Migration by Haptotaxis of a Schwann Cell Tumor Line to the Basement Membrane Glycoprotein Laminin

JAMES B. McCARTHY, SALLY L. PALM, and LEO T. FURCHT
Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota 55455

ABSTRACT Laminin is a large (>850-kdalton) glycoprotein that is localized within basement membranes. Recent work has indicated that this protein is present within the endoneurium of mouse sciatic nerve. Furthermore, it has been shown that a rat Schwannoma cell line, RN22F, produced laminin and that laminin promoted the attachment of these cells to bacterial plastic. This report presents evidence that RN22F cells migrate in vitro to laminin in a concentration-dependent fashion. Laminin was extracted from the mouse EHS tumor and purified by molecular sieve and heparin-agarose affinity chromatography. The migration of Schwannoma cells to laminin, as assessed in a microwell modified Boyden chamber, was inhibited in a dose-dependent manner by affinity-purified antilaminin antibody. Zigmond-Hirsch checkerboard analysis experiments indicated that laminin stimulated both random and directed movement of RN22F cells. Additionally, reversal of the laminin gradient in the chambers also stimulated RN22F migration in a concentration-dependent manner, suggesting that directed migration of RN22F cells was due to a substratum-bound laminin (haptotaxis) as opposed to cell movement in response to fluid-phase laminin (chemotaxis). Binding studies using [^{3}H]laminin demonstrated that laminin bound to the filter surface under the assay conditions used, and support the contention that cells are migrating to substrate-bound material. Furthermore, RN22F cells were shown to migrate on filters coated with laminin in the absence of additional fluid-phase laminin. The magnitude of this response could be altered by changing the relative density of bound laminin. In contrast, fibronectin promoted only marginal migration of RN22F cells. Collectively, these observations indicate that haptotaxis may be a mechanism by which laminin may guide cells during development and raise the possibility that it may be involved in peripheral nervous system myelination.

It is well documented that extracellular matrices play a crucial role in determining the phenotypic behavior of both normal and transformed cells. Furthermore, it is becoming apparent that the altered behavior of cells on various matrices results from selective adhesive interactions of cells with discrete matrix constituents, including collagenous and noncollagenous proteins and proteoglycans (1). Laminin and fibronectin are two high molecular weight noncollagenous matrix glycoproteins that promote the adhesion of cells to other matrix constituents.

Laminin is distributed in basement membranes in adult tissue (2) and is synthesized by various cell types such as epithelial and endothelial cells (3, 4). Laminin consists of disulfide-bonded subunits of 200 and 400 kdaltons (5), binds glycosaminoglycans (6), and promotes cell attachment to type IV collagen (7, 8). The cell adhesion protein fibronectin has wide distribution in loose connective tissue, basement membranes, and body fluids (reviewed in 9, 10). Many of the functions of fibronectin seem due to molecular associations with collagens, glycosaminoglycans, or cell surfaces. Recent studies on the cellular adhesion-promoting capacity of laminin and fibronectin on regenerating murine liver cells (8) and rat hepatocytes (7) indicate that these proteins promote the attachment of cells via different cellular receptors.

The directed movement of cells is an essential prerequisite for the various developmental, inflammatory, and reparative processes that occur in vivo. Much information has been gained in understanding the processes that govern the nonrandom distribution of cells (see reviews 11–13). The specific directional migration of cells in response to established

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gradients of soluble attractant molecules has been termed chemotaxis. Presumably, the migration of inflammatory cells such as neutrophils and mononuclear phagocytes into wounds and other lesions involves a chemotactic response by these cells. Specific attractants for these cell types include complement components, bacterial factors, and lymphocyte-derived products. It has also been reported that fibronectin is chemotactic in vitro for noninflammatory cells such as fibroblasts (14), neural crest cells (15), and Schwann cells (16). The addition of fibronectin to both normal and transformed cells in culture has been shown to increase cell motility, as assayed by the formation of phagokinetic tracks (17). These data indicate that fibronectin may be important in regulating cell movement during both reparative and developmental processes.

Alternative mechanisms for directing cellular migration have been proposed, including directed cellular movement based on the formation of adhesion gradients in response to substratum-bound constituents. This type of cell movement has been termed haptotaxis (18), and has been studied by employing surfaces that have been coated with metal deposits to alter the relative "wettability," or charge, of the substrate (19, 20). Studies by Rich and Harris (21) have demonstrated that inflammatory (macrophage) and noninflammatory (fibroblasts) cells can move as a result of differential adhesiveness. Additionally, neutrophils have been shown to respond to substratum-bound attractants such as casein (22). Surface-bound albumin has been reported to play a role in synthetic peptide-induced neutrophil chemotaxis (23). Letourneau has shown that haptotaxis is important in determining the direction of axonal growth on a major matrix constituent, collagen (24).

There are an increasing number of reports that suggest roles for extracellular matrix proteins such as laminin and fibronectin in the development and function of the central and peripheral nervous systems. Surfaces coated with fibronectin promote neurite outgrowth (25). Fibronectin has recently been reported to promote Schwann cell growth and motility in vitro (16). Antilaminin antibodies have been used to localize laminin around axons in normal liver (26). Most recently, work in our laboratory has shown that laminin is present in the endoneurium of mouse peripheral sciatic nerve and that fibronectin is associated with the perineurium (27). This same study indicated that the RN22F Schwannoma cell line produced laminin and fibronectin, and attached preferentially to laminin-coated bacterial plastic as compared with such surfaces coated with fibronectin. The present data extend these findings to demonstrate that RN22F cells preferentially migrate in a directed manner in vitro to laminin when compared with fibronectin. Furthermore, the results collectively indicate that movement of cells in this system occurs primarily in response to laminin which has bound to the filter surface and, as such, represents migration by haptotaxis.

MATERIALS AND METHODS

Cells: The cells used in these experiments were the RN22F line, originally derived from a Schwannoma by Dr. S. E. Pfeiffer, and were a gift from Dr. J. R. Sheppard, Dight Institute of Genetics, University of Minnesota. The cells were grown in Dulbecco's modified Eagle's medium (GIBCO Laboratories, Grand Island, NY) containing 10% heat-inactivated horse serum (Sterile Systems, Inc.; Logan, UT).

Isolation of Proteins: Laminin was isolated from the mouse EHS tumor as described previously (27). A weight extinction coefficient for laminin was determined to be $A^{1\%}1\ cm_{280} = 8.3$. This value was used to calculate laminin concentrations in these studies.

Fibronectin was prepared from fresh frozen human plasma using a gelatin-agarose affinity column technique and ion exchange column as previously described (28). The concentration of fibronectin in the preparation was determined assuming $A^{1\%}1\ cm_{280} = 12.8$ (29). Samples of both proteins were analyzed using discontinuous polyacrylamide slab gels containing SDS that were formulated as previously described (28).

Cell Migration Assay: Migration of the Schwannoma cell line was assayed using a modified blind well microchamber assembly (Neuroprobe, Inc., Cabin John, MD). The use of this chamber for the measurement of polymorphonuclear leukocyte migration has been described previously (30). The assays for cell migration utilized 8-μm pore size Nucleopore polycarbonate filters without polyvinylpyrrolidone ([PVP[1]]; Neuroprobe, Inc., [31]). In experiments in which gelatin coating of the filters was necessary, PVP filters of the same pore size were used. The gelatin coating procedure was based on previous work designed to examine Schwann cell migration (16).

The cells for the assay were obtained by brief exposure (usually 30 s to 1 min) of subconfluent cultures of RN22F cells to 0.05% trypsin with 0.5 mM EDTA. The reaction was terminated by the addition of an equal volume of Dulbecco's modified Eagle's medium containing 2% horse serum. Cells were pelleted at 400 *g* for 10 min and washed once with serum-free DME. Cells obtained in this manner were resuspended in Dulbecco's modified Eagle's medium with 44 mM $NaHCO_3$ and 15 mM HEPES, pH 7.2 to a final concentration of 3×10^5 cells/ml. Protein samples to be tested for the stimulation of migratory activity were diluted in this same medium to appropriate concentrations, and 25 μl of these samples were dispensed into blind well portions of the microchamber. Filters were overlayed onto the wells, the chambers assembled, and 50 μl of cell suspension were added to each top well of the assembly. Chambers were incubated for 4 h at 37°C in a humidified incubator with 5% CO_2. The filters were removed and cells on the filter were fixed and stained with Diff-Quick (Dade Diagnostics, Aguada, PR). The filters were cut in half and mounted with the bottom side down (containing migrated cells) onto 24 × 50-mm glass coverslips. Cells that had not migrated were removed from the upper surface with cotton swabs. After air drying, the coverslips were mounted onto glass slides and migration was quantitated by counting the migrated cells in 20 randomly selected high power (magnification, × 400) fields. Each sample was tested in triplicate and data represent the mean plus or minus the standard error of the mean (± SEM). Zigmond-Hirsch checkerboard analysis (32) was performed by incubating the cells in the chamber with various doses of laminin either on the bottom of the filter (to establish a positive gradient), on top of the filter (to establish a reversed gradient), or on both sides of the filter in equal concentrations (to examine accelerated random movement). Statistical significance of data was determined with a one-tailed Student's t-test.

Antiserum Preparation: Antiserum was produced in New Zealand white rabbits and was passed through successive affinity columns of basement membrane proteoglycan, type-IV collagen, and fibronectin. Antibody against laminin was then affinity purified over a laminin-Sepharose column, and concentrated to a final concentration of 170 μg/ml. The reactivity and specificity of the antibody was verified using an enzyme-linked immunoadsorbent assay, where it had a titer of 1:24,000 against laminin and no reaction against fibronectin or Type-IV collagen at 1:100.

Determination of Laminin on Filters: Laminin samples were prepared and dispensed into chemotactic chambers as for the cell migration assay. Chambers were assembled and incubated in the absence of cells for 4 h at 37°C in 5% CO_2-humidified atmosphere. Filters were removed, rinsed extensively in PBS, fixed for 30 min in 3% formalin, and rinsed in PBS with 0.05% Tween 20 (PBS-Tween). The filters were then incubated in a 1:100 dilution of rabbit antilaminin, rabbit antifibronectin, or normal rabbit serum for 45 min at room temperature. After washing in PBS-Tween, the filters were incubated in a 1:500 dilution of goat anti-rabbit immunoglobulin G conjugated with peroxidase (Cappel Laboratories, Cochranville, PA) for 45 min. After a final wash in PBS-Tween, bound antifibronectin or antilaminin was visually detected by incubating the filters in 12 μg/ml 3-3′diaminobenzidine as described (33). Filters were then inspected for the accumulation of peroxidase reaction product, indicating the presence of the antigen. The binding of radioactive laminin was quantified on filters following incubation in the chambers. Laminin isolated from the EHS tumor was radioactively labeled using the reductive methylation procedure of Jentoft and Dearborn (34). This preparation was diluted to concentrations used to stimulate migration (1.87–120 μg/ml) and incubated for various times in the chamber in the absence of cells. The filters were removed, rinsed extensively in PBS to remove unbound protein, and the areas of the filter exposed to protein were cut out and put into 3 ml of Aquasol II (New England Nuclear, Boston, MA). Bound radioactivity was quantified in a Beckman LS230 liquid scintillation counter (Beckman Instruments, Inc.,

[1] *Abbreviations used in this paper*: PVP, polyvinylpyrrolidone.

Fullerton, CA). The amount of laminin bound to the filter was calculated by the following formula:

$$\frac{\text{CPM } ^3\text{H on filter}}{\text{CPM total available } ^3\text{H}} \times \text{Amount protein in well} = \text{Amount protein bound to filter}$$

Precoating of Filters with Laminin: PVP-free 8.0-μm pore filters were precoated with 120 μg/ml laminin prior to the migration assay. The filters were coated on the lower surface only by floating the filter overnight on a solution of 120 μg/ml laminin in 0.1 M carbonate buffer, pH 9.6 at 37°C. Additional filters were immersed and incubated to coat both filter surfaces with laminin. Control filters were incubated in carbonate buffer in the absence of laminin. The filters were washed extensively in PBS prior to use in the migration chambers.

RESULTS

Migration of Schwannoma Cells to Laminin and Fibronectin

The data in Fig. 1 *a* show the migration of Schwannoma cells to the lower surface of PVP-free polycarbonate filters in response to laminin and fibronectin. The cells responded significantly to the lowest level of laminin tested (1.87 μg/ml) and the response increased in a concentration-dependent manner, with the peak response greater than 60-fold over control at 15.0 μg/ml of laminin. There was virtually no detectable migration in the absence of laminin. Concentrations of laminin that were in excess of that required to elicit the maximal response promoted a progressive decrease in cellular migration. In contrast, the response of these cells to fibronectin was significantly lower than the response to laminin. A slight increase (two- to fourfold) in RN22F migration compared with controls was observed at the higher concentrations of fibronectin tested (30 and 60 μg/ml), with essentially no cellular movement detectable at protein concentrations below this level.

The migration of these cells on gelatin-coated filters was also examined, to determine whether differences in migration observed between laminin and fibronectin might be due to an improper conformation of fibronectin bound on PVP-free filters (Fig. 1 *b*). These data demonstrate that migration of RN22F to fibronectin on gelatin-coated filters is somewhat improved (four- to sixfold) compared with cellular migration to fibronectin on a PVP-free filters, although the difference was judged not significant. Migration to laminin was somewhat less on gelatin-coated filters compared with that observed on PVP-free filters, although it is still significantly greater than the response to fibronectin in either situation. As in the case of PVP-free filters, concentrations of laminin higher than that required to promote a maximal response caused a progressive decrease in RN22F migration on gelatin-coated filters.

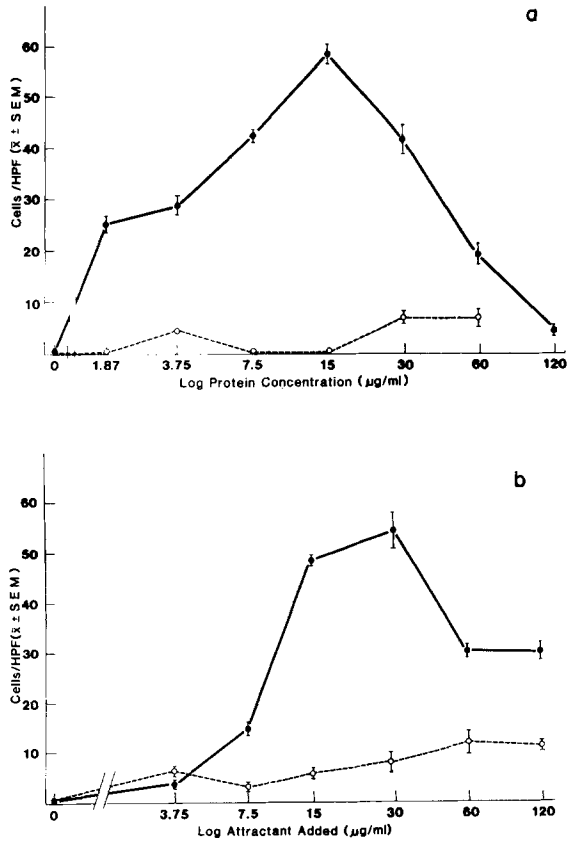

FIGURE 1 Migration of RN22F cells to dilutions of laminin and fibronectin. Cells were prepared as described, and incubated for 4 h in the presence or absence of laminin (●) or fibronectin (○). (*a*) RN22F migration on PVP-free polycarbonate surfaces. (*b*) RN22F migration on polycarbonate filter surfaces coated with gelatin. Determinations were made in triplicate and are expressed as the mean number of migrated cells per high-power field plus or minus the standard error of the mean (x̄ ± SEM). × 400.

Effect of Antilaminin Antibody on Migration

The addition of various dilutions of affinity-purified antilaminin antibody inhibited laminin induced migration of RN22F in a concentration-dependent manner (Fig. 2). The presence of the antibody appeared to have no obvious effect on the attachment of the cells to the upper surface of the filters (judged visually). Antibody alone in the lower well caused no migration of RN22F cells.

Checkerboard Analysis of Cellular Migration to Laminin

Checkerboard analysis of directed RN22F cell migration to

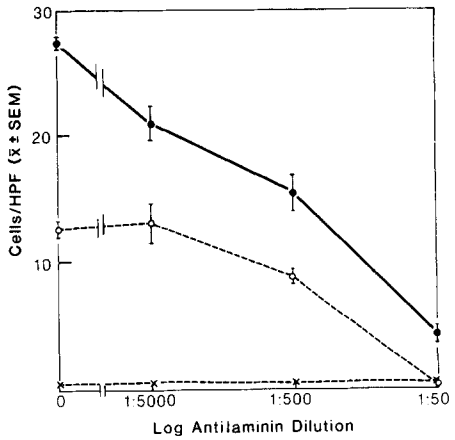

FIGURE 2 Effects of antilaminin antibody on Schwannoma migration. Protein samples were diluted and preincubated briefly with or without various concentrations of affinity-purified antilaminin antibody as indicated in the figure. These samples were then tested as described in the migration assay. Cellular migration to laminin concentrations of 3.1 μg/ml (●) and 1.5 μg/ml (○) is shown. Migration in response to antilaminin alone (×) is also shown. Determinations were made in triplicate and data are represented as the mean number of migrated cells per high-power field plus or minus the standard error of the mean (x̄ ± SEM). × 400.

increasing concentrations of laminin below the filters without attractant above (positive gradient) is depicted in the far left (bold face vertical column) of Fig. 3. The peak response occurred between 15 and 30 μg of protein, with a progressive decrease in migration observed as the laminin concentration was increased past this point. Random migration, which is the migration observed when the concentration of putative attractant is equal on both sides of the filter, is represented along the boldface diagonal. Laminin promoted accelerated random movement of RN22F cells, but this was quite reduced in comparison with the directed movement observed with stimulation only from the bottom (far side) of the filter. Maximal stimulation of directed migration was observed with 15 and 30 μg/ml laminin and was approximately twofold higher when compared with migration in the presence of these concentrations of laminin presented on both sides of the filter. Of particular interest in this experiment is that migration of these cells occurred when the gradient of laminin was completely reversed (i.e., attractant added with the cells on the top or near side of the filter, in the absence of additional attractant on the bottom of the filter). The data are shown by horizontal boldface type in Fig. 3. In contrast to results with positive gradients, the migration of RN22F cells in response to reversed gradients of laminin continued to increase throughout the concentration tested, with a plateau at the highest levels of laminin examined (60, 120 μg/ml).

Binding of Laminin and Fibronectin to Filters

The binding of both laminin and fibronectin to the PVP-free filters under the conditions used in the assay could be demonstrated qualitatively. Qualitative analysis of laminin and fibronectin binding to filters was performed using antibodies against the proteins followed by a peroxidase-mediated colorimetric reaction as described above. Visual inspection of the filters indicated that both laminin and fibronectin bound in equivalent amounts to the filters in an apparent concentration-dependent manner (data not shown). The binding of [^{3}H]laminin to the filters was also examined. The data shown in Fig. 4 represent the amount of [^{3}H]laminin bound to the filter following 90 min of incubation. Concentrations of [^{3}H]laminin were equivalent to those used for the migration assay. These results indicate that laminin binds to the filters in a concentration-dependent manner. The most rapid changes in binding occur at low levels of protein. The binding of laminin to PVP-free filters saturates with an apparent plateau at higher amounts of protein (1.5 to 3.0 μg protein). The binding of [^{3}H]laminin was also examined at earlier time points of 30 and 60 min. These studies indicate that laminin binds to the filters within 30 min, and that binding does not appear to increase dramatically from 30 to 90 min of incubation (not shown).

Laminin Concentration (μg/ml)

near \ far	0	7.5	15	30	60	120
0	**0.4**	**14.8**	**18.8**	**31.2**	**40.2**	**44.2**
7.5	**35.2**	**33.4**	41.6	33.0	39.2	31.4
15	**66.6**	35.2	**34.2**	45.2	46.6	29.4
30	**65.4**	32.2	35.8	**36.4**	24.8	27.2
60	**38.6**	35.4	27.2	24.6	**22.6**	29.6
120	**25.2**	18.4	21.8	21.6	15.8	**13.2**

Laminin Concentration (μg/ml)

FIGURE 3 Checkerboard analysis of laminin induced Schwannoma migration. Dilutions of laminin were prepared and added to either the lower or the upper wells at final concentrations indicated in italics in the table. Samples were dispensed into the lower wells, the chambers were assembled, and cells were added to the upper wells in the presence or absence of various concentrations of laminin as indicated. Boldface vertical data indicate migration of RN22F cells to concentrations of laminin in the lower wells in the absence of additional protein with the cells (directed migration). Schwannoma migration in the presence of equal concentrations of laminin above and below the filter is indicated by boldface diagonal data (increased random migration). Migration of RN22F in the presence of concentrations of laminin added with the cells without additional protein in the lower wells is shown in horizontal boldface data (reversed gradient migration). Determinations were made in triplicate and data represent the mean number of cells per high-power field. Standard errors of the means (not shown) were <10% of the mean values. × 400.

Migration of RN22F Cells on Laminin-coated Filters

The data in Fig. 5 show that RN22F cells will migrate in significant numbers on filter surfaces precoated with laminin in the absence of additional soluble laminin. Significant migration occurred when the filter was coated on the distal side only. This level of migration is reduced by ~50% when the same concentration of laminin was used to coat both the upper and lower filter surface.

DISCUSSION

The results of this study indicate that laminin promotes both directed and accelerated random migration of the RN22F Schwannoma cell line. Laminin preparations promote the movement of Schwannoma cells on gelatin-coated polycarbonate filters as well as on non gelatin-coated filters that lack the wetting agent, PVP. A previous report measuring neutrophil migration on PVP-free polycarbonate filters indicated that cells adhered well to PVP-free filters and that such surfaces afforded more reliable migration data than surfaces

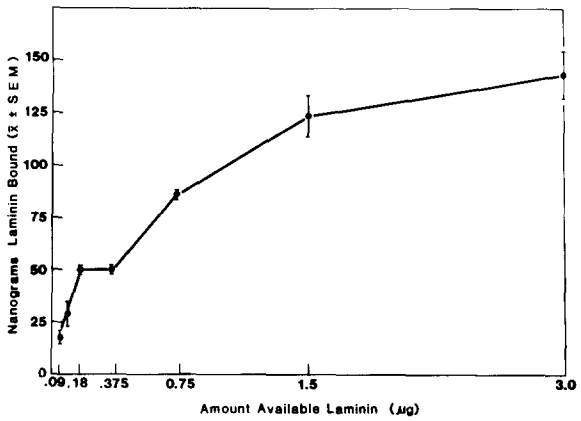

FIGURE 4 Binding of [^{3}H]laminin to PVP-free filters. Dilutions of [^{3}H]laminin were incubated in assembled chambers in the absence of cells for 90 min. Determinations of radioactivity bound to the filter were made in duplicate and are represented as the mean amount of bound laminin plus or minus the standard error of the mean ($\bar{x}$ ± SEM).

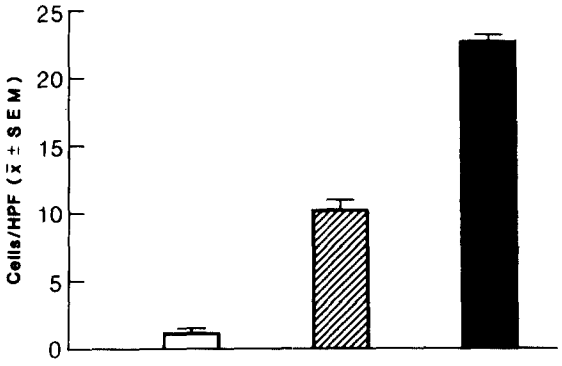

FIGURE 5 Response of RN22F cells to laminin-coated filter surfaces. Filters were precoated with 120 μg/ml laminin, as described, on either the far (distal) side (solid bar), both sides (hatched bar), or not at all (clear bar). Filters were washed and tested for support of RN22F migration in the absence of additional soluble attractant. Determinations were made in triplicate and values represent mean number of migrated cells per high power field plus or minus the standard error of the mean ($\bar{x} \pm$ SEM).

coated with PVP (31). We chose PVP-free filters for the current studies because the cells responded better on this filter surface compared with gelatin-coated surfaces. The reason for this difference could be due to less adherence of RN22F cells or to less binding of laminin to gelatin-coated filters compared with PVP-free surfaces. The addition of antilaminin antibody inhibited migration of Schwannoma cells. The lack of an obvious effect (judged visually) of this antibody on attachment suggested that the cells may attach to the filter surface by multiple mechanisms.

Checkerboard analysis (32) indicated that the response of these cells to laminin was complex. The data indicated that the cells migrated in the greatest numbers when attractant was added only on the far side of the filter (directed migration). Presentation of levels of attractant only from the far side caused progressively increased migration to a maximal level which was followed by a decline in the response. This type of deactivating response has been reported for other cell types and attractants. The cells were also observed to migrate in response to challenge with increasing laminin concentrations in the absence of an established gradient (increased random migration). This migration was approximately half that observed for directed migration. These two features of the checkerboard analysis are in agreement with similar studies on other cell types and chemoattractants (11, 14, 32, 35). In contrast to these reported studies, however, RN22F cells were observed to also migrate in a concentration-dependent manner to attractant added only on the near side of the filter, i.e., with the cells (reversed gradient). Response of the cells to a reversed gradient continued to increase to a point, past which a plateau was observed. We considered that migration in response to progressively increasing reversed gradients of attractant might indicate that laminin promoted negative migration (i.e., movement away from a stimulus) of RN22F cells. However, we would expect a negative stimulant to promote migration of cells in greater numbers when presented in a completely reversed manner compared with a completely directed (positive) manner, and this was not observed for laminin-induced RN22F cell movement.

There are several reasons that we conclude that RN22F cells are responding to substratum-bound laminin in this system (haptotaxis) as opposed to a fluid-phase gradient (chemotaxis). Immunolocalization studies (not shown) and studies of [^{3}H]laminin binding to the filter surface indicate that laminin binds rapidly in a concentration-dependent, saturable manner to PVP-free filter surfaces under the conditions used for the assay. Additionally, precoating of the filter with laminin prior to the assay was sufficient to promote RN22F migration in the absence of a further soluble stimulus. Importantly, it was observed that migration over coated filter surfaces could be affected by the way in which the filter was coated. Thus, precoating the filter only on the lower side promoted approximately twice the migration towards that surface as coating both sides with the same concentration of laminin. Interestingly, this difference was the same as that observed between directed and accelerated random migration in the checkerboard experiment. It therefore appears that RN22F cells can detect and respond to alterations in the distribution and amount of bound laminin. Finally, the difference in the response profiles of directed and reversed migration is consistent with the hypothesis that laminin binds to the filter surface prior to RN22F movement. RN22F migration against the gradient may indicate that these cells do not respond significantly to soluble laminin. The "reversed gradient" migration of the cells may reflect movement along laminin as it binds increasingly to the distal filter surface. Higher starting levels of attractant would diffuse more rapidly across the pore, bind to the lower surface, and promote progressive increases in cell movement, which was observed. Alternatively, presentation of increasing levels of laminin on the distal side, to form a positive gradient, would coat the filter in a progressively more uniform manner prior to the settling of cells on the upper filter surface. This increased uniformity would be predicted to cause relative decreases in cell migration, as indicated by results using a precoated filter. Thus, decreases in directed cell movement at high levels of attractant may reflect both cellular deactivation and more uniform binding of laminin.

Previous studies have addressed a role for substratum-bound attractants and neutrophils (22, 23). In this study, we believe that it is reasonable to distinguish the RN22F response as haptotaxis, in contrast to chemotaxis. As initially defined, haptotoaxis did not involve specific attractants but rather addressed nonspecific (i.e., charge) alterations in the substratum. In this case we conclude that specific attraction of the cells occurs as a result of substrate alterations due to laminin binding. The observation that laminin promotes the adhesion of these (27) as well as other (7, 8) cells indicates that adhesion gradients could be established as a result of the interaction of RN22F cells with bound laminin. Movement of cells across bound laminin could thus be accomplished by accumulation of receptors at the "leading edge" of the cell. Additionally, localized membrane-associated enzymes could digest and remove substratum-bound laminin at selective sites and thus contribute to the continued formation of adhesion gradients on this substrate. Obviously, further work is necessary to determine to what extent these as well as other factors contribute to laminin-induced RN22F migration.

In contrast to the results with laminin, fibronectin was observed to produce only a nominal increase in the migration of RN22F cells on PVP-free and gelatin-coated surfaces. Preliminary experiments in our lab (not shown) indicate that RN2 cells, the line of origin for the RN22F line, do respond in a highly significant manner to fibronectin (and laminin). The lower response of the RN22F cell to fibronectin thus

distinguishes it from the RN2 and normal Schwann cells (16). The difference in response of RN22F cells to laminin and fibronectin extends previous work from our laboratory (27) on the RN22F cell line. The preference of these cells for laminin compared with fibronectin is consistent with the idea that these proteins may interact with cell surface via different mechanisms, as has been proposed for other cell types (7).

Finally, by extending these findings, a role for laminin in the development and regeneration of the peripheral nervous system is implied, especially with respect to the proper establishment of myelin sheaths. An extensive study on in vivo myelination of peripheral nerve (36) demonstrated that migration of Schwann cells along the axon occurred prior to myelination. More recent work by Bunge and Bunge (37) also indicated that portions of elongating dorsal root axons, not in contact with a substrate, were associated with Schwann cells that were irregularly distributed along such portions of these axons. When proper contact was established between these axons and a collagen matrix, the Schwann cells were observed to migrate normally along the axon and to proceed with the myelination process. The ability of laminin to interact with glycosaminoglycans and/or collagens that accumulate in the matrix could provide a mechanism by which laminin directs the haptotactic migration of Schwann cells in vivo. As previously discussed (27), RN22F cells undergo a morphologic alteration after attachment to laminin. One can speculate that this morphological change is due to a haptotactic response and that such a mechanism could be involved in vivo in the progressive spiraling of the Schwann cell membrane around the axon during myelination.

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