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Dystroglycan Is Involved in Laminin-1-Stimulated Motility of Müller Glial Cells: Combined Velocity and Directionality Analysis

ELÖD MÉHES,[1] ANDRÁS CZIRÓK,[2] BALÁZS HEGEDÜS,[2] BÁLINT SZABÓ,[2] TAMÁS VICSEK,[2] JAKOB SATZ,[3] KEVIN CAMPBELL,[3] AND VERONIKA JANCSIK[1]*

[1]*Department of Anatomy and Histology, Faculty of Veterinary Science, Szent István University, Budapest, Hungary*

[2]*Department of Biological Physics, Faculty of Sciences, Eötvös University, Budapest, Hungary*

[3]*Howard Hughes Medical Institute, University of Iowa, Roy J. and Lucille A. Carver College of Medicine, Iowa City, Iowa*

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ABSTRACT We investigate the role of dystroglycan, a major laminin-1 receptor and central member of the dystrophin–glycoprotein complex, in the laminin-1 induced motility of cultured Müller glial cells. Binding of laminin-1 to dystroglycan was prevented by IIH6, a function-blocking monoclonal antibody against α-dystroglycan. As an alternative means of inhibition, we used heparin to mask the dystroglycan binding site of the laminin-1, known to overlap with heparin binding sites. Cell motility was characterized in a two-dimensional motility assay based on computer-controlled videomicroscopy and statistical analysis of cellular trajectories. We obtained data on both the cell velocity and the diffusion index, a measure of direction-changing frequency. Both means of inhibition of dystroglycan function led to a significant decrease in the ability of laminin-1 to stimulate cell migration. At the same time, dystroglycan function does not appear to be involved in laminin-1-dependent increase in process dynamism and direction-changing activity.

INTRODUCTION

Laminin is known to increase the motility of various cell types (Martin and Timpl, 1987), including Schwann cells (McCarthy et al., 1983; Milner et al., 1997) and retinal Müller glial cells (Mehes et al., 2002). Laminin was also shown to promote axonal growth in retinal ganglion cell cultures (Smalheiser et al., 1984; Cohen and Johnson, 1991) and to increase the process dynamism of cultured Müller cells (Mehes et al., 2002). Müller cells are macroglial cells of the retina playing crucial role in maintenance of retinal ionic and metabolic homeostasis. Spanning the entire thickness of the retina, Müller cells are characterized by extensive processes ensheathing individual synapses thus contributing to high signal-to-noise ratio of retinal neurotransmission. In the inner part of the retina, Müller glial process endfeet join to form a functional barrier, the inner limiting membrane, separating the neural retina from the vitreous body. Across this barrier, K^+ ions are released into the vitreal space through inwardly rectifying K^+ channels, contributing to K^+ spatial buffering (Reichenbach and Robinson, 1995).

The extracellular matrix (ECM) at the inner limiting membrane (ILM) of the retina is enriched in laminin-1,

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*Correspondence to: Veronika Jancsik, Department of Anatomy and Histology, Faculty of Veterinary Science, Szent István University, H-1400 Budapest P.O. Box 2, Hungary. E-mail: vjancsik@univet.hu

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consisting of α1, β1, and γ1 subunits. Other laminins, such as laminin-5 and two novel isoforms, laminin-14 and -15, are expressed in the inter-photoreceptor matrix and the ECM between photoreceptors and interneurons, but not in the ILM. Because of their expression pattern during retinal development, they are implied in photoreceptor differentiation (Libby et al., 2000). Besides the retina, laminin-1 is localized primarily in epithelial basement membranes and involved in epithelial morphogenesis (Ekblom et al., 2003). Laminin-rich migratory routes contribute to important guidance mechanisms during embryonic development (Duband and Thiery, 1987; Morissette and Carbonetto, 1995; Libby et al., 1996).

Two main types of laminin receptors have become known thus far: integrins and dystroglycan, a heterodimer consisting of an extracellular α and a transmembrane β subunit (Ibraghimov-Beskrovnaya et al., 1992; Dedhar and Hannigan 1996). Studies with embryonic stem cells indicate that both dystroglycan (DG) and β1 integrins are required for and play distinct consecutive roles in laminin-1 assembly on the cell surface (Henry and Campbell, 1999; Henry et al., 2001; Tsiper and Yurchenco, 2002). Müller glial endfeet at the laminin-1-rich inner limiting membrane of the retina were found to be enriched in DG (Claudepierre et al., 2000b), as well as α1, α2, and β1 integrins (Brem et al., 1994).

Integrins are one of the most studied receptor families (Critchley, 2000). After ligand-induced dimerization, they are known to build an intracellular complex (Katz et al., 2000). Depending on the physical properties of the substrate, binding of talin, vinculin, and α-actinin is followed by recruitment of regulatory molecules such as p125FAK, PAK, and paxillin. The complex is linked to the actin cytoskeleton via talin and α-actinin.

Relatively less is known about dystroglycan function: DG is known to bind a number of extracellular matrix constituents, such as laminin-1 (Ibraghimov-Beskrovnaya et al., 1992), laminin-2, perlecan (Talts et al., 1999), and agrin (Henry and Campbell, 1999). The ECM-DG binding leads to phosphorylation of tyrosine residues within the C-terminal domain of β-DG (James et al., 2000). These phosphorylation events appear to regulate the assembly of the dystrophin glycoprotein complex (DGC): incorporation of either dystrophin or utrophin (Sotgia et al., 2001; Ilsley et al., 2001, 2002), which in turn serve as a bridge for dystrobrevins and syntrophins (Durbeej and Campbell, 2002). The DGC can also bind proteins involved in signal transduction, including nitrogen oxide synthase (Gorecki et al., 1997), and p125FAK (Cavaldesi et al., 1999). Additional signaling pathways could be activated through recruitment of adaptor proteins such as Grb2. Recent studies demonstrate binding of β-DG to MEK2 and its active downstream effector, ERK, localized in adhesion complexes (Spence et al., 2004). An interesting interplay was implicated between signaling pathways mediated by DG and integrins (Ferletta et al., 2003).

This work is aimed at understanding the role of the DGC in the regulation of cell motility. In particular, we investigate the DG-mediated contribution to the laminin-1-stimulated motility response of cultured primary Müller cells. Müller cells are known to express many components of the DGC, including DG, Dp71f, the splice variant of the 71-kDa dystrophin protein, utrophin, α-dystrobrevin-1, α1-syntrophin, and δ-sarcoglycan (Claudepierre et al., 1999, 2000a,b). The DG-laminin-1 interaction was perturbed either by the function-blocking antibody IIH6 against the extracellular α-DG subunit (Ervasti and Campbell, 1991) or by masking the DG binding site on laminin-1 with heparin. Using computer-controlled videomicroscopy and statistical motility analysis, we demonstrate that both perturbations result in significant reduction of the laminin-1-stimulated motility. At the same time, DG does not appear to be involved in the laminin-1-stimulated direction-changing activity of migrating cells.

MATERIALS AND METHODS

Cell Cultures

Primary Müller glial cultures were established as described by Hicks and Courtois (1990) from retinae of 10-day-old Wistar rats by cutting retinae into small pieces under sterile conditions, followed by incubation in DMEM (GIBCO-BRL Life Technologies, Rockville, MD) supplemented with 10% fetal calf serum (FCS) (Sigma, St. Louis, MO) at 37°C in 5% CO_2, in tissue culture-grade 60-mm Petri dishes (Sigma). FCS was heat-inactivated at 56°C for 20 min. The medium was changed on the fourth day after Müller cells had migrated out of the retinal pieces and adhered to the culture surface. Residual retinal pieces were discarded upon change of medium. The medium was changed every 3 days thereafter.

The purity of the cultures was checked as described earlier (Mehes et al., 2002). In brief, cultures were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) (pH 7.4) and immunolabeled for vimentin intermediate filaments (anti-vimentin Clone V9; Boehringer-Mannheim GmbH, Germany) yielding strong characteristic immunoreaction in situ only in Müller cells within the retina. Cultures were also immunolabeled for Dp71f, the spliced 71-kDa dystrophin protein (using mAb 5F3; Fabbrizio et al., 1994), similarly expressed only by Müller cells within the retina (Claudepierre et al., 1999). All cells were positive for both markers. Cultures were negative for glial fibrillary acidic protein (GFAP) immunolabeling (anti-GFAP; Boehringer-Mannheim) indicating absence of astrocytes. In all cases, immunolabeling was visualized by fluorescein-labeled secondary antibody (fluorescein anti-mouse IgG (H+L), Vector Laboratories, Burlingame, CA), all according to the manufacturers' instructions. Müller cells were cultured for 8 days; the cells were then incubated with 5 mM EDTA in PBS (0.1 M phosphate, 0.9% NaCl, pH 7.4) for 5 min then washed

TABLE 1. Summary of Experimental Settings

Surface treatment	Untreated	Laminin-1	Laminin-1 + heparin	Heparin		
No. of cultures	2	2	2	2		
No. of cells tracked	205	208	277	206		
No. of positions recorded	14230	10333	14043	14513		
Surface treatment + antibody	Untreated	Untreated + IIH6	Untreated + XA7	Laminin-1	Laminin-1 + IIH6	Laminin-1 + XA7
No. of cultures	4	4	4	4	4	4
No. of cells tracked	136	128	77	203	216	113
No. of positions recorded	13679	11911	6349	11601	13986	7316

in DMEM 10% FCS. Cells were seeded at a density of 1×10^4 cells/cm^2 in 24-well tissue culture plates (Greiner).

Two sets of experiments were carried out. In the first, four experimental settings were used for videomicroscopy: cells were seeded in wells pretreated with laminin-1, or laminin-1 and heparin, or heparin, or PBS alone. In the second, six experimental settings were used: cells were seeded on either laminin-1-pretreated or untreated surfaces either in the presence or absence of antibodies. Two antibodies, IIH6 and XA7, were always used in parallel experiments separately (Table 1).

Preincubation of Culture Surface

The bottom of 24-well plates was pre-incubated with or without 20 μg/ml laminin-1 (EHS laminin; Sigma) in PBS for 24 h at 37°C. Wells were then incubated with 5,000 IU/ml Sodium-heparin (Becton Dickinson Vacutainer Systems Eur., France) in PBS for 20 min at room temperature. Incubations were followed by washing steps with PBS. Wells were thus coated either with laminin-1 only, or laminin-1 and heparin, or heparin only. Some wells were left non-incubated for control. Coating with laminin-1 or heparin was checked by atomic force microscopy. All cultures were kept in a medium containing 10% fetal bovine serum. It is known that serum contains molecules supporting cell attachment such as vitronectin. Therefore, these molecules attach to the surface of all cultures including the culture on surface without pre-incubation.

Inhibition of Dystroglycan–Laminin-1 Binding by Antibody

IIH6, a function-blocking monoclonal IgM class antibody against α-dystroglycan (Ervasti and Campbell, 1991; Durbeej et al., 1995; Brown et al., 1999, was used at a concentration of 60 μg/ml in the cell culture medium, which is sufficient for prevention of dystroglycan–laminin-1 binding (unpublished data). XA7, a monoclonal IgM class antibody against intracellular ryanodine receptor was used at the same concentration as negative control (Campbell et al., 1987), as it fails specifically to recognize cell surface receptors (also termed "negative control antibody" in Results).

Atomic Force Microscopy

Atomic force microscopy (AFM) was used to test whether laminin-1, heparin, or serum proteins covers the surface evenly after incubation of glass coverslips with 20 μg/ml laminin-1 for 1 h, or 5,000 IU/ml heparin for 20 min, or DMEM 10% FCS for 20 min, all followed by multiple washing steps in PBS. Glass coverslips were used since the plastic surface of the Petri dish is not suitable for acquisition of high resolution AFM images. After washing in PBS, laminin-1-covered surface was scanned both in PBS, without drying, and in air, after drying, yielding similar images. However, to acquire an AFM scannable surface, heparin covered surface was first fixed for 1 min with 4% paraformaldehyde in PBS and then dried and scanned in air. Serum treated surface was scanned in PBS without drying. The untreated surface was also scanned in air, for comparison. A commercial AFM (TopoMetrix Explorer, Santa Clara, CA) was used in contact mode with soft silicon nitride cantilevers (Thermomicroscopes, coated sharp microlevers, model MSCT-AUHW, with typical force constant 0.03 N/m, 20 nm nominal radius of curvature) at room temperature with a typical frequency of 5 Hz.

Computer-Controlled Videomicroscopy

Müller glial cells seeded in 24-well plates were kept in a plate incubator providing a stabilized temperature of 37 ± 0.5 °C, with 100% humidity and 5% CO_2/95% air atmosphere and optical transparency for microscopic observations (Czirok et al., 1998; Hegedus et al., 2000). The incubator was fastened to an inverted phase-contrast microscope (World Precision Instruments PIM, Sarasota, FL) equipped with a 20× objective and a powered stage moved by computer-controlled motors. Images of up to 24 microscopic fields were taken at every 10 min, using a CCD camera (Sanyo VCB-3170P, Sanyo, Japan) connected to the frame grabber card (Matrox Meteor, Matrox Electronic Systems, Canada) of a PC running under Linux operating

system. The stored jpeg (768 × 576 pixels, over 400 × 300 μm area) images were submitted to analysis then compressed into mpeg files thus creating accelerated time-lapse movies. Cells living in the incubator were monitored for up to 24 h. Experimental data are summarized in Table 1.

Cell Tracking

Images from the phase contrast microscope were analyzed individually with of a cell-tracking program (Xtrack, developed for this purpose in our laboratory) enabling manual marking of individual cells and recording their position parameters into data files. The precision of this tracking procedure is estimated to be 5 μm, to be compared with the average cell diameter (~50 μm). In the following, the position of the i-th cell at time t is denoted by $x_i(t)$.

Cell Velocity

The velocity, $v_i(t)$, of a given cell i at time t was calculated as

$$v_i(t) = |x_i(t + \Delta t) - x_i(t)|/\Delta t$$

with a suitably chosen Δt. For Δt that is too small, the error of the tracking procedure can mask the actual cell velocities. For Δt that is too large, we miss the "instantaneous" feature of the velocity values. Thus, we selected Δt = 4h, where the typical displacements are larger than 20 μm, hence larger than the error of the tracking procedure. To characterize the motility of an ensemble of cells, the average velocity v(t) and the velocity distribution function F(v) (Czirok et al., 1998) were calculated. Average velocity is given by

$$v(t) = 1/N(t)\Sigma_{i=1}^{N(t)} v_i(t)$$

where the summation goes over each N(t) cell in the cell population. The distribution function F(v) gives the probability that for a randomly chosen i and t the velocity $v_i(t)$ is larger than v.

Diffusibility

To characterize the persistence of cell motility, the average distance of cell migration, d(τ) (Stokes et al., 1991), was calculated for a range of elapsed time lengths τ as

$$d(\tau) = \langle |x_i(t + \tau) - x_i(t)| \rangle_{i,t}$$

where the average $\langle \cdots \rangle_{i,t}$ is taken over each possible choice of t and i. As the functional form of d(τ) is characteristic of the quality of the motion (linear, random walk, bounded diffusion), a diffusion index α(τ) was derived as

$$\alpha(\tau) = [\ln d(\tau)]'$$

with the prime denoting (numerical) derivative with respect to lnτ.

Statistical Analysis

Statistical quantities [F(v), d(τ),α (τ)] were calculated for each individual field analyzed. The resulting quantities were assigned to one of the groups depending on the type of experimental condition.

The groups were characterized (see Figs. 3 and 4) by the averages of the above statistical quantities, calculated for various parameter (v or τ) values.

The significance of the difference between the groups was established by Wilcoxon tests ($P < 0.05$). In the cases of d(τ) and α (τ), the corresponding values obtained from the various fields were used, for each τ. Thus, the relevant sample size was equal to the number of microscopic fields investigated. The F(v) distribution functions were compared using the velocity data $[v_i(t)]$ of the individual cells, from which the distribution functions were constructed.

RESULTS

The functional role of dystroglycan–laminin binding was studied in a motility assay. Müller cells, known to express dystroglycan (DG) on their cell membrane and respond to laminin-1 (Mehes et al., 2002), were obtained from rat retinae. Sister cell cultures were seeded on four types of substrates: laminin-1, heparin, the combination of both, as well as untreated substrate. Some of the cultures were exposed either to the function-blocking α-DG antibody IIH6 or the intracellular ryanodine receptor antibody XA7, which does not recognize any epitope on the cell surface, as a negative control. Subsequently, cells were tracked by videomicroscopy and their migration trajectories subjected to statistical analysis. (supplementary videos at http://angel.elte.hu/cellmotility/laminin)

Preincubation with laminin-1 or heparin, as well as the adsorption of serum proteins from the culture medium resulted in full surface coverage by these proteins with frequent aggregates, demonstrated by atomic force microscopy performed on glass substrates. Topographical differences of ~40 nm indicated a continuous multi-layered laminin-1 coverage for areas corresponding to a typical cell size.

Inhibition of Laminin–Dystroglycan Binding Reduces the Motogenic Effect of Laminin-1

The migration trajectories of 30 randomly chosen Müller cells were established from the recordings of

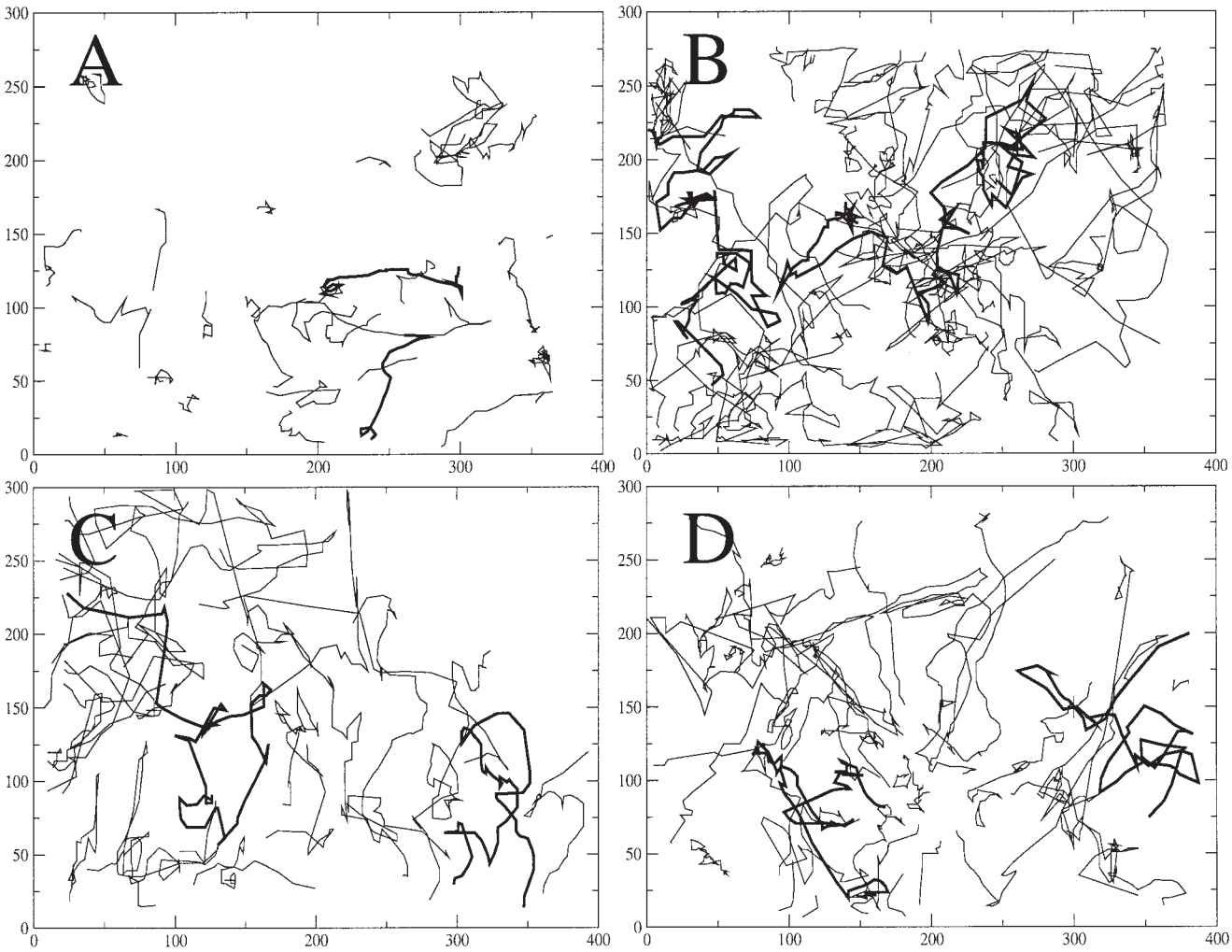

Fig. 1. Trajectories of Müller cells migrating for 20 h on untreated (**A**), or laminin-1-covered (**B**), or laminin-1-covered and heparin-masked surface (**C**), or laminin-1-covered surface in the presence of anti-DG antibody IIH6 (**D**), observed by videomicroscopy. Trajectories of 30 randomly selected cells are shown for each field. Two representative trajectories are plotted with thick line in all panels. Note the difference between trajectory lengths in D compared with B and the high number of turns in B–D. Scales are plotted in microns (μm).

each culture, displaying the usual fluctuating, irregular migratory activities (Fig. 1). As described earlier, Müller cells on laminin-1 substrate exhibit faster and less persistent motion than corresponding cells migrating on untreated surfaces. The increased frequency of direction-changing is intrinsic, as the laminin-1 coverage appears to be even on the scale of the cells.

To characterize the various cultures, we calculated average velocities, which include each cell in the entire observation period (Table 1). The effect of heparin masking or the antibodies was then quantified as changes in the average velocities compared with the corresponding sister culture on either laminin-1 or untreated surface (Fig. 2). The data show that both heparin masking and the blocking of DG function results in a laminin-specific reduction of cell motility: Müller cells migrating on either laminin-1 substrates in the presence of IIH6 or laminin-1 masked with heparin were all characterized by lower motility than cells on laminin-1. Their motility, however, was still higher than that of cells on an untreated surface. Heparin pre-treatment or IIH6 antibodies in the absence of laminin-1 did not result in significant changes in cell velocities. Finally, the negative control antibody XA7 had no effect on either laminin-1 or untreated substrates.

To investigate whether the observed effects were uniformly distributed in the entire cell population, we determined F(v), the cumulative distribution function of cell velocities, for the various sister cultures. F(v) gives the empirical probability of the event that the velocity of a randomly selected cell at an arbitrary time point will be greater than v. The velocity distributions obtained from six different experimental settings are shown in Figure 3. As is often the case for the migration of surface-attached cells (Czirok et al., 1998), F(v) is well approximated by an exponential distribution. As compared with cells on an untreated surface, cells migrating on laminin-1 either in the absence or presence of the negative control antibody have significantly in-

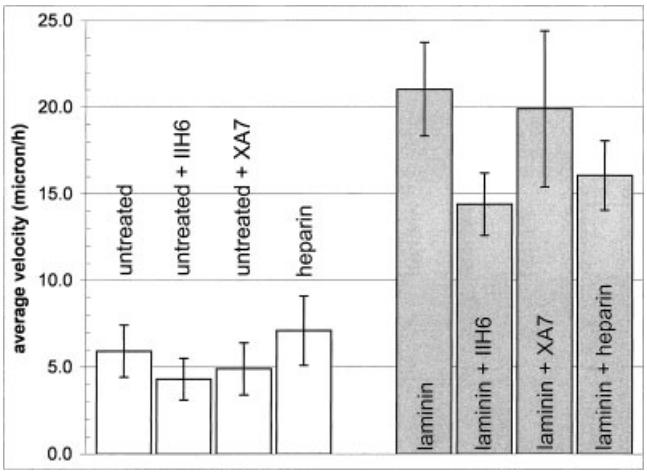

Fig. 2. Average velocities of Müller cells on different substrates. Note the similar reduction in velocity for cells on laminin-1 in the presence of anti-DG antibody (laminin + IIH6) and cells on laminin-1 masked with heparin (laminin + heparin).

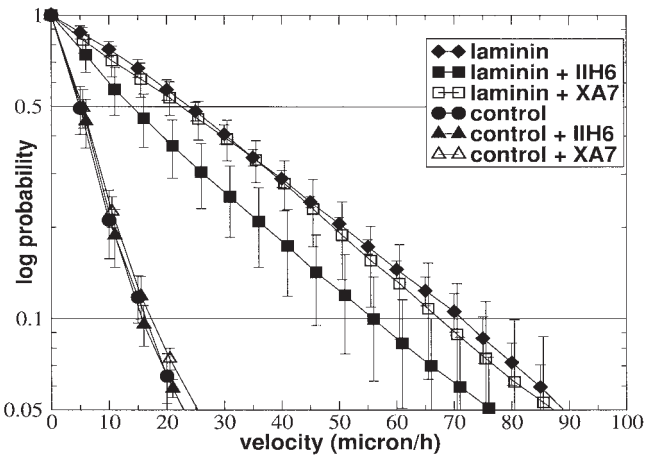

Fig. 3. Cumulative distribution function of cell velocities of Müller cells migrating on an untreated or laminin-1-covered surface in the presence or absence of anti-DG antibody (IIH6). Data from cells in the presence of the negative control antibody (XA7) not recognizing any epitope on cell surface are also shown for comparison. Probability of the event that a cell exceeds the velocity limit scaled on the horizontal axis is plotted in logarithmic scale on the vertical axis.

creased velocities. Anti-DG antibody significantly slows down the cells on laminin-1, but they are still significantly faster than cells on an untreated surface (in the presence or absence of any of the antibodies). The distribution of velocities remained exponential in all cases, suggesting that in each case the whole cell population was affected.

Laminin-Stimulated Direction-Changing Activity Is Independent of DG Function

To characterize the long-time features of motility, the average net displacement of cells, $d(\tau)$, was calculated in time frames with various lengths, τ, ranging

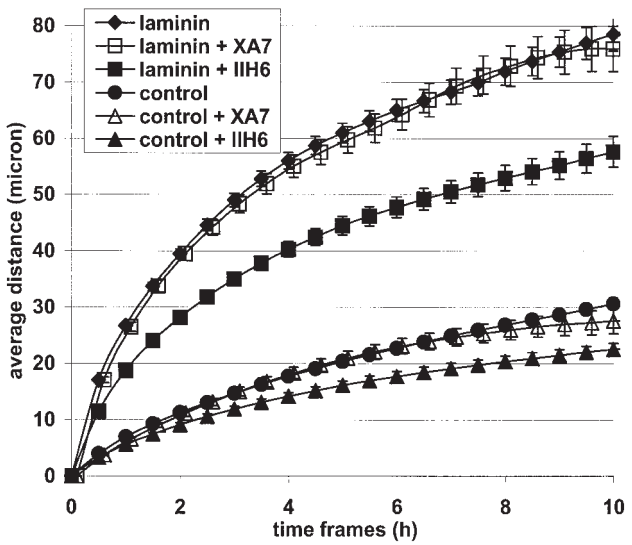

Fig. 4. Relationship of distance taken by cells and time frames of migration on untreated or laminin-1-covered surfaces in the presence or absence of anti-DG antibody (IIH6). Data from cells in the presence of the negative control antibody (XA7) is also shown for comparison. Note that distances of cells on laminin-1 + IIH6 are significantly shorter than distances on laminin-1 alone but significantly longer than distances on an untreated surface, in all time frames.

from 10 min to 10 h. Net displacements of each cell in the culture within all possible time frames of length τ were included in the average. As indicated in Figure 4, in agreement with the velocity data in Figure 3, cells on laminin-1 have significantly larger displacements in each time frame as compared with cells on an untreated surface. There is no significant difference between displacements of cells in the presence or absence of the negative control antibody, regardless of the surface coverage, i.e., laminin-1 or untreated surface. However, displacements of cells on laminin-1 in the presence of anti-DG antibody fall between the values of cells on laminin-1 and cells on an untreated surface with significant differences among these three groups.

The frequency of direction changes is a further important characteristic feature of cell trajectories and cell motility. The graph of the $d(\tau)$ function is characteristic of the persistence of the trajectory segments (Stokes et al., 1991). This is because cells with more frequent turns, and thus more curvy trajectories, will inevitably reach less far due to accumulation the distance decreasing effect of turns. In particular, the $d(\tau)$ function would be linear ($d(\tau) \sim \tau$) for an imaginary population of cells, each migrating in a straight line without changing direction. In contrast, $d(\tau) \sim \sqrt{\tau}$ for a mathematical random walk, where the direction of each step is an independent random variable. Finally, if a cell cannot freely migrate away from its position, $d(\tau)$ saturates for large τ values as $d(\tau) = \text{const}$. Thus, to address the impact of DG function on motion persistence, logarithmic derivatives, i.e., local power-law fits as consecutive slope values of the double-logarithmic plot, were calculated from the $d(\tau)$ functions. This quantity, the diffusion index $\alpha(\tau)$, is independent of cell

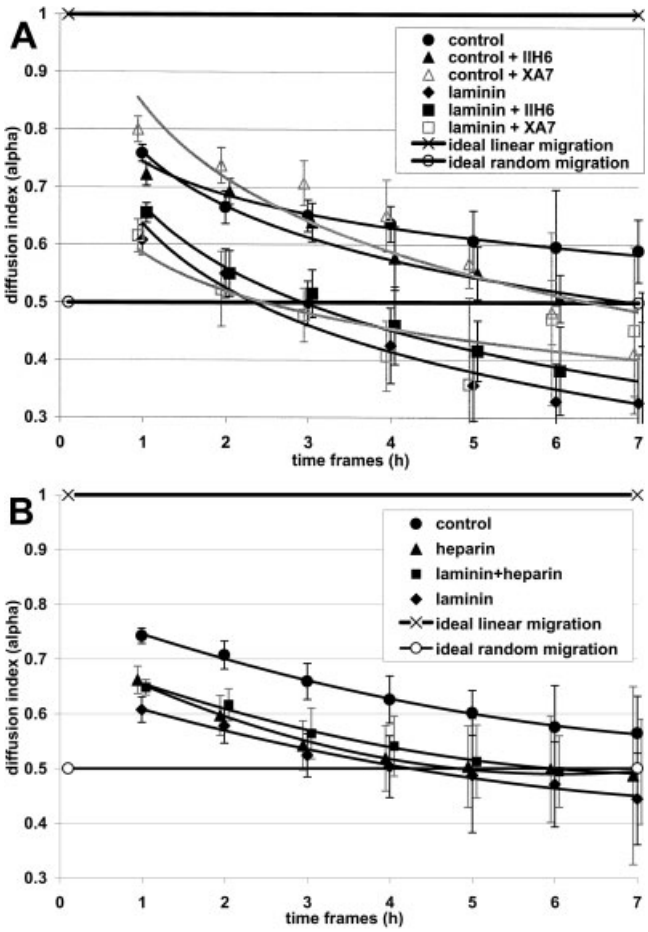

Fig. 5. Direction changing activity of Müller cells migrating on different substrates. Diffusion index curves of cells on an untreated or laminin-1-covered surface are shown in the presence or absence of anti-DG (IIH6) or "negative control" (XA7) antibodies (**A**). Curves of cells on laminin-1 or laminin-1 + heparin or heparin or untreated surfaces (**B**). Lines corresponding to linearly or randomly migrating imaginary cell populations with diffusion index (α) values of 1 or 0.5, respectively, are also shown. Curves representing Müller cells migrating on laminin-1 are approaching and reach the 0.5 value for α in shorter time frames of migration (persistence time) than the curve of control cells. Note the similar curves for laminin-1 and laminin-1 + IIH6 (A) and laminin-1 and laminin-1 + heparin (B). For a further explanation of diffusion index (α), see Materials and Methods or Results.

velocities and clearly separates the above three idealized cases yielding $\alpha = 1$ for the linearly directed migration, $\alpha = 1/2$ for random walk and $\alpha = 0$ for the "bound" cells.

As shown in Figure 5, the diffusion index for cells on laminin-1 is approaching ½, the value for ideal random migration, in shorter time frames and α values are significantly lower in all time frames as compared with cells on untreated surfaces. The time frame where the diffusion index curve of a given cell population reaches ½ is referred to as persistence time. In this time frame, the trajectory of an average cell of the population already contains a sufficient number of turns to be considered a random walk.

Neither of the diffusion index curves representing the two means of disruption of DG-laminin binding, IIH6 antibodies or heparin masking, differ significantly from the corresponding curve of cells on laminin-1 (Fig. 5A,B), indicating unaltered turning frequency. However, they are all clearly distinct from the curves of cells on an untreated surface. The presence of the negative control antibody has no significant impact on the diffusion index curves of cells on either laminin-1 or untreated substrates. Interestingly, pure heparin coverage also results in similar curves as laminin-1 with identical persistence times.

DISCUSSION

Dystroglycan–Laminin-1 Binding Increases Cell Motility

The presence of laminin-1 significantly increases the motility of Müller cells in our in vitro assay, as demonstrated earlier (Mehes et al., 2002). To determine whether dystroglycan, a major laminin receptor, is involved in the mediation of the motogenic effect, we perturbed binding of DG to laminin-1 by IIH6 antibodies (Ervasti and Campbell, 1991; Durbeej et al., 1995; Brown et al., 1999). IIH6 specifically blocks the laminin-1-binding site on the α-DG subunit, while the binding of laminin-1 to other receptors remains unperturbed. At the applied concentrations, the antibody is expected to saturate the available DG epitopes, (unpublished data) resulting in a complete block of DG receptor function. In our analysis of the motion statistics of a large number of cells (cell velocities in Figs. 2 and 3, average distances of migration in Fig. 4), we demonstrated that inhibition of DG-laminin-1 binding substantially reduces, but does not fully eliminate the laminin-1-stimulated motility. This result indicates that dystroglycan is involved in relaying the motility stimulating effect of laminin-1, but other laminin receptors, presumably integrins, also participate in the process.

Heparin Competes With Dystroglycan for Laminin-1 Binding

Heparin can bind the laminin-1 heterotrimer at a number of binding sites (Sorokin et al., 1992; Colognato-Pyke et al., 1995; Colognato and Yurchenco, 2000). The dystroglycan binding site is localized in one of the five C-terminal globular domains, termed LG4 domain, of the laminin α-1 chain. The LG4 domain is part of the E3 laminin fragment, and contains three heparin binding sites, mapped at amino acid positions 2766-2770, 2791-2793, 2819-2820 of the mouse laminin α-1 subunit (Andac et al., 1999), within the overlapping larger DG binding region. One of the heparin binding sites is also a DG binding site as mutation in the shared DG/heparin binding motif (KRK, in positions

2791–2793) was shown to perturb both heparin and DG binding, the latter being indispensable in embryonic differentiation (Li et al., 2002). None of the heparin binding sites, however, overlaps with any known integrin binding site. Heparin, therefore, is hypothesized to compete with dystroglycan for the same binding domain, whereas no such competition is evident between heparin and the integrins. This hypothesis is further supported by our data, which show that heparin-masking of laminin-1 results in the same motile behavior as the functional blocking of the dystroglycan receptor by IIH6 antibodies.

Reduction in the motility stimulating effect of laminin-1 by masking its dystroglycan binding site with heparin may have physiological relevance; i.e, heparan sulfate proteoglycans (HSPGs), such as agrin and perlecan, can indeed bind to the dystroglycan binding site and mask it from dystroglycan in vivo (Hohenester et al., 1999). The retinal basal lamina contains both agrin and perlecan besides laminin-1 (Dong et al., 2002) and the interplay of these molecules may have a role in stabilizing the Müller glial endfeet. These processes are tightly joined at the basal lamina and form the inner limiting membrane, a barrier structure separating the neural retina from the vitreous body. At the outer limiting membrane, Müller cell processes and photoreceptors are joined by junction structures and embedded in the inter-photoreceptor matrix, containing laminin-5 and laminin-14 and -15. As a similar interplay of EMC molecules, HSPGs expressed in spatially restricted manner have been shown to contribute to the guidance of retinal ganglion cell axons growing along a laminin-1-rich route to the optic chiasm in mouse or to the tectum opticum in *Xenopus* embryos (Chung et al., 2001; Irie et al., 2002).

Heparin coverage alone also brought similar changes in migration pattern of Müller cells as laminin-1 without increasing cell velocity (Figs. 2 and 5B), indicating that heparin directly influences the cell migration pattern by means not yet clarified. The mechanism of this effect may be the binding of heparin to vitronectin (Edens et al., 2001), a serum protein in the culture medium and an important factor for integrin-mediated cell attachment to the substrate (Schvartz et al., 1999).

Dynamics of Process Formation and Direction Changes

Cell migration is controlled by a vast network of proteins coupled to various biophysical processes (Lauffenburger and Horwitz, 1996; Borisy and Svitkina, 2000): ECM receptors function both as physical adhesion sites as well as initiators of intracellular signaling events. At the molecular level, lamellipodium formation is regulated by downstream targets of ECM receptor signaling, such as WASP, Arp2/3, and fascin (Welch, 1999). These proteins promote actin polymerization into a sheet-like network, as opposed to growing filopodia characterized by actin polymerized into fibers. The direction changes of cell migration are due to initiation of new cell processes or lamellipodia. These structures are unstable, unless adhesion sites are formed in sufficient number. In that case, the process can become a new leading edge, which results in an altered migration direction selected on seemingly random basis. For certain cell types the leading edge appears to be stabilized by an autocrine positive feedback loop involving epidermal growth factor signaling, which is clearly independent of the regulation of cell speed (Maheshwari and Lauffenburger, 2001).

Müller cells also exhibit random migration: cell trajectories are similar to Brownian motion when observed in longer time frames (Figs. 1 and 5). Irrespective of cell velocity, the more frequent the direction changes are, the shorter time frame (persistence time) is required for cell trajectories to exhibit random walk-like properties. Our results show that dystroglycan is involved in the complex regulation of cell velocity as inhibition of the binding of laminin-1 to dystroglycan reduces laminin-stimulated cell velocity. At the same time, dystroglycan does not appear to be involved in the regulation of process dynamism and directional stability of Müller cell migration.

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