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RESEARCH ARTICLE

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Glial cell proliferation in the spinal cord after dorsal rhizotomy or sciatic nerve transection in the adult rat

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Abstract Proliferation of glial cells is one of the hallmarks of CNS responses to neural injury. These responses are likely to play important roles in neuronal survival and functional recovery after central or peripheral injury. The boundary between the peripheral nervous system (PNS) and CNS in the dorsal roots, the dorsal root transitional zone (DRTZ), marks a distinct barrier for growth by injured dorsal root axons. Regeneration occurs successfully in the PNS environment, but ceases at the PNS-CNS junction. In order to understand the role of different glial cells in this process, we analysed the proliferation pattern of glial cells in central (CNS) and peripheral (PNS) parts of the dorsal root and the segmental white and grey spinal cord matter after dorsal rhizotomy or sciatic nerve transection in adult rats 1–7 days after injury. Monoclonal antibody MIB-5 or antibodies to bromodeoxyuridine were used to identify proliferating cells. Polyclonal antibodies to laminin were used to distinguish the PNS and CNS compartments of the dorsal root. Dorsal root lesion induced glial cell proliferation in the CNS as well as PNS beginning at 1 day, with peaks from 2 to 4 days postoperatively. After sciatic nerve injury, cell proliferation occurred only in the CNS, was minimal at 1 day, and peaked from 2 to 4 days postoperatively. Double immunostaining with specific glial cell markers showed that after dorsal root transection 60% of the proliferating cells throughout the postoperative period examined were microglia, 30% astrocytes and 10% unidentified in the CNS, while in the PNS 40% were Schwann cells, 40% macrophages and 20% unidentified. After sciatic nerve injury virtually all proliferating cells were microglia. These findings indicate that non-neuronal cells in the CNS and PNS are extremely sensitive to the initial changes which occur in the degenerating dorsal root axons, and that extensive axonal degeneration is a prerequisite for astroglial and Schwann cell, but not microglial cell, proliferation.

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Introduction

Injury to peripheral sensory nerve axons results in a series of structural and molecular changes in the affected sensory ganglion cell bodies. These changes include a downregulation of molecules associated with neuronal stability, such as high molecular weight neurofilament proteins, and an upregulation of molecules associated with axonal sprouting and elongation, such as GAP43, actin and tubulin (for references see Aldskogius and Svensson 1993). In addition to the perikaryal changes, there are also prominent changes in the central terminals of peripherally axotomized sensory ganglion cells, so-called transganglionic changes. These changes appear to involve a retraction or degeneration of some terminals (for references see Aldskogius et al. 1992), as well as sprouting of others (Shortland and Woolf 1993). Concomitantly with the transganglionic neuronal changes there is proliferation of microglial cells and hypertrophy of astrocytes in the corresponding projection territories (for references see Aldskogius and Kozlova 1998).

The transganglionic neuronal growth response is of interest since it occurs in a CNS environment which is usually known for not being conducive to axon growth. Likewise, the transganglionic non-neuronal responses are intriguing for two reasons. First, because they develop shortly after axotomy, at a time when the central processes can be assumed to be structurally intact. Second, the time courses of neuronal and non-neuronal responses raise the issue of what role the latter may have in inducing, promoting or inhibiting the transganglionic growth response.

Following injury to the central processes of sensory ganglion cells, the segment of the axon which has lost

contact with the ganglion cell body undergoes a complete disintegration, Wallerian degeneration. In the CNS, microglial cells proliferate along these degenerating axons and transform into phagocytes, while astrocytes hypertrophy and develop tightly packed filament-rich processes (for references see Aldskogius and Kozlova 1998). These two events underlie the formation of a glial scar in the location of the affected nerve fibres.

When traced centrally from the ganglion, the central processes of sensory neurones lie at first in a peripheral environment containing Schwann cells and a well developed basal lamina (Fraher and Kaar 1986). Towards the proximal part of the dorsal rootlet they traverse the interface between the CNS and peripheral nervous system (PNS), the dorsal root transitional zone (DRTZ), which extends distally from the spinal cord into the rootlet (Fraher 1997). Injured sensory axons grow readily in the PNS compartment of the dorsal root, but cease to grow at the junction between the PNS and CNS. This abortive regeneration is believed to be a consequence of specific growth-inhibiting properties of the DRTZ astrocytes and their extracellular environment. Sensory axons form synapse-like profiles with astrocytes outlining the boundary between the PNS and CNS (Carlstedt 1985; Liuzzi and Lasek 1987), and appear to be inhibited by proteoglycans secreted by DRTZ astrocytes (Pindzola et al. 1993; Smith Thomas et al. 1994).

The interaction between growing axons and the DRTZ reflects the contrasting behaviour of injured axons in the PNS compared with the CNS environment. Elucidating the cellular and molecular mechanisms underlying the non-permissive nature of the DRTZ will therefore provide information relevant to our understanding of why injured axons fail to grow in the mature CNS. One of the most striking signs of robust and early glial cell activation is proliferation. The present study was undertaken to elucidate the time course of glial cell proliferation and the nature of the dividing cells in the DRTZ, the PNS compartment of the dorsal root as well as the spinal cord grey and white matter following dorsal root injury or peripheral nerve injury.

Materials and methods

Animals and surgery

Altogether 43 adult female Sprague-Dawley rats (150–200 g, Charles River, Sweden) were used for the study; three of them were intact control animals. The use of animals was approved by the regional ethics committee for animal research, and followed the guidelines of the national agency for research on animals. All operative procedures were carried out on deeply anaesthetized animals (chloral hydrate, 35 mg/100 g body weight, intraperitoneally).

Twenty animals were subjected to dorsal rhizotomies. The left L4 and L5 dorsal roots were exposed via a hemilaminectomy and transected immediately proximal to the corresponding ganglion, i.e. about 30–35 mm from the spinal cord. In another 20 animals, the left sciatic nerve was transected at midthigh level. The operated rats were allowed to survive for 1, 2, 3, 4 or 7 days postoperatively (n=4 for each survival time), at which time they were perfused with saline (37°C) followed by 4% (w/v) paraformaldehyde in 0.15 M phosphate buffer (pH 7.3–7.4, 4°C) containing 14% (v/v) of a saturated picric acid solution.

In one of the animals from each of the different experimental groups and one intact control animal the thymidine analogue bromodeoxyuridine (BrdU) was administered 2 h prior to sacrifice. These animals were reanaesthetized, the atlanto-occipital membrane and dura mater were opened and 600 µl of a solution consisting of BrdU (10 mg/ml saline, Sigma, USA) and 5-fluoro-2-deoxyuridine (1 mg/ml saline, Sigma, USA) was injected over a period of 15 min into the cerebellomedullary and lumbar cisterns. The latter was reached by maneuvering a fine catheter (outer diameter 0.61 mm) connected to a Hamilton syringe epidurally from the cerebellomedullary cistern about 90 mm caudally (cf. Persson et al. 1995). BrdU injected rats were maintained on anaesthesia for 2 h, when they were perfused as described above.

After perfusion the L4 and L5 spinal cord segments were removed and postfixed in the same fixative for 2 h and washed overnight (4°C) in 0.15 M phosphate buffer with 10% (w/v) sucrose.

Immunohistochemistry

Fourteen-micrometre horizontal serial cryosections were made through the combined L4 and L5 segments, including the attached dorsal roots, and processed for immunofluorescence. All sections were incubated with 1% bovine serum albumin (BSA, Amersham, UK) and 0.3% Triton-X 100 (Sigma, USA) in phosphate-buffered saline (PBS) for 1 h at room temperature, then incubated with the appropriate primary antibodies. Sections from three operated animals with dorsal rhizotomy and sciatic nerve transection, respectively, from each postoperative survival time, and two intact control animals were used for quantitative assessment of cell proliferation. These sections were all incubated with antibody MIB-5, which recognizes a cyclin-dependent kinase (mouse monoclonal, 1:25, Immunotech, France) for 36 h at 4°C. To distinguish the PNS and CNS compartments of the root, one out of four sections in this series was double labelled with antibodies to laminin (rabbit polyclonal, 1:1000, Sigma, USA). To determine the identity of the MIB-5 labelled cells, the remaining three sections were double labelled with markers for astrocytes, Schwann cells and microglia/macrophages, respectively, i.e. anti-GFAP (anti-glial fibrillary acidic protein; rabbit polyclonal, 1:1000, DAKO, Denmark), anti-S100 (A and B; rabbit polyclonal, 1:1000, DAKO, Denmark), or antibodies to the lectin Griffonia Simplicifolia Agglutinin isolectin B4 (GSA-IB4; goat polyclonal, 1:1000, Vector, USA). Incubations with these primary antibodies were done overnight at 4°C.

Sections from an additional set of animals (n=1 for each operation and postoperative survival time) were used for demonstrating BrdU labelled cells. These sections were treated with 0.1 M NaOH (2 min) and phosphate buffer (pH 8.5, 30 s) to denature DNA, prior to incubation with anti-BrdU (mouse monoclonal, 1:1, Amersham, UK) overnight at 4°C. This series of sections was also double labelled with laminin and glial cell markers in the way described above.

After rinsing, immunoreactivity was visualized with fluorescent secondary antibodies. Sections were incubated for 1 h at room temperature with Texas-red-tagged donkey anti-mouse (1:40, Jackson ImmunoResearch, USA) or fluorescein (FITC)-tagged goat anti-mouse (1:40, DAKO, Denmark) immunoglobulins to visualize MIB-5 immunoreactivity (IR), or with rhodamine (TRITC)- or FITC-tagged swine anti-rabbit immunoglobulins (1:40, DAKO, Denmark) to visualize laminin-IR. Sections incubated with anti-GFAP or anti-S100 were incubated for 1 h at room temperature in rhodamine (TRITC)- or FITC-tagged secondary antibodies (swine anti-rabbit, 1:40, DAKO, Denmark). To identify GSA-IB4 positive cells the sections were first incubated with MIB-5 as described above, and after a rinse in PBS incubated with the lectin GSA-IB4 (10 µg/ml, Vector, USA) overnight at 4°C, rinsed in PBS, incubated with goat anti-GSA-IB4 overnight at 4°C, and finally incubated with Cy^3-conjugated donkey anti-goat IgG (1:400, Jackson Immunoresearch, USA) for 1 h at room tem-

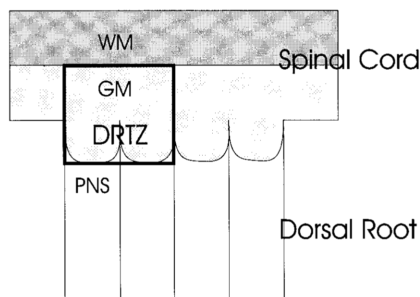


Fig. 1 Scheme showing the areas in which proliferating cells were calculated: the dorsal root transitional zone (*DRTZ*), grey matter (*GM*) and white matter (*WM*) of the L4 spinal cord segment and dorsal root as well as in the PNS compartment immediately adjacent to the DRTZ (*PNS*). *Bold lines* indicate orientation of counting frame for obtaining cell numbers in the DRTZ and GM

perature. After final rinses, sections were mounted in glycerol containing the anti-fading agent *p*-phenylenediamine (PPD, Sigma, USA), and viewed under a Nikon Eclipse fluorescence microscope.

The specificity of the immunolabeling was validated by omission of the primary antibodies.

Quantitative analysis

Estimations of the total number of proliferating cells were done on sections double labelled with MIB-5 and anti-laminin (every fourth section) separately for the following areas: spinal cord white matter (WM), grey matter (GM), DRTZ and the peripheral compartment of the dorsal root immediately adjacent to the DRTZ (PNS) (cf. Fig. 1). The number of MIB-5 positive cells was calculated at an objective magnification of ×20 in an ocular frame (area 0.34 mm^2). In order to count cells in the four different areas (see above), the frame was oriented in the following ways (cf. Fig. 1). DRTZ and GM: the frame was oriented so that half of it included adjacent DRTZ areas, including the thin line of Lissauer's tract, and the other half the adjacent spinal cord grey matter. WM: half of the frame was placed across the left dorsal funiculus. PNS: the frame was placed in the PNS compartment, immediately distal to the DRTZ. In order to obtain representative data from each case, counts were made at different rostrocaudal as well as dorsoventral levels of the L4–L5 dorsal root-spinal cord junctions. Altogether, counts were made in six complete frames from each area. The cell numbers in all the frames within each tissue compartment were added to give an estimate of the total number of labelled cells within all six frames per specimen. These data were used for comparing the size of the proliferating cell populations along the postoperative survival time.

To estimate the proportion of cells of different identity, the number of cells double labelled with MIB-5 and either anti-GFAP, anti-GSA-IB4 or anti-S100 was calculated separately for each region, DRTZ, GM, WM and PNS, in the way described above in each of the three subsequent sections. Given an area of 0.34 mm^2 for each of the six analysed frames, these calculations yielded the number of cells/2 mm^2.

The quantitative data on numbers of proliferating cells following dorsal rhizotomy and sciatic nerve transection were analysed with Student's *t*-test for each survival time and area examined.

Results

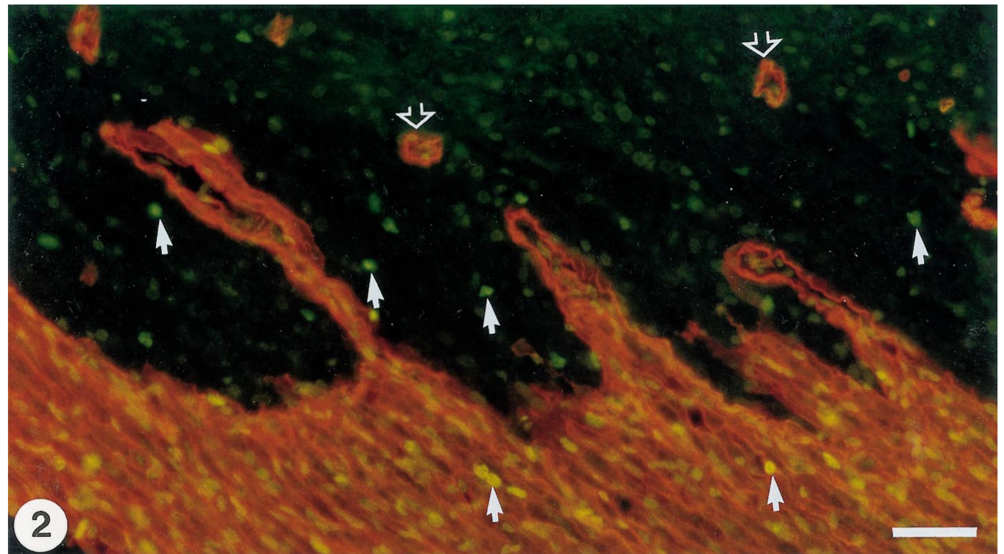
General observations

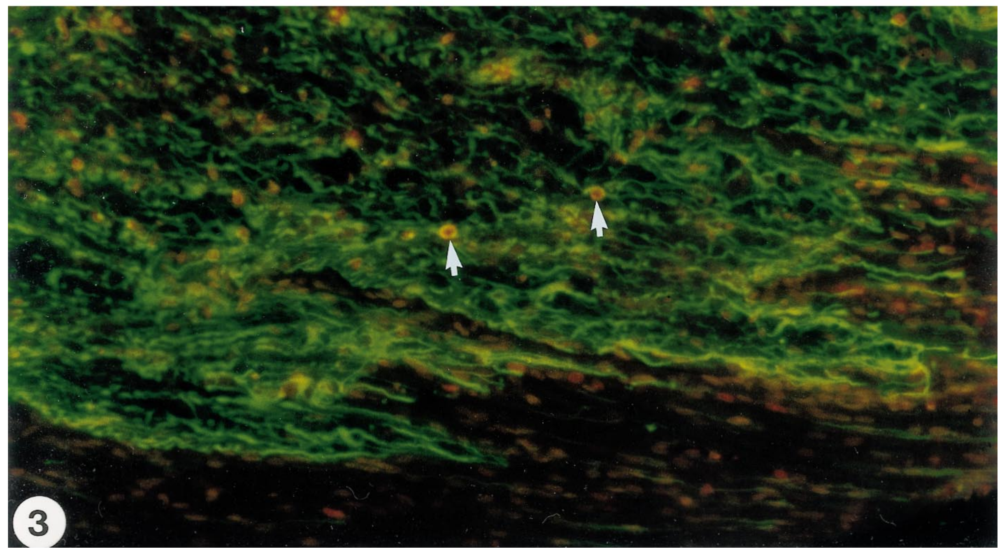
We made two kinds of injuries, dorsal rhizotomy and sciatic nerve transection, and analysed glial cell proliferation along the degenerating primary sensory afferents in the peripheral compartment of the dorsal root as well as at various levels along their entry into the spinal cord white and grey matter after the different survival times. Dorsal root lesion induced glial cell proliferation in the CNS as well as PNS (Fig. 2), including astrocytic proliferation (Fig. 3), while sciatic nerve injury caused cell proliferation almost exclusively in the CNS (Fig. 4). Although quantitative comparisons were not made, the pattern of labelling with BrdU and MIB-5 showed good agreement. With MIB-5 marker were visualized astrocytes and microglia in grey matter in the CNS (Fig. 5) and with BrdU marker, Schwann cells in the PNS (Fig. 6). No cells labelled with the proliferation markers were observed in intact animals or on the unoperated sides at any postoperative survival time.

Dorsal root transection

Glial cell proliferation was analysed in four areas after different survival times: DRTZ, GM, WM and PNS. In all areas, cells labelled with MIB-5 or anti-BrdU were first observed 1 day after injury (Fig. 7). In the DRTZ (Fig. 7a), the number of MIB-5 positive cells peaked at 2 and 4 days, and by 7 days after lesion they were almost absent. In the GM (Fig. 7b) and the WM (Fig. 7c), the number of proliferating cells peaked at 2 days, was still high at 3 and 4 days after injury, but only a few were seen at 7 days postlesion. In the PNS (Fig. 7d), only a few MIB-5 positive cells were observed at 1 day postoperatively. Numerous labelled cells were found from 2 to 4 days, but by 7 days after injury their number had declined markedly.

In the DRTZ (Fig. 8a) and the WM (Fig. 8c), the majority (about 60–65%) of proliferating cells during the first 4 days were double labelled with antibodies to the lectin GSA-IB4, indicating that they were microglial cells or macrophages. A smaller proportion (about 20%) were double labelled during the same period with the astrocyte marker GFAP (Fig. 3). About 10% of cells were not labelled with any of the cell markers used. In the GM (Fig. 8b), at 1 day postlesion most of the proliferating cells were labelled with GSA-IB4, but a few GFAP-IR proliferating cells were observed as well. The number of proliferating GFAP-positive cells increased markedly at 2 and 3 days postlesion and during this period was the major part of the entire proliferating cell population (Fig. 5a). At longer postoperative survival times the number of GFAP-IR proliferating cells decreased substantially, and GSA-IB4 labelled cells predominated among the MIB-5 positive cells (cf. Fig. 5b). In the PNS (Fig. 8d), double labelling analysis showed colocalization between





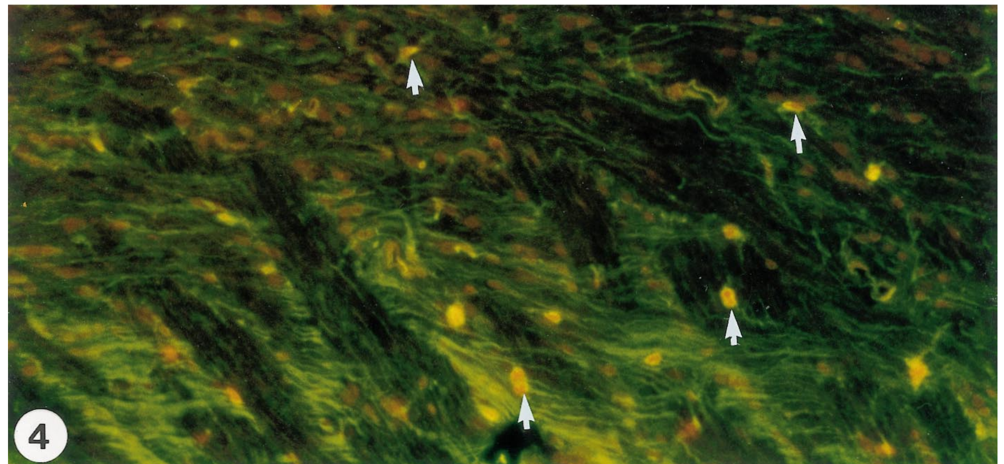


Fig. 2 Immunostaining with laminin (*red*) and BrdU (*green*) in the DRTZ 2 days after dorsal root transection. Laminin is found only in the PNS and around blood vessels in the CNS (*open arrows*). BrdU positive cells are found in the CNS as well as the PNS (*arrows*). *Bar* 50 μm

Fig. 3 Double immunostaining with MIB-5 (*red*) and anti-GFAP (*green*) in the L4 spinal cord DRTZ 3 days after dorsal root cut. GFAP immunoreactivity is present only in the CNS. Some of the MIB-5 positive cells are labelled with anti-GFAP (*arrows*). *Bar* as in Fig. 2

Fig. 4 Double immunostaining with MIB-5 (*red*) and anti-GSA-IB4 (*green*) in the DRTZ 3 days after sciatic nerve cut. Note almost all MIB-5 positive cells are colabelled with GSA-IB4 (*arrows*). The presence of GSA-IB4 labelled fibres coursing through the area is due to lectin binding to a subset of non-myelinated primary sensory axons. *Bar* as in Fig. 2

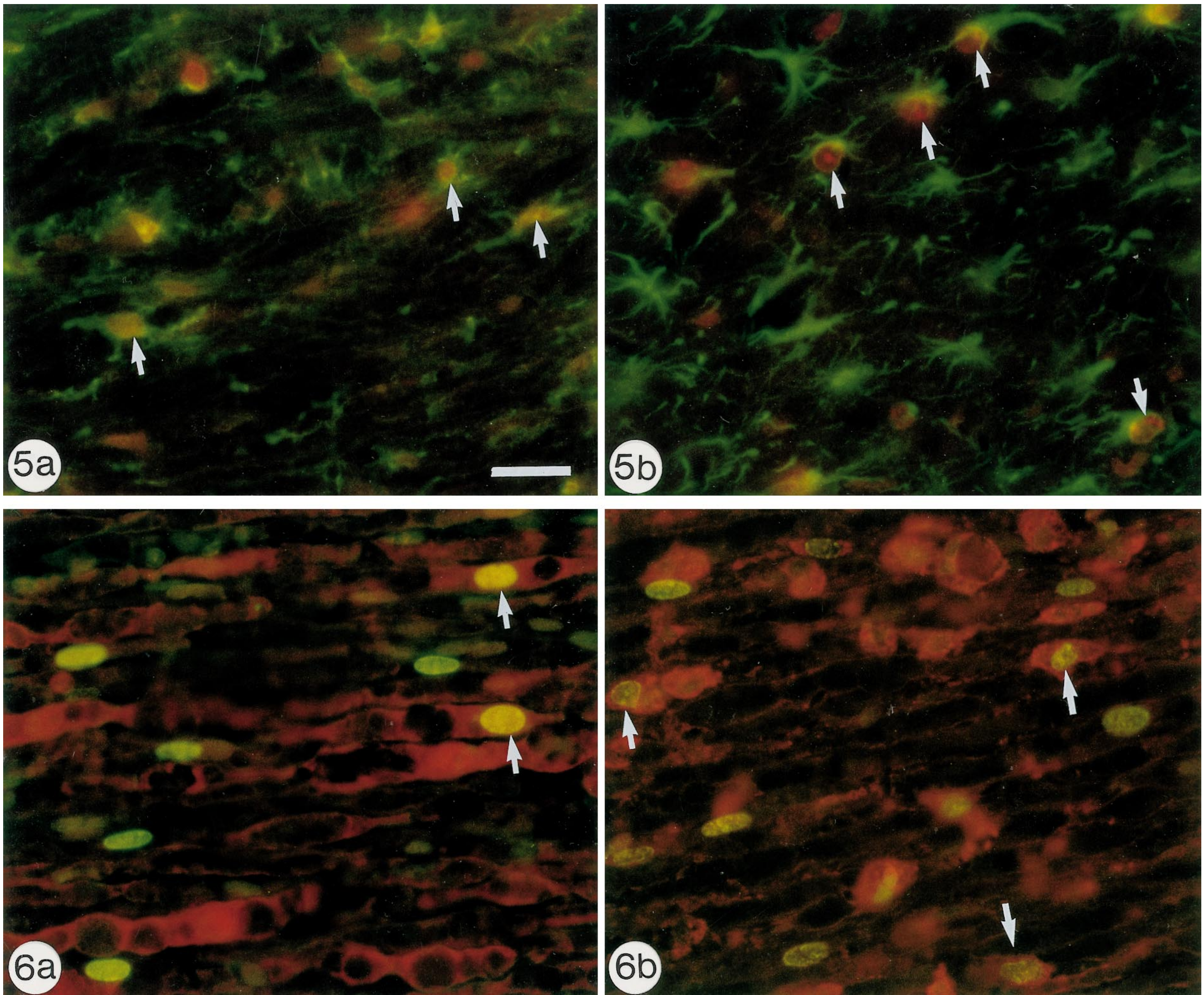


Fig. 5 Double immunostaining with MIB-5 (*red*) and anti-GFAP (**a** *green*) or anti-GSA-IB4 (**b** *green*) in the L4 spinal cord grey matter (GM) 3 days after dorsal root transection. Numerous MIB-5 positive cells are labelled with anti-GFAP (**a** *arrows*) or anti-GSA-IB4 (**b** *arrows*). *Bar* 25 μm

Fig. 6 Proliferating cells in the PNS immediately distal to the DRTZ 4 days after dorsal root transection. **a** shows double immunostaining with anti-BrdU (*green*) and the Schwann cell marker anti-S100 (*red*), while **b** shows double immunostaining with anti-BrdU (*green*) and the macrophage marker anti-GSA-IB4 (*red*). The vast majority of the BrdU positive cells are labelled with either anti-S100 (**a** *arrows*) or anti-GSA-IB4 (**b** *arrows*). *Bar* as in Fig. 5

MIB-5 and S100 (about 40%; Fig. 6a), and between MIB-5 and GSA-IB4 (about 40%; Fig. 6b), indicating that Schwann cells and macrophages contributed in roughly equal proportions to the proliferating cell population. About 20% of the proliferating cells in the PNS were not labelled with any of the markers used.

Sciatic nerve injury

After sciatic nerve transection, occasional MIB-5-IR cells were observed in the DRTZ (Fig. 7a), WM (Fig. 7b) and GM (Fig. 7c) 1 day after injury. A substantial increase in MIB-5 positive cells occurred at 2 and 3 days postoperatively. Already at 4 days there was a sharp decline in their number, and by 7 days they were almost completely absent. Only a few proliferating cells were found in the PNS (Fig. 7d). Virtually all MIB-5 IR cells in the DRTZ, WM and GM were double labelled with GSA-IB4 (Fig. 4). None of them showed colocalization with anti-GFAP. In the PNS compartment of the dorsal root, the few cells labelled with MIB-5 were not labelled with any of the markers used.

Discussion

In order to obtain a comprehensive picture of the pattern of cell proliferation after sensory axon injury, we analy-

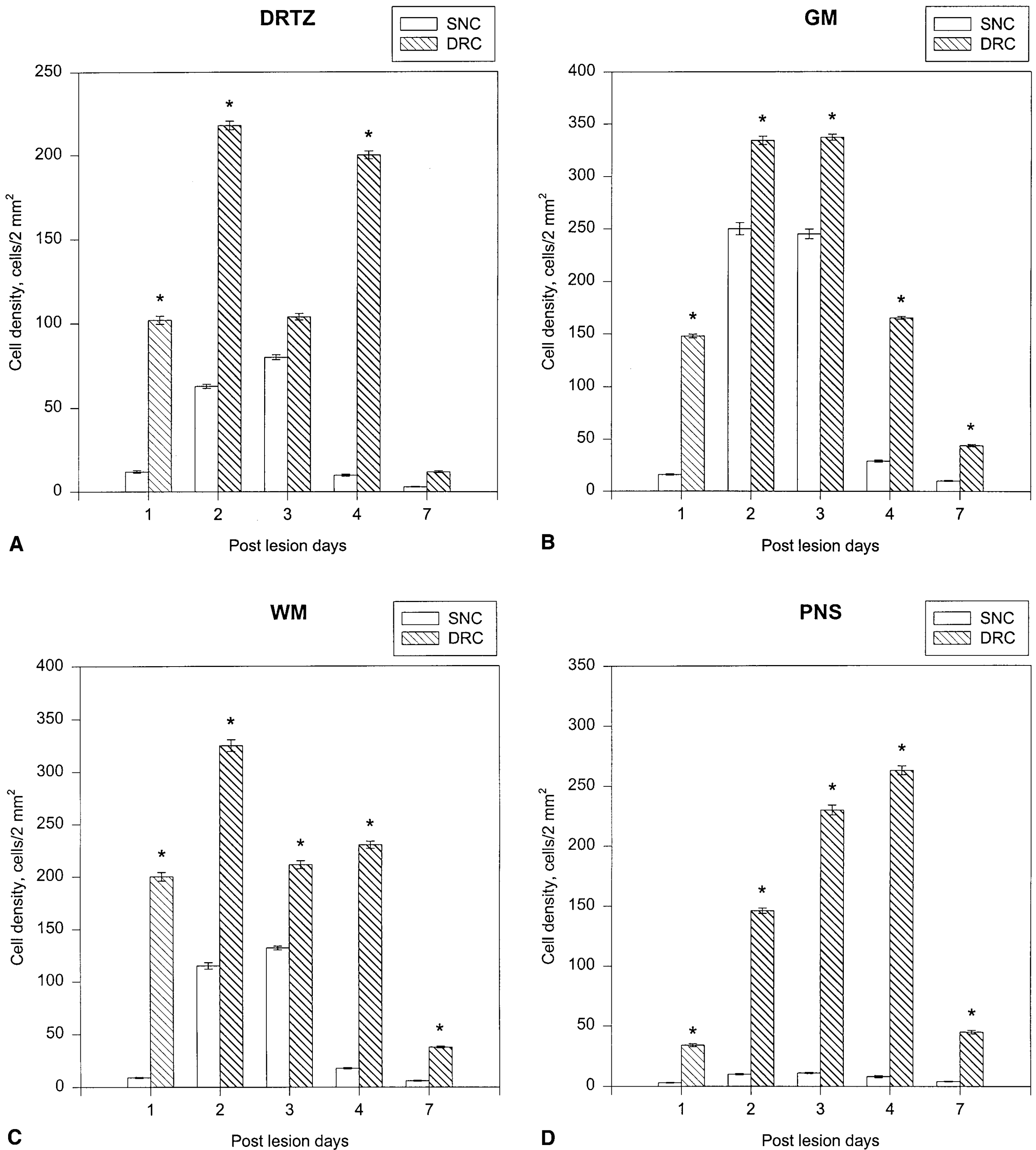


Fig. 7 Mean number (±SEM) of MIB-5 labelled cells/unit area (n=3) in the DRTZ **(a)**, GM **(b)** and WM **(c)** of the L4 spinal cord segment, and PNS **(d)** at different postoperative survival times after dorsal root (*DRC*) or sciatic nerve transection (*SNC*). *Asterisk* indicates statistical significance between data from DRC and SNC ($P<0.01$)

sed this pattern along the four distinct areas which the central processes of dorsal root ganglion cells encounter during their course from the PNS to their termination sites in the spinal cord grey matter. As a marker for our quantitative analysis of proliferating cells we used antibody MIB-5, which recognizes a cyclin-dependent kinase involved in regulating the mitotic cycle. Recent studies have demonstrated the feasibility of MIB-5 immunocytochemistry for identifying proliferating cells in

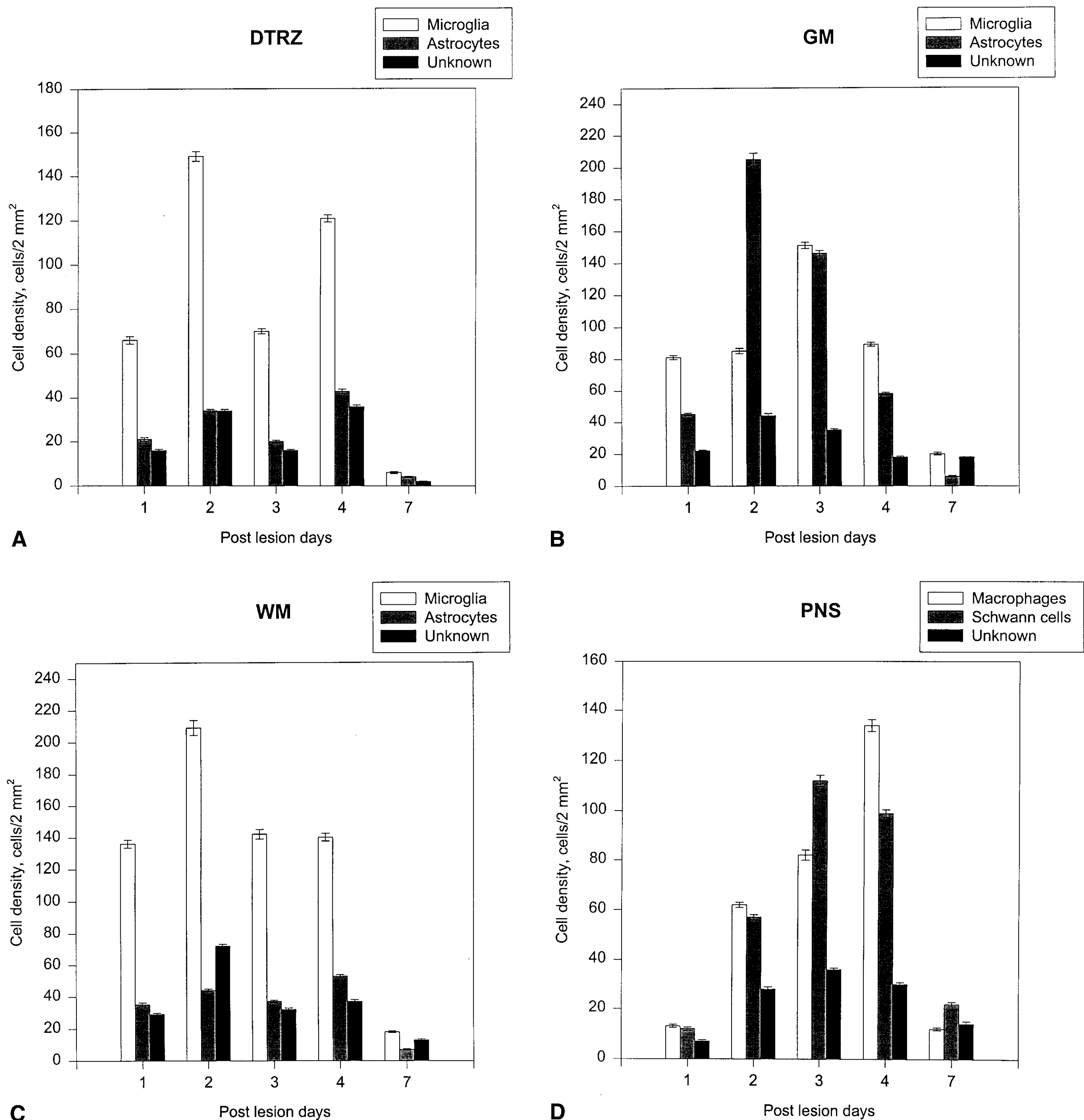


Fig. 8 Mean number (±SEM) of different glial cell types/unit area labelled with antibody MIB-5 in the DRTZ **(a)**, GM **(b)** and WM **(c)** of the L4 spinal cord segment, and PNS **(d)** at different survival times after dorsal root transection

the nervous system (Graeber et al. 1998; Tao and Aldskogius, unpublished observations). By using antibodies to laminin we were able to unambiguously distinguish the PNS (laminin-positive) and the CNS (laminin-negative) compartments of the dorsal roots. Our findings demonstrate striking differences in glial cell proliferation depending on whether axon injury occurs peripheral or central to the dorsal root ganglion.

Dorsal root transection

Dorsal root transections were made immediately proximal to the L4 and L5 dorsal root ganglia, i.e. about 30–35 mm distal to the areas of examination. The observed glial cell proliferation therefore reflects a response to pure Wallerian degeneration, and not to direct trauma to the dorsal root entry site. After dorsal rhizotomy there was a proliferation response in all CNS areas examined, already within 1 day postoperatively. Cells labelled with MIB-5 or BrdU at this stage were almost all GSA-IB4 positive, indicating that they are microglia or macrophages. Existing evidence strongly indicates that

peripheral macrophages are not entering the CNS at a distance from the site of injury, perhaps because of the lack of expression of critical adhesion molecules in the endothelium of the CNS vasculature (Castano et al. 1996). We therefore refer to the GSA-IB4 labelled cells in the CNS as microglia, although a contribution of peripheral macrophages cannot be completely excluded. At a later stage, a large proportion of proliferating cells was identified as microglia as well, but many cells, particularly in the spinal cord grey matter, were now labelled with anti-GFAP, the marker for astrocytes. Our findings in the spinal cord grey and white matter are in agreement with a previous study, in which microglia as well as astrocytes were labelled with the thymidine analogue bromodeoxyuridine (BrdU) in the spinal cord dorsal horn after dorsal root injury (Liu et al. 1998).

Wallerian degeneration in the DRTZ is accompanied by a marked peripheral extension of GFAP-rich processes (Kozlova et al. 1995, 1997), and the gradual development of a "scar" composed of tightly packed astrocytic processes in the degenerating area of the dorsal funiculus (for references see Aldskogius and Kozlova 1998). From our data we infer that this hypertrophy of white matter astrocytes is the result of a combination of astroglial proliferation and hypertrophy. The early and massive proliferation of astrocytes in the spinal cord grey matter is likely to be associated with the rapid anterograde degeneration of primary sensory terminals and preterminal axons (Coimbra et al. 1984). Astrocytes are closely interrelated with these structures and are therefore in a unique position to respond promptly to the disappearance of synaptic complexes. Previous studies have shown that astrocytes participate together with microglia in phagocytosis of disintegrating terminals and axons (Kapadia and LaMotte 1987; Cheng et al. 1994; Bechmann and Nitsch 1997).

Anterograde degeneration of myelinated axons is a protracted process. After the initial fragmentation and disintegration of the axon, the surrounding myelin collapses and forms myelin ovoids, which appear to be removed exclusively by microglia (for references see Aldskogius and Kozlova 1998). Proliferation of microglia in the DRTZ and spinal cord occurs rapidly; yet previous observations indicate that these cells develop into phagocytes much slower than macrophages in the PNS (Avellino et al. 1995). Furthermore, even after their transformation into phagocytes, microglial cells show an inadequate response compared to peripheral macrophages (Reichert and Rotshenker 1996). As a consequence, myelin debris remains in the CNS for a considerable length of time.

In the PNS dorsal rhizotomy induced proliferation of predominantly Schwann cells and macrophages, as evidenced by double labelling with anti-S100 and GSA-IB4. Previous information on the proliferation response of non-neuronal cells to Wallerian degeneration in the PNS appears to derive exlusively from studies on injured peripheral nerve. Our findings from the degenerating dorsal root are in general agreement with these previous data (Brück 1997). By extrapolation from degenerating peripheral nerve we suggest that Schwann cells as well as macrophages participate in the removal of degenerating axons and myelin in the PNS compartment of the dorsal root. The origin of the proliferating macrophages is not completely clear. Intact dorsal roots contain a small contingent of resident macrophages (Avellino et al. 1995). Circulating macrophages readily invade degenerating peripheral nerve (Brück 1997), and may be an alternative or additive source of proliferating macrophages.

About 10% of the MIB-5 positive cells in the CNS, and about 20% in the PNS, were not labelled with any of the glial markers used. One possibility is that these cells are still too undifferentiated to have developed a phenotype recognized by our markers. Alternatively, the unidentified cells may represent a category of cells to which appropriate markers were not applied. There are no previous reports of oligodendrocyte proliferation after distant axon injury, indicating that this is not a source of the unidentified cells in the CNS. In the PNS, it is possible that, e.g., connective tissue cells contribute to the proliferating cell population (Fu and Gordon 1997).

Sciatic nerve injury

Peripheral nerve injury induces proliferation of microglial cells, but not astrocytes, in the DRTZ, WM and GM. Previous studies using ^{3}H-thymidine (Murray et al. 1990) or BrdU (Persson et al. 1995) to label proliferating cells have shown similar results with regard to the grey and white matter. Virtually no proliferating cells were observed in the degenerating PNS compartment. These marked differences compared with dorsal root injury clearly reflect the absence of axon degeneration after peripheral nerve injury at these early postlesion stages. The induction of a microglial proliferation accompanies the early transganglionic changes in the central terminal of axotomized sensory ganglion cells. A similar microglial response occurs in the vicinity of motoneurons after motor axon injury (for review see Aldskogius and Kozlova 1998). These observations suggest the presence of common motor and sensory neuron "signal(s)" in the mediation of microglial cell proliferation after peripheral nerve injury.

Peripheral sensory nerve injury eventually leads to degeneration and loss of primary sensory axons and terminals in the affected projection territories in the CNS (Aldskogius et al. 1992). These changes occur considerably later than the period of microglial cell proliferation. Therefore, transganglionic degeneration appears not to be a major factor underlying this proliferation. Furthermore, several previous ultrastructural studies have documented extensive degeneration and loss of primary sensory terminals following peripheral nerve injury with no or minimal phagocytosis of microglia (Castro-Lopes et al. 1990; Persson et al. 1991). The functional signifi-

cance of rapid microglial proliferation in this situation is therefore obscure.

Concluding comments

Results from previous studies indicate that microglia and astrocytes mutually influence each other when the normal CNS homeostasis is disturbed. Activated microglia release factors which promote astroglial cell proliferation and hypertrophy. Putative such factors include interleukin-1 (Da Cunha et al. 1993; Giulian et al. 1994) and interleukin-6 (Suzumura et al. 1996; Klein et al. 1997). On the other hand, astrocytes have been implicated as a source for microglial growth factors such as colony stimulating factor (CSF)-1 (Fujita et al. 1996; Lee et al. 1993, 1994). Thus, the proliferative patterns described here in the various CNS locations are likely to reflect not only interactions between the injured neurons and surrounding non-neuronal cells, but also between the non-neuronal cells residing in the affected regions. The lesion paradigms used here are attractive experimental models for exploring the mechanisms and functional significance of these interactions for axon growth and neural plasticity.

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