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Colloquium Paper

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Does a neuroimmune interaction contribute to the genesis of painful peripheral neuropathies?

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ABSTRACT **Painful peripheral neuropathies are precipitated by nerve injury from disease or trauma. All such injuries will be accompanied by an inflammatory reaction, a neuritis, that will mobilize the immune system. The role of the inflammation itself is difficult to determine in the presence of structural damage to the nerve. A method has been devised to produce a focal neuritis in the rat sciatic nerve that involves no more than trivial structural damage to the nerve. This experimental focal neuritis produces neuropathic pain sensations (heat- and mechano-hyperalgesia, and cold- and mechano-allodynia) in the ipsilateral hind paw. The abnormal pain sensations begin in 1–2 days and last for 4–6 days, with a subsequent return to normal. These results suggest that there is a neuroimmune interaction that occurs at the outset of nerve injury (and perhaps episodically over time in slow developing conditions like diabetic neuropathy) that produces neuropathic pain. The short duration of the phenomena suggest that they may prime the system for more slowly developing mechanisms of abnormal pain (e.g., ectopic discharge in axotomized primary afferent neurons) that underlie the chronic phase of painful neuropathy.**

Painful peripheral neuropathies begin with nerve injury caused by disease or trauma. This injury will result in an inflammatory reaction, a neuritis, that will mobilize the immune system. It is important to note that this will occur not only in cases of injury caused by infection and autoimmune disorder (e.g., herpes zoster and Guillain-Barré syndrome) but also in cases of sterile injury because cellular debris is an inflammatory and immune stimulus. It is difficult to study the role of the inflammation and the immune system response when it presents together with structural damage to axons because the structural damage itself gives rise to pathogenic mechanisms that lead to pain: for example, ectopic discharge in injured nociceptors. We have devised a method for producing a focal neuritis in the rat that is accompanied by little or no structural injury to the nerve (1). We find that this neuritis produces neuropathic pain sensations in the ipsilateral hind paw, even though the inflammation is at mid-thigh level.

We have used adult male Sprague–Dawley rats. The neuritis is produced by loosely wrapping the nerve at mid-thigh level with hemostatic oxidized cellulose (Oxycell, Parke-Davis) that then is saturated with an inflammatory stimulus. The Oxycell does not constrict the nerve; it serves merely as a sponge for the inflammatory stimulus. As the stimulus, we have used both λ carrageenan and complete Freund's adjuvant with about equal effects; the results described below were obtained with complete Freund's adjuvant. As a control, we (*i*) have treated the opposite nerve with Oxycell saturated with saline (*ii*) and have examined animals with unilateral Oxycell/saline treatment. These control procedures do not evoke abnormal pain sensations in the hind paw. As a control for the general effects of a painful thigh, and for the possibility of a systemic response to the inflammatory stimulus, we created an experimental unilateral myositis by implanting Oxycell/complete Freund's adjuvant in a pocket made in biceps femoris at the same level as the nerve treatment. Animals with the myositis did not have abnormal pain responses in the hind paw.

Rats with the focal neuritis have heat- and mechano-hyperalgesia and cold- and mechano-allodynia on the ipsilateral hind paw. Responses from the contralateral hind paw are normal regardless of whether the contralateral side is untreated or treated with Oxycell/saline.

Heat-hyperalgesia was measured with the paw-flick method of Hargreaves *et al.* (2). Abnormal sensitivity was noted within 1–2 days of treatment and reached peak severity 3–4 days after treatment. Responsiveness returned to normal within 5–6 days. The maximum severity of the heat-hyperalgesia was slightly less than what we have seen with an experimental traumatic nerve injury [the chronic constriction injury (CCI) model of Bennett and Xie (3)]. All animals with the neuritis developed obvious heat-hyperalgesia.

Mechano-hyperalgesia was measured with the pin-prick method, and mechano-allodynia was measured with the von Frey hair method as described by Tal and Bennett (4). Mechano-hyperalgesia and mechano-allodynia were noted within 1–2 days of treatment, reached peak severity after 3–4 days, and resolved to normal within 5–6 days. The maximum severity of both was comparable to that seen in CCI rats (3). All animals with the neuritis developed obvious mechano-hyperalgesia and mechano-allodynia.

Cold-allodynia was assayed with a slight modification of the method described by Choi *et al.* (5): 0.15 ml of acetone is sprayed onto the plantar hind paw while the animal stands on a floor made of screening. On our own forearm skin, this stimulus produces a strong but non-painful cooling sensation (as the acetone evaporates). Normal rats either ignore the stimulus, or it produces a very brief and small withdrawal reflex. Neuropathic animals react with a large and prolonged withdrawal response (painful peripheral neuropathy patients with cold-allodynia complain that this stimulus produces a severe burning pain sensation). Approximately one-half of the neuritis rats displayed neuropathic responses to cold. This is in contrast to the CCI model, in which nearly every rat has an abnormal response. When present, cold-allodynia was detected within 2–3 days of treatment, peaked within 3–4 days, and resolved within 4–5 days. Light- and electron-microscopic analyses of the treated region of the nerve harvested at the time of peak symptom severity (3–4 days after treatment)

Abbreviation: CCI, chronic constriction injury.

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showed that some cases had no detectable axonal injury. In one case only (of three that were examined), we detected ≈20 degenerating axons confined to a small patch just below the epineurium. The presence of a few tens of degenerating axons is trivial and is very highly unlikely to produce the marked signs of neuropathic pain that were present in every animal. In all cases, there were clear signs of an endoneurial inflammatory reaction (even though the inflammatory stimulus was applied to the outside of the nerve). The spaces between axons was greater than normal, indicating an edematous reaction, and immune cells (macrophages, polymorphonuclear leukocytes, and monocytes) were present throughout the endoneurial compartment. Immunocytochemical staining identified CD4 and CD8 T-lymphocytes amongst this infiltrate. The T-cells surrounded the nerve on the outside surface of the epineurium (to be expected because this was where the inflammatory stimulus was applied), but they were also within the nerve. The endoneurial cells were most abundant toward the center. This suggests that the cells within the nerve arrived via the endoneurial vasculature because, if they had migrated from the outside, they would have been concentrated just beneath the epineurium.

The neuropathic pain produced by the neuritis lasted for only a few days. It therefore cannot be the sole mechanism for chronic painful peripheral neuropathies. It is possible, however, that it is of clinical importance. First, there may be conditions under which nerves are chronically inflamed or suffer from repeated episodes of inflammation. Of importance, the inflammation may be in structures near nerves that expose the nerve as an ''innocent bystander'' to an inflammatory milieu. For example, in diabetes, nerves may experience repeated episodes of inflammation as the underlying microvascular and metabolic disease processes wax and wane. Nerves that are near tumors may be bathed in an inflammatory soup of tumor-products and inflammatory cytokines directed against the malignancy. It is noteworthy that the nucleus pulposus is a very potent inflammatory stimulus (6), so that a dorsal root lying near a leaky intervertebral disc may be exposed to an inflammatory environment. Second, in those conditions under which the neuritis is likely to be acute (e.g., post-herpetic neuralgia), the neuritis-evoked neuropathic pain may prime the nervous system such that pathogenic mechanisms that develop later (e.g., spontaneous ectopic discharge in injured nociceptors, sprouting sympathetic efferent axons in the dorsal root ganglia, A-β low-threshold mechanoreceptors invading laminae I and II) are able to produce chronic neuropathic pain.

Current work with this model is attempting to determine the relative roles of the inflammatory mediators derived from the cycloxygenase and lipoxygenase cascades and the pro-inflammatory cytokines. We have not found any effect in the neuritis model with indomethacin, suggesting that the arachidenic acid pathways are not involved (J.-E. Baños, S. Shiiba, and G.J.B., unpublished results). It has already been shown that tumor necrosis factor α is found in CCI nerves and that tumor necrosis factor α injected into the nerve produces neuropathic pain symptoms (7). Sommers *et al.* (8) have shown that inhibition of tumor necrosis factor α release with thalidomide reduces neuropathic pain in the CCI model. We have replicated the results of Sommers *et al.* in CCI rats but have not found any effect of thalidomide in the neuritis model (J.-E. Baños, S. Shiiba, and G.J.B., unpublished results). The difference may be attributable to differing immune system responses—primarily to cellular debris in the case of the CCI model but to bacterial epitopes in the neuritis model. We have found that other immune suppressants are effective in the neuritis model; for example, cyclosporin A works well (S. Shiiba, J.-E. Baños, and G.J.B., unpublished results).

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