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Lumbar Nerve Root Injury Induces Central Nervous System Neuroimmune Activation and Neuroinflammation in the Rat

Relationship to Painful Radiculopathy

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Study Design. These studies were designed to examine the role of central neuroimmune activation and neuroinflammation in a rat model of lumbar radiculopathy.

Objectives. In the present study the authors investigated the role of neuroimmune activation using immunocytochemistry to detect expression of major histocompatibility complex Class II, cluster determinant 4, intracellular adhesion molecule-1 (ICAM-1), and platelet endothelial cellular adhesion molecule-1 (PECAM-1). The role of central neuroinflammation was investigated using radiation bone marrow chimeric rats.

Summary of Background Data. The pathologic mechanisms resulting in painful lumbar radiculopathy secondary to nerve root injury remain obscure. There is a growing body of evidence that central neuroimmune activation and neuroinflammation may play a key role in the initiation and maintenance of various pain states, including lumbar radiculopathy.

Methods. Male Holtzman rats undergoing mechanical sensitivity testing were divided into two groups: a sham group and a chromic gut suture group. Animals were killed on day 14 post surgery. Male Holtzman rats, used to detect cluster determinant 4, major histocompatibility complex Class II, and CAM spinal expression, were divided into three groups: a normal group, a sham surgery group, and a chromic group. The male Brown Norway rats used to make the radiation bone marrow chimeras were divided into two groups: a sham group and a chromic group. Animals were killed at 1, 3, 7 or 14 days following surgery.

Results. Nerve root injury in the rat produced increased spinal major histocompatibility complex Class II, cluster determinant 4, ICAM-1, and PECAM-1 immunoreactivity and increased bilateral sensitivity to tactile stimuli. Leukocyte trafficking into the spinal parenchyma was observed, which increased over time after nerve root injury.

Conclusions. The presence of bilateral mechanical allodynia and spinal neuroimmune changes following nerve root injury supports the hypothesis that central sensitization through activation of immune mediators, coupled with macrophage traffic across the blood–brain barrier, plays a key role in the development and maintenance of radicular pain. [Key words: CD4, ICAM-1, low back pain, MHC Class II, microglia, PECAM-1, radiation bone marrow chimeras] **Spine 2002;27:1604–1613**

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Chronic low back pain with or without radiculopathy is a frequently occurring condition in Western society with a lifetime prevalence of 60%–80%.[13,46] The financial burden to society as a result of lost productivity and the cost of treatment is enormous.[46] Despite a plethora of experimental and clinical data concerning lumbar radiculopathy (LR),[26,29,37] the broad range of treatments available have not made a significant impact on the course of pain progression or decreased the incidence of LR in the United States.

A variety of pain states, including referred pain, mirror pain and neuropathic pain, are thought to be centrally mediated.[7] LR may also be the result of similar central nervous system (CNS) mechanisms. Experimental evidence suggests that spinal cord neuroplasticity and neuroimmune processes are major contributors in the initiation and maintenance of chronic pain after central or peripheral nerve damage.[17,30,38] Many different chemical mediators have been implicated in the enhanced facilitation of central pain pathways: neurotransmitters, such as substance P and *N*-methyl-D-aspartate,[22,50] inflammatory mediators including prostaglandins,[42] and more recently, proinflammatory cytokines.[11,16,48] It is unlikely that any one of these mediators in isolation is responsible for exaggerated nociceptive modulation after peripheral nerve or nerve root injury. Rather, they most likely act in concert at peripheral and central locations to bring about CNS changes, leading to a variety of pain states.

Interest in CNS immune responses has grown rapidly in recent years with the recognition of the role of CNS inflammation and immune responses in the etiology of neurologic disorders, such as the AIDS dementia complex, Alzheimer's disease, cerebral infarction, Parkinson's disease, traumatic brain and spinal cord injury, and demyelinating diseases, such as multiple sclerosis.[41] Similarly, the role of central neuroimmune activation and neuroinflammation in chronic pain is currently an area

of intense interest.[48] Neuroimmune activation involves endothelial cells, microglia, and astrocytes. Activation of these cells leads to subsequent production of cytokines, chemokines, and the expression of surface antigens that enhance the central immune cascade and render cells immunocompetent. The hallmark of the inflammatory component of an innate immune response is the infiltration and/or migration of cells to the site of injury. Neuroinflammation can be defined as the infiltration of leukocytes into the perceived site of injury in response to damage of the nervous system. Together, neuroimmune activation and neuroinflammation following nerve root injury may generate a vicious cycle that directly sensitizes dorsal horn neurons and/or induces the generation of other algesic mediators.[12] For example, there is mounting evidence that cytokines induce the release or expression of cyclooxygenase (COX)-2, inducible nitric oxide (NO) synthase, and substance P as well as enhancing capsaicin sensitivity.[23,24,34,43] Similarly, activated glial cells synthesize proinflammatory cytokines, proteases, NO, excess glutamate, superoxide anions, hydrogen peroxide, eicosanoids, and other toxins that act by way of the *N*-methyl-D-aspartate receptors.[5,6,25,31,35] Therefore, cytokines can induce or amplify the activation of CNS elements in a way that may indirectly enhance spinal sensitization.

Previous studies have reported the role of glial activation and increases in spinal cord expression of the proinflammatory cytokine interleukin-1β in a model of LR in the rat.[18] From these data we further postulated that increased spinal production and expression of specific cytokines may induce cellular adhesion molecules and initiate cell trafficking into the spinal parenchyma after injury. To better understand these central processes in LR, we adopted two approaches in the LR model to examine both neuroimmune activation and neuroinflammatory responses. Initially, spinal cord expression of major histocompatibility complex (MHC) Class II and CD4 molecules and intracellular adhesion molecule (ICAM)-1 and platelet endothelial cellular adhesion molecule (PECAM)-1 was characterized. Although these immunologically important membrane glycoproteins and cellular adhesion molecules (CAMs) are pivotal components in the immune cascade, little is known about their spinal cord expression following injury to lumbar nerve roots.

In a complementary study radiation bone marrow chimeric rats were used to determine whether leukocyte traffic into the CNS is possible. To produce the chimeric rats, bone marrow cells are harvested from one strain of rat, termed the donor strain. A second and different strain of rat, termed the recipient strain, is lethally irradiated. These recipient rats are systemically injected with the bone marrow cells from the donor strain; without this bone marrow transplant, the recipient rats would not survive. The rats are then left to mature to repopulate their immune cells. Using antibodies that are specific to the donor strain, it is possible to determine whether cells of the donor origin are able to cross the blood–brain barrier into the CNS of the recipient. Assessment of the distribution and magnitude of these cells throughout the L5 region of the spinal cord in response to a selective L5 nerve root injury is also possible.

The rodent model of LR used in the present study is well established and was developed to mimic the chemical and mechanical components involved in clinical radiculopathy secondary to herniated nucleus pulposus.[17,18] Behavioral and immunocytochemical data from the current study support the hypothesis that central neuroimmune activation and neuroinflammation are important contributors in the genesis of LR and its behavioral sequelae. The elucidation of these central immune responses and their association to pain may lead to the development of new and more effective treatments for LR than those currently available.

Materials and Methods

Animals. All rats, including Brown Norway (BN), Lewis × BN F_1 hybrids, and Holtzman strains (Harlan Sprague Dawley, Indianapolis, IN), were housed individually under USDA and AAALAC-approved conditions with 12–12 hours light–dark cycle and free access to food and water. All experimental procedures were approved by the Dartmouth College Institutional Animal Care and Use Committee. Experiments using radiation bone marrow chimeric rats were performed with male BN × Lewis (BN × L) F_1 hybrids and male BN rats. The BN × L F_1 hybrids served as bone marrow donors. Radiation bone marrow chimeras were produced as previously detailed.[19] Briefly, 2-month-old BN rats were lethally irradiated (1000 rad) and reconstituted with 10^8 bone marrow and spleen cells from the F_1 hybrids *via* tail vein injection within 2–4 hours of irradiation. These chimeric rats were allowed to mature for 2 months to repopulate their somatic tissues with donor-derived cells before undergoing the LR surgery.

Surgical Procedure. All surgical procedures were performed under inhalation anesthesia (induced at 4% and maintained at 2% halothane in 100% O_2). The LR model has been previously described.[17] Briefly, a sham surgery consisted of a left L5 hemilaminectomy to expose, without any manipulation of, the left L5 nerve root. In the chromic gut suture surgery, the exposed left L5 nerve root was ligated with two loose 5–0 chromic gut ligatures following application of five 3-mm pieces of 4–0 chromic gut suture to the nerve root proximal to the dorsal root ganglion. The muscle layers and skin incision were closed with 3–0 Ethibond polyester suture and staples, respectively. The male Holtzman strain of rats undergoing mechanical sensitivity testing was divided into two groups: 1) a sham surgery group (n = 4) and 2) a chromic group (n = 7). All animals were killed on day 14 post surgery. The male Holtzman strain of rats used to detect CD4, MHC Class II, and CAM expression was divided into three groups: 1) a normal group (n = 2) in which no procedure was performed, 2) a sham surgery group (n = 4), and 3) a chromic group (n = 12). One sham animal and three chromic animals were killed on days 1, 3, 7, or 14 post surgery for spinal cord assessment. The radiation bone marrow chimeric rats were divided into two groups: 1) a sham group (n = 4) and 2) a chromic group (n = 16). Of these animals, one sham

Table 1. CD4, ICAM-1, and PECAM-1 Immunoreactivity in the Lumbar Spinal Cord for Normal, Sham, and Chromic Groups

		CD4 Expression		ICAM Expression		PECAM Expression	
Treatment	Day	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra
Normal		+	+	+	+	+	+
Sham	1	+	+	+	+	+	+
	3	+	+	+	+	+	+
	7	++	+	+	+	+	+
	14	++	+	+	+	+	+
Chromic	1	+	+	+	+	+	+
		+	+	+	+	+	+
		+	+	+	+	+	+
	3	+	+	+	+	+	+
		+	+	+	+	+	+
		+	+	+	+	+	+
	7	+++	++	+	+	+++	++
		+++	++	++	+	+++	++
		+++	++	++	+	++	++
	14	+++	++	++	+	+++	++
		+++	++	+	+	++	++
		+++	++	++	+	+++	++

+ = baseline staining; ++ = mild response; +++ = moderate response; ++++ = intense response; Ipsi = ipsilateral; Contra = contralateral.

animal and four chromic animals were killed at 1, 3, 7, or 14 days following surgery for spinal cord assessment.

Mechanical Allodynia. Animals were tested for 3 days before surgery to acclimate them to the behavioral testing apparatus and to obtain baseline values. All behavioral testing was performed by an investigator blinded to the surgical procedure. Mechanical allodynia was measured as the frequency of foot withdrawals elicited by a defined non-noxious, mechanical stimulus.[9] In each blinded testing session, rats were subjected to three sequential series of 10 tactile stimulations to the plantar surface of the ipsilateral (nerve root injured) hind paw and contralateral hind paw using 2-g and 12-g von Frey filaments (Stoelting, Wood Dale, IL). Mechanical allodynia was assessed by recording the total number of responses elicited during three successive trials (10 stimulations/each filament) separated by at least 10 minutes for a total possible score of 30. After surgery mechanical allodynia was assessed up to 14 days.

Histologic Preparation and Immunocytochemistry. Under deep anesthesia (100 mg/kg sodium pentobarbital, intraperitoneally), rats were killed by transcardiac perfusion with 200 mL of phosphate-buffered saline followed by 100 mL of 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline, pH 7.4. After perfusion and laminectomy the L5 nerve roots were verified and traced to their site of entry into the spinal cord. Appropriate L5 spinal cord segments were harvested and cryoprotected for 3–4 days in 30% sucrose/phosphate-buffered saline at 4 C. The segments were then freeze-mounted in OCT embedding medium on cork blocks for cryostat sectioning.

Immunocytochemistry was performed by the avidin-biotin technique (Vector Labs, Burlingame, CA) on free floating 20-μm sections. Elimination of the primary antibody was performed in each run as a negative control. The culture supernatants containing the following monoclonal antibodies were used: OX-6 (anti-MHC Class II), W3/25 (anti-CD4), TLD-4C9 (anti-ICAM-1), TLD-4E8 (anti-PECAM-1), OX-42 (CR3/CD11b), and ED-2. Lumbar spinal cord sections of the radiation bone marrow chimera rats were stained with the culture supernatant containing a donor antibody, I1–69 (anti-MHC Class I).

Assessment of spinal cord staining was performed by an investigator blinded to the surgical groups. After staining of lumbar spinal cord, L5 sections from each animal were surveyed under low (10×) and medium (40×) magnification using an Olympus bright field microscope. A scoring system for CD4 immunoreactivity was based on a previously established scale (Table 1).[9] An analogous scoring system for ICAM-1 and PECAM-1 was developed for this study (Table 1). The final score was based on the average reactivity of all sections for each animal. This semiquantitative scoring allowed comparison between the normal, sham, and chromic groups. Individual cell profiles containing positive immunoreactive MHC Class II and MHC Class I in the chimeric rats were counted in each tissue.

Statistical Analysis. All data obtained from the observations of mechanical sensitivity are presented as the mean response of all animals per treatment group ± SEM. To compare the time-dependent curves among the groups, a repeated analysis of variance with a Bonferroni multiple comparison was used. Additionally, the data from the tactile stimulation were analyzed by a one-way analysis of variance at each time point. $P < 0.05$ for intergroup differences was defined as significant.

Results

Mechanical Allodynia

Baseline (prelesion) responsiveness was minimal as confirmed by testing sessions before the surgery. Mechanical allodynia was observed in both ipsilateral and contralateral hind paws in all animals in the chromic gut suture group. Overall, mechanical allodynia was significantly greater in the ipsilateral hind paw (Figure 1A) than the contralateral (unoperated) hind paw (Figure 1B) when tested with both the 2-g and 12-g von Frey filaments (analysis of variance; $P < 0.001$) (2-g data not shown).

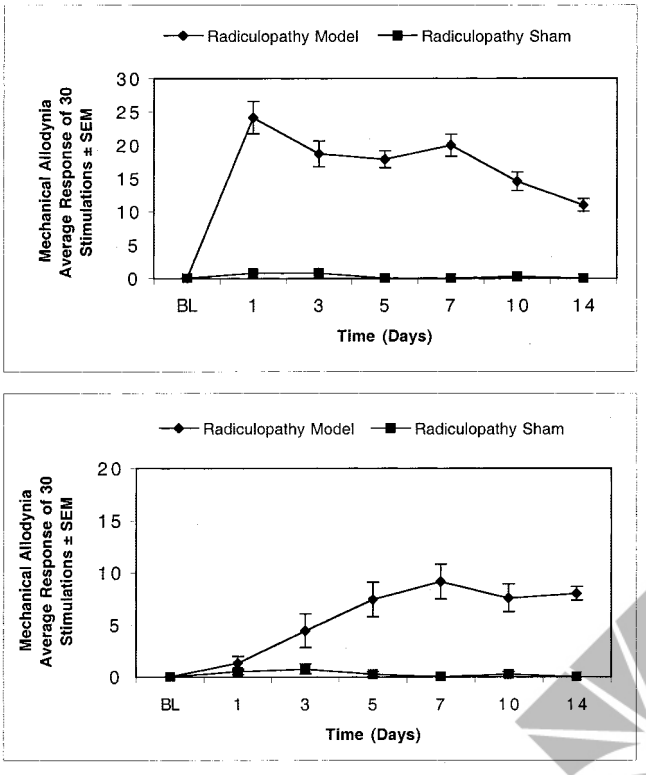

Figure 1. Time course for ipsilateral **(A)** and contralateral **(B)** hind paw mechanical allodynia using 12-g von Frey filaments following both ligation of the left L5 nerve root with chromic gut sutures and exposure of the left L5 nerve root. Average foot lift response frequency ± SEM to 30 stimulations is depicted.

There was a sharp initial increase in mechanical allodynia on day 1 following surgery for the ipsilateral hind paw. In contrast, the maximal response in the contralateral hind paw was not seen until day 7. The sham group did not display significant mechanical allodynia in either hind paw over baseline testing (Figure 1).

The typical mechanical allodynia response was observed at 1, 3, 7, and 14 days post surgery in the radiation bone marrow chimeric rats that underwent LR surgery. Rats in the chimeric sham group did not show any increase in mechanical allodynia over their baseline measurements using both 2-g and 12-g von Frey filaments (results not shown).

Spinal CD4, MHC Class II, and CAM Immunoreactive Expression

Diffuse CD4 immunoreactivity of cells of microglial morphology was detected throughout the spinal gray matter of the chromic group. The staining in this group was slightly more intense on the ipsilateral than the contralateral side. The CD4 immunoreactivity increased over time, with maximum expression at days 7 and 14 post surgery (Table 1). Sham animals displayed some increase over the normal group, but compared with the chromic group, immunoreactivity was minimal (Figure 2A–C). CD4 immunoreactivity colocalized with OX-42 immunoreactivity on specific cells as demonstrated in Figure 3C.

MHC Class II immunoreactivity was restricted to cells with morphology similar to that of activated microglia with bushy, perineuronal appearance. Immunoreactivity was increased in all regions of the spinal cord gray matter in the chromic group (Figure 2F) compared with the

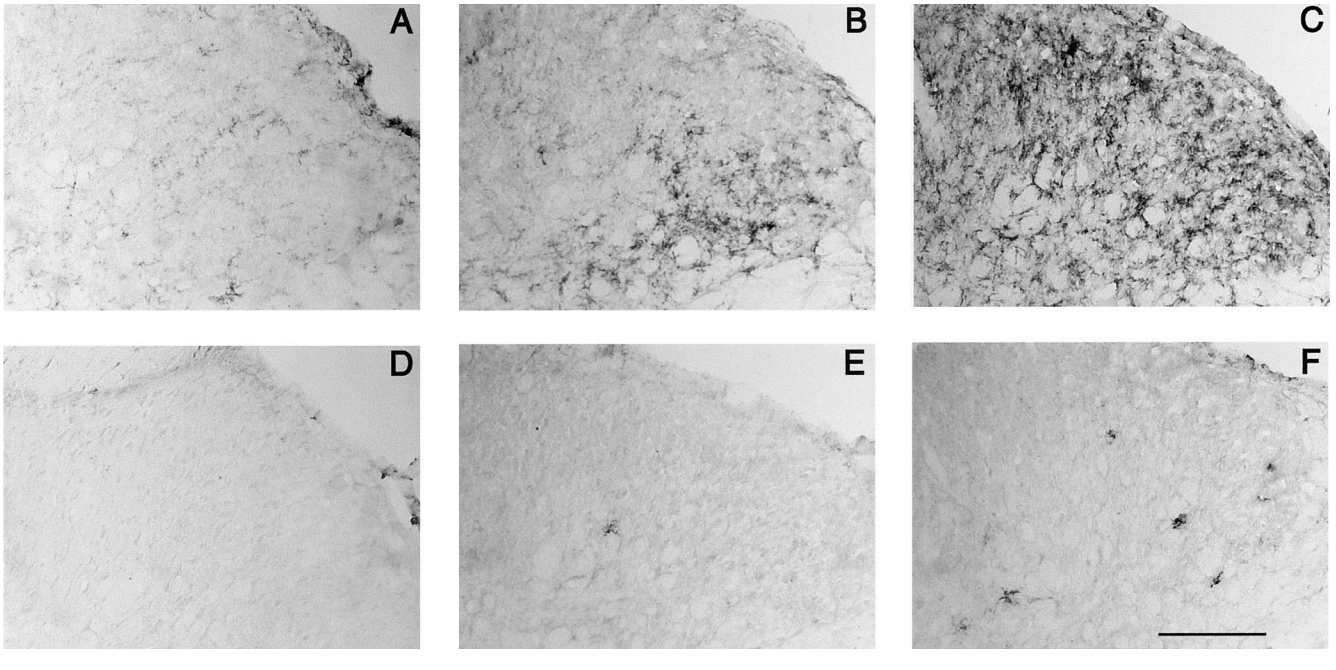

Figure 2. Representative photomicrographs of the ipsilateral lumbar dorsal horn in sham, normal, and chromic groups. CD4 immunoreactivity in the normal group **(A)**, the sham group **(B)**, and the chromic group **(C)**. MHC Class II immunoreactivity is depicted in the normal group **(D)**, the sham group **(E)**, and the chromic group **(F)**. Bar = 250 μm.

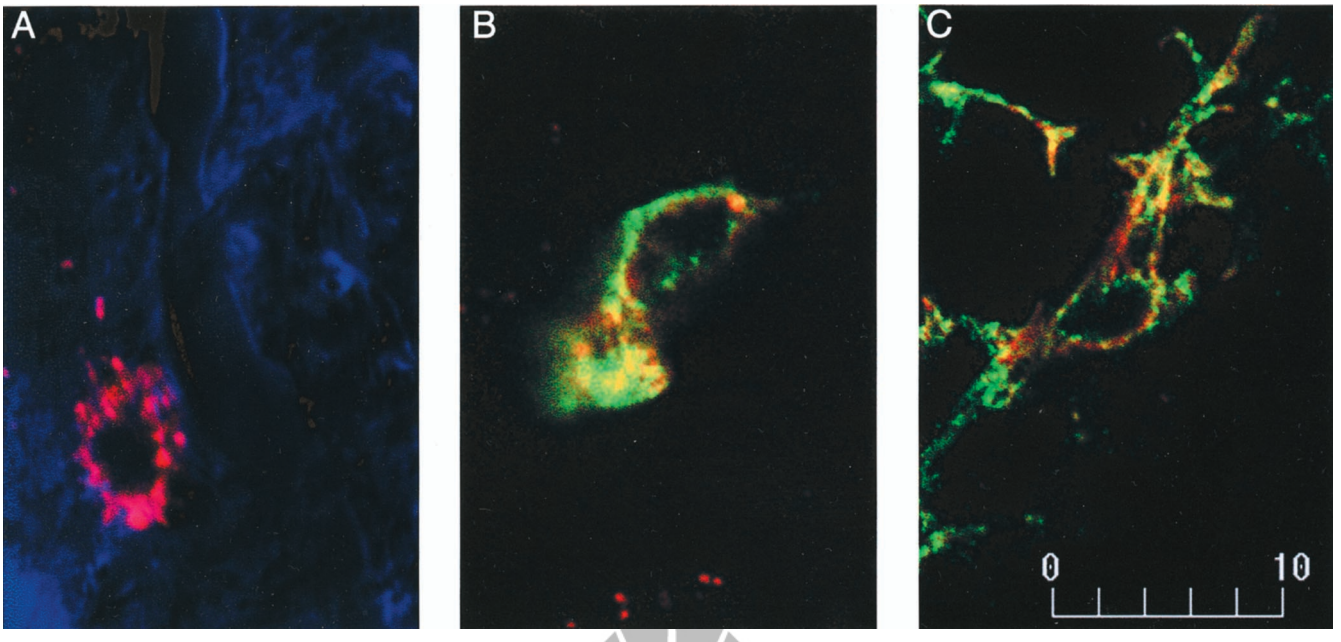

Figure 3. Representative confocal photomicrograph depicting perivascular expression of I1–69-like immunoreactivity **(A)**, coregionalization of I1–69 with ED-2 immunoreactive staining **(B)**, and colocalization of CD4 and OX-42 immunoreactivity **(C)** in the ipsilateral dorsal horn in the LR model. Bar = 10 μm.

sham and normal groups (Figure 2D, E). The increase was greatest in the ipsilateral ventral horns. Expression of MHC Class II on both the ipsilateral and contralateral side was maximal on day 14 (Table 2).

There was a paucity of ICAM-1 immunoreactivity in the normal and sham groups (Figure 4A, B). The chromic group (Figure 4C) exhibited a slight temporal increase in ICAM immunoreactivity compared with the sham or normal. ICAM-1 immunoreactivity was restricted to cells along vascular channels with a morphology resembling endothelial cells and was seen throughout the gray matter of the spinal cord sections. PECAM-1 immunoreactivity was generally more intense than that of ICAM-1. Again, a definite vascular pattern of immunoreactivity was observed, and the cells displaying immunoreactivity resembled endothelial or perivascular cells (Figure 4D, E). Minimal immunoreactivity was observed in normal groups; expression in the sham group was slightly increased over the normal. PECAM-1 immunoreactivity was seen throughout the spinal cord gray matter in the chromic group and was most intense at days 7 and 14 (Figure 4F).

Table 2. MHC Class II immunoreactivity in the Lumbar Spinal Cord for Normal, Sham, and Chromic Groups

		Substantia Gelatinosa		Nucleus Proprius		Central Canal		Ventral Horn	
Treatment	Day	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra
Normal		0	0	0	0	0	0	3	3
Sham	1	0	0	0	0	0	2	2	3
	3	2	1	1	0	0	0	7	2
	7	2	1	2	0	2	1	9	5
	14	1	0	0	0	0	0	4	2
Chromic	1	2	2	2	1	1	1	10	8
		2	1	1	0	0	0	7	5
		1	1	1	0	1	0	6	4
	3	3	2	1	1	2	1	12	7
		2	1	1	0	1	0	10	7
		2	1	1	0	1	0	10	6
	7	5	2	2	1	1	0	7	3
		4	1	2	1	1	0	9	5
		3	2	3	1	2	1	12	7
	14	4	2	2	1	2	1	13	3
		4	2	2	1	1	0	17	4
		2	1	1	0	2	1	14	2

Ipsi = ipsilateral; Contra = contralateral. The total number of cells displaying MHC Class II immunoreactivity in the lumbar spinal cord. Cells were counted in the substantia gelatinosa (laminae I and II), the nucleus proprius (laminae III and IV) of the dorsal horn, as well as the central canal (lamina X) and the ventral horn (lamina IX) of the ipsilateral and contralateral sides.

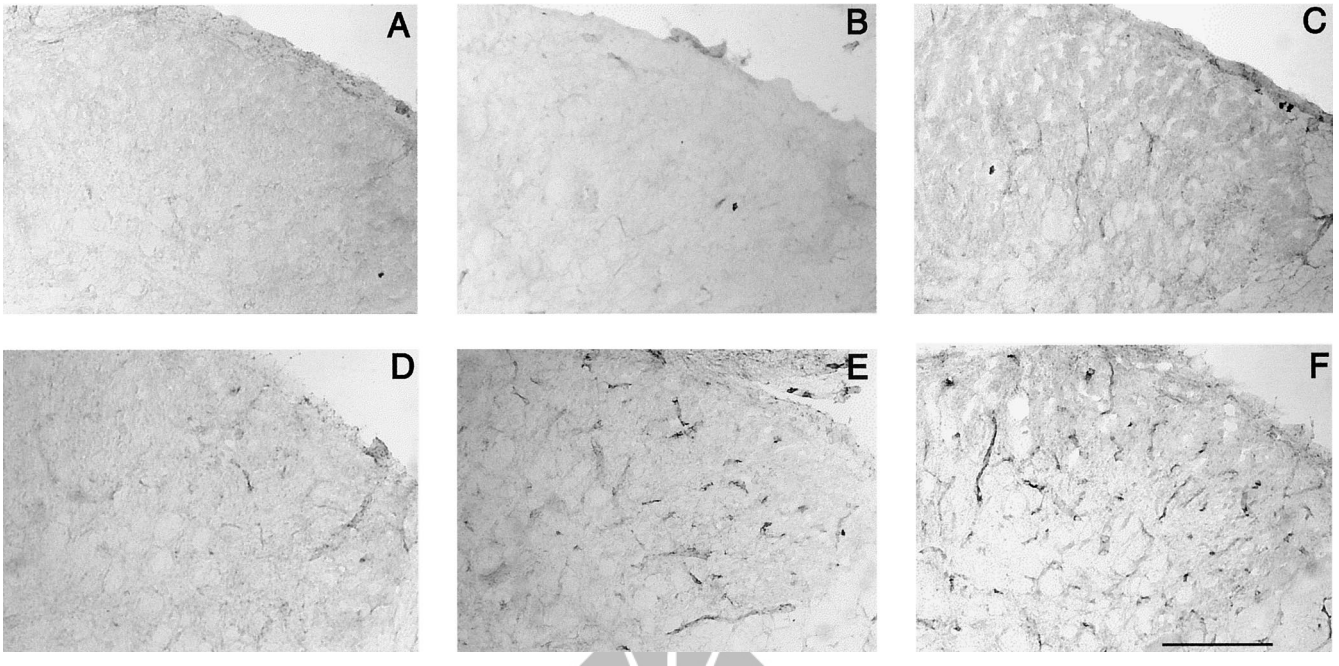

Figure 4. Photomicrographs depicting cellular adhesion molecule expression in the ipsilateral lumbar spinal cord dorsal horn 7 days post surgery. ICAM-1 immunoreactivity in the normal group **(A)** and the sham group **(B)**, compared with the chromic group **(C)**. PECAM-1 immunoreactivity in the normal group **(D)**, the sham group **(E)**, and the chromic group **(F)**. Bar = 250 μm.

Radiation Bone Marrow Chimeras

MHC Class I immunoreactive cells specific solely to the donor strain were present in the lumbar spinal cord in the radiation bone marrow chimeras following lumbar nerve root injury (Figure 3A). These cells were shown to coregionalize with ED-2 immunoreactivity (Figure 3B). In the dorsal horn the number of I1–69-positive cell profiles were approximately the same in both the L5 and L4 regions of the spinal cord. The more sacral section demonstrated a slight decrease of I1–69-positive cells compared with the L5 region. MHC Class I immunoreactive cells were seen throughout both the ipsilateral and contralateral dorsal and ventral horns; however, expression was greatest in the ipsilateral dorsal horn. Immunoreactivity was observed to increase over time, with the greatest expression appearing at day 14 (Figure 5). Sham surgery alone resulted in a slight increase in the ipsilateral dorsal horn of I1–69 immunoreactive cells.

■ Discussion

Recently, the role of CNS neuroimmune activation and neuroinflammation in centrally mediated pain states has been studied in an effort to identify and characterize the components of a CNS immune cascade in response to nerve injury. A further understanding of the inflammatory mediators expressed in the spinal cord following nerve root injury is imperative for the development of novel therapies to treat LR. To this end, we used a rodent model of LR to investigate the correlation between central changes of immune cells and increases in both ipsilateral and contralateral mechanical allodynia. Increases of immune membrane glycoproteins (CD4 and MHC Class II) and cell adhesion molecules (ICAM-1 and PECAM-1), which are integral to the immunologic activation of tissue, were found to increase in response to nerve root injury. Leukocyte trafficking into the spinal cord in response to a focal lumbar nerve root injury, as determined by donor MHC Class I cells present in the chimera spinal cord, was also discovered.

Central Sensitization in Lumbar Radiculopathy

Central sensitization is an enhanced responsiveness of the CNS to afferent input and is defined as a decreased threshold, an increased response to suprathreshold stimuli, and ongoing spontaneous activity in the dorsal horn. It has been reported following nerve root injury both in clinical and experimental conditions: mechanical allodynia is seen occasionally following disc prolapse or posterior decompression surgery[32] where excessive manipulation of the nerve root has occurred. Using the rat LR model, bilateral mechanical allodynia was observed, supporting centrally mediated sensitization in this condition.

The clinical phenomenon of mirror pain also supports central mediation of pain in LR. Mirror pain is best illustrated as the phenomenon of allochiria following unilateral cordotomy for intractable pain. After this procedure the pain reappears on the contralateral side of the body. Mirror pain is also observed in patients with radiculopathy who complain of bilateral leg or arm pain. It is well established that various kinds of hind limb tissue or nerve injury in animal models produce behavioral responses (allodynia and thermal hyperalgesia) in both the ipsilateral and contralateral hind paws.[8,51,52] Increases in contralateral dorsal horn metabolism using 2-deoxy-

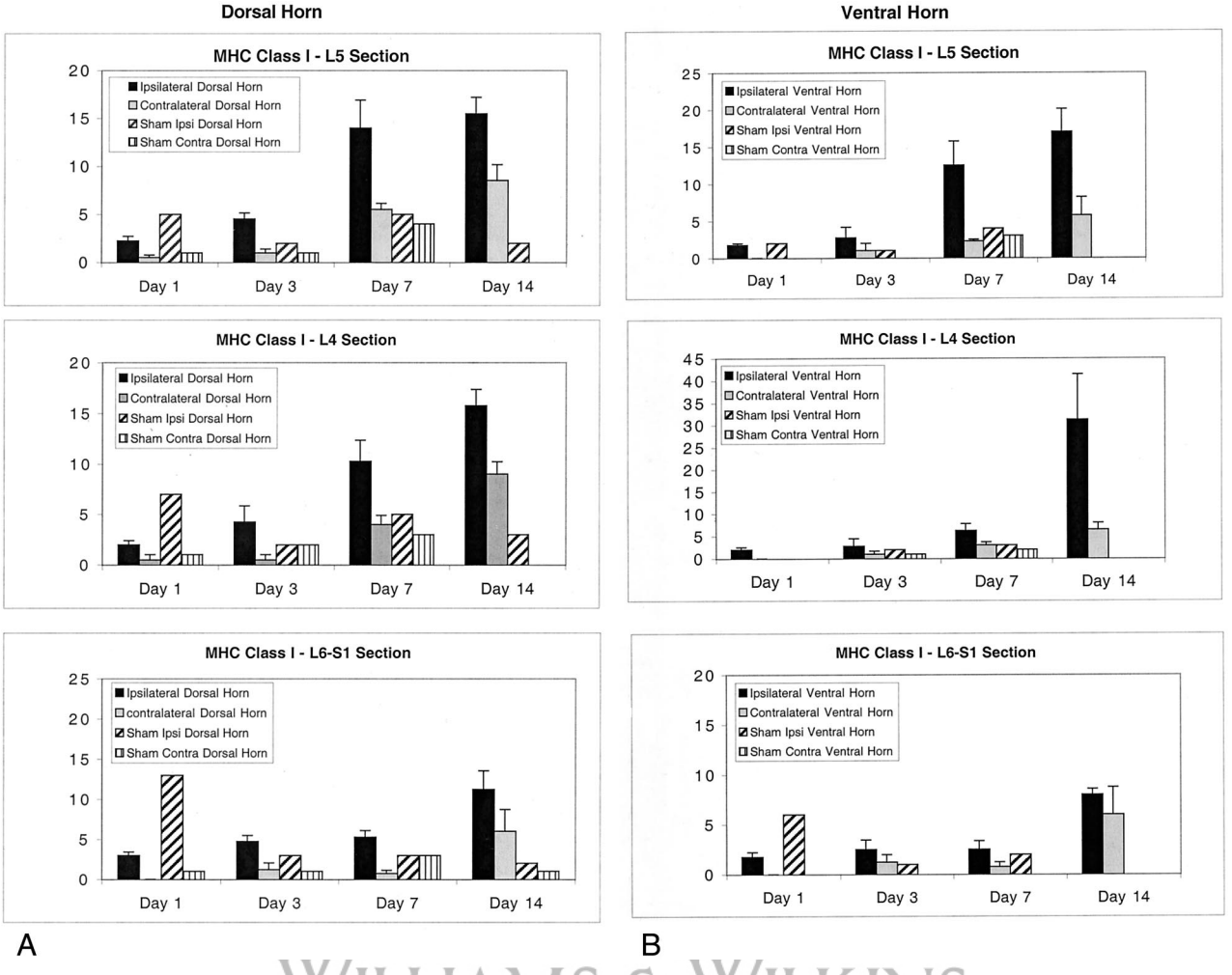

Figure 5. Bar graphs depicting average number of I1–69-like immunoreactive cells at L5, above (L4) and below (L6–S1) lumbar levels of dorsal horns **(A)**, and ventral horns **(B)** in sham and chromic groups at days 1, 3, 7, or 14 post surgery.

glucose,[1] increases in the bilateral spinal expression of dynorphin,[47] and proinflammatory cytokines and glial activation[17] after root or peripheral nerve injury support the theory that central changes result from varying nerve or nerve root lesions.

The mode of activation of contralateral dorsal horn neurons has been investigated to understand mirror pain. The possibility of a subsidiary pathway was put forward by Nathan in 1956.[33] Since then, a number of investigators have supported this theory. Projection of primary afferents to the contralateral dorsal horn has been described.[36] Bilateral projections from primary afferents have also been reported in some spinal cord segments.[10,28] Impulses may also be relayed *via* supraspinal centers bypassing the ipsilateral dorsal horn.[28] Mirror pain and the presence of contralateral pain-associated behaviors, as observed in the LR model in this study, further support a central role in nociceptive processing in LR.

Spinal MHC Class II and CD4 Expression

In the CNS both MHC Class I[20] and MHC Class II[27] basal expression is minimal compared with other tissues. In addition, the CNS is not surveyed by circulating lymphocytes to the same degree as extracerebral tissues.[21,39] This low expression of MHC and reduced immune surveillance are thought to contribute to the immune-privileged status that may confer some level of protection for nonrenewable CNS neurons. MHC Class II is expressed on antigen-presenting cells for T cells. The antigen-presenting cells of the normal CNS are perivascular cells and, to a limited extent, microglia.[15,49] Glia do not normally express MHC Class II, but expression can be induced by cytokines.[27,40] In this study the presence of MHC Class II on activated microglia further supports the notion that central neuroimmune processes are involved in persistent painful LR. It has been demonstrated that intrathecally administered methotrexate, a potent immunosuppressive agent, attenuates mechanical allodynia and reduces spinal MHC Class II expression in this model.[18] In addition, MHC Class II deficient mice display reduced mechanical allodynia following an L5 spinal nerve transection.[45] These data implicate a role of microglial expression of MHC Class II in the resultant behavioral hypersensitivity following lumbar nerve root injury.

The membrane glycoprotein CD4 is expressed on T helper cells, macrophages, and microglia.[3] CD4, like MHC Class II, is an integral part of antigen recognition because it is expressed on T lymphocytes that react with antigen presented in association with MHC Class II. More recently, it has been appreciated that CD4-bearing microglia may function as crucial mediators of indirect neuronal damage and are detected in a variety of neurodegenerative diseases.[3] In the present study we have demonstrated that lumbar root injury resulted in enhanced CD4 immunoreactivity in the gray matter of the spinal cord compared with sham and normal animals. The morphology of the cells exhibiting CD4 identifies them as microglia. The exact role of CD4 on microglia is presently unknown, but certainly its increased expression suggests that microglia become activated and may exhibit increased immunocompetence following a nerve root injury.

Spinal ICAM-1 and PECAM-1 Expression

Increases in both spinal ICAM-1 and PECAM-1 expression following nerve root injury were demonstrated. Cellular adhesion molecules within the CNS are thought to be pivotal to cell migration into the CNS and the development of a variety of inflammatory diseases in the nervous system. Whereas there are low levels of constitutive expression of ICAM-1 on some of the larger venules within the CNS,[14] massive upregulation of ICAM-1 has been reported in a wide array of inflammatory conditions, including autoimmune, viral, bacterial, and parasitic conditions. Modulation of ICAM expression correlates with disease time course in experimental allergic encephalitis. Cannella et al have shown that the upregulation of ICAM precedes the perivascular cuffing that occurs in this condition and the onset of clinical disease.[4] Recently, the use of ligands to block ICAM-1 led to the amelioration of experimental allergic encephalitis, presumably by blocking the entry of T cells into the CNS which are thought to initiate the autoimmune response in experimental allergic encephalitis.[2] Nerve root injury may produce a cascade of events resulting in the upregulation of ICAM-1 which, in turn, seems to enable the entry of hematogenous cells into the CNS, contributing to neuroinflammation, the development of central sensitization, and LR.

PECAM-1, an immunoglobulin supergene family adhesion molecule like ICAM-1, is important in leukocyte transmigration during inflammation.[40] PECAM-1 in the CNS is predominately expressed in endothelial cells of the blood–brain barrier. Upregulation of both ICAM-1 and PECAM-1 expression following nerve root injury may promote the entry of immune cells into the CNS and contribute to the development of neuroinflammation and sensitization as well as the initiation and maintenance of LR.

Cell Trafficking

This is the first demonstration of leukocyte trafficking into the spinal cord in response to a focal nerve root injury that results in behavioral sensitivity reminiscent of radicular pain. The trafficking of cells from the periphery into the CNS closely parallels the temporal increase in mechanical allodynia following the nerve root injury. Using colabeling confocal microscopy, we demonstrated that these cells coregionalized with ED-2 at 7 days after injury. However, in some cells the I1–69 immunoreactivity resembled infiltrating macrophages with distinct morphology from perivascular cells. This finding has been corroborated in a separate study characterizing the donor I1–69 staining in a peripheral nerve injury using radiation bone marrow chimeric rats.[44] Whether this influx of hematogenous cells represents an epiphenomenon induced by endothelial cell activation or is an essential element in the production of central pain remains to be determined.

The sharp initial increase in ipsilateral hind paw mechanical allodynia followed by a delayed contralateral increase 5 days following surgery suggests that separate mechanisms are involved in the maintenance of ipsilateral and contralateral mechanical allodynia. Cellular infiltration that increases over time after injury may provide the impetus for maintained central sensitization through continued algesic mediator production by activated macrophages and microglia. Factors secreted by these activated cells include interleukin-1, tumor necrosis factor complement proteins, and hydrolytic enzymes that propagate the inflammatory response.

■ Conclusion

Through their expression on activated microglia, CD4 and MHC Class II render these glial cells immunocompetent and, thus, play an essential role in the immune response of the CNS to injury. Further, cell adhesion molecules ICAM-1 and PECAM-1 expressed in microvasculature of the spinal cord are required for recruitment of elements of the immune system through the blood–brain barrier into the CNS. In the present study we present novel data demonstrating enhanced expression of immune membrane glycoproteins MHC Class II and CD4 and the cell adhesion molecules ICAM-1 and PECAM-1 in the spinal cord following root injury resembling the ensuing clinical pathology of herniated nucleus pulposus. The upregulation of these molecules is temporally and anatomically associated with the presence of mechanical allodynia following a root injury. Importantly, these changes, as well as the microglial and endothelial activation described herein, were demonstrated in nociceptive-relevant laminae in the dorsal horn of the spinal cord relative to afferent sensory fibers. The evidence of macrophage trafficking into the CNS in response to a nerve root injury further implicates inflammatory mechanisms in the initiation and maintenance of central sensitization, the pathologic correlate to persistent pain states. Combining these experimental results and findings from other laboratories with the clinical findings supporting central sensitization as discussed herein, a theory emerges favoring a potential role of cen-

tral pain immune processing in radiculopathy. Further basic science and clinical research may better elucidate these mechanisms, eventually realizing superior treatment options for those with chronic radicular pain.

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■ Key Points

- Central sensitization plays a role in initiating and maintaining nerve root injury-induced hypersensitivity, as shown by the immediate onset of mechanical allodynia in the ipsilateral hind paw and the delayed response in the contralateral hind paw.
- The enhanced expression of MHC Class II and CD4 immunoreactivity by microglia that correlated with an increase in mechanical allodynia over time supports the role of central neuroimmune activation in radicular pain.
- Cellular adhesion molecules ICAM-1 and PECAM-1 may contribute to lumbar radiculopathy by aiding in leukocyte migration and infiltration into the CNS.
- Using radiation bone marrow chimeric rats, peripheral leukocytes infiltrate into the CNS in response to nerve root injury, implicating a role of neuroinflammation in persistent pain states.

References

1. Aloisi AM, Porro CA, Cavazzuti M, et al. 'Mirror pain' in the formalin test: behavioral and 2-deoxyglucose studies. Pain 1993;55:267–73.
2. Archelos JJ, Jung S, Maurer M, et al. Inhibition of experimental autoimmune encephalomyelitis by an antibody to the intercellular adhesion molecule ICAM-1. Ann Neurol 1993;34:145–54.
3. Buttini M, Westland CE, Masliah E, et al. Novel role of human CD4 molecule identified in neurodegeneration. Nat Med 1998;4:441–6.
4. Cannella B, Cross AH, Raine CS. Upregulation and coexpression of adhesion molecules correlate with relapsing autoimmune demyelination in the central nervous system. J Exp Med 1990;172:1521–4.
5. Chao CC, Hu S. Tumor necrosis factor-alpha potentiates glutamate neurotoxicity in human fetal brain cell cultures. Dev Neurosci 1994;16:172–9.
6. Chao CC, Hu S, Ehrlich L, et al. Interleukin-1 and tumor necrosis factor-alpha synergistically mediate neurotoxicity: involvement of nitric oxide and of *N*-methyl-D-aspartate receptors. Brain Behav Immunol 1995;9:355–65.
7. Coderre TJ, Katz J, Vaccarino AL, et al. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. Pain 1993;52:259–85.
8. Coderre TJ, Melzack R. Increased pain sensitivity following heat injury involves a central mechanism. Behav Brain Res 1985;15:259–62.
9. Colburn RW, DeLeo JA, Rickman AJ, et al. Dissociation of microglial activation and neuropathic pain behaviors following peripheral nerve injury in the rat. J Neuroimmunol 1997;79:163–75.
10. Culberson JL, Haines DE, Kimmel DL, et al. Contralateral projection of primary afferent fibers to mammalian spinal cord. Exp Neurol 1979;64:83–97.
11. DeLeo JA, Colburn RW, Rickman AJ. Cytokine and growth factor immunohistochemical spinal profiles in two animal models of mononeuropathy. Brain Res 1997;759:50–7.
12. DeLeo JA, Yezierski RP. The role of neuroinflammation and neuroimmune activation in persistent pain. Pain 2001;90:1–6.
13. Deyo RA, Tsui-Wu YJ. Descriptive epidemiology of low-back pain and its related medical care in the United States. Spine 1987;12:264–8.
14. Engelhardt B, Conley FK, Butcher EC. Cell adhesion molecules on vessels during inflammation in the mouse central nervous system. J Neuroimmunol 1994;51:199–208.
15. Ford AL, Goodsall AL, Hickey WF, et al. Normal adult ramified microglia separated from other central nervous system macrophages by flow cytometric sorting: phenotypic differences defined and direct ex vivo antigen presentation to myelin basic protein-reactive CD4+ T cells compared. J Immunol 1995;154:4309–21.
16. Gatti S, Bartfai T. Induction of tumor necrosis factor alpha mRNA in the brain after peripheral endotoxin treatment: comparison with interleukin-1 family and interleukin-6. Brain Res 1993;624:291–4.
17. Hashizume H, DeLeo JA, Colburn RW, et al. Spinal glial activation and cytokine expression after lumbar root injury in the rat. Spine 2000;25:1206–17.
18. Hashizume H, Rutkowski, MD, Weinstein JN, et al. Central administration of methotrexate reduces mechanical allodynia in an animal model of radiculopathy/sciatica. Pain 2000;87:159–69.
19. Hickey WF, Kimura H. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. Science 1988;239:290–2.
20. Hickey WF, Vass K, Lassmann H. Bone marrow-derived elements in the central nervous system: an immunohistochemical and ultrastructural survey of rat chimeras. J Neuropathol Exp Neurol 1992;51:246–56.
21. Hickey WF. Leukocyte traffic in the central nervous system: the participants and their roles. Semin Immunol 1999;11:125–37.
22. Hokfelt T, Elde R, Johansson O, et al. Immunohistochemical evidence for separate populations of somatostatin-containing and substance P-containing primary afferent neurons in the rat. Neurosci 1976;1:131–6.
23. Huang ZF, Massey JB, Via DP. Differential regulation of cyclooxygenase-2 (COX-2) mRNA stability by interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF-alpha) in human in vitro differentiated macrophages. Biochem Pharmacol 2000;59:187–94.
24. Inoue A, Ikoma K, Morioka N, et al. Interleukin-1 beta induces substance P release from primary afferent neurons through the cyclooxygenase-2 system. J Neurochem 1999;73:2206–13.
25. Kalaria, RN, Cohen DL, Premkumar DR. Cellular aspects of the inflammatory response in Alzheimer's disease. Neurodegeneration 1996;5:497–503.
26. Kawakami M, Weinstein JN, Spratt KF, et al. Experimental lumbar radiculopathy: immunohistochemical and quantitative demonstrations of pain induced by lumbar nerve root irritation of the rat. Spine 1994;19:1780–94.
27. Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci 1996;19:312–8.
28. Linderoth B. Dorsal column stimulation and pain: experimental studies of putative neurochemical and neurophysiological mechanisms [published thesis]. Stockholm: Karolinska Institute, 1992:67.
29. McCarron RF, Wimpee MW, Hudkins PG, et al. The inflammatory effect of nucleus pulposus: a possible element in the pathogenesis of low-back pain. Spine 1987;12:760–4.
30. Meller ST, Gebhart GF. Spinal mediators of hyperalgesia. Drugs 1994;47(suppl):10–20.
31. Mallat M, Chamak B. Brain macrophages: neurotoxic or neurotrophic effector cells? J Leukocyte Biol 1994;56:416–22.
32. Nakamura SI, Myers RR. Injury to dorsal root ganglia alters innervation of spinal cord dorsal horn lamina involved in nociception. Spine 2000;25:537–42.
33. Nathan PW. Reference of sensation at the spinal level. J Neurol Neurosurg Psychiatry 1956;19:88–100.
34. Nicol GD, Lopshire JC, Pafford CM. Tumor necrosis factor enhances the capsaicin sensitivity of rat sensory neurons. J Neurosci 1997;17:975–82.
35. Nottet HS, Jett M, Flanagan CR, et al. A regulatory role for astrocytes in HIV-1 encephalitis: an overexpression of eicosanoids, platelet-activating factor, and tumor necrosis factor-alpha by activated HIV-1-infected monocytes is attenuated by primary human astrocytes. J Immunol 1995;154:3567–81.
36. Ogawa T, Kanazawa I, Kimura S. Regional distribution of substance P, neurokinin alpha and neurokinin beta in rat spinal cord, nerve roots and dorsal root ganglia, and the effects of dorsal root section or spinal transection. Brain Res 1985;359:152–7.
37. Olmarker K, Meyers RR. Pathogenesis of sciatic pain: role of herniated nucleus pulposus and deformation of spinal nerve root and dorsal root ganglion. Pain 1998;78:99–105.
38. Patterson PH, Nawa H. Neuronal differentiation factors/cytokines and synaptic plasticity. Cell 1993;72(suppl):123–37.
39. Rall GF. CNS neurons: the basis and benefits of low class I major histocompatibility complex expression. Curr Top Microbiol Immunol 1998;232:115–34.
40. Roitt I, Brostoff J, Male D. Immunology, 5th ed. London: Mosby, 1998.
41. Ruffolo R, Fuerstein GZ, Hunter AJ, et al. Inflammatory cells and mediators in CNS diseases. Netherlands: Harwood Academic, 1999;

42. Saal JS, Franson R, Dobrow R, et al. High levels of inflammatory phospholipase A2 activity in lumbar disc herniations. Spine 1990;15:674–8.
43. Serou MJ, DeCoster MA, Bazan NG. Interleukin-1 beta activates expression of cyclooxygenase-2 and inducible nitric oxide synthase in primary hippocampal neuronal culture: platelet-activating factor as a preferential mediator of cyclooxygenase-2 expression. J Neurosci Res 1999;58:593–8.
44. Sweitzer SM, Hickey WF, Rutkowski MD, et al. Focal peripheral nerve injury induces leukocyte trafficking into the CNS: relationship to neuropathic pain. Pain. In press.
45. Sweitzer SM, White KM, Dutta C, et al. The differential role of spinal MHC Class II and cellular adhesion molecules in inflammatory versus neuropathic pain. J Neuroimmunol 2002;125:82–93.
46. van Tulder MW, Koes BW, Bouter LM. A cost-of-illness study of back pain in The Netherlands. Pain 1995;62:233–40.
47. Wagner R, DeLeo JA, Coombs DW, et al. Spinal dynorphin immunoreactivity increases bilaterally in a neuropathic pain model. Brain Res 1993;629:323–6.
48. Watkins LR, Maier SF, Goehler LE. Immune activation: the role of proinflammatory cytokines in inflammation, illness responses and pathological pain states. Pain 1995;63:289–302.
49. Wekerle H, Linington C, Lassman H, et al. Cellular immune reactivity within the CNS. Trends Neurosci 1986;9:271–7.
50. Wiertelak EP, Furness LE, Watkins LR, et al. Illness-induced hyperalgesia is mediated by a spinal NMDA-nitric oxide cascade. Brain Res 1994;664:9–16.
51. Willenbring S, DeLeo JA, Coombs DW. Differential behavioral outcomes in the sciatic cryoneurolysis model of neuropathic pain in rats. Pain 1994;58:135–40.
52. Woolf CJ. Long term alterations in the excitability of the flexion reflex produced by peripheral tissue injury in the chronic decerebrate rat. Pain 1984;18:325–43.

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