol.* 21:119–131.
10. Lam, W. C., E. J. Delikatny, F. W. Orr, J. Wass, J. Varani, and P. A. Ward. 1981. The chemotactic response of tumor cells. *Am. J. Pathol.* 104:69–76.
11. Osaki, T., K. Yoshida, K. Oshijsma, and H. Hayashi. 1971. Studies on the mechanisms of invasion in cancer. II. In vivo effects of a factor chemotactic for cancer cells. *Int. J. Cancer.* 7:93–100.
12. Furcht, L. T. 1982. Structure and function of the adhesive glycoprotein fibronectin. *In* Modern Cell Biology. B. Satir, editor. Alan R. Liss Inc., New York. 1:53–117.
13. Yamada, K. M. 1983. Cell surface interactions with extracellular materials. *Annu. Rev. Biochem.* 52:761–799.
14. Terranova, V. P., and D. H. Rohrbach, and G. R. Martin. 1980. Role of laminin in the attachment of PAM 212 (epithelial) cells to basement membrane collagen. *Cell.* 22:719–726.
15. Couchman, J. R., M. Höök, D. A. Rees, and R. Timpl. 1983. Adhesion, growth, and matrix production by fibroblasts on laminin substrates. *J. Cell Biol.* 96:177–183.
16. Oliver, N., R. F. Newby, L. T. Furcht, and S. Bourgeois. 1983. Regulation of fibronectin biosynthesis by glucocorticoids in human fibrosarcoma cells and normal fibroblasts. *Cell.* 33:287–296.
17. Gauss-Müller, V., H. K. Kleinman, G. R. Martin, and E. Schiffman. 1980. Role of attachment factors and attractants in fibronectin chemotaxis. *J. Lab. Clin. Med.* 96:1071–1080.
18. Greenburg, J. H., S. Seppa, H. Seppa, and A. Tyl Hewitt. 1981. Role of collagen and fibronectin in neural crest cell adhesion and migration. *Dev. Biol.* 87:259–276.
19. Repesh, L. A., T. J. Fitzgerald, and L. T. Furcht. 1982. Fibronectin involvement in granulation tissue and wound healing in rabbits. *J. Histochem. Cytochem.* 30:351–358.
20. Grinnell, F., K. E. Billingham, and L. Burgess. 1981. Distribution of fibronectin during wound healing *in vivo. J. Invest. Dermatol.* 76:181–189.
21. Donaldson, D. J., and J. T. Mahan. 1983. Fibrinogen and fibronectin as substrates for epidermal cell migration during wound closure. *J. Cell Sci.* 62:117–127.
22. Schor, S. L., A. M. Schor, and G. W. Bazill. 1981. The effects of fibronectin on the migration of human foreskin fibroblasts and Syrian hamster melanoma cells into three-dimensional gels of native collagen fibres. *J. Cell Sci.* 48:301–314.
23. McCarthy, J. B., S. L. Palm, and L. T. Furcht. 1983. Migration of haptotaxis of a Schwann cell tumor line to the basement membrane glycoprotein laminin. *J. Cell Biol.* 97:772–777.
24. Rogers, S. L., P. C. Letourneau, S. L. Palm, J. B. McCarthy, and L. T. Furcht. 1983. Neurite extension by peripheral and central nervous system neurons in response to substratum-bound fibronectin and laminin. *Dev. Biol.* 98:212–220.
25. Baron-van Evercooren, A., H. K. Kleinman, S. Ohno, P. Marangos, I. P. Schwartz, and M. Dubois-Dala. 1982. Nerve growth factor, laminin, and fibronectin promote neurite growth in human fetal sensory ganglion cultures. *J. Neurosci. Res.* 8:179–193.
26. Terranova, V. P., L. A. Liotta, R. G. Russo, and G. R. Martin. 1982. Role of laminin in the attachment and metastasis of murine tumor cells. *Cancer Res.* 42:2265–2269.
27. Varani, J., E. J. Lovett, J. P. McCoy, S. Shibata, D. E. Maddox, I. J. Goldstein, and M. Wicha. 1983. Differential expression of a laminin like substance by high- and low-metastatic tumor cells. *Am. J. Pathol.* 111:27–34.
28. Terranova, V. P., C. N. Rao, T. Kalebic, I. M. Margulies, and L. A. Liotta. 1983. Laminin receptor on human breast carcinoma cells. *Proc. Natl. Acad. Sci. USA.* 80:444–448.
29. Malinoff, H. L., and M. S. Wicha. 1983. Isolation of a cell receptor protein for laminin from murine fibrosarcoma cells. *J. Cell Biol.* 96:1475–1479.
30. Smith, D. E., and L. T. Furcht. 1982. Localization of two unique heparin binding domains of human plasma fibronectin with monoclonal antibodies. *J. Biol. Chem.* 257:6518–6523.
31. Palm, S. L., and L. T. Furcht. 1982. Production of laminin and fibronectin by Schwannoma cells: cell-protein interactions in vitro and protein localization in peripheral nerve in vivo. *J. Cell Biol.* 96:1218–1226.
32. Mosseson, M. W., and A. Umfleet. 1970. The cold-insoluble globulin of human plasma. *J. Biol. Chem.* 245:5728–5736.
33. Jentoft, N., and D. G. Dearborn. 1979. Labeling of proteins by reductive methylation using sodium cyanoborohydride. *J. Biol. Chem.* 254:4359–4365.
34. Zigmond, S. H., and J. G. Hirsch. 1973. Leukocyte locomotion and chemotaxis: new methods for evaluation and demonstration of a cell derived chemotactic factor. *J. Exp. Med.* 137:387–410.
35. Ward, P. A., and E. L. Becker. 1968. The deactivation of rabbit neutrophils by chemotactic factor and the nature of the activatable esterase. *J. Exp. Med.* 127:693–709.
36. Becker, E. L. 1972. The relationship of the chemotactic behavior of the complement-derived factors, C3a, C5a, and C567, and a bacterial chemotactic factor to their ability to activate the proesterase 1 of rabbit polymorphonuclear leukocytes. *J. Exp. Med.* 135:376–387.
37. Grinnell, F. 1980. Fibroblast receptor for cell-substratum adhesion: studies on the interaction of baby hamster kidney cells with latex beads coated by cold insoluble globulin (plasma fibronectin). *J. Cell Biol.* 86:104–112.
38. Carter, W. G., H. Rauvala, and S.-I. Hakomori. 1981. Studies on cell adhesion and recognition. II. The kinetics of cell adhesion and cell spreading on surfaces coated with carbohydrate-reactive protein (glycosidases and lectins) and fibronectin. *J. Cell Biol.* 88:138–148.
39. Carter, S. B. 1967. Haptotaxis and the mechanism of cell motility *Nature (Lond.)* 213:256–260.
40. Kleinman, H., R. J. Klebe, and G. R. Martin. 1981. Role of collagenous matrices in the adhesion and growth of cells. *J. Cell Biol.* 88:473–485.
41. Klebe, R. J., K. L. Bentley, and R. C. Schoen. 1981. Adhesive substrates for fibronectin. *J. Cell Physiol.* 109:481–488.
42. Seppä, H. E. J., K. M. Yamada, S. T. Seppä, and M. H. Silver. 1981. The cell binding fragment of fibronectin is chemotactic for fibroblasts. *Cell Biology International Reports.* 5:813–819.
43. Rao, N. C., S. H. Barsky, V. P. Terranova, and L. A. Liotta. 1983. Isolation of a tumor cell laminin receptor. *Biochem. Biophys. Res. Commun.* 111:804–808.
44. Brown, S. S., H. L. Malinoff, and M. S. Wicha. 1983. Connectin: cell surface protein that binds both laminin and actin. *Proc. Natl. Acad. Sci. USA.* 80:5927–5930.
45. Liotta, L. A., S. Abe, P. Gehron-Robey, and G. R. Martin. 1979. Preferential digestion of basement membrane collagen by an enzyme derived from a metastatic tumor. *Proc. Natl. Acad. Sci. USA.* 76:2268–2272.
46. Sloane, B. F., J. R. Dunn, and K. V. Honn. 1981. Lysosomal cathepsin B: correlation with metastatic potential *Science (Wash. DC).* 212:1151–1153.
47. Liotta, L. A., K. Tryggvason, S. Garbisa, P. Gehron-Robey, and J. C. Murray. 1980. Interaction of metastatic tumor cells with basement membrane collagen. *In* Cancer Campaign. E. Grundman, editor. GustavFischer Verlag, New York. 21–31.
48. Kalebic, T., S. Garbisa, B. Glaser, and L. A. Liotta. 1983. Basement membrane collagen: degradation by migrating endothelial cells. *Science (Wash. DC).* 221:281–283.