Neuropathic Pain: The Immune Connection

Evidence for a role of the immune system in neuropathy and neuropathic pain is increasing.\(^1\) It is now estimated that half of all clinical cases of neuropathic pain are associated with infection or inflammation of peripheral nerves rather than with nerve trauma.\(^2\) Animal models have now been developed to explore the nociceptive impact of immune activation in and around nerve trunks, dorsal root ganglia (DRG), and dorsal roots. Such models reveal that immune activation at any of these sites is hyperalgesic, and that the immune system actively participates in creating and maintaining neuropathic pain of diverse etiologies.

This issue of Pain: Clinical Updates is a brief survey of the immune system’s involvement in hyperalgesia (pain amplification). The roles of the immune system in pain will be illustrated using examples of trauma to or inflammation of peripheral nerves, antibody attack upon peripheral nerves, and immune attack upon the blood supply to peripheral nerves or upon DRG and spinal roots.

Painful Neuropathy Involving Nerve Trauma and Inflammation

Neuropathic pain may arise after frank nerve trauma, and almost all animal models of neuropathic pain are based on traumatic injury to peripheral nerves. Physical damage to nerves alters functioning within nociceptive pathways and changes pain perception. Immune activation takes place whenever there is damage to peripheral nerves or associated tissues. Yet neuropathic pain may also occur without traumatic nerve injury, following peripheral immune activation and inflammation. Novel animal models of inflammatory neuropathic conditions provide insight into both types of etiology.

From animal models of both traumatic and inflammatory neuropathies, a consistent picture is beginning to emerge for immune involvement in nociception. In both traumatic and inflammatory models, the key immune cells involved at the level of the peripheral nerve are neutrophils and macrophages recruited into the affected area from the general circulation, together with a host of locally resident cells. Cells resident within peripheral nerves include fibroblasts, endothelial cells, Schwann cells, mast cells, macrophages, and dendritic cells.\(^3\) While cells such as fibroblasts and endothelial cells are not commonly thought of as immune cells, they can function like immune cells in many ways. For example, activation of fibroblasts causes them to release an array of molecules involved in host defense, including chemok attractants, which recruit immune cells (primarily neutrophils and macrophages) from the circulation into the nerve; proinflammatory cytokines, which orchestrate the early immune response by communicating between immune cells; and nitric oxide and reactive oxygen species, which kill pathogens such as viruses and bacteria. However, the effects of proinflammatory cytokines, nitric oxide, and reactive oxygen species extend beyond killing pathogens; these fibroblast-derived agents can also directly increase nerve excitability, damage myelin, and alter the blood-nerve barrier.\(^4\)
This latter effect leads to nerve swelling and infiltration with immune cells, antibodies, and other immune molecules. Across several animal models, the importance of proinflammatory cytokines in the creation and maintenance of neuropathic pain is most consistently seen in tumor necrosis factor, interleukin-1, and interleukin-6. Nitric oxide, reactive oxygen species, and complement have also been implicated in the few models in which they have so far been tested. The potential involvement of other substances released after trauma or during inflammation (acids, proteases, digestive enzymes, and chemoattractant molecules) has not yet been assessed, other than in a study noting that decreasing pH (increasing acidity) near peripheral nerves enhances pain.

It is also clear from studies of traumatic and inflammatory neuropathies that immune activation is not restricted to the periphery. Rather, spinal cord immune involvement also occurs in the form of glial activation. While a discussion of immune activation in the central nervous system is beyond the scope of this review, spinal cord glia are becoming increasingly recognized as powerful modulators of spinal pain transmission.

---

**Immune activation may occur with or without antecedent trauma. Novel animal models of pain offer insight into both etiologies.**

---

**Painful Neuropathy from Antibody Attack on Peripheral Nerves**

A second way that immune activation can contribute to neuropathic pain is via antibody attack on peripheral nerves. Most humans do not have antibodies in their bloodstream directed against peripheral nerves. Even when such antibodies are present, there may be no clinical consequence if the blood-nerve barrier is intact.

Antibodies exert two distinct types of effects on nerves. First, they bind to nerve cell membranes and alter ion channel function. Second, IgM, IgG1, and IgG3 antibodies activate the complement cascade. A variety of cells in peripheral nerve make complement, including activated immune cells and Schwann cells. Notably, antibodies are not the only triggers for complement cascade activation; this pathway can also be activated by various viruses, yeasts, and bacteria, as well as by exposure to the peripheral nerve protein P0.

Complement activation disrupts the blood-nerve barrier, recruits macrophages and neutrophils from the general circulation into the nerve, disrupts Schwann cell function, creates membrane attack complexes that punch holes in nerves, and facilitates macrophage destruction of antibody-bound sites. Macrophages further amplify this damage by releasing injurious molecules such as reactive oxygen species, nitric oxide, proteases, acids, eicosanoids, and proinflammatory cytokines. Which components of this antibody-induced cascade of events are directly involved in the creation of neuropathic pain is unknown, but several are excellent candidates.

Antibodies that attack peripheral nerves often arise by molecular mimicry. Such antibodies are generated to recognize portions (“epitopes”) of the external surface of viruses, bacteria, and cancer cells, but may also attack similarly shaped regions on the surface of normal nerves. Epitopes of pathogens or cancer cells (e.g., small-cell lung carcinoma, melanoma, and neuroblastoma) that are recognized as “non-self” stimulate the host to produce antibodies that specifically bind to them. If these “non-self” epitopes are sufficiently similar to epitopes expressed on peripheral nerve, the antibodies may bind to and damage peripheral nerves as well. Autoantibodies—antibodies that attack “self”—trigger autoimmune neuropathies. Once the autoantibodies have been released by the long-lived immune cells that generate them, killing the inciting pathogen does not relieve the neuropathic symptoms.

Antibodies to peripheral nerve can also arise after nerve trauma exposes peripheral nerve proteins (P0 and P2, among others), normally buried within the myelin sheath, to immune surveillance. P0 and P2 are recognized as “non-self” when they are newly exposed by nerve damage, hence generating an immune response.

Antibodies may also be directed against pathogens that have invaded the nerve. In this case, the primary immune response triggered by antibodies targets not the nerve itself, but rather the “non-self” pathogens within the nerve bundle. Substances released during the immune attack upon the pathogen—proinflammatory cytokines, nitric oxide, reactive oxygen species, and degradative enzymes—can produce “innocent bystander” damage to the structure and function of surrounding nerve fibers.

Animal models for studying pain secondary to anti-nerve antibodies have met with variable degrees of success. Experimental allergic neuritis generates antibodies against the peripheral nerve proteins P0 and P2 to model pathology associated with Guillain-Barré syndrome. Unfortunately, the model is so severe as to block conduction in peripheral nerves, negating its usefulness for studying neuropathic pain associated with autoimmune attack. While variable degrees of conduction blocks with associated paralysis and autonomic dysfunction are hallmarks of Guillain-Barré syndrome, one should not overlook the fact that at least 70% of patients with this syndrome suffer from neuropathic pain. Models are needed to help us understand neuropathic pain in Guillain-Barré syndrome.

A new animal model appears appropriate for this goal. In this rat model, antibody against the peripheral nerve ganglioside GD2 is administered intravenously, producing allodynia and electrophysiological signs of peripheral nerve hyperexcitability. This model is based on two clinical observations. First, gangliosides had been administered to patients in an attempt to aid recovery from various neurological disorders. However, some of these patients developed antibodies against gangliosides that attacked their peripheral nerves, causing symptoms of Guillain-Barré syndrome, including pathological pain. Later, intravenous monoclonal antibodies directed against the GD2 ganglioside were tested in clinical trials as a therapeutic approach for melanoma, small-cell lung carcinoma, and neuroblastoma. In response, patients developed a demyelinating neuropathy affecting sensory and motor nerves throughout the body (i.e., sensorimotor demyelinating polynuropathy). This neuropathy was associated with aching or burning pain, severe shooting pain, intense mechanical allodynia, moderate-to-severe abdominal pain, joint pain, moderate-to-severe extremity pain, headache, sensations having unusually unpleasant qualities (dysesthesias), and muscle pain (myalgia). Subsequent studies revealed that anti-GD2 reacted with peripheral nerve myelin sheaths and DRG neuronal cell bodies. Future studies of this model may clarify which immunological changes are responsible for creating the neuropathic pains observed.
Painful Neuropathy from Immune Attack upon Peripheral Nerve Blood Vessels

Neuropathic pain can also result from immune attack upon peripheral blood vessels, called vasculitic neuropathy. While blood vessels of nerves can occasionally be specifically targeted, far more common is a diffuse attack upon vessels throughout the body. Induction of vasculitis in the blood vessels supplying peripheral nerves results in widespread occlusion of these small blood vessels; increased adherence of immune cells to vessel walls; and disruption of the blood-nerve barrier, leading to edema and immune cell migration into the extracellular space within such nerves. Indeed, vasculitic neuropathic pain is thought to be a downstream consequence of ischemia resulting from blood vessel injury, intravascular clotting, and necrosis. There are multiple candidate immune mediators for this process, each of which has known pain-enhancing effects: autoreactive antibodies, reactive oxygen species, degradative enzymes, and complement.

No animal model yet exists for immune attack on blood vessels. However, if localized ischemic damage of the nerve and consequent immune activation are the causes of vasculitic neuropathic pain, a recently developed animal model may advance future studies. This model employs ischemia of peripheral nerves to provoke immune activation in the affected region, mechanical allodynia, and thermal hyperalgesia. Although there is no immune attack on the nerve blood vessels per se, clots form within the nerves’ blood vessels. The resultant nerve damage is followed by macrophage infiltration and activation. Future studies using this model may clarify the role of immune-derived substances in the resultant pain state.

Antibodies exert two distinct types of effects on nerves. First, they bind to nerve cell membranes and alter ion channel function. Second, IgM, IgG1, and IgG3 antibodies activate the complement cascade.

Pain from Immune Effects on Dorsal Root Ganglia and Dorsal Roots

The impact of immune activation upon pain enhancement is not restricted to peripheral nerves but affects the cell bodies of sensory neurons as well. DRG, like peripheral nerves, contain many immune cells in proximity to the cell bodies of sensory neurons. Such cells include glial-derived satellite cells, dendritic cells, macrophages, and endothelial cells. Indeed, each neuronal cell body in the DRG is encapsulated by a layer of satellite cells that can regulate its activity. These glial-derived cells, when activated, can rapidly release extracellular excitatory amino acids and L-arginine, the substrate for neuronal nitric oxide production. In addition, peripheral nerve injury stimulates activated satellite cells (and most likely the adjoining macrophages) to release proinflammatory cytokines and a variety of growth factors in close proximity to DRG neurons.

Pain and hyperalgesia can occur with protrusion of the nucleus pulposus so that it touches the DRG and dorsal root. While pressure alone has often been considered the major cause of such pain, growing evidence implicates immune-derived substances as well. Herniated disks are reacted to as “foreign” and evoke an autoimmune inflammatory response. They generate a variety of pain-producing substances including nitric oxide, proinflammatory cytokines, cyclooxygenase-2, phospholipase A2, thromboxanes, leukotrienes, and prostaglandins. These substances are produced not only by infiltrating macrophages, but also by histiocytes, fibroblasts, endothelial cells, and chondrocytes within the disk. In addition, herniated disks produce elevated levels of matrix metalloproteinases, which catalyze the release of proinflammatory cytokines into the extracellular fluid. Furthermore, herniated disks become hyper-responsive to inflammatory stimuli. For example, in preclinical studies they show exaggerated release of nitric oxide, proinflammatory cytokines, and prostaglandins in inflammatory situations. Such evidence suggests that disk-associated proinflammatory substances may be a major factor in the genesis of back pain after disk herniation, and may help to explain favorable responses to injection of glucocorticoids epidurally or around the root irritated by disk herniation.

Painful Neuropathy from Immune Attack upon Peripheral Nerve Blood Vessels

No animal model yet exists for immune attack on blood vessels. However, if localized ischemic damage of the nerve and consequent immune activation are the causes of vasculitic neuropathic pain, a recently developed animal model may advance future studies. This model employs ischemia of peripheral nerves to provoke immune activation in the affected region, mechanical allodynia, and thermal hyperalgesia. Although there is no immune attack on the nerve blood vessels per se, clots form within the nerves’ blood vessels. The resultant nerve damage is followed by macrophage infiltration and activation. Future studies using this model may clarify the role of immune-derived substances in the resultant pain state.

Antibodies exert two distinct types of effects on nerves. First, they bind to nerve cell membranes and alter ion channel function. Second, IgM, IgG1, and IgG3 antibodies activate the complement cascade.

Pain from Immune Effects on Dorsal Root Ganglia and Dorsal Roots

The impact of immune activation upon pain enhancement is not restricted to peripheral nerves but affects the cell bodies of sensory neurons as well. DRG, like peripheral nerves, contain many immune cells in proximity to the cell bodies of sensory neurons. Such cells include glial-derived satellite cells, dendritic cells, macrophages, and endothelial cells. Indeed, each neuronal cell body in the DRG is encapsulated by a layer of satellite cells that can regulate its activity. These glial-derived cells, when activated, can rapidly release extracellular excitatory amino acids and L-arginine, the substrate for neuronal nitric oxide production. In addition, peripheral nerve injury stimulates activated satellite cells (and most likely the adjoining macrophages) to release proinflammatory cytokines and a variety of growth factors in close proximity to DRG neurons.

Pain and hyperalgesia can occur with protrusion of the nucleus pulposus so that it touches the DRG and dorsal root. While pressure alone has often been considered the major cause of such pain, growing evidence implicates immune-derived substances as well.
Trauma to sensory neurons. Immune cells (e.g., macrophages, T-lymphocytes, mast cells, and dendritic cells), immunocompetent cells that can release classic immune-cell factors (e.g., endothelial cells, fibroblasts, and Schwann cells), and glial cells (DRG satellite cells, spinal cord astrocytes, and microglia) are all potential participants in nociceptive activation.

Equally important is the potential targeting of immune mechanisms for future analgesic therapies that rely upon mechanisms not yet harnessed by nonsteroidal anti-inflammatory drugs, coxibs, or glucocorticoids. Elucidation of peripheral and central immune cell involvement in pain of diverse etiologies offers hope for novel therapeutic agents.

Another major point is the pervasiveness of proinflammatory cytokines in diverse neuropathic pain conditions. There are multiple sites and situations where these immune-derived proteins—tumor necrosis factor, interleukin-1, and interleukin-6—are correlated with and probably causative of neuropathic pain conditions. Some of these situations currently have substantial experimental support, such as the hyperalgesic effects of proinflammatory cytokines at sites of peripheral nerve trauma and herniated disks. Some are unexpected and little studied, such as the phenotypic shift of immune cells during chronic inflammation. This phenotypic shift in receptor expression permits these cells, for the first time, to be activated by catecholamines so as to release proinflammatory cytokines. If such a shift were to occur in “sympathetically maintained” pain states, we could predict that proinflammatory cytokines released from this previously unrecognized source may contribute to such pain.

The final point is that investigation of immune and glial involvement in neuropathic pain is in its infancy. It is already clear, however, that research on immunology has great importance for the understanding and ultimate control of clinical pain.

References


Linda R. Watkins and Steven F. Maier
Department of Psychology and the Center for Neuroscience
University of Colorado at Boulder
Boulder, CO 80309-0345 USA
Tel: 303-492-7034
Email: lwatkins@psych.colorado.edu