

A role for the polysialic acid – neural cell adhesion molecule in PDGF-induced chemotaxis of oligodendrocyte precursor cells

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Summary

Directed migration of oligodendrocyte precursor cells (OPCs) is important for myelin formation and repair but the mechanisms of directional control are poorly understood. Here we have tested the role of polysialic acid-neural cell adhesion molecule (PSA-NCAM) in the directional migration of OPCs towards platelet-derived growth factor (PDGF). Using a Boyden microchemotaxis chamber and the Dunn direct viewing chamber, we show that in concentration gradients of PDGF, PSA-positive OPCs polarize and efficiently migrate towards the source of PDGF (chemotaxis). The loss or inactivation of the polysialic tail of NCAM leads to an altered pattern of OPC migration in response to PDGF gradients. Cells under these conditions, while being polarized and migrating, show no

bias of displacement towards the source of PDGF and make random turns. By contrast, directed migration of OPCs towards basic fibroblast growth factor was not affected by the removal of PSA. Moreover, inactivation of PSA does not interfere with the random migration pattern of cells in uniform concentrations of PDGF (chemokinesis). These results suggest that PSA-NCAM is specifically involved in establishing the directionality of OPC migration in response to the concentration gradient of PDGF, but it is not essential for cell motility per se.

Key words: PSA-NCAM, PDGF, Oligodendrocyte precursor, Cell migration, Chemotaxis, Chemokinesis

Introduction

During development, directed migration of oligodendrocyte precursor cells (OPCs) is essential for myelin formation in the CNS. OPCs are generated in the ventricular zone and then migrate over considerable distances to their destinations (for a review, see Lee et al., 2000). OPCs may also be guided to sites of injury in the adult brain where they contribute to myelin repair (Blakemore and Keirstead, 1999; Zhang et al., 1999). Although much has been learned about the molecular control of OPC motility, the mechanisms by which the direction of motility is established remains poorly understood. This is an important issue because random movement has little physiological significance; successful myelination or wound healing in the CNS requires a precisely timed concerted migration of OPCs in a precise direction.

Molecules that are involved in the control of OPC motility include growth factors, such as platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and hepatocyte growth factor (Armstrong et al., 1990; Milner et al., 1997; Yan and Rivkees, 2002). Of particular interest is the ability of OPCs to migrate towards a chemical gradient of PDGF, i.e. chemotaxis (Armstrong et al., 1990) *in vitro*, to stimulate motility of these cells in the optic nerve and to maintain OPCs in a bipolar migratory state (Noble et al., 1988; Milner et al.,

1997). Evidence indicates that the chemokine CXCL1 and its receptor CXCR2 inhibit OPC migration and play an essential role in glial patterning during embryonic development (Tsai et al., 2002). Recent studies have identified netrin-1 and the class 3 semaphorins as guidance factors for OPCs in the embryonic optic nerve (Sugimoto et al., 2001; Spassky et al., 2002; Tsai et al., 2003). Cell adhesion molecules such as integrins, cadherins and the neural cell adhesion molecule (NCAM), in particular its embryonic polysialylated isoforms (PSA-NCAM), have also been implicated in the control of OPC migration (Wang et al., 1994; Milner et al., 1996; Blaschuk et al., 2000). In the prevailing view of PSA-NCAM functions, the large negatively charged PSA chain is thought to be a spacer that reduces adhesion forces between cells, thereby allowing dynamic changes in membrane contacts and promoting morphological plasticity and cell movement (Rutishauser et al., 1988; Acheson et al., 1991). Consistent with this view, migratory OPCs express PSA-NCAM, whereas nonmigratory mature oligodendrocytes and astrocytes exhibit the adult, less-sialylated isoforms of NCAM (Trotter et al., 1989). Impaired migration of precursor cells has been reported in the NCAM knockout mouse or after removing or blocking PSA in diverse models (Ono et al., 1994; Wang et al., 1994; Hu et al., 1996; Wang et al., 1996; Chazal et al., 2000).

However, the exact role of PSA-NCAM in cell migration remains unclear.

We have previously shown that PSA-NCAM was required for the migration of OPC-like cells from neurohypophyseal explants in culture (Wang et al., 1994; Wang et al., 1996). More recently, using an *in vitro* scratch wound assay, we have provided evidence for a role of PSA in the migration of OPCs from the cerebral cortex (Barral-Morran et al., 2003). We showed that scratching a confluent monolayer of primary glial cells resulted in a rapid migration of OPCs into the wounded area. The treatment of wounded cultures with the enzyme Endo N, which specifically removes PSA from the cell surface, led to a significant decrease in OPC migration into the wounded area and completely blocked the wound closure. A simple explanation for these observations could be that the removal of the PSA tail from NCAM leads to an increase in adhesive forces, thereby inhibiting cell movement. Surprisingly, our time-lapse video analysis showed that OPCs in the presence of Endo N remained motile, but did not move away from the monolayer. We speculated that the loss of PSA from the cell surface could affect the directional responses of cells to guidance cues.

In the present study, we tested this hypothesis by focusing on the chemotactic responses of purified OPCs to concentration gradients of PDGF. We show that removing PSA from the cell surface or blocking PSA functions leads to a significant inhibition of PDGF-induced transfilter migration of OPCs in a Boyden microchemotaxis chamber. We also show, using the direct viewing Dunn chamber, that the directed migration of cells in a PDGF concentration gradient was abrogated after the removal of PSA. However, PSA-NCAM was not required for random migration. We propose that PSA-NCAM participates in establishing the directionality of OPC movement in response to PDGF gradients.

Materials and Methods

Purification and culture of OPCs

OPCs were purified by Percoll gradient centrifugation following the procedure described by Lubetzki et al. (Lubetzki et al., 1991) and Avellana-Adalid et al. (Avellana-Adalid et al., 1996). Briefly, the cerebral cortices from newborn Sprague-Dawley rats were first dissociated mechanically as described (Kiss et al., 1994). The obtained cell suspension was then trypsinized by 0.0025% trypsin-EDTA for 20 minutes at 37°C and filtered through 40 µm filters. After washing, cells were layered on a preformed Percoll gradient (23,500 g for 30 minutes at 4°C) and centrifuged for 20 minutes at the same speed. The fraction containing OPCs was recovered, washed, resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 4.5 g/l glucose, 10% fetal calf serum (FCS), penicillin and streptomycin (Sigma) (DMEM/10% FCS), and seeded onto poly-L-lysine- or laminin- (Sigma Saint Louis, Missouri) coated glass coverslips (2.7×2 cm) in 35 mm Falcon culture dishes (Falcon Plymouth, England). The cells were first layered in 100 µl of DMEM/10% FCS for 1 hour then plated in OPC culture medium [DMEM supplemented with 50 µg/ml transferrin, 100 µM putrescin, 30 nM sodium selenite, 1 mM sodium pyruvate, 10 ng/ml biotin, 20 nM progesterone, 180 U/ml insulin and 4.5 g/l glucose, 5 ng/ml PDGF, 10 ng/ml neurotrophin-3 (NT3)] and maintained in culture for 5-7 days (Milner et al., 1996). Culture medium was replaced every 2 days. All cultures were kept in a humidified incubator with an atmosphere containing 5% CO₂ at 37°C.

Antibodies and immunocytochemistry

Mouse monoclonal antibody (Ab) A2B5 (hybridoma supernatant, ATCC, Rockville, MD, 1:5 dilution) specific to cell-surface ganglioside epitopes was used to identify OPCs (Eisenbarth et al., 1979). Men B (Meningococcus group B) is a mouse IgM monoclonal antibody (mAb) (1:500 dilution) that recognizes specifically α 2-8-linked PSA with chain length superior to 12 residues (Rougon et al., 1986). Another anti-PSA Ab, mouse IgG m735 (2 µg/ml) (kindly provided by Rita Gerardy-Schahn, Hannover, Germany) was used to block the function of PSA at the cell surface during migration assay. Anti-GalC (Ranscht et al., 1982) mouse IgM mAb (culture supernatant, 1:5 dilution) was used to label differentiated oligodendrocytes. Neurons were identified by mAb directed against β-tubulin isotype III (1:400 dilution) (Sigma, St Louis, MO). Astrocytes were identified by labeling with a rabbit polyclonal Ab to glial fibrillary acidic protein (GFAP) (Dakopatts, Copenhagen, Denmark, 1:200 dilution). Texas Red-phalloidin (5 U/ml) and Oregon Green-DNase I (10 µg/ml) were used for F-actin and G-actin staining and purchased from Molecular Probes.

OPC cultures were fixed in cold (4°C) 4% paraformaldehyde in 0.1 M phosphate buffer overnight, then washed three times with phosphate-buffered saline (PBS), pH 7.2, and incubated with the primary Abs for 2 hours at room temperature or overnight at 4°C. Primary Abs directed against cell-surface antigens (A2B5, PSA, Gal C) were diluted in PBS/0.5% bovine serum albumin (BSA), and Abs against intracellular antigens were diluted in PBS/0.5% BSA/0.3% Triton X-100. The bound Abs were revealed with fluorescein-conjugated sheep anti-mouse Ig (dilution 1:80; Boehringer Mannheim Biochemicals, Germany) or rhodamine-conjugated sheep anti-rabbit IgG (dilution 1:40; Boehringer Mannheim Biochemicals, Germany) secondary Abs (diluted in PBS/0.5% BSA solution). Cells were examined with a fluorescence microscope (Axiophot; Zeiss, Oberlochen, Germany). Controls treated with nonspecific mouse IgM, or IgG pre-immune sera or secondary Abs alone showed no staining. In double immunolabeling experiments, the use of only one primary Ab followed by the addition of both anti-mouse FITC- and anti-rabbit TRITC-conjugated secondary Abs resulted in only single labeling.

For F-actin and G-actin staining, fixed OPCs were first permeabilized in 0.1% Triton X-100 for 10 minutes, washed in PBS and incubated in PBS/0.5% BSA/0.3% Triton X-100 for 2 hours at room temperature or overnight at 4°C. Cells were then incubated with a mixture of Texas Red-phalloidin and Oregon Green-DNase I for 15 minutes, washed with PBS and mounted. The staining was examined and photographed with a confocal microscope (LSM 510, Zeiss, Germany).

Viability of cells after treatment

The viability of cells under different conditions was determined by trypan blue exclusion. OPCs were grown in medium containing PDGF (5 ng/ml) and NT3 (10 ng/ml). After 5-7 days, the culture medium was replaced by fresh medium (control) or fresh medium containing Endo N (1:2000) or anti-PSA Ab (2 µg/ml) and the cells were incubated for 20 hours (with Endo N) or for 16 hours (with anti-PSA Ab). PDGF at 1 or 5 ng/ml and cytosine arabinoside (Ara-C) (Sigma Saint Louis, Missouri) at 5×10⁻⁶ M were included in the medium. The viability of cells was assessed and compared with control.

OPC differentiation assay after Endo N treatment

To exclude the possibility that the different migratory response to PDGF after Endo N treatment results from the cell differentiation, OPCs at 5-7-day culture were trypsinized, seeded in poly-L-lysine precoated glass coverslips. After incubation in PDGF (1 or 5 ng/ml), Ara-C (5×10⁻⁶ M), Endo N (1:2000) or without Endo N (control) for 20 hours, cells were processed for immunostaining with Abs against

A2B5, GFAP, Gal C and β -tubulin (not shown). The proportion of different cell types was determined by cell counting.

Migration assays

Boyden microchemotaxis chamber

Migration of OPCs under different conditions was first assayed in a microchemotaxis chamber (Armstrong et al., 1990; Behar et al., 1999). Briefly, a single cell suspension (50 μ l of 10^6 cells/ml) from 5-7-day cultures of OPCs was seeded in the upper chamber of each well on a poly-L-lysine precoated PVP-free polycarbonate membrane (8 μ m pore size, Poretics Products, Osmonics, Laboratory & Specialty Products Group, USA) and 30 μ l of OPC medium/0.5% FCS/Ara-C containing PDGF (recombinant human platelet-derived growth factor AA, purchased from Roche Molecular Biochemicals, Mannheim, Germany) or bFGF (Roche Molecular Biochemicals, Mannheim, Germany) was put in the lower compartment. Ara-C (5×10^{-6} M) was systematically included in the medium to stop cell proliferation. [Twenty-hour 5-bromo-2-deoxyuridine (BrdU) incorporation showed that more than 90% of OPCs at 5-7-day cultures were proliferative, whereas the presence of Ara-C decreased the dividing OPCs to less than 4.5%.] Cells were incubated for 16 hours at 37°C in a 5% CO₂ humidified incubator. After incubation, the chamber was disassembled, the upper side of the filter was wiped off and cells attached to the lower side were fixed in 4% formaldehyde/0.1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, then stained for 30 minutes in 0.1% cresyl violet and counted at 630 \times magnification in 20 randomly selected microscopic fields in six wells (each condition was run in six wells in each assay). Random cell motility (chemokinesis) was assessed by adding PDGF to both upper and lower wells of the chamber. As control, the migration of OPCs was assessed in OPC medium/0.5% FCS and Ara-C without PDGF.

To assess the effect of PSA on OPC migration, OPC cultures were incubated with either Endo N (1:2000-5000) for 6 hours or with anti-PSA Ab m735 (2 μ g/ml) for 30 minutes before the migration assay. Complete removal of PSA from the cell surface was confirmed by immunostaining. The same amount of Endo N or anti-PSA Ab was present in both the lower and upper wells of the Boyden microchemotaxis chamber during the migration assays.

Dunn chamber

OPC chemotaxis was directly viewed and recorded in stable concentration gradients of PDGF using the Dunn chemotaxis chamber (Weber Scientific International, Teddington, UK) (Zicha et al., 1991; Allen et al., 1998). This device is made from a Helber bacteria counting chamber by grinding a circular well in the central platform to leave a 1 mm wide annular bridge between the inner and the outer well. Chemoattractants added to the outer well of the device diffuse across the bridge to the inner blind well of the chamber and form a gradient. This apparatus allows the direction of migration in relation to the direction of gradient to be determined. In these experiments, the outer well of the Dunn chamber was filled with OPC medium containing 100 ng/ml PDGF and the concentric inner well with only medium. PDGF added to the outer well diffuses across the bridge to the inner well of the chamber and form a linear steady gradient within about 30 minutes of setting up the chamber with the half-life of the gradient (i.e. the time for its slope value to halve) about 30 hours (Zicha et al., 1991). Coverslips with OPCs were inverted onto the chamber and cell migration was recorded through the annular bridge between the concentric inner and outer wells. Data were recorded every 10 minutes for 6 hours using a Zeiss 10 \times objective via an Hamamatsu CCD video camera using Openlab software. For chemokinesis experiments, PDGF (5 ng/ml) was added in both outer and inner wells of the Dunn chamber. To evaluate the role of PSA on OPC migration, cells were pretreated with Endo N for 24 hours and Endo N was included in both outer and inner wells during recording.

Endo N-treated cells can re-express PSA in 16 hours and these cells were used for recording to study the reversibility of chemotaxis.

The directionality of cell movement was analyzed using scatter diagrams of cell displacement. The diagrams are oriented so that the position of the outer well of the chamber is vertically upwards (y direction). Each point represents the final positions of the cells at the end of the recording period where the starting point of the migration is fixed at the intersection of the two axes.

To determine the efficiency of forward migration during the 6 hour recording period, the forward migration index (FMI) was calculated as described previously (Foxman et al., 1999):

$$\text{FMI} = \frac{\text{Forward progress}}{\text{Total path length}}$$

Forward progress = net distance the cell progressed in the forward (towards the outer well) direction;

Total path length = total distance the cell traveled through the field.

FMI values are negative when cells move away from the source of PDGF (the outer well of the Dunn chamber).

The cell speed was calculated for each lapse interval recorded during the 6-hour period.

Analysis of actin cytoskeleton

OPCs were grown in 5 ng/ml PDGF, 10 ng/ml NT3 for 6 days, and then in 0.1% FCS for 12 hours in the presence or absence of Endo N. After starvation, cells were incubated for 10 minutes with medium, or with 0.5 and 5 ng/ml PDGF, fixed and fluorescently labeled with Oregon Green DNase I for G-actin and Texas Red-phalloidin for F-actin. Photographs were taken with a Zeiss confocal microscope.

Results

PSA-NCAM modifies PDGF-induced migration of OPCs in a microchemotaxis chamber

To test the role of PSA-NCAM in migratory responses of OPCs to PDGF, we set up a Boyden chamber-type assay using purified OPCs (Armstrong et al., 1990; Behar et al., 1999). Because PDGF stimulates both migration and proliferation of OPCs, Ara-C (5×10^{-6} M) was systematically included in the medium during the migration assay.

OPCs were isolated and purified from newborn rat cerebral cortex using Percoll gradient centrifugation as previously described (Lubetzki C et al., 1991; Avellana-Adalid et al., 1996). More than 90% of the cells in these preparations were A2B5+ and PSA-NCAM+ OPCs (Fig. 1). OPCs migrated through the filter at a low rate in the absence of PDGF (400 ± 15 cells per mm², $n=10$ independent experiments) (Fig. 2A). The addition of 1-5 ng/ml PDGF to the lower wells of the chamber (chemotaxis condition) resulted in a significant increase in OPCs migrated through the filter (Fig. 2A). The effects were dose dependent and the increase was over sevenfold at a PDGF concentration of 5 ng/ml. Migration induced by PDGF was significantly reduced if the growth factor was added to both top and bottom chambers, thereby eliminating the chemical gradient (random migration or chemokinesis condition) (Fig. 2B). These data confirm previous reports (Armstrong et al., 1990; Behar et al., 1999) that transfilter migration of OPCs in response to PDGF is due, at least in part, to chemotaxis.

To investigate the role of PSA-NCAM in this process, cell migration was assayed in the presence of Endo N, which selectively removes PSA from NCAM (Vimr et al., 1984). In

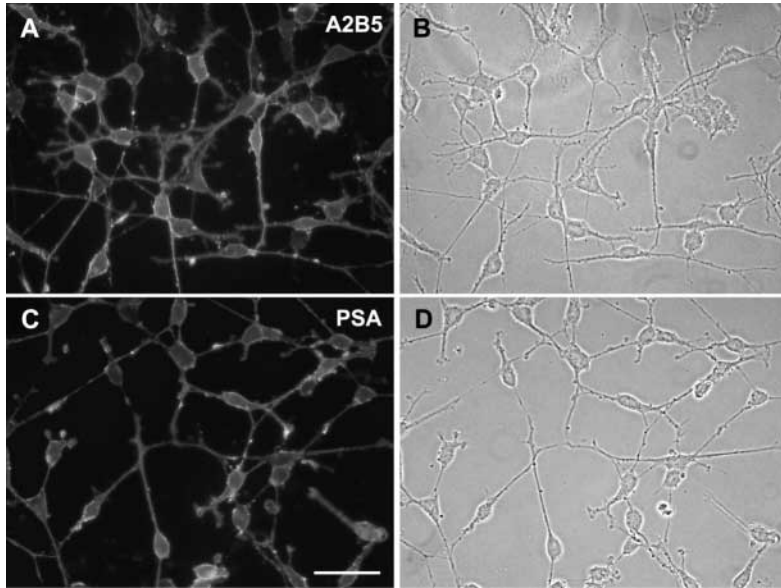


Fig. 1. Characterization of purified OPCs. Cells were purified by Percoll gradient centrifugation and cultured in the presence of PDGF (5 ng/ml) and NT3 (10 ng/ml) for 5 days. Immunofluorescence staining shows that most cells exhibit A2B5 (A) and PSA (C) immunoreactivity. (B,D) Phase contrast photographs corresponding to A and C, respectively. Bar, 40 μ m.

preliminary experiments, we verified that Endo N treatment for 2–20 hours in the presence of 1–5 ng/ml PDGF was sufficient to completely remove PSA immunoreactivity without any visible effect on NCAM immunoreactivity on OPCs. Consistent with previous reports (Wang et al., 1994), Endo N did not influence the survival of OPCs and did not cause visible changes in cell morphology. As shown in Fig. 2A, addition of Endo N to the medium produced a significant reduction in the migration of OPCs in response to PDGF when the growth factor was added only to the bottom chamber. The reduction

of cell migration towards PDGF at 5 ng/ml was over 50%. OPCs are known to differentiate into oligodendrocytes, or type-2 astrocytes depending on the culture conditions (Lillien et al., 1988). Because these cells are less motile than OPCs, we explored whether Endo N treatment could induce differentiation of OPCs that could cause the observed migration deficit. We observed that after 20 hours of Endo N treatment the percentage of A2B5+/GFAP–OPC, GalC+ oligodendrocytes and GFAP+ astrocytes did not change compared with controls (not shown).

In a second approach to establish a role for PSA-NCAM in PDGF-dependent migration of OPCs, an anti-PSA Ab m735 (2 μ g/ml) was used to block the function of PSA on NCAM. The specificity of this Ab has been extensively characterized in previous studies (Frosch et al., 1985). This antibody specifically binds to polysialic acid with eight or more residues (Frosch et al., 1985). OPCs were plated in the presence of m735 to the upper wells of the chemotaxis chamber and the same concentration of m735 was present in lower wells with PDGF. As shown in Fig. 2A, in the presence of anti-PSA Ab, the migration of OPCs was significantly inhibited. No alteration of the OPC migration towards the gradient of PDGF was observed when nonimmune serum was used at the same concentration (data not shown). The cell viability was not affected by the application of anti-PSA Ab as monitored by trypan blue exclusion (not shown).

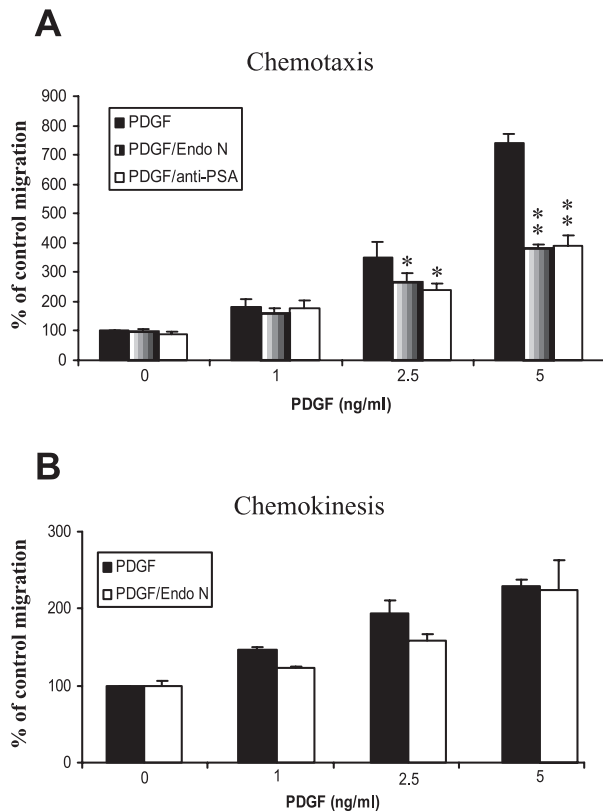


Fig. 2. Transfilter chemotaxis (A) and chemokinesis (B) of OPCs in response to PDGF. Purified OPCs were placed in the upper wells of the Boyden chamber, and cells that had migrated to the lower side of the filter were counted. (A) OPCs showed chemotaxis to PDGF placed in the lower well. This chemotaxis was inhibited by Endo N or by anti-PSA Ab. For Endo N-treated groups, cells were treated with Endo N for 6 hours before they were prepared for migration assay. In addition, Endo N was present in both upper and lower wells during the migration assay to prevent the recovery of PSA expression. For inactivation of PSA functions, anti-PSA Ab (2 μ g/ml) was also applied to both upper and lower wells of the chemotaxis chamber. Values are expressed as a percentage of control value, i.e. the migration of OPCs without PDGF (100% = 400 ± 15 cells per mm^2). Data represent the mean \pm s.e.m. from at least three independent experiments. * $P < 0.05$ and ** $P < 0.01$ by two-tailed unpaired t-test, compared with PDGF alone at the same concentrations. (B) PSA removal from the cell surface has no effect on random motility of OPCs stimulated by PDGF (chemokinesis). The same concentrations of PDGF were applied to both the upper and lower wells of the microchemotaxis chamber, so as to eliminate the gradients of PDGF. Chemokinesis of OPCs increases in the presence of PDGF, but PSA removal from the cell surface does not affect this process. Data represent the mean \pm s.e.m. from at least three independent experiments.

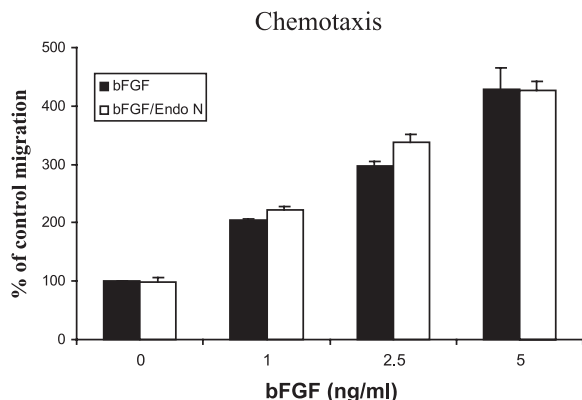


Fig. 3. Endo N treatment has no effect on bFGF-induced chemotaxis of OPCs. The experiments were carried out as in Fig. 2. Data represent the mean \pm s.e.m. from at least three independent experiments.

We also tested the effect of Endo N on OPC chemokinesis after the addition of the same concentration of PDGF to both upper and lower wells of the chambers. Fig. 2B shows that random motility was increased in the presence of PDGF by 50–100% compared with controls (without PDGF). This effect was not modified by Endo N treatment.

Because bFGF has been shown to induce chemotaxis of OPCs (Milner et al., 1997; Fok-Seang et al., 1998) as does PDGF, we examined whether the Endo N-induced deficit in migration could be a PDGF-specific effect or whether migration towards bFGF was also PSA-NCAM dependent. As shown in Fig. 3, the migration rate was increased by bFGF in a dose-dependent manner, but to a less extent than by the same concentration of PDGF, especially at 5 ng/ml. The results also show that bFGF-stimulated OPC migration was not affected by the removal of PSA from the cell surface. Thus, Endo N-induced deficit in directional migration appears to be specific for PDGF-induced chemotaxis.

Together, these experiments show that the loss or inactivation of PSA-NCAM leads to impaired transfilter chemotaxis of OPCs in response to PDGF. They also suggest that PSA-NCAM is not required for chemokinesis of OPCs.

PSA-NCAM is required for the directional migration of OPCs in response to PDGF

In the above experiments, it was impossible to visualize OPCs while they were responding to PDGF with or without PSA-NCAM, and the evaluation of chemotaxis has been deduced from the final distribution of cells at a defined timepoint. To observe the migratory behavior of cells directly by time-lapse video microscopy, we took advantage of the direct-viewing Dunn chemotaxis chamber (Zicha et al., 1991; Allen et al., 1998). This apparatus allows for the direct monitoring of cell locomotion and the analysis of migration speed, turning behavior and directionality of migration. It has been established that chemoattractants added to the outer well of the Dunn chamber diffuse across the bridge to the inner well of the chamber (Fig. 4) and form a linear steady gradient within about 30 minutes of setting up the chamber (Zicha et al., 1991; Webb et al., 1996). The gradient remains stable for

about 30 hours thereafter (Zicha et al., 1991; Webb et al., 1996). To study the chemotaxis of OPCs, the outer well of the Dunn chamber was filled with medium containing different concentrations of PDGF and the concentric inner well was filled with medium only. Coverslips with OPCs were inverted onto the chamber and cell locomotion (Fig. 4A–C) was recorded over one part of the bridge region. In preliminary experiments, we determined that concentration gradients established with 100 ng/ml PDGF induced strong positive chemotaxis of OPCs, and a period of 6 hours was chosen to assess cell migration. The scatter diagram of cell displacement after 6 hours in Fig. 4D shows a strong directional bias towards the source of PDGF. Consistent with previous observations, OPCs had an elongated bipolar shape, exhibited a growth-cone-like structure at the end of the main processes and often showed preferential alignment along the axis of the PDGF gradient (Fig. 5A). By comparison, when PDGF was added to both inner and outer wells (chemokinesis conditions), cells remained motile and traveled about the same distance as during chemotaxis, but the population as a whole showed no clear preference in displacement (Fig. 4E).

The addition of Endo N to the medium significantly altered the pattern of migration of OPCs in response to PDGF gradient. Cells under these conditions, while being polarized and migrating, showed no bias of displacement towards the source of PDGF (Fig. 4F). This effect was observed on poly-L-lysine-coated as well as on laminin-coated coverslips.

Examination of the individual cell tracks indicates that while the great majority of cells under control conditions maintained the direction of migration up the PDGF gradient, cells without PSA-NCAM made random turns and lost the directionality of migration towards the source of PDGF gradient (Fig. 5A,B). This observation has been confirmed by the quantitative analysis of cell migration. To measure the efficiency of directed cell migration, we calculated each cell's forward migration index (FMI) – that is, the ratio of the most direct distance the cell progressed towards the gradient source to its total path length (Foxman et al., 1999). The FMI of cells subjected to linear gradient of PDGF was significantly larger than that of cells exposed to Endo N or to a uniform concentration of PDGF (chemokinesis) (Fig. 6). However, the average velocity of cells, 40–45 $\mu\text{m}/\text{hour}$, was similar under all three conditions (Fig. 6). We then investigated whether higher concentrations of PDGF could reverse the effect of Endo N on the directionality of migration of OPCs. Our results revealed that the directionality of migration was completely recovered (Fig. 4I, Fig. 6), and normal chemotaxis of OPCs was observed when PDGF was used at 400 ng/ml.

We also tested the reversibility of the effect of Endo N. Cultures were treated with the enzyme in the presence of PDGF and Ara-C for 24 hours, and then washed and maintained in medium with PDGF, NT3 and Ara-C without enzyme for an additional 16 hours. Cells were then exposed to PDGF gradient for 6 hours. In agreement with previous observations, we found that Endo N-treated OPCs re-expressed PSA at the cell surface 16 hours after Endo N was washed out (not shown). Moreover, these cells migrated up the PDGF gradient similarly to control non-Endo N-treated cells (Fig. 7). The average FMI of 55 analyzed cells was not significantly different from that of control cells (Fig. 6). Therefore, we conclude that Endo N does not induce irreversible changes in the ability of cells to

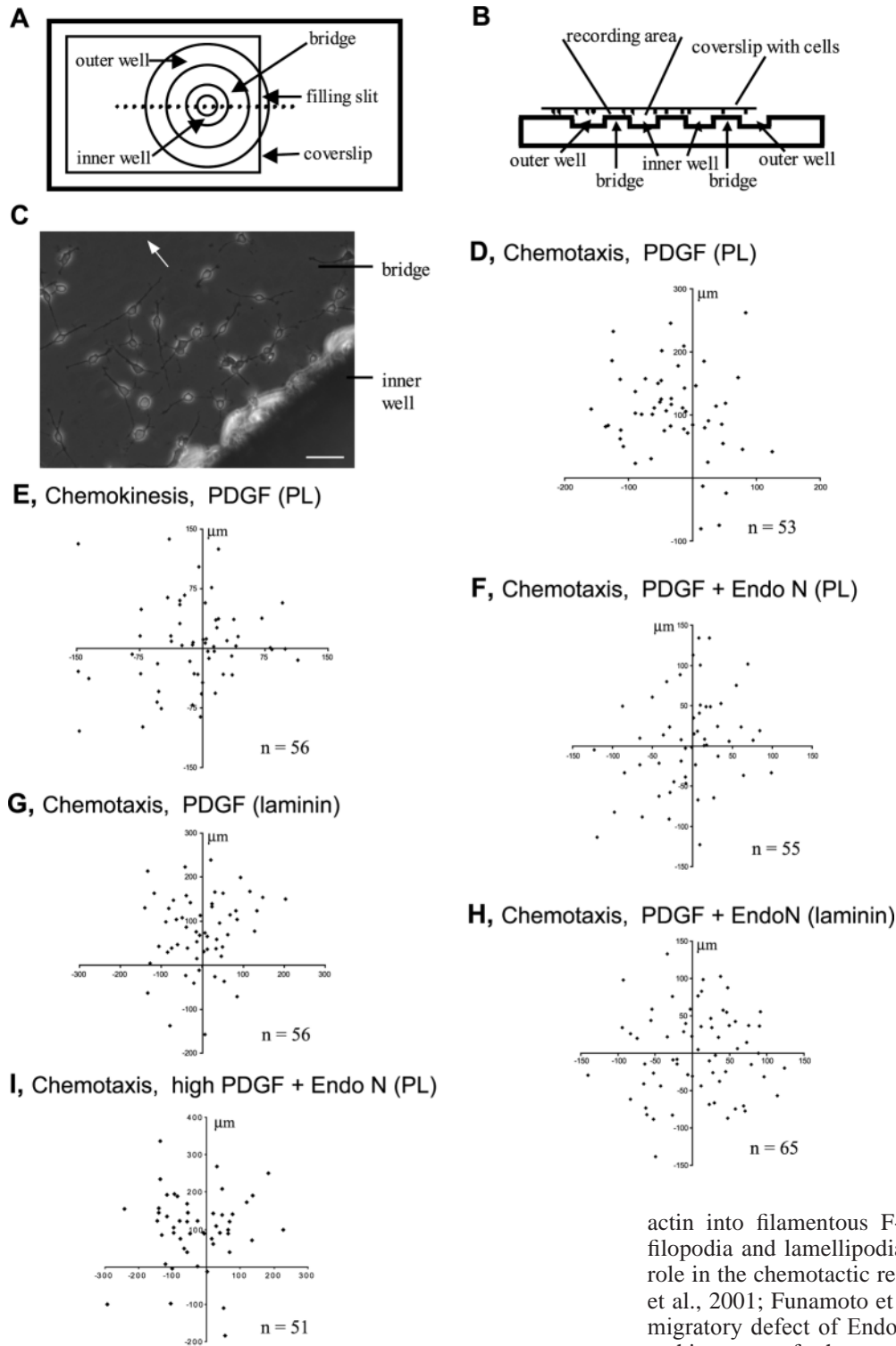


Fig. 4. Effect of Endo N on the directional migration of OPCs in response to PDGF. Top view (A) and cross section (B) of Dunn chamber (drawn after Allen et al., 1998) with the overlying coverslip show the position of the inner well, bridge and outer well of the chamber can be observed under phase-contrast optics (as shown in C). Cell migration was recorded automatically by time-lapse frame grabbing and the migration tracks were plotted in scatter diagrams (D-I) as described in Materials and Methods. The starting point for each cells is at the intersection between X and Y axes (0, 0), and data points indicate the final positions of individual cells at the end of the 6 hour recording period. Chemotaxis on poly-L-lysine (PL) (D-F,I) or laminin (G,H) was tested by placing PDGF at 100 ng/ml (D-H) or 400 ng/ml (I) in the outer well. The direction of the gradient is vertically upwards. In the presence of PDGF gradient, OPCs migrate toward PDGF and display clear directionality of migration (D,G,I). For chemokinesis (E), equal amounts of PDGF (5 ng/ml) were added in both inner and outer wells of the chamber. After PSA removal by Endo N treatment, these cells lose the chemotactic response to PDGF and migrate randomly (F,H). Arrow in C indicates the direction of the outer well of the Dunn chamber. Bar, 50 μm .

chemotax in response to PDGF. This is consistent with our observation in the Boyden chamber that Endo N treatment does not induce irreversible changes in the differentiation state of OPCs under our experimental conditions.

These experiments thus reveal that the PSA chain on NCAM is not required for the motility of OPCs, but it is essential for the directional movement in response to concentration gradients of PDGF.

Lamellipodial extension in response to a low concentration of PDGF is reduced by PSA removal

Cell migration involves the polymerization of monomeric G-actin into filamentous F-actin, which is a prerequisite for filopodia and lamellipodia formation and plays an important role in the chemotactic responses of cells (Hall, 1998; Chung et al., 2001; Funamoto et al., 2002). To better understand the migratory defect of Endo N-treated OPCs, we examined the architecture of the cytoskeleton and the formation of lamellipodia in response to PDGF stimulation by fluorescent staining. Oregon Green DNase I was used for G-actin staining and Texas Red-phalloidin for F-actin staining. In agreement with a previous report (Simpson and Armstrong, 1999), when cells were maintained in the absence of PDGF for 12 hours, F-actin in OPCs was predominantly expressed in growth-cone-like structures, lamellipodia and in the cell periphery (Fig. 8A). Fluorescence labeling indicative for G-actin was present both in cytoplasm and in cellular extensions. Under

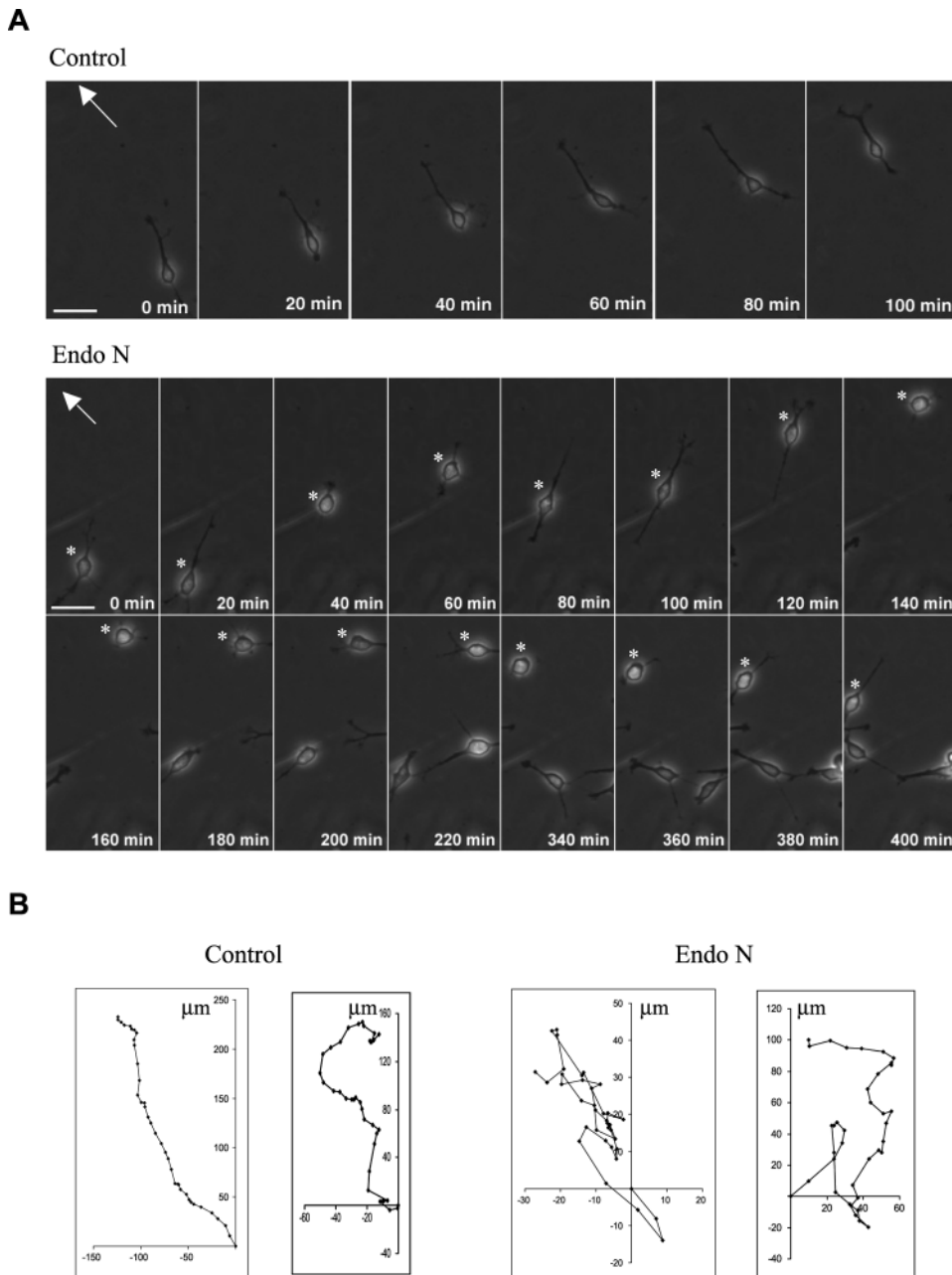


Fig. 5. Migration tracks of control and Endo N-treated OPCs.

(A) Representative time-lapse sequences of OPC migration under control conditions and in the presence of Endo N. Arrows indicate the source of PDGF. It is evident that control cells maintain the directionality of migration towards the source of PDGF, whereas Endo N-treated cells make random turns. Asterisks indicate migrating cells.

(B) The migration tracks of two representative control cells and two Endo N-treated cells in the presence of PDGF gradient, taking the starting point for each cell at the intersection between X and Y axes (0, 0) and the source of PDGF at the top. Bars, 30 μm.

Discussion

Directed migration of OPCs is critically important for myelin formation and repair in the CNS and thus much effort has been devoted to identify molecules that control locomotion (Noble et al., 1988; Milner et al., 1997), adhesion (Trotter et al., 1989; Wang et al., 1994; Milner et al., 1996; Wang et al., 1996; Blaschuk et al., 2000) and guidance (Sugimoto et al., 2001; Spassky et al., 2002; Tsai et al., 2003) of these cells. To migrate directionally, OPCs must sense accurately guidance cues, establish polarity, extend forward protrusion and organize differential adhesion to create traction at the front and detachment at the rear. Despite its importance, our understanding of how these cells establish and maintain directionality of migration remains fragmentary. The present study was carried out to elucidate the role of the adhesion molecule PSA-NCAM in this process. We used in

these conditions no obvious differences between control and Endo N-treated cells were revealed by phalloidin staining of actin filaments (Fig. 8A,B). However, although a low concentration (0.5 ng/ml) of PDGF caused substantial extension of lamellipodia at the cell periphery of control cells (Fig. 8C,E), lamellipodia formation was dramatically reduced in Endo N-treated OPCs after 10 minutes stimulation (Fig. 8D,F). By contrast, no difference was observed between control and Endo N-treated cultures in terms of lamellipodia formation in response to higher concentrations of PDGF (5 ng/ml) (Fig. 8G,H). These results confirm our observations in the Dunn chamber that Endo N-treated cells are able to extend filopodia and lamellipodia in response to PDGF. They also suggest that after removal of PSA, OPCs are less responsive to PDGF.

vitro models that allowed us to focus on directional migration responses of OPCs to PDGF in the absence of many other factors, such as cell-to-cell and cell-to-matrix interactions, which undoubtedly contribute to the migration of these cells in vivo. Our experiments revealed that PSA-NCAM is required for the directional migration of OPCs in response to concentration gradients of PDGF. However, PSA-NCAM was not essential for cell locomotion, indicating that this molecule is not a component of the basic cellular machinery for movement/adhesion. These findings support the contention that PSA-NCAM in OPCs is part of a regulatory network required for the adequate sensing and response to guidance cues.

To explore the role of PSA-NCAM in migratory responses of OPCs to PDGF, we adapted previously described chemotaxis chambers. Using a Boyden microchemotaxis

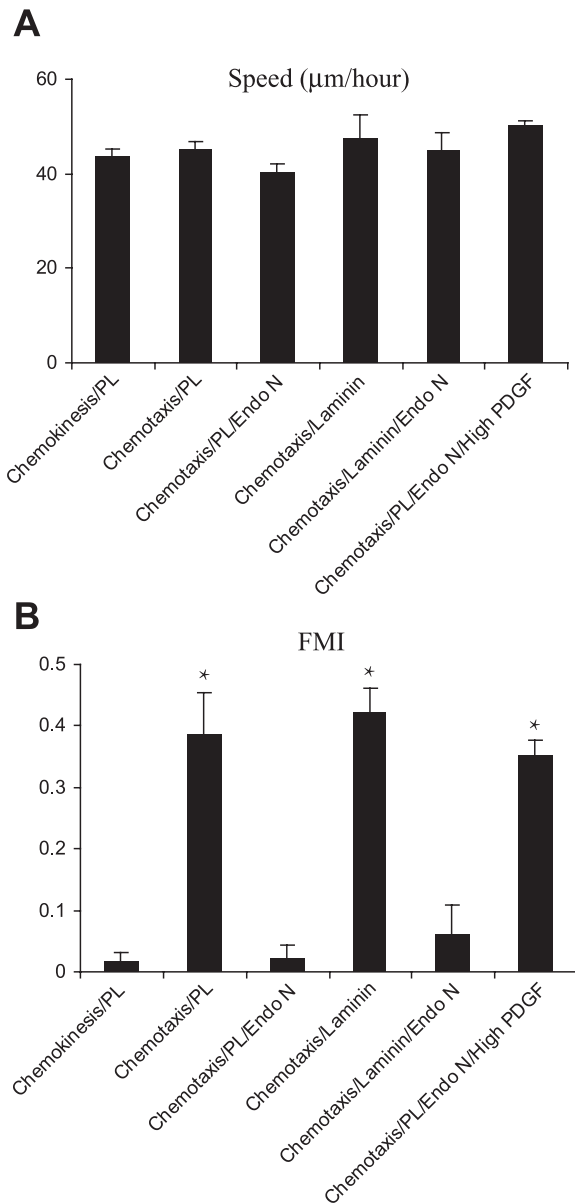
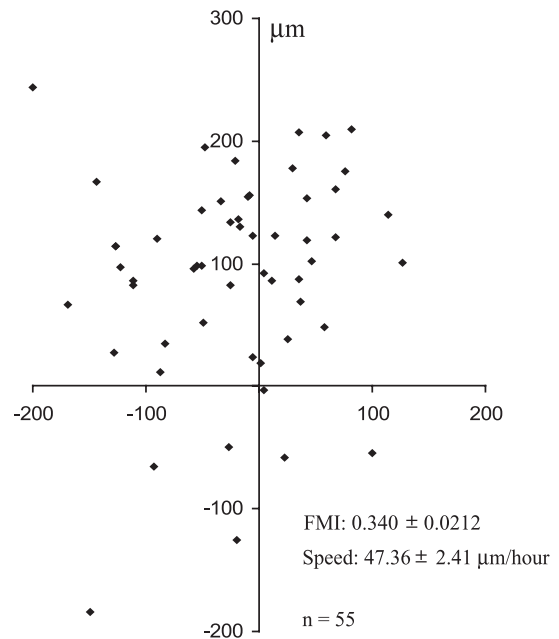


Fig. 6. The migration speed ($\mu\text{m}/\text{hour}$) and FMI values under different conditions. The cell migration speed was calculated for each lapse interval and the mean speed was derived for a period of 6 hours. Data are shown as mean \pm s.e.m. from at least three independent experiments (the numbers of the counted cells were indicated in Fig. 4). FMI values can be either positive or negative, depending on the direction in which cells migrate (see Materials and Methods). PL, poly-L-lysine. * $P < 0.01$ by two-tailed unpaired t-test, significantly different from chemokinesis or chemotaxis with Endo N.

chamber, we confirmed the findings of Armstrong et al. (Armstrong et al., 1990) and Simpson and Armstrong (Simpson and Armstrong, 1999) that A2B5 and PSA-NCAM-positive OPCs are migratory and respond positively to PDGF. Recently, Spassky et al. (Spassky et al., 2001) reported the existence of PDGF α -negative and -positive subpopulations of OPCs in the telencephalon in vivo. However, the OPCs we used in this study were grown and expanded with PDGF-A, and most of these cells are positive for the PDGF α receptor by



Experimental Protocol

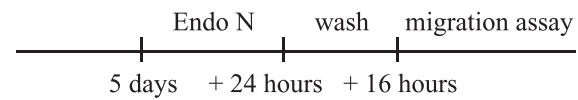


Fig. 7. Scatter diagram showing the reversibility of the effect of Endo N treatment on OPC chemotaxis. Before the migration assay in Dunn chamber, cultures were treated with Endo N in the presence of Ara-C (5×10^{-6} M) and PDGF (5 ng/ml) for 24 hours, subsequently washed and further cultured in normal medium with PDGF (5 ng/ml), NT3 (10 ng/ml) and Ara-C for 16 hours. The migration assay was performed as described in Materials and Methods. The final positions of cells after 6 hours of migration were indicated with the starting point for each cell at (0, 0), with the source of PDGF (100 ng/ml) at the top. Note that cells have normal chemotactic responses to PDGF gradient in contrast to cells kept in the presence of Endo N (Fig. 4). Data from four independent experiments are shown. Speed and FMI represent the mean \pm s.e.m. from these four independent experiments.

immunostaining (not shown). Thus, it is very unlikely that a significant number of PDGF α -negative cells are present in our cultures. In line with earlier studies, we showed that PDGF not only stimulates OPC motility, but also it is able to induce a positive chemotaxis of these cells in concentration gradients. In the present studies we extended these data and found that transfilter migration of OPCs in response to PDGF gradients was significantly decreased when PSA had been destroyed by Endo N. This effect was not due to an altered survival, differentiation or proliferation of cells. Moreover, the effect of PSA removal on cell migration appears to be PDGF specific under these experimental conditions, as FGF-induced OPC migration was unaffected by Endo N. The results obtained in the presence of Endo N was confirmed by a second experimental approach; when transfilter migration was tested in the presence of anti-PSA Ab, we observed a significant

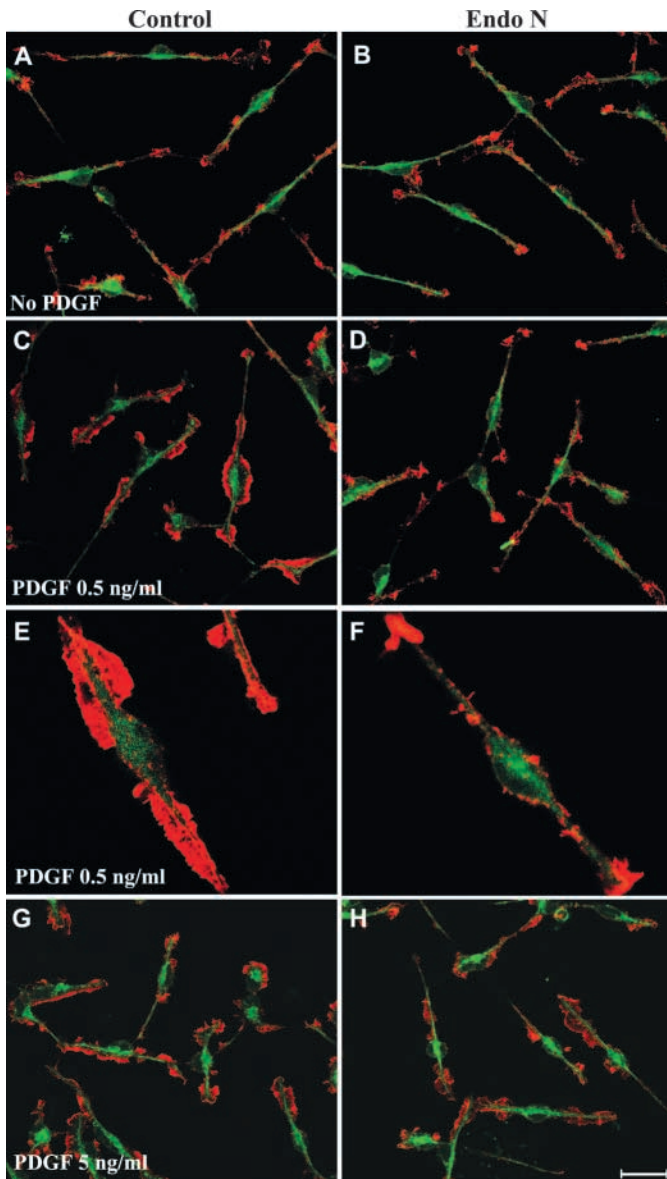


Fig. 8. The effect of PSA removal on PDGF-induced lamellipodial formation. Control and Endo N-treated (12 hours) cells were serum starved and incubated with medium (A,B), 0.5 ng/ml PDGF (C-F) or 5 ng/ml PDGF (G,H) for 10 minutes. Cells were fixed, stained with Texas Red-phalloidin and Oregon Green DNase I to detect F-actin fibers and G-actin, respectively. Cells were examined by fluorescence confocal microscopy. Bar, 20 μ m in A-D,G and H; 8 μ m in E and F.

decrease in OPC migration in response to gradients of PDGF but not to bFGF. Intriguingly, we found that Endo N did not modify cell migration under conditions of chemokinesis, when PDGF was present in both the upper and lower wells of the Boyden microchemotaxis chamber. Thus, PSA-NCAM seems to be specifically required for OPC chemotaxis but not for cell locomotion. This conclusion is in agreement with the observation of Decker et al. (Decker et al., 2000) that removal of PSA with Endo N does not modify the migration rate of OPCs in uniform concentrations of PDGF. It also gives support and extends our previous results in a scratch wound assay

where Endo N modified the directionality of OPCs but not cell motility (Barral-Moran et al., 2003). Finally, these observations confirm the notion that directionality of cell movement can be independently regulated from cell locomotion (Allen et al., 1998; Parent and Devreotes, 1999). For example, disruption of specific signaling elements such as phosphoinositide 3-kinases (PI 3-kinases), PTEN (phosphatase and tensin homolog), FAK (focal adhesion kinase) in diverse cell types leads to deficient chemotaxis without affecting random cell migration (Chung et al., 2001; Funamoto et al., 2002).

The direct viewing Dunn chamber has provided us with an insight into the mechanism of the migratory defect of OPCs after removal of PSA from the cell surface. When control non-Endo N-treated cells were maintained in uniform concentrations of the growth factor, they were polarized and displayed a bipolar elongated shape with two main processes. As described previously (Schmidt et al., 1997; Simpson and Armstrong, 1999), at the tip of processes, growth-cone-like structures were formed similarly to those observed on neurons (Miller et al., 1992; Grabham and Goldberg, 1997). Once the bipolar morphology had been established, cells started to move randomly with a mean speed of about 43.6 μ m/hour. When OPCs were exposed to linear gradients of PDGF, the leading processes became stable over a substantial period of time and cells showed persistence in migration in the direction established by the orientation of the leading process. A significant proportion of the population migrated along and up the gradient of PDGF. The results shown in the scatter diagrams showed that in the presence of Endo N there was no biased displacement of cells in the direction of PDGF gradient. However, it was also evident that this was not due to an abrogation of cell migration. Cell locomotion appeared normal and the mean displacement speed was not significantly different from that of control non-Endo N-treated cells. In addition, we also observed that cells established polarized morphology just as under control conditions. However, closer examination revealed that PSA-negative cells did not persist in the direction of migration, the leading processes changed frequently and cells made random turns. The migratory behavior of cells became reminiscent of that observed in uniform concentration of PDGF. The effect of PSA removal on the migration of cells was identical on poly-L-lysine as well as on laminin-coated coverslips. Together, these observations suggest that the PSA moiety on NCAM affects the directional migration of OPCs without modifying their ability to polarize and to generate traction.

The directed migration of cells in response to soluble guidance cues is a complex process that requires the acquisition of spatial and temporal asymmetry between the front and the rear of the cell (Lauffenburger and Horwitz, 1996). Lamellipodia and filopodia at the leading front extend the cells towards the stimulus and make new connections with the substratum. These events involve complex dynamic rearrangements of the actin cytoskeleton, which is locally controlled by incoming guidance information via the activation of G protein receptor systems, PI 3-kinase lipid products and the Rho family of GTPases (Hall, 1998). The mechanisms by which PSA-NCAM modify this process remain to be determined. In the present study we observed that the pattern of actin organization was similar in control and Endo N-treated cells in the absence of PDGF. However, removal of PSA

significantly reduced lamellipodia formation in response to low concentrations (0.5 ng/ml) of PDGF, raising the possibility that under these conditions OPC cells are less responsive to PDGF. By contrast, Endo N-treated OPCs displayed an apparently normal capacity for lamellipodia extension in the presence of higher concentrations (5 ng/ml) of PDGF. These results concur with our time-lapse video data, showing that the directionality of migration of Endo N-treated OPCs recovered in the presence of high concentrations (400 ng/ml) of PDGF. These observations raise the intriguing possibility that PSA-NCAM could modify the ability of cells to sense accurately growth factor gradients. This hypothesis is consistent with previous studies showing that the PSA chain of NCAM is required for adequate responses of neurons to BDNF (Muller et al., 2000; Vutskits et al., 2001) and ciliary neurotrophic factor (CNTF) (Vutskits et al., 2003).

In conclusion, the current studies suggest a potential role for PSA-NCAM in regulating the directional migration of OPCs. It is intriguing that in these simple in vitro models the loss or inactivation of PSA leads to such a specific deficit in the directionality of migration without affecting the basic locomotion of OPCs. In these models, directional migration of OPCs has been elicited by a single chemoattractant PDGF. In vivo, it is more likely that these cells are guided by a multitude of overlapping signals. It is unlikely that PSA-NCAM expression in vivo represents an all-or-none molecular switch for the directional migration of OPCs. Indeed, Ono et al. (Ono et al., 1997) reported that OPC migration in the chick optic nerve was unaffected by the removal of PSA from NCAM, although these findings might be explained by the existence of PDGF α receptor-negative cells (Spassky et al., 2001). It is more likely that PSA-NCAM takes part in a complex molecular network that allows the continuous and subtle modulation of the guiding process of OPC migration. Understanding this process in detail will be crucial for the successful use of OPCs to treat damaged myelin in the CNS.

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References

- Acheson, A., Sunshine, J. L. and Rutishauser, U. (1991). NCAM polysialic acid can regulate both cell-cell and cell-substrate interactions. *J. Cell Biol.* **114**, 143-153.
- Allen, W. E., Zicha, D., Ridley, A. J. and Jones, G. E. (1998). A role for Cdc42 in macrophage chemotaxis. *J. Cell Biol.* **141**, 1147-1157.
- Armstrong, R. C., Harvath, L. and Dubois-Dalq, M. E. (1990). Type 1 astrocytes and oligodendrocyte-type 2 astrocyte glial progenitors migrate toward distinct molecules. *J. Neurosci. Res.* **27**, 400-407.
- Avellana-Adalid, V., Nait-Oumesmar, B., Lachapelle, F. and Baron-Van Evercooren, A. (1996). Expansion of rat oligodendrocyte progenitors into proliferative "oligospheres" that retain differentiation potential. *J. Neurosci. Res.* **45**, 558-570.
- Barral-Moran, M. J., Calaora, V., Vutskits, L., Wang, C., Zhang, H., Rougon, G. and Kiss, J. Z. (2003). Contribution of PSA-NCAM to wound healing of glial monolayer. *J. Neurosci. Res.* **72**, 679-690.
- Behar, T. N., Scott, C. A., Greene, C. L., Wen, X., Smith, S. V., Maric, D., Liu, Q. Y., Colton, C. A. and Barker, J. L. (1999). Glutamate acting at NMDA receptors stimulates embryonic cortical neuronal migration. *J. Neurosci.* **19**, 4449-4461.
- Blakemore, W. F. and Keirstead, H. S. (1999). The origin of remyelinating cells in the central nervous system. *J. Neuroimmunol.* **98**, 69-76.
- Blaschuk, K. L., Frost, E. E. and ffrench-Constant, C. (2000). The regulation of proliferation and differentiation in oligodendrocyte progenitor cells by alpha V integrins. *Development* **127**, 1961-1969.
- Chazal, G., Durbec, P., Jankovski, A., Rougon, G. and Cremer, H. (2000). Consequences of neural cell adhesion molecule deficiency on cell migration in the rostral migratory stream of the mouse. *J. Neurosci.* **20**, 1446-1457.
- Chung, C. Y., Potikyan, G. and Firtel, R. A. (2001). Control of cell polarity and chemotaxis by Akt/PKB and PI3 kinase through the regulation of PAKs. *Mol. Cell* **7**, 937-947.
- Decker, L., Avellana-Adalid, V., Nait-Oumesmar, B., Durbec, P. and Baron-Van Evercooren, A. (2000). Oligodendrocyte precursor migration and differentiation: combined effects of PSA residues, growth factors, and substrates. *Mol. Cell Neurosci.* **16**, 422-439.
- Eisenbarth, G. S., Walsh, F. S. and Nirenberg, M. (1979). Monoclonal antibody to a plasma membrane antigen of neurons. *Proc. Natl. Acad. Sci. USA* **76**, 4913-4917.
- Fok-Seang, J., DiProspero, N. A., Meiners, S., Muir, E. and Fawcett, J. W. (1998). Cytokine-induced changes in the ability of astrocytes to support migration of oligodendrocyte precursors and axon growth. *Eur. J. Neurosci.* **10**, 2400-2415.
- Foxman, E. F., Kunkel, E. J. and Butcher, E. C. (1999). Integrating conflicting chemotactic signals. The role of memory in leukocyte navigation. *J. Cell Biol.* **147**, 577-588.
- Froscher, M., Gorgen, I., Boulnois, G. J., Timmis, K. N. and Bitter-Suermann, D. (1985). NZB mouse system for production of monoclonal antibodies to weak bacterial antigens: isolation of an IgG antibody to the polysaccharide capsules of Escherichia coli K1 and group B meningococci. *Proc. Natl. Acad. Sci. USA* **82**, 1194-1198.
- Funamoto, S., Meili, R., Lee, S., Parry, L. and Firtel, R. A. (2002). Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. *Cell* **109**, 611-623.
- Grabham, P. W. and Goldberg, D. J. (1997). Nerve growth factor stimulates the accumulation of beta1 integrin at the tips of filopodia in the growth cones of sympathetic neurons. *J. Neurosci.* **17**, 5455-5465.
- Hall, A. (1998). G proteins and small GTPases: distant relatives keep in touch. *Science* **280**, 2074-2075.
- Hu, H., Tomaszewicz, H., Magnuson, T. and Rutishauser, U. (1996). The role of polysialic acid in migration of olfactory bulb interneuron precursors in the subventricular zone. *Neuron* **16**, 735-743.
- Kiss, J. Z., Wang, C., Olive, S., Rougon, G., Lang, J., Baetens, D., Harry, D. and Pralong, W. F. (1994). Activity-dependent mobilization of the adhesion molecule polysialic NCAM to the cell surface of neurons and endocrine cells. *EMBO J.* **13**, 5284-5292.
- Lauffenburger, D. A. and Horwitz, A. F. (1996). Cell migration: a physically integrated molecular process. *Cell* **84**, 359-369.
- Lee, J. C., Mayer-Proschel, M. and Rao, M. S. (2000). Gliogenesis in the central nervous system. *Glia* **30**, 105-121.
- Lillien, L. E., Sendtner, M., Rohrer, H., Hughes, S. M. and Raff, M. C. (1988). Type-2 astrocyte development in rat brain cultures is initiated by a CNTF-like protein produced by type-1 astrocytes. *Neuron* **1**, 485-494.
- Lubetzki, C., Goujet-Zalc, C., Gansmuller, A., Monge, M., Brillat, A. and Zalc, B. (1991). Morphological, biochemical, and functional characterization of bulk isolated glial progenitor cells. *J. Neurochem.* **56**, 671-680.
- Miller, M., Bower, E., Levitt, P., Li, D. and Chantler, P. D. (1992). Myosin II distribution in neurons is consistent with a role in growth cone motility but not synaptic vesicle mobilization. *Neuron* **8**, 25-44.
- Milner, R., Edwards, G., Streuli, C. and ffrench-Constant, C. (1996). A role in migration for the alpha V beta 1 integrin expressed on oligodendrocyte precursors. *J. Neurosci.* **16**, 7240-7252.
- Milner, R., Anderson, H. J., Rippon, R. F., McKay, J. S., Franklin, R. J., Marchionni, M. A., Reynolds, R. and ffrench-Constant, C. (1997). Contrasting effects of mitogenic growth factors on oligodendrocyte precursor cell migration. *Glia* **19**, 85-90.
- Muller, D., Djebbara-Hannas, Z., Jourdain, P., Vutskits, L., Durbec, P., Rougon, G. and Kiss, J. Z. (2000). Brain-derived neurotrophic factor restores long-term potentiation in polysialic acid-neural cell adhesion molecule-deficient hippocampus. *Proc. Natl. Acad. Sci. USA* **97**, 4315-4320.
- Noble, M., Murray, K., Stroobant, P., Waterfield, M. D. and Riddle, P. (1988). Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. *Nature* **333**, 560-562.
- Ono, K., Tomaszewicz, H., Magnuson, T. and Rutishauser, U. (1994). NCAM mutation inhibits tangential neuronal migration and is phenocopied by enzymatic removal of polysialic acid. *Neuron* **13**, 595-609.

- Ono, K., Yasui, Y., Rutishauser, U. and Miller, R. H.** (1997). Focal ventricular origin and migration of oligodendrocyte precursors into the chick optic nerve. *Neuron* **19**, 283-292.
- Parent, C. A. and Devreotes, P. N.** (1999). A cell's sense of direction. *Science* **284**, 765-770.
- Ranscht, B., Clapshaw, P. A., Price, J., Noble, M. and Seifert, W.** (1982). Development of oligodendrocytes and Schwann cells studied with a monoclonal antibody against galactocerebroside. *Proc. Natl. Acad. Sci. USA* **79**, 2709-2713.
- Rougon, G., Dubois, C., Buckley, N., Magnani, J. L. and Zollinger, W.** (1986). A monoclonal antibody against meningococcus group B polysaccharides distinguishes embryonic from adult N-CAM. *J. Cell Biol.* **103**, 2429-2437.
- Rutishauser, U., Acheson, A., Hall, A. K., Mann, D. M. and Sunshine, J.** (1988). The neural cell adhesion molecule (NCAM) as a regulator of cell-cell interactions. *Science* **240**, 53-57.
- Schmidt, C., Ohlemeyer, C., Labrakakis, C., Walter, T., Kettenmann, H. and Schnitzer, J.** (1997). Analysis of motile oligodendrocyte precursor cells in vitro and in brain slices. *Glia* **20**, 284-298.
- Simpson, P. B. and Armstrong, R. C.** (1999). Intracellular signals and cytoskeletal elements involved in oligodendrocyte progenitor migration. *Glia* **26**, 22-35.
- Spassky, N., Heydon, K., Mangatal, A., Jankovski, A., Olivier, C., Queraud-Lesaux, F., Goujet-Zalc, C., Thomas, J. L. and Zalc, B.** (2001). Sonic hedgehog-dependent emergence of oligodendrocytes in the telencephalon: evidence for a source of oligodendrocytes in the olfactory bulb that is independent of PDGFRalpha signaling. *Development* **128**, 4993-5004.
- Spassky, N., de Castro, F., le Bras, B., Heydon, K., Queraud-LeSaux, F., Bloch-Gallego, E., Chedotal, A., Zalc, B. and Thomas, J. L.** (2002). Directional guidance of oligodendroglial migration by class 3 semaphorins and netrin-1. *J. Neurosci.* **22**, 5992-6004.
- Sugimoto, Y., Taniguchi, M., Yagi, T., Akagi, Y., Nojyo, Y. and Tamamaki, N.** (2001). Guidance of glial precursor cell migration by secreted cues in the developing optic nerve. *Development* **128**, 3321-3330.
- Trotter, J., Bitter-Suermann, D. and Schachner, M.** (1989). Differentiation-regulated loss of the polysialylated embryonic form and expression of the different polypeptides of the neural cell adhesion molecule by cultured oligodendrocytes and myelin. *J. Neurosci. Res.* **22**, 369-383.
- Tsai, H. H., Frost, E., To, V., Robinson, S., Ffrench-Constant, C., Geertman, R., Ransohoff, R. M. and Miller, R. H.** (2002). The chemokine receptor CXCR2 controls positioning of oligodendrocyte precursors in developing spinal cord by arresting their migration. *Cell* **110**, 373-383.
- Tsai, H. H., Tessier-Lavigne, M. and Miller, R. H.** (2003). Netrin 1 mediates spinal cord oligodendrocyte precursor dispersal. *Development* **130**, 2095-2105.
- Vimr, E. R., McCoy, R. D., Vollger, H. F., Wilkison, N. C. and Troy, F. A.** (1984). Use of prokaryotic-derived probes to identify poly(sialic acid) in neonatal neuronal membranes. *Proc. Natl. Acad. Sci. USA* **81**, 1971-1975.
- Vutskits, L., Djebbara-Hannas, Z., Zhang, H., Paccaud, J. P., Durbec, P., Rougon, G., Muller, D. and Kiss, J. Z.** (2001). PSA-NCAM modulates BDNF-dependent survival and differentiation of cortical neurons. *Eur. J. Neurosci.* **13**, 1391-1402.
- Vutskits, L., Gascon, E. and Kiss, J. Z.** (2003). Removal of PSA from NCAM affects the survival of magnocellular vasopressin- and oxytocin-producing neurons in organotypic cultures of the paraventricular nucleus. *Eur. J. Neurosci.* **17**, 2119-2126.
- Wang, C., Rougon, G. and Kiss, J. Z.** (1994). Requirement of polysialic acid for the migration of the O-2A glial progenitor cell from neurohypophyseal explants. *J. Neurosci.* **14**, 4446-4457.
- Wang, C., Pralong, W. F., Schulz, M. F., Rougon, G., Aubry, J. M., Pagliusi, S., Robert, A. and Kiss, J. Z.** (1996). Functional N-methyl-D-aspartate receptors in O-2A glial precursor cells: a critical role in regulating polysialic acid-neural cell adhesion molecule expression and cell migration. *J. Cell Biol.* **135**, 1565-1581.
- Webb, S. E., Pollard, J. W. and Jones, G. E.** (1996). Direct observation and quantification of macrophage chemoattraction to the growth factor CSF-1. *J. Cell. Sci.* **109**, 793-803.
- Yan, H. and Rivkees, S. A.** (2002). Hepatocyte growth factor stimulates the proliferation and migration of oligodendrocyte precursor cells. *J. Neurosci. Res.* **69**, 597-606.
- Zhang, S. C., Ge, B. and Duncan, I. D.** (1999). Adult brain retains the potential to generate oligodendroglial progenitors with extensive myelination capacity. *Proc. Natl. Acad. Sci. USA* **96**, 4089-4094.
- Zicha, D., Dunn, G. A. and Brown, A. F.** (1991). A new direct-viewing chemotaxis chamber. *J. Cell Sci.* **99**, 769-775.