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Presence of spinal B7.2 (CD86) but not B7.1 (CD80) co-stimulatory molecules following peripheral nerve injury: role of nondestructive immunity in neuropathic pain

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Abstract

Previous work in our laboratory demonstrated spinal neuroimmune activation and leukocyte trafficking into the central nervous system (CNS) parenchyma in a rat model of neuropathic pain. Recent studies suggest that co-stimulatory molecules B7.1 (CD80) and B7.2 (CD86) play a differential role in the effect of beneficial versus deleterious CNS autoimmune responses. In the present study, we determined the lumbar spinal expression of the co-stimulatory molecules B7.1 and B7.2 in a rat model of neuropathy. We observed intense B7.2 microglial immunoreactivity in the lumbar spinal cord following the injury but no expression of B7.1. These data suggest a role of protective CNS autoimmunity following peripheral nerve injury.

Keywords: Neuroimmune activation; Autoimmunity; Neuropathy; Chronic pain; Mechanical allodynia

1. Introduction

It is recognized that the central nervous system (CNS) mounts an immunologic response to various stressors including ischemia, bacterial pathogens and peripheral nerve injury. This response may either be maladaptive leading to subsequent neural degeneration and demyelination or neuroprotective. Previous work in our laboratory demonstrated that an L5 spinal nerve transection, a rodent model of neuropathic pain, elicits marked CNS neuroimmune activation as well as leukocyte trafficking into the CNS parenchyma (DeLeo and Yezierski, 2001; DeLeo et al., 1997; Sweitzer et al., 2002a). Recent studies suggest that co-stimulatory molecules B7.1 (CD80) and B7.2 (CD86) play a differential role in the effect of beneficial versus deleterious autoimmune responses (Badie et al., 2002; Bechmann et al., 2001). Proinflammatory cytokines are thought to be activated by autoimmune $CD4^+$ T cells, which can cause demyelinating diseases such as multiple sclerosis (MS) and Alzheimer's disease. Work by Bechmann et al. (2001) demonstrated that B7.2 was associated with nondestructive autoimmunity, while the presence of B7.1 can lead to destructive autoimmunity.

It is known that B7.1 is responsible for stimulating the T_H1 response pathway, which deals with cell-mediated response through the release of pro-inflammatory cytokines such as IL-2, IFN-γ and TNF-α. Conversely, B7.2 mediates the T_H2 response pathway, leading to a release in anti-inflammatory cytokines such as IL-4, IL-5 and IL-10 (Bottomly, 1988). It has been observed that T_H1 cells downregulate the constitutive expression of B7.2 and induce B7.1 expression while T_H2 cells do not induce B7.1 activation (Wolf et al., 2001). In experimental autoimmune encephalomyelitis (EAE), T cells appear to be the main cell type expressing B7 co-stimulatory molecules (Cross et al., 1999). In the same murine model, astrocytes and endothelial cells did not express B7 immunoreactivity (Cross and Ku, 2000). In contrast to CNS autoimmune disease, axonal degeneration appears not to induce microglial B7.1 expression (Bechmann et al., 2001).

We hypothesize that beneficial CNS autoimmunity may ensue following a peripheral nerve injury as a mechanism to prevent excessive neuronal cell death. However, in this process, neuronal sensitization can occur due to the production and release of proinflammatory cytokines affecting the

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neuronal/glial milieu. This manifests as heightened sensitivity to non-noxious stimuli or chronic pain states. In the present study, we determined the lumbar spinal expression of the co-stimulatory molecules B7.1 and B7.2 as a result of a peripheral nerve injury resulting in behavioral hypersensitivity, suggestive of neuropathic pain.

2. Materials and methods

All experimental procedures were approved by the Dartmouth College Institutional Animal Care and Use Committee. All surgical procedures were performed under inhalation anesthesia: 4% halothane for induction and 2% for maintenance with O_2 as the carrier. Animals were divided into three groups: (1) a neuropathy group with an L5 peripheral spinal nerve transection on the left side, ($n=4$/time point), (2) a sham group with the L5 nerve exposed only ($n=1$/time point) and (3) normal, uninjured rats ($n=2$). Spinal nerve transection and sham surgeries were performed as previously described (Rutkowski et al., 2000). For the spinal nerve transection surgery, an incision was made, and the muscle was separated and retracted to expose the left transverse process which was then partially removed. The L5 spinal nerve was gently separated from the L4 spinal nerve and transected. Surgical procedures for the sham group involved the exposure of the L5 spinal nerve, without any manipulation or transection. Following surgery, wounds were irrigated with sterile saline and closed using 3-0 polyester suture for the fascia and surgical staples for the skin. All animals recovered in room air.

Mechanical allodynia was assessed using 2- and 12-g von Frey filaments (Stoelting, Wood Dale, IL) on the ipsilateral hind paw. Allodynia was measured as the number of hind paw withdrawals elicited by a defined non-noxious mechanical stimulus (Colburn et al., 1997). Allodynia was quantified by a tester blinded to the injury type and treatment. Rats were tested on days 1, 3, 5, 7, 10 and 14.

On day 7 or 14 postsurgery, rats were anesthetized by sodium pentobarbital injection (75 mg/kg, i.p.) and transcardially perfused with 150 ml phosphate-buffered saline (PBS) followed by 75 ml 4% paraformaldehyde in 0.1 M PBS. In a separate group of rats, complete Freund's adjuvant (CFA) (50 μl, intraplantar) was injected and used as a positive control for co-stimulatory antibodies. Following perfusion, lumbar spinal cord sections were harvested by laminectomy and postfixed for 2 h in 4% paraformaldehyde followed by 5 days in 30% sucrose/PBS. Tissue was then freeze-mounted in OCT embedding medium on cork blocks for cryostat sectioning.

Immunohistochemistry was performed on 20-μm free-floating L4–L5 spinal sections utilizing an avidin–biotin complex technique (Vector Laboratories, Burlingame, CA) as previously described (Colburn et al., 1997). Monoclonal antibodies to B7.1, B7.2 (BD Pharmingen, San Diego, CA) and OX-42 (gift of William F. Hickey) were used at a 1:10 dilution. Elimination of the primary antibody was performed in each run as a negative control. For confirmation of co-labeling of B7.2 and OX-42 on microglia cells, a separate immunohistochemical run was performed where antibodies to OX-42 and B7.2 were sequentially applied and visualized with Vector SG Blue (OX-42) and DAB (B7.2). Immunohistochemistry was scored blinded to experimental conditions. At least five spinal sections were used to determine scoring for each animal. The following general scoring categories were used for anti-B7.1 and B7.2 immunoreactivity: (•) baseline levels, (+) mild, (++) moderate, (+++) intense immunoreactivity. Data were analyzed for significance with a one-way ANOVA and post hoc Bonferroni analysis (STATA 5.0, Stata, College Station, TX). Significance was defined at $p<0.05$.

3. Results

Baseline behavioral responsiveness was minimal as confirmed by testing sessions prior to surgery. Mechanical allodynia was observed in the ipsilateral hind paw in all animals in the neuropathy group using both the 2- (results not shown due to the identical trend) and 12-g von Frey

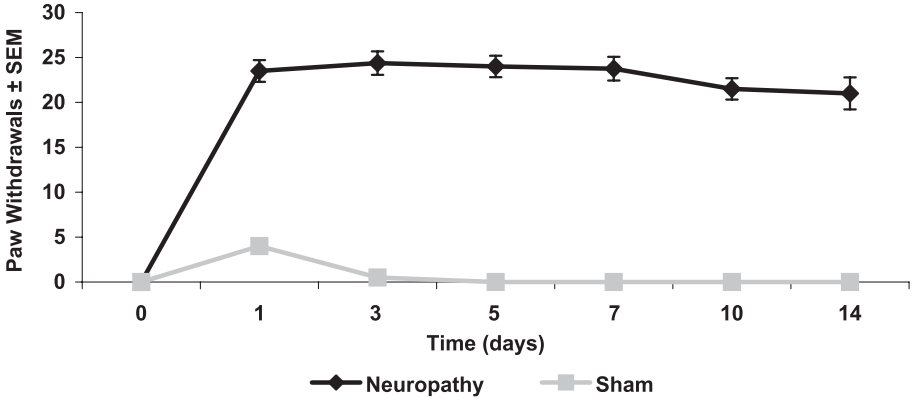

Fig. 1. Mechanical allodynia as measured by foot lift response frequency to stimulation with 12-g von Frey filament is depicted over time following L5 spinal nerve transection ($n=4$/time point) or sham surgery ($n=1$/time point). Responses are reported as the mean of 30 stimulations ± S.E.M.

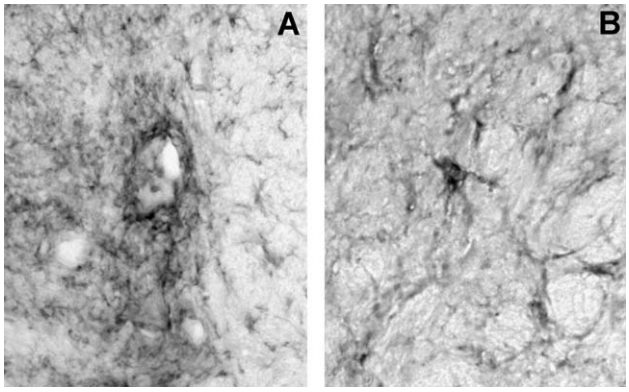

Fig. 2. Representative photomicrograph depicting B7.2 immunoreactivity in the lumbar spinal cord at postoperative day 7. (A) B7.2 positive cells surround motor neurons in the ventral horn, and (B) B7.2 positive cells in the dorsal horn are identical to microglia in morphology.

filaments (Fig. 1). There was a sharp initial increase in mechanical allodynia on day 1 following surgery for the ipsilateral hind paw. The sham surgery group displayed a slight, but not statistically significant increase in mechanical allodynia on day 1, which returned to baseline by day 5 (Fig. 1).

Positive B7.1 and B7.2 immunoreactivity was observed in the spleen of the CFA stimulated group (photomicrograph not shown). Extensive B7.2 staining was observed in the red pulp and moderate staining in the marginal zone (identical to Damoiseaux et al., 1998).

B7.1 positive immunoreactivity was not observed in the lumbar spinal cord in normal, sham surgery or following L5 spinal nerve transection. B7.2 positive immunoreactivity in the ipsilateral dorsal and ventral horns was increased over normal levels in the neuropathy group. Accumulation of B7.2 expressing cells was observed around ventral horn motor neurons (Fig. 2A). There was minimal to no staining in the white matter. B7.2 immunoreactive expression was greatest at day 7 and slightly decreased by day 14, though still elevated over normal and sham animals (Table 1; Fig. 3). The morphological expression of the B7.2 immunoreactivity was identical to ramified microglia (Fig. 2B). This was confirmed by performing co-labeling immunohistochemistry in which antibodies to OX-42 and B7.2 were applied to the same sections using Vector Blue for OX-42 staining and DAB for B7.2 staining. Representative photomicrographs shown in Fig. 4 demonstrate complete co-labeling with these two antibodies when B7.2 is expressed (top panel) in the ipsilateral dorsal horn following peripheral nerve injury. The bottom panel illustrates only OX-42 immunoreactivity in the contralateral

Table 1
B7.2 immunoreactivity in the lumbar spinal cord for normal, sham and neuropathy groups

Postoperative time	Treatment	B7.2 (CD86) staining[a]			
		Ipsilateral		Contralateral	
		DH	VH	DH	VH
Normal		•	•	•	•
		•	•	•	•
Day 7	sham	•	+	•	•
	neuropathy	+++	+++	+	++
		+++	+++	•	++
		+++	+++	+	++
		++	++	•	+
Day 14	sham	•	•	•	•
	neuropathy	++	++	+	+
		+	++	•	+
		+	++	+	++
		+	++	•	+

[a] Staining key: baseline staining (•), mild response (+), moderate response (++) and intense response (+++).

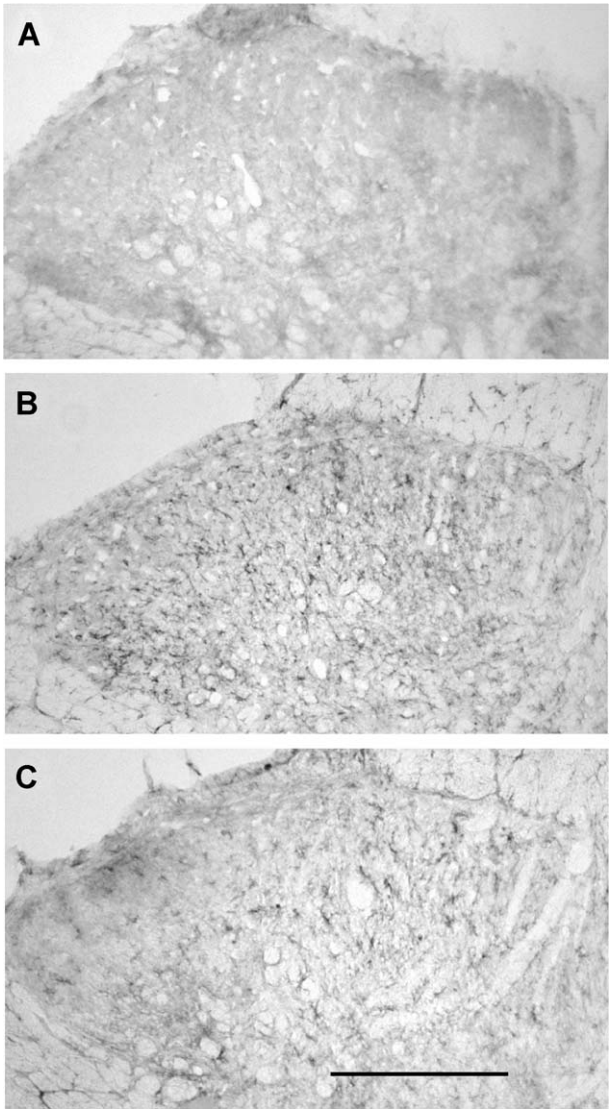

Fig. 3. Representative photomicrographs depicting B7.2 immunoreactivity in lumbar spinal cord dorsal horns in (A) sham, day 7 postoperative, (B) neuropathy, day 7 postoperative, and (C) neuropathy, day 14 postoperative. Scale bar = 250 μm.

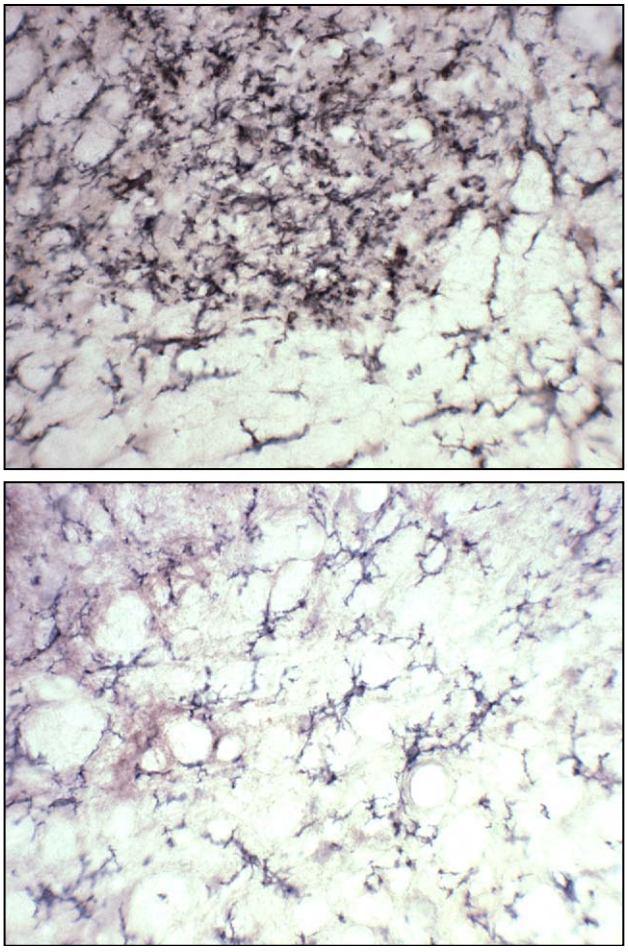

Fig. 4. Representative photomicrographs demonstrating co-labeling of OX-42 and B7.2 immunoreactivity in the ipsilateral lumbar spinal cord dorsal horns following peripheral nerve injury in the top panel. The bottom panel demonstrates only OX-42 staining in the same section of the contralateral lumbar spinal dorsal horn.

dorsal horn of the same section. There was no evidence of phagocytic microglia or T cell expression of B7.2 immunoreactivity.

4. Discussion

We report a differential spinal expression of the costimulatory molecules, B7.1 and B7.2 following a peripheral nerve injury that results in behavioral hypersensitivity, a pathologic correlate to clinical neuropathic pain. It has been previously demonstrated that B7.1 is associated with destructive immunity as seen in MS and EAE. The concept that T cells can be protective through a cascade of events that entails microglial activation, induction of MHC-II, B7.2 expression that leads to infiltration of neurotrophin-secreting T cells, is mimicked in the spinal cord following peripheral nerve injury (Colburn et al., 1997; Sweitzer et al., 2002a,b). The extensive upregulation of B7.2 in the lumbar spinal cord following a peripheral nerve injury suggests a role of protective CNS immunity in neuropathic pain. In fact, the degree of neuronal death has been reported to be very limited in the superficial dorsal horn of the spinal cord following peripheral nerve injury (Whiteside and Munglani, 2001).

We hypothesize that following a peripheral nerve injury, within minutes and hours spinal glia become activated, upregulating their expression of specific surface antigens and proinflammatory cytokines (Tanga et al., 2004; DeLeo et al., 1997). These cytokines, namely, IL-1, IL-6 and TNF induce cellular adhesion molecule (CAM) expression (Sweitzer et al., 2002b). These CAMs, by nature of their action, enable T cell and/or macrophage migration into the parenchyma of the CNS (Sweitzer et al., 2002a). This cell migration propagates the immune response further by increasing cytokine expression and MHC-II expression rendering microglia and astrocytes immunocompetent. In the presence of this ongoing inflammatory response, both infiltrating cells and endogenous glia become immune activated. These proinflammatory cytokines can amplify and sustain inflammatory responses that in turn affect neural function. For example, cytokines induce COX expression which leads to production of algesic mediators, such as prostaglandins. In addition, it is known that cytokines also induce substance P and NO release. If the organism is unable to turn off or downregulate this neuroinflammatory process, persistent neuropathic pain may ensue.

In summary, the finding that B7.2 is selectively upregulated in the spinal cord following a peripheral nerve injury supports the presence of nondestructive CNS immunity. However, this neuroimmune response associated with glial activation and leukocyte trafficking may provide and maintain a hypersensitive state that manifests as debilitating chronic pain states. The challenge for pain therapy, therefore, remains to blunt only that aspect of the CNS immune sensitizing response and not perturb neuroprotective autoimmunity.

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