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Pain hypersensitivity in rats with experimental autoimmune neuritis, an animal model of human inflammatory demyelinating neuropathy

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Abstract

Experimental autoimmune neuritis (EAN) is a T cell mediated autoimmune disease of the peripheral nervous system that serves as an animal model of the acute inflammatory demyelinating polyradiculoneuropathy in Guillain-Barre syndrome (GBS). Although pain is a common symptom of GBS occurring in 55–85% of cases, it is often overlooked and the underlying mechanisms are poorly understood. Here we examined whether animals with EAN exhibit signs of neuropathic pain including hyperalgesia and allodynia, and assessed their peripheral nerve autoimmune inflammation. We immunized Lewis rats with peripheral myelin P2 peptide (amino acids 57–81) emulsified with complete Freund's adjuvant, or with adjuvant only as control. P2-immunized rats developed mild to modest monophasic EAN with disease onset at day 8, peak at days 15–17, and full recovery by day 28 following immunization. Rats with EAN showed a significant decrease in withdrawal latency to thermal stimuli and withdrawal threshold to mechanical stimuli, in both hindpaws and forepaws, during the course of the disease. We observed a significant infiltration of T cells bearing αβ receptors, and a significant increase in antigen-presenting cells expressing MHC class II as well as macrophages, in EAN-affected rats. Our results demonstrate that animals with active EAN develop significant thermal hyperalgesia and mechanical allodynia, accompanied by pronounced autoimmune inflammation in peripheral nerves. These findings suggest that EAN is a useful model for the pain seen in many GBS patients, and may facilitate study of neuroimmune mechanisms underlying pain in autoimmune neuropathies.

Keywords: Neuropathic pain; Hyperalgesia; Allodynia; Autoimmune neuropathy; EAN; GBS; Inflammatory response; Sciatic nerve; Rat

1. Introduction

Autoimmune diseases of the nervous system including multiple sclerosis (MS) and Guillain-Barre Syndrome (GBS) are debilitating neurologic diseases, which cause not only demyelination with impaired motor function, but also abnormal sensory phenomena including moderate to severe pain (Pentland and Donald, 1994; Moulin et al., 1997). GBS is a human acute inflammatory demyelinating neuropathy caused by an autoimmune attack on the peripheral nervous system, and is characterized by motor disorders such as weakness or paralysis, as well as variable sensory disturbances (Hughes et al., 1999). GBS consists of several pathological subtypes including acute inflammatory demyelinating polyradiculoneuropathy (AIDP), acute motor and sensory axonal neuropathy (AMSAN) and acute motor axonal neuropathy (AMAN) (Hughes et al., 1999; Winer, 2001; Hughes and Cornblath, 2005). The most common underlying subtype of the syndrome is AIDP with prominent lymphocytic infiltration of the peripheral nervous system, primary macrophage penetration of the Schwann cell basal lamina and invasion of normal myelin (Hughes et al., 1999). The incidence of GBS is between 1 and 2 per 100,000 with more men affected than women (Hughes and Cornblath, 2005). Pain is a common symptom of GBS occurring in 55–85% of cases and includes paraesthesia/dysesthesia, backache and sciatica, neck pain associated with meningism, muscle pain, joint pain, visceral pain and other discomfort (Pentland and Donald, 1994; Moulin et al., 1997). An even higher incidence of pain is observed in cases of

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children with GBS (Nguyen et al., 1999). Many patients with GBS describe the pain as distressing, horrible or excruciating, and require aggressive treatment (Moulin et al., 1997). Despite the fact that a high percentage of patients with MS or GBS experience intense pain, the symptom is often overlooked (Howarth, 2000), animal models are lacking (Watkins and Maier, 2002) and the mechanisms underlying such pain are poorly understood. Aicher et al. have recently addressed the issue of MS-related pain by demonstrating that mice with experimental autoimmune encephalomyelitis, an experimental model of MS, show decreased tail withdrawal latencies to noxious radiant heat during the chronic phase of the disease (Aicher et al., 2004).

Experimental autoimmune neuritis (EAN) is a T cell-mediated acute demyelinating inflammatory disease of the peripheral nervous system, and is considered to be an animal model of AIDP, the most common form of GBS (Hahn, 1996). EAN can be induced in susceptible animals by active immunization with peripheral nerve myelin, purified peripheral myelin proteins P0, P2 or PMP-22, or synthetic immunogenic peptides of these proteins (Waksman and Adams, 1955; Brostoff et al., 1972; Kadlubowski and Hughes, 1979; Milner et al., 1987; Shin et al., 1989; Gabriel et al., 1998). Animals develop a monophasic disease characterized by weight loss, slowing of nerve conduction, ascending progressive paresis and spontaneous recovery. The pathogenesis of EAN comprises breakdown of the blood-nerve barrier, immunoglobulin leakage, infiltration of peripheral nerve tissue by activated T cells and macrophages, and focal demyelination of nerve roots (Hahn, 1996). The close similarities between EAN and AIDP make EAN a potential model for studies of the mechanisms underlying pain in GBS. However, studies of pain in the EAN model have not been performed so far.

Recent studies on immune mechanisms in neuropathic pain due to peripheral nerve injury or inflammation have demonstrated the importance of inflammatory and immune cells in the development and maintenance of persistent pain (Watkins and Maier, 2002; Marchand et al., 2005; Moalem and Tracey, 2006; Myers et al., 2006). For example, lesion of the ventral roots of spinal nerves without any damage to sensory axons (Li et al., 2002; Sheth et al., 2002) or application of an inflammatory stimulus such as zymosan (Chacur et al., 2001), carrageenan or complete Freund's adjuvant (Eliav et al., 1999) around intact sciatic nerve initiates immune activation followed by allodynia and hyperalgesia. Peripheral nerve injury induces activation of resident mast cells and infiltration of neutrophils and macrophages, followed by recruitment of T cells to the nerve. Stabilization of mast cells (Zuo et al., 2003), depletion of neutrophils (Perkins and Tracey, 2000) and macrophages (Liu et al., 2000) and absence of functional T cells (Moalem et al., 2004; Kleinschnitz et al., 2006) all result in reduced hyperalgesia, indicating the importance of each cell type to neuropathic pain behavior. Based on these observations, we hypothesized that an autoimmune inflammatory response in the peripheral nervous system induces increased neuropathic pain sensitivity. Thus, in the present study we induced EAN in rats by active immunization, compared the responsiveness of animals with EAN and control animals to thermal and mechanical noxious stimuli, and assessed the associated inflammatory response in their peripheral nerves.

2. Methods

2.1. Animals

Male Lewis rats (Animal Resources Center, Perth, Australia) at 6–8 weeks of age were used. Lewis rats were chosen since they reliably develop EAN (Hoffman et al., 1980). The animals were maintained at 23 °C on a 12:12 h light-dark cycle with light onset at 06:00 a.m. They were housed in groups of six in large plastic cages with soft bedding and with food and water available ad libitum. The animals were allowed to habituate to the housing facilities for 1 week before experiments began. Behavioral studies were performed in the same room where animals were housed and at around the same time of the day (9:00 a.m.–1:00 p.m.) by the same experimenter. Food and water were placed within easy access to animals experiencing motor impairment. All procedures were approved by the Animal Care and Ethics Committee of the University of New South Wales and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain. All efforts were made to minimize the number of animals used and their suffering.

2.2. Experimental design

Three treatment groups were used:

1. Rats that were immunized with self antigen (immunogenic P2 peptide of peripheral myelin, amino acids 57–81) emulsified with complete Freund's adjuvant (CFA).
2. Rats that were immunized with saline emulsified with CFA, as control.
3. Rats that were immunized with saline emulsified with incomplete Freund's adjuvant (IFA), as control.

All the rats were scored daily for clinical signs of EAN, as described below. In one experiment, rats were sacrificed on day 17 post-immunization (disease peak) to check inflammation in their sciatic nerves using immunohistochemistry ($N = 3$ rats per group). In another experiment, pain behavior was evaluated (as described below) 3 times a week and compared amongst the different treatment groups ($N = 6$ rats per group). Separate sets of animals were utilized for immunohistochemistry and for measurements of pain behavior.

2.3. Active induction of EAN

Lewis rats were immunized by subcutaneous injection at the base of the tail with 200 μl of inoculum containing P2 antigen (neuritogenic P2 peptide-amino acids 57–81, synthesized by Auspep Pty Ltd, Australia) or no antigen (control). The antigen was dissolved in saline (2 mg/ml) and emulsified with an equal volume of incomplete Freund's adjuvant (Difco Laboratories, Detroit, MI) supplemented with desiccated 1 mg/ml *Mycobacterium tuberculosis* (strain H37 RA, Difco Laboratories). The IFA with the *Mycobacterium tuberculosis* is defined as CFA. Final doses in the inoculum were 0.1 mg *Mycobacterium tuberculosis* and 0.2 mg P2 antigen.

2.4. Animal monitoring (EAN evaluation)

EAN clinical scores were assessed immediately before immunization (day 0) and every day thereafter until day 30. Body weight was assessed once before immunization and 3 times a week after immunization. Severity of disease was graded as follows: 0 = no illness; 1 = tail weakness/paralysis; 2 = slight hind leg paraparesis; 3 = hind leg paralysis; 4 = complete paralysis.