Signal Transduction for Chemotaxis and Haptotaxis by Matrix Molecules in Tumor Cells

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Abstract. Transduction of signals initiating motility by extracellular matrix (ECM) molecules differed depending on the type of matrix molecule and whether the ligand was in solution or bound to a substratum. Laminin, fibronectin, and type IV collagen stimulated both chemotaxis and haptotaxis of the A2058 human melanoma cell line. Peak chemotactic responses were reached at 50–200 nM for laminin, 50–100 nM for fibronectin, and 200–370 nM for type IV collagen. Checkerboard analysis of each attractant in solution demonstrated a predominantly directional (chemotactic) response, with a minor chemokinetic component. The cells also migrated in a concentration-dependent manner to insoluble step gradients of substratum-bound attractant (haptotaxis). The haptotactic responses reached maximal levels at coating concentrations of 20 nM for laminin and type IV collagen, and from 30 to 45 nM for fibronectin. Pretreatment of cells with the protein synthesis inhibitor, cycloheximide (5 μg/ml), resulted in a 5–30% inhibition of both chemotactic and haptotactic responses to each matrix protein, indicating that de novo protein synthesis was not required for a significant motility response. Pretreatment of cells with 50–500 μg/ml of synthetic peptides containing the fibronectin cell-recognition sequence GRGDS resulted in a concentration-dependent inhibition of fibronectin-mediated chemotaxis and haptotaxis (70–80% inhibition compared to control motility); negative control peptide GRGES had only a minimal effect. Neither GRGDS nor GRGES significantly inhibited motility to laminin or type IV collagen. Therefore, these results support a role for the RGD-directed integrin receptor in both types of motility response to fibronectin.

After pretreatment with pertussis toxin (PT), chemotactic responses to laminin, fibronectin, and type IV collagen were distinctly different. Chemotaxis to laminin was intermediate in sensitivity; chemotaxis to fibronectin was completely insensitive; and chemotaxis to type IV collagen was profoundly inhibited by PT. In marked contrast to the inhibition of chemotaxis, the haptotactic responses to all three ligands were unaffected by any of the tested concentrations of PT. High concentrations of cholera toxin (CT; 10 μg/ml) or the cAMP analogue, 8-Br-cAMP (0.5 mM), did not significantly affect chemotactic or haptotactic motility to any of the attractant proteins, ruling out the involvement of cAMP in the biochemical pathway initiating motility in these cells. The sensitivity of chemotaxis induced by laminin and type IV collagen, but not fibronectin, to PT indicates the involvement of a PT-sensitive G protein in transduction of the signals initiating motility to soluble laminin and type IV collagen. The insensitivity of haptotaxis to PT indicates that for these two attractants, chemotaxis and haptotaxis are induced by distinct transduction mechanisms.

INVASION and metastatic spread of malignant neoplasms are associated with active locomotion of tumor cells. This locomotion occurs at the site of the primary growth (46), as well as during entrance and egress from blood vessels (44). Many factors derived from host tissues have been shown to stimulate motility of tumor cells in vitro, including factors derived from resorbing bone (32), from liver and lung (16), from the fifth component of human complement (35), and certain growth factors (43). In addition, an autocrine motility factor (AMF)[1] produced by a human melanoma cell line has been found to stimulate the intrinsic motility of the same cells (25). These factors are believed to influence both the extent and the direction of tumor cell movement in vivo, facilitating penetration of host tissue barriers and serving to "home" tumor cells to specific target organs. Components of the extracellular matrix (ECM) includ-

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1. *Abbreviations used in this paper*: AMF, autocrine motility factor; CT, cholera toxin; DPBS, Dulbecco's phosphate-buffered saline; ECM, extracellular matrix; 8-Br-cAMP, 8-bromoadenosine 3′:5′ cyclic monophosphate; G proteins, guanine nucleotide-binding proteins; HPF, high power field; IGF-I, insulin-like growth factor I; PT, pertussis toxin.

ing laminin, fibronectin, type IV collagen, thrombospondin, and serum spreading factor (vitronectin), as well as their proteolytic digestion products, also stimulate the in vitro migration of a variety of normal cells and tumor cells (1, 14, 26–29, 39, 47, 54). The intact ECM molecules are distinct from the other motility-stimulating factors in that they are present in vivo as insolubilized structures mediating cell attachment, although soluble forms of fibronectin and vitronectin are also present in plasma. Interactions of tumor cells with the ECM at multiple stages in the metastatic cascade (24) could considerably influence invasion and metastasis by contributing to the movement of tumor cells through vascular basement membrane and interstitial stroma. The highly aggressive human melanoma cell line A2058 has been found to migrate in response to several diverse attractant molecules (25, 43, 47, 54). We used this cell line to study the nature of the motility responses to laminin, fibronectin, and type IV collagen, three ECM proteins encountered by tumor cells during metastatic spread.

The term "chemotaxis" has been used to describe tumor cell motility and is presumed to refer to directed migration towards a soluble attractant, although this has not always been shown directly. Alternatively, tumor cell motility toward substratum-bound, insolubilized laminin and fibronectin has been termed "haptotaxis" (26, 27). However, the distinction between chemotaxis and haptotaxis to ECM components has not always been made clear, and uncertainty exists as to whether there are fundamental differences between the two processes. Recently, Taraboletti et al. (47) established that separate domains on the NH_2- and COOH-terminal regions of the thrombospondin molecule are responsible for chemotaxis and haptotaxis, respectively, and that the two processes could be differentially inhibited by antibodies to the relevant sites. This suggests that these functionally distinct domains stimulate motility through separate receptors on the cell surface. The potential importance of a chemotactic response to ECM components is apparent when considering that during the process of tumor invasion and metastasis, proteolytic degradation results in solubilization of ECM components (23). As a result, tumor cells could conceivably detect and respond to the soluble fragments as well as to the insoluble intact matrix molecules. Therefore, chemotaxis and haptotaxis to ECM components could represent two separate and distinguishable means by which tumor cells penetrate basement membranes and interstitial stroma.

Tumor cell migration is believed to result from the interaction of the attractant molecule with its specific receptor, as is the case for leukocytes. The signal transduction pathways that initiate tumor cell migration are among the least understood aspects of invasion and metastasis. The migratory response of the human melanoma cell line A2058 to its AMF is known to be extremely sensitive to pertussis toxin (PT) pretreatment, which in other cells is known to ADP-ribosylate and inactivate certain receptor coupled guanine nucleotide-binding proteins (G proteins) (42). However, migration of the same cell line to insulin-like growth factor I (IGF-I) is completely insensitive to PT pretreatment, distinguishing the immediate postreceptor signal transduction events in the chemotactic responses to the two attractants (43). In this study, we assessed the migratory responses of the A2058 cell line to laminin, fibronectin, and type IV collagen presented to the cells both as soluble (chemotaxis) and as insoluble, substratum-bound attractants (haptotaxis). To determine if a toxin-sensitive G protein is involved in the transduction of these motility signals, we also tested the sensitivity of each response to PT pretreatment of the cells. To assess a possible role of RGD-directed integrin receptors in these ECM-induced motile responses, we pretreated cells with soluble RGD-containing peptides. Finally, to examine the possibility that the cells were secreting a substance that could affect cell motility, we determined the effect of pretreating the cells with the protein synthesis inhibitor, cycloheximide.

Materials and Methods

Laminin was purified from the Engelbreth-Holm-Swarm tumor as described (50). The isolated laminin (M_r 900,000) was checked for purity by examination on 5% SDS-PAGE run under reducing conditions. Fibronectin (M_r 440,000) and type IV collagen (M_r 540,000) were purchased from Collaborative Research, Inc. (Bedford, MA). Purity was verified by PAGE and rotary shadowing EM (performed by Inger Margulies, Laboratory of Pathology, National Cancer Institute, National Institutes of Health). The type IV collagen contained both the 7S amino-terminal domain and the globular NCI carboxyl-terminal domain. PT and cholera toxin (CT) were from List Biological Laboratories, Inc. (Campbell, CA). The Nuclepore membranes (polyvinyl-pyrrolidone-free) and the 48-well chemotaxis chamber were purchased from Neuro Probe, Inc. (Cabin John, MD). Cycloheximide and 8-bromo-adenosine 3′:5′-cyclic monophosphate (8-Br-cAMP) were from Sigma Chemical Co. (St. Louis, MO).

Cell Culture

The human melanoma cell line, A2058 (51), was maintained in culture in DME supplemented with 10% FBS. Medium and serum were from Biofluids, Inc. (Gaithersburg, MD).

Cell Motility Assays

Cells in logarithmic phase of growth were detached by brief exposure to 0.05% trypsin/0.02% EDTA and allowed to regenerate for 1 h in serum-containing medium. The cells were then centrifuged at 800 *g* for 5 min and resuspended at 2×10^6/ml in serum-free DME containing 0.1% BSA. In experiments with PT and CT, cells were preincubated with the toxins for an additional 2 h at room temperature. For PT, chemotaxis to AMF was run as a positive control to test the toxin efficacy. In experiments with synthetic peptides, cycloheximide and 8-Br-cAMP, cells were preincubated with each agent for 1 h. For each set of experiments, untreated control cells were preincubated under the same conditions as the treated cells. Motility assays were carried out in the presence of the toxins, peptides, and pharmacological agents.

Determination of Haptotaxis. Haptotaxis was assayed in triplicate using modified Boyden chambers, with 8-μm pore size polycarbonate Nuclepore filters (13-mm-diam). Filters were floated overnight at 37°C on a solution of laminin, fibronectin, or type IV collagen diluted to the indicated concentration in DPBS, similar to the method described by McCarthy and Furcht (27). Negative control filters were floated on Dulbecco's PBS (DPBS) alone. Chambers were assembled with serum-free DME containing 0.1% BSA in the lower compartment. All filters were washed five times with DPBS, dried, and placed in the chamber so that the side of the filter with the higher concentration of protein faced the lower compartment. After addition of 4×10^5 cells to the upper compartment, chambers were incubated in a humidified atmosphere at 37°C for 5 h. At the end of the assay, filters were removed, fixed and stained with Diff-Quik (Baxter Scientific, McGaw Park, IL), and then mounted on glass slides. Cells that had migrated were quantitated by light microscopy under high power field (HPF) (500×). For each replicate, the number of cells in five randomly chosen HPFs was determined and the counts were averaged.

Determination of Chemotaxis. Chemotaxis was assayed in triplicate using 48-well microchemotaxis chambers as described previously (13) with 8-μm pore size polycarbonate Nuclepore filters. Filters were precoated on both sides to enhance cell adherence by soaking overnight in solutions of gelatin (100 μg/ml in 0.02 M acetic acid for chemotaxis to laminin and in

some cases to type IV collagen), type IV collagen (40 μg/ml in 0.1 M acetic acid for chemotaxis to fibronectin), or fibronectin (10 μg/ml in cyclohexylaminopropane sulfonic acid [CAPS] buffer for chemotaxis to type IV collagen). ECM components to be tested as chemoattractants were diluted to the indicated concentrations into serum-free DME containing 0.1% BSA and added to the lower wells, while the upper wells received 1.1 × 10^5 cells. The chambers were incubated at 37°C for 4 h. Filters were then processed and migration quantitated as for haptotaxis. Untreated cells, as well as cells from every treatment group, were tested for their unstimulated random motility in response to DME with 0.1% BSA in the lower wells.

Stimulated motility represents the total motile response minus the unstimulated random motility. In haptotaxis, random motility did not exceed two to four cells per HPF. Average unstimulated random motility in chemotaxis was 10 cells per HPF with laminin as attractant, 22 cells per HPF with fibronectin, and 2-10 cells per HPF with type IV collagen.

Preparation of Synthetic Peptides

The two peptides, GRGDS and GRGES, were synthesized using a peptide synthesizer (model 9600; Biosearch, San Rafael, CA). The Biosearch preprogrammed t-Boc Merrifield solid-phase protocol was used in these preparations.

Results

Haptotactic Migration of A2058 Cells to ECM Components

A2058 cells migrated over a step-density gradient of substratum-bound laminin, type IV collagen, and fibronectin in the absence of soluble attractant. With each ECM protein, increasing the gradient resulted in a higher level of migration until a plateau was reached, which could result from saturation of the filters at the higher coating concentrations. Fig. 1 shows representative concentration-response curves of stimulated motility with each attractant protein. The haptotactic responses to laminin and to type IV collagen reached a peak when concentrations of ~20 nM were used to coat the filters. Increasing coating concentrations to 100 nM did not result in significant change in migration levels. With fibronectin, the peak response occurred at the slightly higher coating concentration of 30–45 nM and, as with laminin and type IV collagen, remained at this level when up to fivefold of that coating concentration was used. With each protein, the maximal response was >50-fold above unstimulated random motility. In a separate set of controls, migration over a gradient of an unrelated, nonmatrix protein was assessed by coating filters on the lower side with BSA exactly as done for laminin, fibronectin, and type IV collagen. Over a coating concentration range of 1.5–735 nM, BSA did not significantly stimulate haptotactic response (not shown).

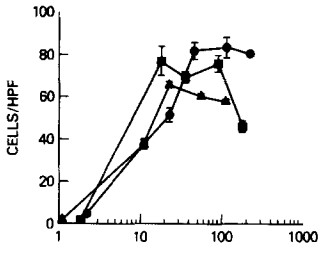

Figure 1. Concentration curves of A2058 haptotactic response to ECM components. Cell motility was assessed in modified Boyden chambers. Polycarbonate filters were precoated with a step gradient of increasing concentrations of laminin (▲), type IV collagen (■), or fibronectin (●). Filters were placed in the chambers with the higher protein side facing the lower well. After addition of 4 × 10^5 cells to the upper compartment, chambers were incubated at 37°C for 5 h. Data represent the mean of triplicate determinations for a single representative experiment of migrated cells per HPF ($\bar{x}$ ± SEM). All experiments were repeated five to seven times. Nonspecific random migration was subtracted out for each data point.

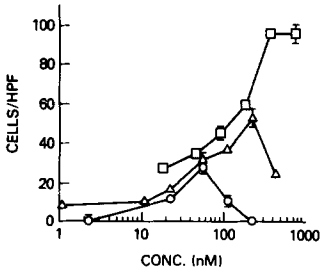

Figure 2. Concentration curves of A2058 chemotactic response to ECM components. Cell motility was assessed in 48-well microchemotaxis chambers. Increasing concentrations of laminin (△), type IV collagen (□), and fibronectin (○) were diluted in DME supplemented with 0.1% BSA and added to the lower wells. Polycarbonate filters were precoated on both sides with a noninteracting adhesive protein (see Materials and Methods). After addition of 1.1 × 10^5 cells to the upper compartments, chambers were incubated at 37°C for 4 h. Data represent the mean of triplicate determinations for a single representative experiment of migrated cells per HPF ($\bar{x}$ ± SEM). All experiments were repeated five to seven times. Nonspecific random migration was subtracted out for each data point.

Chemotactic Migration of A2058 Cells to ECM Components

A2058 cells also migrated in a concentration-dependent manner to soluble laminin, fibronectin, and type IV collagen. In Fig. 2, representative concentration-response curves of stimulated motility are shown. Concentrations of laminin >1 nM stimulated migration over background levels, with a peak in response occurring in the range of 50 to 200 nM (sixfold above unstimulated motility). Higher concentrations of fibronectin (20–50 nM) were required to stimulate migration, but the maximal response (at 50–100 nM), although still significant, was only 2- to 4.5-fold increased above unstimulated motility. Migration to both laminin and fibronectin declined at concentrations higher than those which elicited a maximal response, resulting in bimodal curves. Type IV collagen elicited a peak response at 200–370 nM, which was 50-fold above unstimulated motility, and substantially higher than the peak responses to laminin and fibronectin. Over the concentration range tested, a decline in motility response to type IV collagen either was not observed or was very slight.

Checkerboard analysis (Figs. 3, 4, and 5) was performed with each protein in solution to assess the random (chemokinetic) or directed (chemotactic) nature of the tumor cell migratory response. Chambers were assembled with different ratios of the attractant above and below the filter. The squares below the diagonal reflect responses to a positive gradient, whereas those above the diagonal reflect responses to a negative gradient. The values along the diagonal indicate random motility to uniform concentrations of soluble attractant added to both sides of the chamber. Checkerboard analysis indicated that laminin stimulated both random and directed motility (Fig. 3), although directed motility was stimulated to a greater extent (maximum of sixfold increase over background compared with a fourfold increase for random migration). Type IV collagen also stimulated both chemotaxis and chemokinesis (Fig. 4), with the chemotactic response predominating (values below the diagonal). Fibro-

LAM (μg/ml) IN LOWER CHAMBER \ LAM (μg/ml) IN UPPER CHAMBER	0	25	50	100	200
0	8.6 +/-1.4	11.0 +/-0.5	9.5 +/-0.9	10.4 +/-0.8	15.2 +/-1.8
25	33.6 +/-1.2	24.1 +/-2.1	28.8 +/-3.8	25.5 +/-0.2	33.3 +/-0.9
50	47.2 +/-1.7	31.1 +/-3.0	34.5 +/-2.5	28.1 +/-1.8	29.5 +/-1.6
100	54.2 +/-2.8	30.4 +/-2.6	32.9 +/-3.2	28.5 +/-0.4	25.7 +/-2.1
200	46.5 +/-1.3	28.5 +/-2.9	22.9 +/-0.8	23.6 +/-1.7	19.7 +/-1.3

Figure 3. Checkerboard analysis of the chemotactic activity of laminin (*LAM*). Varying concentrations of soluble laminin were added to the upper chamber with the cells or to the lower chamber, as indicated. Motility response is expressed as the mean of triplicate determinations of migrated cells per HPF ($\bar{x} \pm$ SEM).

FBN (μg/ml) IN LOWER CHAMBER \ FBN (μg/ml) IN UPPER CHAMBER	0	10	25	50	100
0	8.6 +/-1.4	4.4 +/-1.2	2.8 +/-0.5	5.0 +/-0.8	0.3 +/-0.1
10	36.9 +/-1.2	27.9 +/-0.8	18.1 +/-2.3	9.3 +/-0.2	0.2 +/-0.2
25	41.2 +/-1.3	24.3 +/-0.4	17.3 +/-1.7	8.9 +/-0.9	0.8 +/-0.2
50	27.9 +/-1.7	12.1 +/-0.7	9.5 +/-0.5	6.7 +/-0.2	1.1 +/-0.1
100	13.2 +/-0.7	9.3 +/-0.9	3.9 +/-0.6	1.5 +/-0.4	0.7 +/-0.3

Figure 5. Checkerboard analysis of the chemotactic activity of fibronectin (*FN*). Varying concentrations of soluble fibronectin were added to the upper chamber with the cells or to the lower chamber, as indicated. Motility response is expressed as the mean of triplicate determinations of migrated cells per HPF ($\bar{x} \pm$ SEM).

nectin (Fig. 5) stimulated directed motility with only a minor chemokinetic effect at the lower uniform concentrations (along the diagonal). No significant migration to a negative gradient of attractant was observed with any of the proteins.

To confirm that the attractants were remaining in solution under conditions of the assay, two sets of controls were carried out. In the first set, laminin was iodinated to a specific activity of 4 μCi/μg with immobilized lactoperoxidase (Enzymobeads; Bio-Rad Laboratories, Richmond, CA). Iodinated laminin (25 × 10^3 cpm) was mixed with unlabeled laminin to a final concentration of 100 μg/ml and added to the lower well of the chamber. The chamber was assembled with a gelatin-coated filter and incubated as for chemotaxis, with BSA-DME in the upper well. At the end of the incubation, the radioactivity associated with the liquid phase (both upper and lower wells) and the filter (after washing) was quantitated, and 70–80% of the radioactivity initially added to the lower well was in the liquid phase. Only 5–7% was associated with the filter, and the remaining 10–13% of radioactivity was left behind on the chamber. In the second set of controls, unlabeled laminin and fibronectin were diluted into BSA-DME to concentrations stimulating chemotaxis, and aliquots of each were placed in the lower wells of separate chambers which were then overlaid with the appropriate filter. Chambers were incubated as for chemotaxis, with buffer in the upper wells. At the end, the solutions in the lower wells were diluted 1:2 in sample buffer (0.01 M Tris-HCl, 2% SDS, 10% glycerol, 7% β-mercaptoethanol, 0.001% bromphenol blue, pH 6.8) and electrophoresed under reducing conditions in a 6% SDS-polyacrylamide gel. Equal volumes of the laminin and fibronectin solutions that had not been incubated were also electrophoresed for comparison. After Coomassie staining, the intensity of protein stain was roughly equivalent in the paired samples before and after incubation, demonstrating that the laminin and fibronectin which had been incubated in the chambers had remained in solution (not shown).

TYPE IV COLLAGEN (μg/ml) IN LOWER CHAMBER \ TYPE IV COLLAGEN (μg/ml) IN UPPER CHAMBER	0	50	100	200	400
0	2.4 +/-0.4	3.4 +/-0.8	4.1 +/-0.2	5.5 +/-0.9	6.8 +/-1.7
50	47.9 +/-3.4	34.4 +/-4.7	30.8 +/-0.3	44.8 +/-2.4	48.6 +/-2.4
100	61.6 +/-1.4	49.1 +/-5.9	43.4 +/-4.0	47.3 +/-3.2	66.2 +/-2.4
200	98.9 +/-1.5	71.2 +/-3.9	66.2 +/-1.0	65.4 +/-1.4	83.3 +/-3.0
400	98.7 +/-4.6	78.6 +/-1.3	69.3 +/-2.0	82.6 +/-5.7	82.0 +/-4.4

Figure 4. Checkerboard analysis of the chemotactic activity of type IV collagen. Varying concentrations of soluble type IV collagen were added to the upper chamber with the cells or to the lower chamber, as indicated. Motility response is expressed as the mean of triplicate determinations of migrated cells per HPF ($\bar{x} \pm$ SEM).

Effect of PT on Motility to ECM Components

PT inactivates certain G proteins by ADP-ribosylation (11),

and is a useful probe to determine the possibility of G protein involvement in a cellular response. The motility response of the A2058 melanoma cell line to its AMF has previously been shown to be sensitive to inhibition by PT, indicating direct participation of a G protein in the signal transduction pathway employed by AMF (42). In contrast, motility of the same cell line in response to IGF-I is completely insensitive to the effects of PT, implicating the involvement of a different signal transduction pathway (43).

In this study, we first tested a wide range of PT concentrations to determine the PT sensitivity of the chemotactic or haptotactic motility responses to the matrix proteins laminin, fibronectin, and type IV collagen, using optimally stimulating concentrations of each protein. Fig. 6 *A* illustrates the effects of PT pretreatment on laminin-stimulated haptotaxis

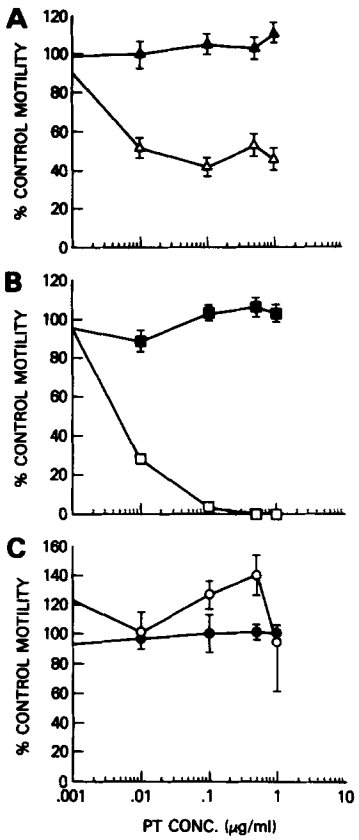

Figure 6. The effect of PT on motility to ECM components. Cells were preincubated with varying concentrations of PT for 2 h at 25°C before assays. Cell chemotactic (*open symbols*) and haptotactic (*closed symbols*) responses were assessed for each ECM component in parallel experiments on the same day. (*A*) Laminin-induced motility responses. The haptotactic response was assessed to a filter-coating concentration of 20 nM. Chemotaxis was measured in response to 110 nM soluble laminin. (*B*) Type IV collagen-induced motility responses. The haptotactic response was assessed to a filter-coating concentration of 20 nM. Chemotaxis was measured in response to 280 nM soluble type IV collagen. (*C*) Fibronectin-induced motility responses. The haptotactic response was assessed to a filter-coating concentration of 45 nM. Chemotaxis was measured in response to 110 nM soluble fibronectin. Results are the mean of triplicate determinations for a single representative experiment and are expressed as percent of control stimulated migration in the absence of PT ($\bar{x} \pm$ SEM). All experiments were repeated three to four times.

Table I. Effect of CT on ECM-induced Motility

	Chemotaxis		Haptotaxis	
Attractant	Concentration*	% of control motility‡	Coating concentration*	% of control motility‡
	nM	%	*nM*	%
Laminin	110	92.5 (10.5)	20	102 (1.6)
Collagen IV	280	85.8 (4.3)	20	101 (5.3)
Fibronectin	60	96.1 (10.0)	45	97.8 (3.8)

* For each attractant, the concentrations that maximally stimulated motility in both types of assay were chosen.
‡ Cells were pretreated with 10 μg/ml CT in 0.1% BSA or 0.1% BSA alone (control) for 2 h before the assay. Results for CT-treated cells are expressed as percent of control motility [$\bar{x} \pm$ (SEM)].

and chemotaxis. The chemotactic response to laminin (110 nM) was moderately sensitive to PT, with 0.01–1.0 μg/ml of PT causing a maximal inhibition of ~50% of the control response. In contrast, the haptotactic response to laminin (coating concentration of 20 nM) was not inhibited by any concentration of PT tested. Haptotactic and chemotactic responses to type IV collagen were also differentially sensitive to inhibition by PT pretreatment of the cells (Fig. 6 *B*). Type IV collagen (280 nM) stimulated a chemotactic response which was profoundly inhibited to <30% of control motility with 10 ng/ml of PT, whereas higher concentrations of the toxin virtually abolished the chemotactic response. In marked contrast, haptotaxis to the same attractant protein (20 nM coating concentration) was completely insensitive to inhibition by PT. For laminin and type IV collagen, the PT sensitivity did not appear to depend on the nature of the coating material because the inhibition by PT was unchanged when either gelatin or fibronectin was used to coat the membranes (results not shown). In addition, cellular adhesion to the gelatin- and fibronectin-coated filters used for chemotaxis to laminin and type IV collagen, respectively, was unaffected by pretreatment with PT, as judged visually. Similarly, pretreatment with PT did not affect adhesion to the coated filters used for haptotaxis. Cell viability, assessed by trypan blue exclusion, was unimpaired at any concentration of PT.

Results with fibronectin (Fig. 6 *C*) differed from those of laminin and type IV collagen. Fibronectin (110 nM) was used as chemoattractant, and a coating concentration of 45 nM was used to precoat filters for haptotaxis. Neither chemotaxis nor haptotaxis to fibronectin was sensitive to the inhibitory effect of PT, suggesting that both types of motility response to this attractant are activated through second messenger pathways which do not involve PT-sensitive transducer G proteins. As found for laminin- and type IV collagen-mediated chemotaxis, the nature of the membrane coating (laminin or type IV collagen coated) did not alter the effect of PT upon fibronectin-mediated chemotaxis.

A second set of experiments was performed using suboptimal concentrations of attractant to see if PT could exert an effect which was obscured at maximal stimulatory concentrations for each matrix protein. In these experiments, the PT concentration was kept constant (0.5 μg/ml) and the attractant concentrations were varied. For haptotaxis, because of the near "all or nothing" nature of the response, generally only one or two suboptimal concentrations could be attained in any assay. However, PT had no significant effect on any of the three ECM components at any coating concentration

(data not shown). Similarly, for chemotaxis, each matrix protein was tested for PT sensitivity over a full range of chemotactic concentrations. PT had no effect on fibronectin-stimulated chemotaxis. In contrast, type IV collagen and laminin were sensitive to the toxin over their full range of concentrations with type IV collagen profoundly inhibited and laminin partially inhibited throughout the assay (results not shown).

Effect of CT and a cAMP Analogue on ECM-induced Motility

PT is known to ADP-ribosylate and inactivate several G proteins including G_i, the inhibitory G protein for adenylate cyclase (10, 11, 31). If the PT effect on chemotaxis were mediated by G_i, treating cells with toxin would abolish both basal- and receptor-mediated inhibition of adenylate cyclase activity, resulting in a net accumulation of cAMP (19, 31). To determine whether cAMP could be contributing to the decreased chemotactic motility of PT-treated cells to laminin and type IV collagen, we tested the effect of another agent known to have a stimulatory effect on adenylate cyclase, CT. Cells were pretreated with CT at a concentration of 10 μg/ml and tested for their motility response to maximally stimulating concentrations of attractant proteins (Table I). It is evident that CT at this concentration does not significantly affect chemotactic or haptotactic motility to any of the attractants (all treatment groups still demonstrated >85% of control motility). Likewise, to test directly the effect of cAMP on motility to the matrix proteins, cells were pretreated with the cAMP analogue, 8-Br-cAMP, which has been shown to cross cell membranes (8, 22). Untreated cells were tested simultaneously as a positive control for each ECM protein. The results (Table II) reveal no significant inhibition or stimulation of any of the chemotactic or haptotactic responses (91–104% of the control response). In addition, unstimulated random motility was also unaffected by the drug (results not shown). These data indicate that laminin, fibronectin, and type IV collagen stimulate motility in these cells through second messenger pathways which are independent of the adenylate cyclase system.

Effect of Peptide GRGDS on Chemotaxis and Haptotaxis to ECM Proteins

Many adhesive proteins in the ECM and in the blood contain the tripeptide arginine-glycine-aspartic acid (RGD) as their cell recognition site, including fibronectin, vitronectin, collagens, thrombospondin, and others. These RGD sequences are recognized by members of a large, structurally related group of cell surface receptors, the integrins, which mediate cell-matrix or cell-cell interactions (38).

Soluble synthetic peptides containing the RGD sequence can inhibit the attachment of cells to a surface coated with fibronectin (33). To determine if the RGD site was necessary to generate a motility response to fibronectin, we pretreated melanoma cells with the peptide GRGDS, negative control peptide GRGES, or media alone. Motility of cells to a maximally stimulating concentration of fibronectin was then as-

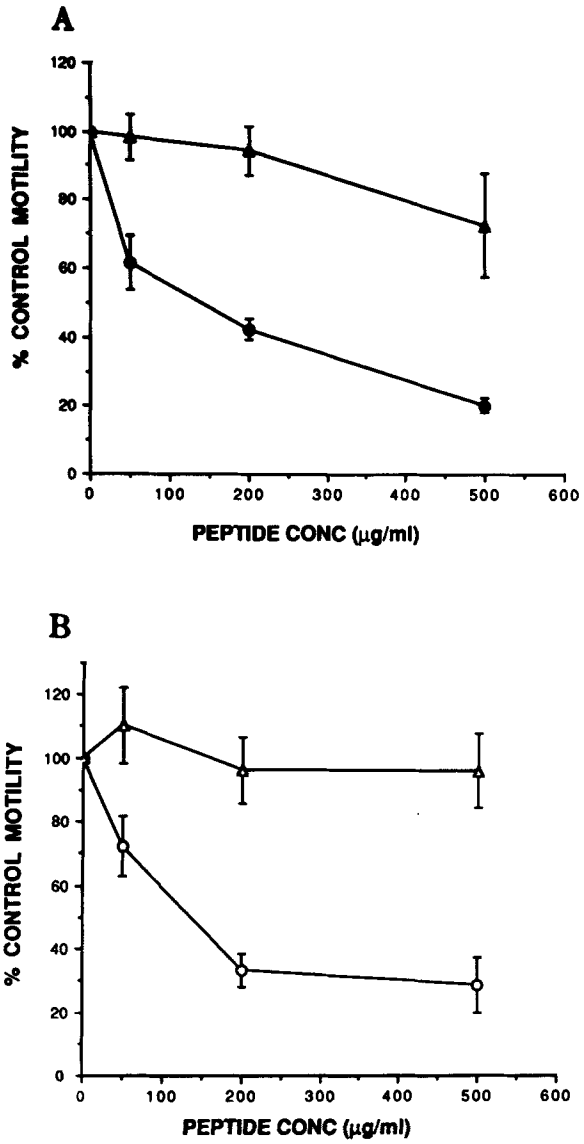

Figure 7. Effect of peptides GRGDS and GRGES on fibronectin-stimulated motility. Cells were preincubated with varying concentrations of GRGDS (*circles*) or GRGES (*triangles*) for 1 h at 25°C before assays. (*A*) The haptotactic responses were assessed to a filter coating concentration of 45 nM. (*B*) Chemotaxis was measured in response to 110 nM soluble fibronectin. Results are the mean of triplicate determinations for a single representative experiment and are expressed as percent of control stimulated migration in the absence of peptides ($\bar{x} \pm$ SEM). These experiments were repeated three to six times.

Table II. Effect of 8-Br-cAMP on ECM-induced Motility

	Chemotaxis		Haptotaxis	
Attractant	Concentration*	% of control motility‡	Coating concentration*	% of control motility‡
	nM	%	*nM*	%
Laminin	100	103 (10.3)	20	95.9 (5.1)
Collagen IV	250	104 (8.0)	20	91.5 (2.8)
Fibronectin	100	92.6 (15.4)	45	104 (7.7)

* For each attractant, the concentrations that maximally stimulated motility in both types of assay were chosen.
‡ Cells were pretreated with 0.5 mM 8-Br-cAMP in 0.1% BSA or 0.1% BSA alone (control) for 1 h before the assay. Results for 8-Br-cAMP-treated cells are expressed as percent of control motility [$\bar{x} \pm$ (SEM)].

sessed in the continued presence of the peptides. Fig. 7 *A* shows the effect of the peptides on haptotaxis to fibronectin. Pretreatment with increasing concentrations of GRGDS resulted in progressively greater inhibition of the haptotactic response. At the highest concentration of peptide tested (500 μg/ml), haptotaxis was reduced to 20% of the control response. This inhibition was accompanied by reduced adherence of cells to the fibronectin-coated filters, as judged visually by the absence of a cell pellet on the top of the filters. In contrast, the presence of negative control peptide GRGES affected haptotaxis only slightly at the highest concentration tested (~80% of control response). This could be attributed to a nonspecific effect of the peptide on the cells. Similarly, pretreatment of cells with GRGDS inhibited chemotaxis to fibronectin in a concentration-dependent manner (Fig. 7 *B*). Maximal inhibition (to 30% of control motility) was achieved with 200 μg/ml of the peptide. No inhibition of chemotaxis was observed when cells were treated with negative control peptide GRGES. Adhesion of cells to type IV collagen-coated filters used for fibronectin chemotaxis appeared to be reduced by ~20% at 500 μg/ml of GRGDS or GRGES, but not by lower concentrations of the two peptides. Pretreatment with the peptides did not significantly impair cellular viability at any of the concentrations tested, as judged by trypan blue exclusion. These results indicate that both chemotaxis and haptotaxis to fibronectin require cellular interaction with the RGD site in the fibronectin molecule.

Although RGD sequences exist in laminin and type IV collagen, they do not appear to represent major cell binding sites. We assessed the effect of the RGD peptides on motility of the cells to maximally stimulating concentrations of laminin and type IV collagen. For comparison, results with fibronectin are also included in Fig. 8 *A*, which illustrates the effect of the peptides on haptotaxis to the ECM proteins. Although GRGDS dramatically inhibited haptotaxis to fibronectin in a concentration-dependent manner, it had a minimal effect on haptotaxis to type IV collagen and laminin. At high concentrations (500 μg/ml), both peptides caused a slight reduction in haptotaxis to type IV collagen. Haptotaxis to laminin was not affected significantly by either peptide. A similar pattern of results was seen with chemotaxis to the ECM proteins (Fig. 8 *B*). Again, the GRGDS peptide strongly inhibited chemotaxis to fibronectin while the negative control peptide had no effect. These same peptides did not inhibit chemotaxis to type IV collagen at all though adhesion to the fibronectin-coated membranes appeared to be decreased. When gelatin-coated filters were used instead of fibronectin-coated filters, the chemotactic response to type IV collagen remained insensitive to the peptide GRGDS. Chemotaxis to laminin was slightly inhibited when cells were treated with 500 μg/ml of either peptide, but not with 200 μg/ml. In conclusion, haptotaxis and chemotaxis to type IV collagen and laminin are not mediated through cellular binding to RGD sequences in these proteins. When a slight inhibitory effect of the peptides was observed, it was seen for both GRGDS and negative control GRGES, indicating a nonspecific inhibition.

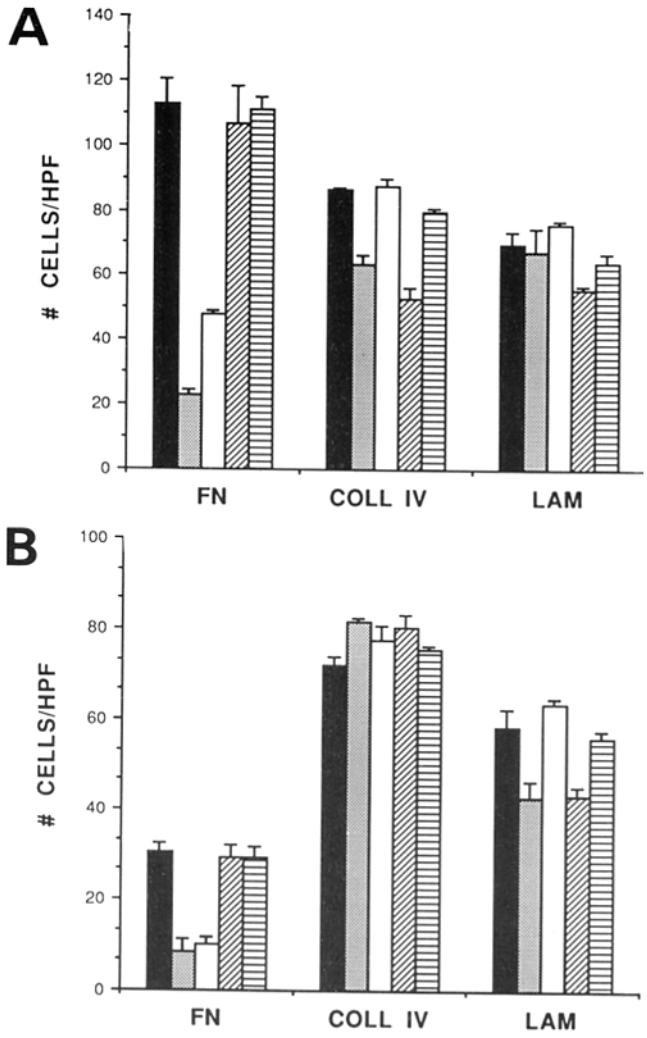

Figure 8. Effect of peptides GRGDS and GRGES on laminin- and type IV collagen-stimulated motility. Cells were preincubated with GRGDS at 200 μg/ml (*stippled*) or 500 μg/ml (*open*), or with GRGES at 200 μg/ml (*diagonal hatching*) or 500 μg/ml (*horizontal hatching*) for 1 h at 25°C before assays. Cells preincubated with DME supplemented with 0.1% BSA were tested concurrently as controls (*solid*). (*A*) Haptotactic responses were assessed to filter-coating concentrations of 20 nM for laminin (*LAM*) and type IV collagen (*COLL IV*) or 45 nM for fibronectin (*FN*). (*B*) Chemotaxis was measured in response to 110 nM soluble laminin, 280 nM soluble type IV collagen, or 110 nM soluble fibronectin. Data represent the mean of triplicate determinations for a single representative experiment of migrated cells per HPF ($\bar{x} \pm$ SEM). Nonspecific random migration was subtracted out for each data point. These experiments were repeated three times.

Effect of Cycloheximide on Chemotaxis and Haptotaxis to ECM Components

To determine whether the A2058 cells were synthesizing proteins which affected the motility responses to the ECM components over the course of a 4–5-h assay, we performed assays in the presence of an inhibitor of protein synthesis. Cycloheximide (5 μg/ml) has been shown to inhibit incorporation of [^{35}S]methionine into A2058 cells (24). When cells were pretreated with this pharmacologic agent, both haptotactic and chemotactic motility responses were slightly inhibited (5–30%) for each ECM protein (Fig. 9). This inhibition appeared to be nonspecific because both haptotactic and chemotactic responses were affected, and each matrix protein was inhibited to a similar degree. However, cyclohex-

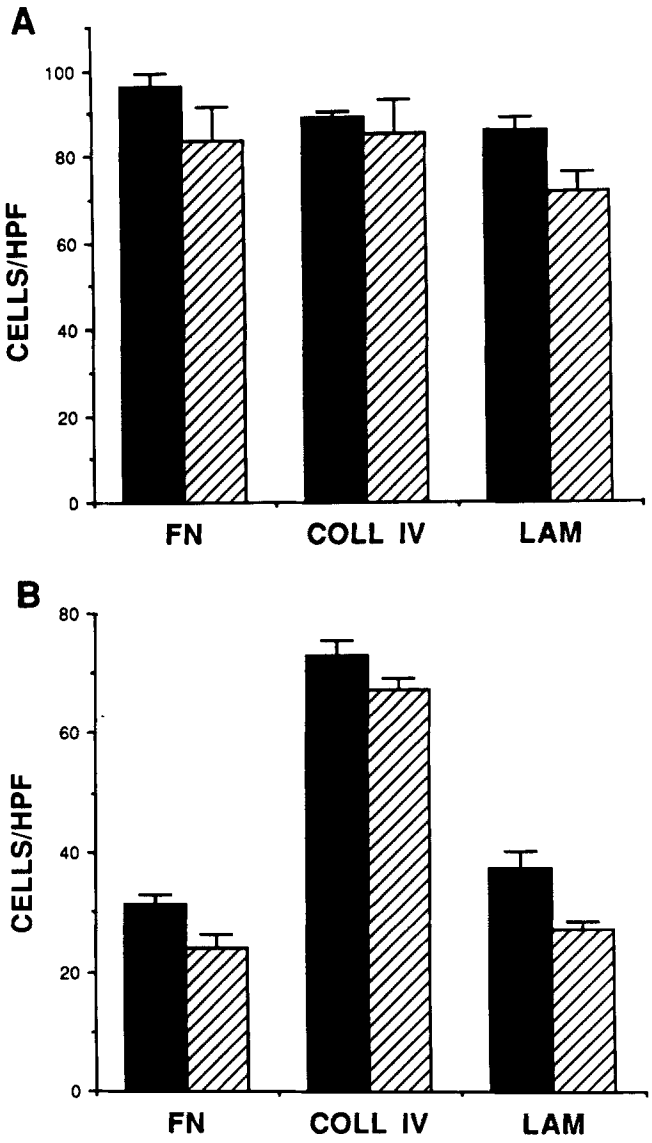

Figure 9. Effect of cycloheximide on motility to ECM components. A2058 cells were pretreated for 1 h at room temperature with cycloheximide at 5 μg/ml (*diagonal hatching*). Identical cells, preincubated only in DME supplemented with 0.1% BSA, were tested concurrently as controls (*solid*). (*A*) Haptotactic responses were assessed to filter coating concentrations of 20 nM for laminin (*LAM*) and type IV collagen (*COLL IV*) or 45 nM fibronectin (*FN*). (*B*) Chemotaxis was measured in response to 100 nM soluble laminin, 250 nM soluble type IV collagen, or 70 nM soluble fibronectin. Data represent the mean of triplicate determinations for a single representative experiment of migrated cells per HPF ($\bar{x} \pm$ SEM). Nonspecific random migration was subtracted out for each data point. These experiments were repeated two times.

imide failed to abolish any of the motility responses. Therefore, the cells do not appear to require de novo protein synthesis to mount a significant chemotactic or haptotactic response to the tested matrix proteins. In addition, we noted that cycloheximide greatly decreased adherence (50–70%, as judged visually) to all the chemotactic membranes (coated with type IV collagen, fibronectin, and gelatin). Because the motility response was still significant, the cells appear to need only minimal adherence in order to produce a chemotactic response.

Like the peptide GRGDS, cycloheximide inhibited fibronectin-induced chemotaxis and haptotaxis in approximately equal proportions (5–24% vs. 14–30%, respectively). To find out whether the effect of GRGDS on fibronectin-induced chemotaxis and haptotaxis could be abolished by inhibition of protein synthesis, we performed a series of experiments in which the cells were pretreated with the peptides alone (GRGDS and GRGES), with cycloheximide alone, or with both cycloheximide plus the peptides. Untreated cells were tested simultaneously as positive controls. The results (Fig. 10) show that GRGDS inhibited both chemotaxis and haptotaxis to fibronectin (60 and 34% inhibition, respectively). Cycloheximide also inhibited both the chemotactic (24%) and haptotactic (14%) responses. When cycloheximide was added to GRGDS, the result appeared to be an additive inhibitory effect (80% inhibition compared with control cells for chemotaxis, 68% for haptotaxis). In contrast, GRGES plus cycloheximide was equivalent to cycloheximide alone. When similar experiments were performed using laminin as the chemotactic or haptotactic agent, GRGDS plus cycloheximide was equivalent to cycloheximide alone without any additive inhibition (results not shown). These experiments were also repeated using type IV collagen as the chemotactic or haptotactic agent. With type IV collagen, there appeared to be a slight increase in the inhibitory effect of cycloheximide plus either GRGDS or GRGES (results not shown). Therefore, the apparent increase in the inhibitory effect was nonspecific.

Discussion

Cells interact with the ECM through specific cell surface re-

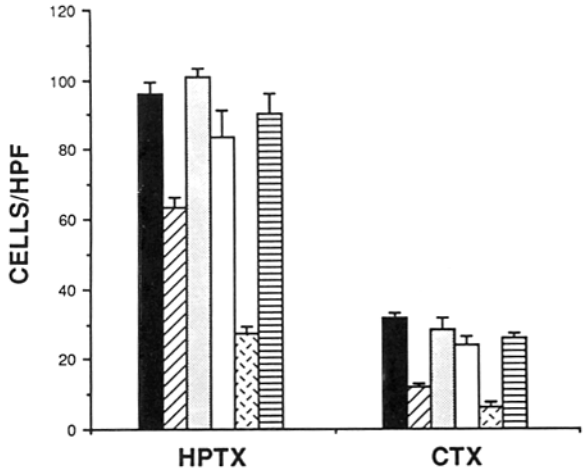

Figure 10. Effect of cycloheximide added to the peptides GRGDS and GRGES on fibronectin-stimulated motility. A2058 cells were pretreated for 1 h at room temperature with GRGDS at 200 μg/ml (*diagonal hatching*), GRGES at 200 μg/ml (*gray*), cycloheximide at 5 μg/ml (*open*), cycloheximide at 5 μg/ml plus GRGDS at 200 μg/ml (*stippled*), or cycloheximide at 5 μg/ml plus GRGES at 200 μg/ml (*horizontal bars*). Identical cells, preincubated only in DME supplemented with 0.1% BSA, were tested concurrently as controls (*solid*). The haptotactic response (*HPTX*) was assessed to a filter coating concentration of 45 nM fibronectin. The chemotactic response (*CTX*) was to 70 nM soluble fibronectin. Data represent the mean of triplicate determinations for a single representative experiment of migrated cells per HPF ($\bar{x} \pm$ SEM). Nonspecific random migration was subtracted out for each data point. These experiments were repeated two to three times.

ceptors. With normal cells, this interaction results in adherence and differentiation (6, 34, 53, 55); migration is stimulated only under specialized conditions such as development (49), wound healing and repair (17), and inflammation (2, 48). The migration of tumor cells in response to ECM proteins also is likely to result from receptor-mediated activation. We tested the ECM components laminin, fibronectin, and type IV collagen and found that all stimulated both the chemotactic and haptotactic motility of the A2058 human melanoma cell line, depending on the conditions of the assay. Presentation of increasing concentration gradients of the soluble attractant proteins fibronectin, laminin, and type IV collagen resulted in a progressive increase in migration until a peak response was reached with bimodal concentration-response curves for laminin and fibronectin. To ensure that a pure chemotactic response was being tested, two modifications of the assay procedure of McCarthy and Furcht (27) were carried out to keep the attractant proteins in solution. First, we precoated the filters on both sides with an adhesive protein (one that is thought not to bind strongly to the attractant) distinct from the attractant protein. Second, 1 mg/ml BSA was added to the medium in which the attractant was diluted, so that an excess of carrier protein would coat any protein-binding sites on the chamber and on the filter surface. Checkerboard analysis of each attractant in solution demonstrated a predominantly directional (chemotactic) response, with a minor chemokinetic component. The melanoma cells also migrated in a concentration-dependent manner to increasing step-density gradients of the insolubilized, substratum-bound attractants. The sensitivity of chemotactic and haptotactic migration to inhibition by PT was assessed for each ECM protein. The chemotactic responses to laminin, fibronectin, and type IV collagen were distinctly different in the degree of inhibition caused by PT. Chemotaxis to type IV collagen was completely inhibited by PT, chemotaxis to laminin was partially inhibited (at most a 50% reduction in stimulated motility), and chemotaxis to fibronectin was unaffected. In contrast to the inhibition of chemotaxis, haptotaxis to all three ECM components was completely insensitive to inhibition by PT. CT and 8-Br-cAMP (10 μg/ml and 0.5 mM, respectively) did not significantly inhibit chemotactic or haptotactic motility. Pretreatment of the cells with 50–500 μg/ml of synthetic peptides containing the fibronectin cell-recognition sequence GRGDS resulted in a concentration-dependent inhibition of fibronectin-mediated chemotaxis and haptotaxis (to 20–30% of control motility); the negative control peptide GRGES had only a minimal effect. Neither of these peptides significantly inhibited motility to laminin or type IV collagen. The protein synthesis inhibitor, cycloheximide, caused a nonspecific 5–30% inhibition of both the chemotactic and haptotactic responses to all three ECM proteins. However, as has been seen for other chemoattractants (7), cycloheximide failed to abolish the significant motility response.

As stated previously, A2058 cells migrated in a concentration-dependent manner to gradients of soluble laminin, fibronectin, and type IV collagen. The concentration-response curves of laminin- and fibronectin-stimulated motility were bimodal with a rather narrow range of concentrations, which elicited a maximal response. This type of curve has been observed previously in the motility response of a Schwann cell tumor line to laminin (27), and of polymorphonuclear leukocytes to certain chemotactic factors. The decline in migratory response to higher than optimal concentrations of attractant may represent down regulation of the chemotactic factor receptors (cellular deactivation) (45) or reduction of the gradient, which is necessary for the stimulation of motility due to more rapid diffusion at high concentrations of attractant (27). Checkerboard analyses demonstrated that laminin and fibronectin stimulated predominantly directional motility responses in A2058 cells, in agreement with previous results with murine melanoma cells (26). Laminin also stimulated random migration, as has been reported previously with a series of laminin-deficient variant cell lines isolated from a heterogeneous murine tumor population (39). Type IV collagen stimulated both significant random and directional motility, although directional motility was more pronounced. Therefore, in the presence of a fluid phase concentration gradient, all three ECM proteins tested have the ability to attract tumor cells. Laminin and type IV collagen also stimulate intrinsic motility when present in more uniform concentrations around the cells.

As with the chemotactic responses, haptotaxis of cells to step-density gradients of the three ECM proteins also increased as higher concentrations of protein were used to coat the filters. When filters were coated with similar concentrations of BSA, a nonmatrix protein, the level of migration observed was no higher than that seen over filters with no protein coating at all (DPBS alone). This indicates that the cells were recognizing and responding to the ECM proteins in a specific manner, and not simply migrating nonspecifically over any protein which would bind to cell surfaces. When comparing the concentration-response curves for haptotaxis and chemotaxis, some differences between the two types of motility responses become evident. There appears to be much less overlap of the three chemotaxis curves compared with the haptotaxis curves. The attractants in solution differed substantially with respect to the magnitude of the peak response elicited; in addition, the concentrations of soluble attractants needed to elicit a peak response varied from 60 nM for fibronectin to 370 nM for type IV collagen. In contrast, the maximal haptotactic responses to the three proteins were very similar, as were the coating concentrations which elicited these responses. Haptotactic responses appeared to be almost "all-or-nothing" in that there were at most only one or two "intermediate" migratory responses before the peak response was reached. This peak migratory response over a broad range of protein concentrations could reflect saturation of potential binding sites on the polycarbonate membranes. These differences between the migratory patterns of haptotaxis and chemotaxis suggest differences in the mechanisms of activation.

In neutrophils, chemotaxis is mediated by specific receptors coupled to G proteins (12, 21, 52). PT, which ADP-ribosylates and inactivates certain G proteins (11, 31), has been shown to inhibit a number of motility responses. Among these are the chemotactic responses of polymorphonuclear leukocytes to FMLP (12). Other studies have shown that pretreatment of T-cell hybridoma cells and lymphosarcoma cells with PT significantly reduces their ability to infiltrate hepatocyte monolayer cultures (36). In addition, Stracke et al. (42) demonstrated that the chemotactic response of the A2058 cells to their AMF was abolished when the cells were pretreated with >10 ng/ml of PT. In these same

cells, however, IGF-I-stimulated chemotaxis was completely insensitive to the effects of PT (43). In one cell type, then, two different ligands can stimulate motility through biochemical pathways that are distinguishable by PT sensitivity. These results suggest that motility responses of the A2058 cells to laminin, fibronectin, and type IV collagen could be distinguishable by PT sensitivity. The second messenger pathways that are activated upon binding of a matrix protein to its receptor are largely unknown. An inhibitory effect of PT on tumor cell motility induced by any of the ECM proteins would implicate the involvement of a second messenger pathway regulated by a G protein. In fact, the chemotactic responses of the cells to the three matrix proteins have a distinctly different profile of inhibition by PT, ranging from lack of inhibition (chemotaxis to fibronectin) to complete sensitivity over a broad range of concentrations (chemotaxis to type IV collagen). Chemotaxis to laminin was intermediate in sensitivity, with, at most, a 50–60% reduction in stimulated motility. These results strongly suggest that, at least for chemotaxis, the three matrix proteins are acting through specific and distinct receptors on the cell surface. More surprising was the fact that migration in response to a single attractant (i.e., type IV collagen) was sensitive to inhibition by PT when the attractant was in the fluid phase, but not when the same attractant was substratum bound. It therefore appears that type IV collagen, and possibly laminin, can stimulate migration through biochemical pathways activated by two distinct signal transduction mechanisms, depending on whether the proteins are presented to the cells in solution or in a solid phase.

Because PT could cause net accumulation of cAMP in the cells, we wanted to rule out the possibility that elevations of cAMP levels were responsible for the inhibition of motility. We found that CT, which is known to ADP-ribosylate the stimulatory G protein (G_s) in an active configuration, had neither an inhibitory nor a stimulatory effect on chemotactic or haptotactic motility to any of the attractants. Similarly, the cAMP analogue, 8-Br-cAMP, which has been shown to enter intact cells (8, 22), neither stimulated nor inhibited the chemotactic or haptotactic motility response to any of the tested ECM proteins. These data agree with previous results (42) demonstrating that G_s and cAMP are not in the biochemical pathway that initiates stimulated motility in these cells.

There are two possible explanations as to how soluble and insoluble laminin and type IV collagen stimulate tumor cell migration through distinct signal transduction pathways. One possibility is that chemotaxis and haptotaxis are mediated through only one receptor, and that there is a bifurcation at the level of the transducer for the soluble and insoluble attractant. Several recent studies have reported that activation of a single receptor can result in split signals (3, 5). In this model, the soluble attractant would activate a PT-sensitive G protein–transduced biochemical pathway generating the motility response; the insoluble substratum-bound attractant would activate the motility response through a different transduction pathway. A more likely explanation for our data is that chemotaxis and haptotaxis to the same ECM protein could be mediated by different cell surface receptors, which recognize different structural domains of the proteins. In this model, the receptor(s) mediating chemotactic migration would be coupled to PT-sensitive G proteins, whereas the receptor(s) mediating haptotactic migration would not. Several recent reports have indicated that the same cellular response can be activated by different agonists acting through separate receptors which are distinct in their mechanisms of signal transduction (4, 15). The possibility of separate receptors for chemotaxis and haptotaxis to the same ECM glycoprotein was raised by the recent work of Taraboletti et al. (47) with thrombospondin. Like other ECM glycoproteins, thrombospondin contains multiple domains through which it mediates cell adhesion and spreading. Through the use of mAbs and protease-derived fragments, it was demonstrated that haptotaxis and chemotaxis of A2058 cells to thrombospondin were each mediated by separate structural domains on the molecule. Because the domains of thrombospondin mediating chemotaxis and haptotaxis were located at opposite ends of the molecule, the implication was that separate receptors on the cell surface recognized each domain.

Laminin, type IV collagen, and fibronectin each contain multiple domains which play roles in cellular adhesion and spreading. For laminin, the specific domains which promote cellular motility are undefined. For type IV collagen, the triple helical domain and perhaps the globular NCI carboxy-terminal domain are involved in cell migration (14). The NCI domain has been shown to promote significant motility only when it is precoated onto the distal surface of polycarbonate membranes (haptotaxis) and not when it is in solution. For fibronectin, a 75-kD tryptic fragment containing the RGDS sequence has been shown to induce motility (29). Our data indicate that, for laminin and type IV collagen, more than one molecular domain is likely to play a role in cellular migration. Which domains are predominantly involved appears to depend on the mode in which the ECM molecule is presented to the cells. The data also confirm that the RGD-containing region of fibronectin is important for both chemotactic and haptotactic migration by A2058 melanoma cells.

As multidomain proteins, the ECM components appear to interact with the cells through multiple cell surface receptors. Each of the three molecules has been reported to bind to integrin-type receptors (9, 18, 20, 37, 41). The specific domains of laminin and type IV collagen that bind to these integrins are not yet defined but are thought to be distinct from RGD sequences present in the molecules. Our data indicate that the peptide GRGDS has little effect on either haptotactic or chemotactic migration to laminin and type IV collagen. Whether the integrin receptors play a role in laminin or type IV collagen-induced motility is still uncertain. In contrast, both types of migration to fibronectin were strongly inhibited by pretreatment of the cells with GRGDS but not the control peptide, GRGES. Furthermore, this inhibitory effect on fibronectin-induced motility was additive to the milder inhibition caused by the protein synthesis inhibitor, cycloheximide. These data indicate that the RGD-directed site may have a long half-life and therefore may not be affected by the relatively brief treatment with cycloheximide. Consequently, the interaction of the cells with the RGDS sequence of fibronectin, presumed to be through the integrin receptor (37), appears necessary to promote both chemotactic and haptotactic motility, although it might not be sufficient. Adhesion at the RGDS site may be necessary to provide traction (in haptotaxis) and/or to place the active site on the ligand in proximity to the relevant receptor(s), leading to locomotion (in chemotaxis).

In conclusion, receptor-mediated migration of a human melanoma cell line to the ECM components laminin, type IV collagen, and fibronectin is activated through distinct signal transduction mechanisms. For laminin and type IV collagen, haptotactic and chemotactic migration to the same molecule is distinguished by sensitivity to PT. These results suggest that separate domains of the proteins, acting through separate cell surface receptors, are responsible for mediating each type of motility response. In solution, laminin or type IV collagen may be able to expose an active chemotaxis-promoting domain to the cells that is not accessible to the cells when the same protein is insolubilized. Conversely, insolubilized laminin or type IV collagen may present certain haptotaxis-promoting domains. In any case, our results illustrate the complexity of tumor cell migration in response to ECM proteins. Tumor cells interact with the ECM at multiple stages during invasion and metastasis (23, 24). Laminin and type IV collagen are normally present as predominantly insoluble structures in host basement membranes. During the initial stages of extravasation, the ability of these proteins to stimulate haptotactic migration of the tumor cells may be more significant. Insoluble fibronectin in the interstitial stroma would then be accessible to the cells, further directing their migration. Tumor cells have been demonstrated to produce certain proteolytic enzymes during invasion, including type IV collagenase (23), cathepsin B (40), and urokinase (a plasminogen activator) (30). Conceivably, then, metastasizing tumor cells could encounter localized pools of matrix proteins in a soluble, partially degraded form that could provide additional stimulation of both random and directional movement of tumor cells. Other soluble chemotactic factors could play a role in the final "homing" of tumor cells to specific target organs. These factors, together with the ubiquitous ECM components, provide aggressive tumor cells with multiple mechanisms allowing migration to distant sites.

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