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THE INJURY RESPONSE OF OLIGODENDROCYTE PRECURSOR CELLS IS INDUCED BY PLATELETS, MACROPHAGES AND INFLAMMATION-ASSOCIATED CYTOKINES

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Abstract—Oligodendrocyte precursor cells recognized with the NG2 antibody respond rapidly to CNS injuries with hypertrophy and upregulation of the NG2 chondroitin sulfate proteoglycan within 24 h. These cells participate in glial scar formation, remaining around the injury site for several weeks. After injury, reactive oligodendrocyte precursor cells increase their production of several chondroitin sulfate proteoglycans, including NG2: this cell type thus represents a component of the inhibitory environment that prevents regeneration of axons in the injured CNS. This study analyzes factors that activate oligodendrocyte precursor cells. Both microglia and astrocytes become reactive around motor neurons following peripheral nerve lesions. We show that oligodendrocyte precursor cells do not hypertrophy or increase NG2 levels after these lesions. Those lesions that cause an oligodendrocyte precursor cell reaction generally open the blood–brain barrier. We therefore opened the blood–brain barrier with microinjections of vascular endothelial growth factor or lipopolysaccharide to the rat and mouse brain, and examined oligodendrocyte precursor cell reactivity after 24 h. Both treatments led to increases in NG2 and hypertrophy of oligodendrocyte precursor cells. Of directly injected blood components serum and thrombin were without effect, while platelets and macrophages activated oligodendrocyte precursor cells. We tested the effects of a range of injury-related cytokines, of which tumor necrosis factor α; interleukin-1; transforming growth factor β; interferon γ had effects on oligodendrocyte precursor cells. Oligodendrocyte precursor cell chemokines, and mitogens did not increase NG2 levels.

Key words: CNS injury, chondroitin sulfate proteoglycans, NG2, platelets, astrogliosis, inflammation.

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Abbreviations: BBB, blood–brain barrier; CSPG, chondroitin sulfate proteoglycan; DAB, diaminobenzidine; dpl, days post-lesion; EGF, embryonic growth factor; hpi, hours post-injection; IFNγ, gamma-interferon; IL, interleukin; IR, immunoreactivity; LPS, lipopolysaccharide; MCP, macrophage chemoattractant protein; MIP, macrophage inflammatory protein; NGS, normal goat serum; OPC, oligodendrocyte precursor cell; PDGF, platelet-derived growth factor; PFA, paraformaldehyde; PRP, platelet-rich plasma; TBS, Tris-buffered saline; TGF, transforming growth factor; TNF, tumor necrosis factor; TNS, Tris non-saline; TRAP, thrombin receptor activator peptide; TXTBS, Tris-buffered saline with 0.02% Triton X-100; VEGF, vascular endothelial growth factor.

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In the adult CNS, antibodies to the chondroitin sulfate proteoglycan (CSPG) NG2 predominantly label a population of glial cells distributed uniformly throughout both the gray and white matter. These cells are antigenically distinct from other glial types [Levine et al., 1993; Nishiyama et al., 1997; Reynolds and Hardy, 1997; Keirstead et al., 1998; McTigue et al., 1998; Butt et al., 1999; Ong and Levine, 1999; Dawson et al., 2003], and may be recognized by their small, irregular cell body and multiple fine, highly branched processes [Levine and Card, 1987]. The capacity for these cells to differentiate into mature oligodendrocytes both *in vitro* upon isolation from rat tissues [Levine and Stallcup, 1987; Stallcup and Beasley, 1987] and *in vivo* during remyelination following experimentally-induced demyelination [Keirstead et al., 1998; McTigue et al., 2001], has led to their general title of oligodendrocyte precursor cells (OPCs) (reviewed by Blakemore and Keirstead, 1999; Reynolds et al., 2001, 2002). More recent evidence suggests these cells may have other functions in the adult CNS: it has become clear that OPCs are involved in aspects of glutamate signaling and homeostasis [Reynolds and Herschkowitz, 1987; Ong and Garey, 1996; Butt et al., 1999; Domercq et al., 1999; Ong and Levine, 1999; Bergles et al., 2000; Dawson et al., 2003].

Following injury or infection to the CNS, OPCs respond rapidly [Levine, 1994; Keirstead et al., 1998; Redwine and Armstrong, 1998; Di Bello et al., 1999; Levine et al., 1998; Levine and Reynolds, 1999; Ong and Levine, 1999; McTigue et al., 2001], displaying within 24 h an increased expression of CSPGs (particularly NG2) and reactive morphology (hypertrophy of the cell body and processes) at the site of tissue damage [Levine, 1994; Jones et al., 2002; Hampton et al., 2004]. Reactive OPCs also undergo rapid cell division within 1–2 mm of the lesion site, accumulating in number for at least 7 days post-lesion (dpl) [Levine, 1994; McTigue et al., 2001; Rhodes et al., 2003; Hampton et al., 2004], where the cells contribute to glial scar formation.

Previous studies have demonstrated the axon growth-inhibitory properties of CSPGs found in the adult rat CNS (reviewed by Asher et al., 2001). Increased CSPG expression by reactive astrocytes and OPCs in the glial scar produces a particularly inhibitory barrier to regenerating axons [e.g. Davies et al., 1997, 1999].

The CSPG NG2 is highly inhibitory to growing axons [Dou and Levine, 1994; Fidler et al., 1999; Chen et al., 2002], and OPCs produce a variety of other inhibitory CSPGs, including neurocan, versican, and phosphacan [Asher et al., 2000, 2002]. The early and rapid increase in

numbers of OPCs concentrated around the site of a CNS lesion therefore represents a significant barrier to axon regeneration. Previous studies have demonstrated that preventing the accumulation of OPCs in CNS injuries through treatment with ethidium bromide [Moon et al., 2000] or cytosine-D-arabinoside [Rhodes et al., 2003] enhances the capacity for lesioned axons to regenerate.

In this study we have sought to identify signals responsible for the early response of OPCs to CNS injury, examining upregulation of NG2 and hypertrophy. We first investigated whether a "closed" activation of the CNS injury response caused by a peripheral nerve lesion, which has been shown to initiate a microglial response but does not open the blood-brain barrier (BBB; see Raivich et al., 1999 for review), was sufficient to activate the OPCs. We compared this to direct trauma to the CNS, which causes an injury response in which the BBB is opened.

Previous studies using such models of direct and indirect trauma, in particular the facial nerve nucleus and elsewhere, have provided much information on the sequence of changes in immune-related proteins, trophic factors and cytokines, and their role in the injury response [e.g. Serpe et al., 1999; Galiano et al., 2001; Kalla et al., 2001; Petitto et al., 2003; reviewed in Raivich et al., 1999]. These studies have not, however, examined the participation of OPCs in the closed lesion injury model, therefore their response to such injuries was examined. We have then gone on to examine factors that might be responsible for the rapid increase in NG2 levels on OPCs in the damaged CNS.

We show that lesions that open the BBB activate OPCs, and identify platelets as a major factor in the blood. In order to identify molecules able to activate OPCs *in vivo*, we made single microinjections of injury-related candidate molecules and measured the resulting changes in NG2 on OPCs.

EXPERIMENTAL PROCEDURES

Surgical procedures and tissue preparation

General procedures and animal care. All animal work was carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. The minimum number of animals was used for the experiments, and suffering was minimized by the use of appropriate analgesia and other measures. Adult female Sprague–Dawley rats (200–220 g) were anesthetized with 5% halothane then placed in a stereotactic frame (with incisor bar set 2.3 mm below the interaural line), where halothane was maintained at 1–2% with 0.6 l/min N_2O and O_2 during surgical procedures. Wounds were sutured [Vicryl 4/0 absorbable sutures]; 4% glucose-0.18% saline was administered s.c. (2.5 ml/flank) and animals were monitored in a heated recovery box until fully conscious.

Sciatic nerve crush. An incision was made through the skin overlying the right femur, and the sciatic nerve freed from the overlying muscle in eight rats. The nerve was crushed just posterior to the greater trochanter using fine needle holders. A band of transparent tissue was seen at the site of the crush, confirming a complete injury; muscle and skin were then sutured. Rats were terminally anesthetized with an i.p. overdose of sodium pentobarbitone [Euthatal; Rhone Merieux, Harlow, UK] after 7 days and perfused through the heart with 200 ml cold PBS prewash (pH 7.4) followed by 200 ml cold 4% paraformaldehyde (PFA). The spine was removed, the cord dissected out and postfixed overnight at 4 °C then transferred to 30% sucrose. Six series of longitudinal sections (40 μm) were taken from the spinal cord between L2 and S2, using a freezing sledge microtome; sections were stored in Tris-buffered saline (TBS) with 0.05% sodium azide at 4 °C.

Microinjections. Adult female rats were anesthetized as described above, and fitted into a stereotactic frame. A midline incision was made along the top of the skull, the skin retracted and the periosteum cleared to expose the bregma. Two holes were drilled at −2.8 mm A and ±2.9 mm L relative to the bregma. A picospritzer was used to inject substances via fine, pulled, siliconized, glass micropipettes lowered to −5.2 mm V relative to the dura. A total of 0.5 μl of each substance was injected over 5 min, the needle was left in place for at least 2 min prior to withdrawal from the brain. Each animal received bilateral injections into the basal ganglia consisting of the substance of interest (into the right hemisphere) and a control (usually vehicle only) injection on the contralateral side (see Table 1); wounds were sutured and postoperative care administered as above. Animals were terminally anesthetized with an overdose of sodium pentobarbitone at 2 or 24 h post-injection (hpi), and perfused as above. The brains were immediately removed and post-fixed overnight in 4% PFA at 4 °C before being transferred to 30% sucrose for cryoprotection. Each brain was sectioned into 10 series of 40 μm coronal sections using a freezing sledge microtome; sections were stored in TBS–azide as above.

Cell culture and preparation for grafting. Cells from the macrophage-like cell line, J774 [American Type Culture Collec-

Table 1. Substances and controls microinjected into the right hemisphere of adult rat basal ganglia (contralateral side)

Substance	Source and concentration	Vehicle/ control	*n*
Blood	Littermate	Saline	3
EGF	R & D Systems, Abingdon, UK; 1 and 200 μg/ml	Saline	3, 3
FGF2	Roche, Welwyn, UK; 10 μg/ml	Saline	3
GRO-α	R & D Systems; 10 μg/ml	PBS	3
IFNγ	R & D Systems; 1 mg/ml	PBS	4
IL-1α	R & D Systems; 25 μg/ml	Saline	3
IL-8	R & D Systems; 10 μg/ml	PBS	3
IL-15	R & D Systems; 5 μg/ml	PBS	3
J774 cells	ATCC, Manassas, VA, USA; 505,000 cells/ml	DMEM	3
LPS	Sigma, Poole, UK; 10 mg/ml	Saline	3
MCP-1	R & D Systems; 10 μg/ml	PBS	3
MIP-2	R & D Systems; 10 μg/ml	PBS	3
PDGF-AB	R & D Systems; 10 and 200 μg/ml	Saline	3, 3
Platelets	Littermates (200,000 cells/ml)	Platelet-poor plasma	4
Rat Ig	Jackson Immunolabs, Soham, UK; 10 μg/ml	Inactivated rat Ig	4
Serum	Littermates	Saline	5
TGFα	R & D Systems; 10 μg/ml	Saline	3
TGFβ_1	R & D Systems; 40 μg/ml	PBS	3
Thrombin	Calbiochem, Nottingham, UK; 10,000 U/ml	PBS	3
TNFα	R & D Systems; 1 mg/ml	Saline	4
TRAP6	Bachem, St. Helens, UK; 500 mM/ml	PBS	3
VEGF	R & D Systems; 1 mg/ml	Saline	4

Total volume of each injection 0.5 μl. DMEM, Dulbecco's modified Eagle medium; PBS, phosphate-buffered saline.