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Immune cell involvement in dorsal root ganglia and spinal cord after chronic constriction or transection of the rat sciatic nerve

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Abstract

Chronic constriction injury (CCI) of the sciatic nerve in rodents produces mechanical and thermal hyperalgesia and is a common model of neuropathic pain. Here we compare the inflammatory responses in L4/5 dorsal root ganglia (DRGs) and spinal segments after CCI with those after transection and ligation at the same site. Expression of ATF3 after one week implied that 75% of sensory and 100% of motor neurones had been axotomized after CCI. Macrophage invasion of DRGs and microglial and astrocytic activation in the spinal cord were qualitatively similar but quantitatively distinct between the lesions. The macrophage and glial reactions around neurone somata in DRGs and ventral horn were slightly greater after transection than CCI while, in the dorsal horn, microglial activation (using markers OX-42(for CD11b) and ED1(for CD68)) was greater after CCI. In DRGs, macrophages positive for OX-42(CD11b), CD4, MHC II and ED1(CD68) more frequently formed perineuronal rings beneath the glial sheath of ATF3+ medium to large neurone somata after CCI. There were more invading MHC II+ macrophages lacking OX-42(CD11b)/CD4/ED1(CD68) after transection. MHC I was expressed in DRGs and in spinal sciatic territories to a similar extent after both lesions. CD8+ T-lymphocytes aggregated to a greater extent both in DRGs and the dorsal horn after CCI, but in the ventral horn after transection. This occurred mainly by migration, additional T-cells being recruited only after CCI. Some of these were probably CD4+. It appears that inflammation of the peripheral nerve trunk after CCI triggers an adaptive immune response not seen after axotomy.

Keywords: Neuroinflammation; Macrophage; Microglia; Astrocyte; OX-42 (CD11b); MHC I; MHC II; ED1 (CD68); CD8+ T-cells; CD4+ macrophages

1. Introduction

Chronic constriction injury (CCI) of the rat sciatic nerve is frequently studied as a model of neuropathic pain, with most lesioned animals demonstrating mechanical allodynia and thermal hyperalgesia within a few days of the lesion (Bennett and Xie, 1988; Schäfers et al., 2003). A series of loose ligatures or a length of tight tubing around the nerve trunk induces local inflammation and swelling that increases the constriction over several days, leading to substantial damage mainly, but not exclusively, to large myelinated axons, and incomplete denervation of the peripheral field (Basbaum et al., 1991; Wakisaka et al., 1991; Munger et al., 1992; Ma and Bisby, 2000). The role of inflammation in the dorsal horn proximal to the lesion has been extensively investigated although whether abnormal pain symptoms arise because of this or other aspects of the inflammatory response is not clear.

Spontaneous and evoked pain after nerve injury are thought to derive from hyperexcitability of primary and/or secondary afferent neurones generated by neurotrophins and proinflammatory cytokines released from activated inflammatory cells (Wieseler-Frank et al., 2005). These include microglia that become activated in the dorsal horn (Eriksson et al., 1993), astrocytes (Tanga et al., 2006) and

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macrophages that invade the lesion site. However, after nerve transection, macrophages also invade the dorsal root ganglia (DRGs) (Hu and McLachlan, 2002; Hu and McLachlan, 2003a) where the neurones that project into the dorsal horn lie. In addition, large DRG somata of muscle afferents discharge spontaneously after lesions (Kajander et al., 1992; Michaelis et al., 2000). Many interventions, including blockade of different cytokines (Wieseler-Frank et al., 2005) or the complement cascade (Twining et al., 2005), or treatment with inhibitors of microglial activation (Raghavendra et al., 2003), reduce or abolish pain behaviour. These findings have led to the view that inflammation is a major factor in generating pain despite evidence that glial activation can also have beneficial consequences (Streit et al., 2004).

Following CCI, changes occur within the dorsal horn, e.g. neuropeptide expression (Ramer et al., 1998), glial activation (Colburn et al., 1997; Zhang and De Koninck, 2006, p. 634) and neuronal hyperexcitability (Hains et al., 2004). Within DRGs projecting in the lesioned nerve, CCI is followed by changes in expression of neuropeptides (Ramer et al., 1998; Schäfers et al., 2003), neurotrophins (Obata et al., 2003b), Nav1.3 channels (Dib-Hajj et al., 1999), tumor necrosis factor-α (TNF-α, Schäfers et al., 2002), extracellular signal-regulated protein kinase (Obata et al., 2004) and p38 mitogen-activated protein kinase (Kim et al., 2002) and sprouting of sympathetic axons (Ramer et al., 1999). Inflammatory responses in DRGs after CCI have not been adequately investigated.

These forms of plasticity also occur after sciatic transection (Wakisaka et al., 1992; McLachlan et al., 1993; Waxman et al., 1994; Michael et al., 1999; Ramer et al., 1999; Zhou et al., 1999; Hu and McLachlan, 2001; Kim et al., 2002; Obata et al., 2003a) when they simply reflect axotomy and the lack of target-derived neurotrophins (e.g. Obata et al., 2004). However, as well as activation of glia (Eriksson et al., 1993) and the complement system (Liu et al., 1998) in the dorsal horn, macrophages and lymphocytes are recruited into the DRGs (Hu and McLachlan, 2002).

Similar plasticity and also pain behaviour have been demonstrated after ventral root transection in which sensory axons are not injured but intact axons are exposed to inflammation during Wallerian degeneration of the motor axons (Li et al., 2002; Xu et al., 2006). Therefore it is still not clear whether the key factors responsible for hyperexcitability and pain are the loss of target-derived neurotrophins, abnormal signalling due to inflammation within the dorsal horn and/or DRGs or factors specific to nerve lesions like CCI and ventral root transection that have a major local inflammatory component around intact sensory axons.

Here, we have compared directly the inflammatory reactions in DRGs and spinal cords of rats with CCI with those elicited by sciatic transection in order to identify whether these injuries induce distinct patterns of neuroinflammation. Immunohistochemistry has been used to identify activating transcription factor 3 (ATF3) in the cell bodies of neurones as a marker of axonal damage (Tsujino et al., 2000). The same technique was applied to investigate the expression of glial fibrillary acidic protein (GFAP) in satellite glia in DRGs and astrocytes in the spinal cord, and of CD68 (detected using ED1), CD163 (with ED2), major histocompatibility complex class I (MHC I) and II (MHC II), CD11b (with OX-42), CD4 and CD8 in macrophages in DRGs and in perivascular and microglial cells in spinal cord. T-lymphocytes expressing the α/β T-cell receptor (TCR) were identified and T-cell expression of CD4 and CD8 examined. Some observations were also made on the distribution of the low affinity nerve growth factor receptor, p75, calcitonin gene-related peptide (CGRP) and tyrosine hydroxylase (TH). Both qualitative and quantitative differences in the inflammatory reactions between the two lesions were revealed. While macrophage invasion of DRGs primarily reflected the extent of neuronal damage, activation of microglia in the dorsal horn was more marked after CCI. Although all motor neurones appeared to be axotomized by the CCI, astrocytic activation was more prominent in the ventral horn after transection. T-lymphocytes invaded both DRGs and spinal cord to a much greater extent after CCI than after transection, suggesting involvement of an adaptive immune response in neuropathic pain.

2. Methods

All experimental procedures were carried out following the Australian Code for the Care and Use of Animals in Research and the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals laid down by the International Association for the Study of Pain (Zimmermann, 1983). All procedures were approved by the Animal Care and Ethics Committees of the University of Sydney and the University of New South Wales.

Experiments were performed on adult male Sprague–Dawley (SD) rats, 250–350 g, obtained from the Animal Resource Centre, Perth, WA, Australia.

For the group subjected to a CCI lesion ($n = 13$), anaesthesia was induced with 5% halothane in 100% oxygen and maintained with 1.0–1.5% halothane in 100% oxygen via a face-mask. Four loosely tied ligatures (5–0 chromic catgut) were applied 1 mm apart around the right sciatic nerve above its trifurcation. Each ligature was tightened in order to 'reduce the diameter of the nerve and retard, but not interrupt, the epineural circulation' (Bennett and Xie, 1988). The wound was sutured and treated with antibiotic powder. Amoxycillin was administered (150 mg/kg, i.m.) prior to recovery from anaesthetic. Before and after the CCI lesion, the animals were tested each day for changes in mechanical and thermal sensitivity (allodynia and hyperalgesia) using standard techniques (Monassi et al., 2003) and observations were made each day of gait, resting posture and autotomy of the hindclaws, as described by Bennett and Xie (1988). A second group of 5 rats were anaesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg) administered i.p. and the left sciatic nerve was ligated and transected just above its trifurcation. The wound was closed after local treatment with terramycin powder. A third group of 5 age-matched naïve animals served as controls.

After post-operative survival for one week, or in 3 CCI animals for 10 weeks, the animals were deeply anaesthetized with pentobarbitone (100 mg/kg i.p.) and perfused through the descending thoracic aorta with normal saline containing heparin (10,000 IU/L) and sodium nitrite (10 mg/L) followed by Zamboni's fixative. Tissues were dissected and post-fixed overnight before washing in phosphate buffered saline (PBS) and infiltrating with 30% sucrose in PBS. Ganglia and nerve trunks taken from both