

# Beyond Neurons: Evidence That Immune and Glial Cells Contribute to Pathological Pain States

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**Watkins, Linda R., and Steven F. Maier.** Beyond Neurons: Evidence That Immune and Glial Cells Contribute to Pathological Pain States. *Physiol Rev* 82: 981–1011, 2002; 10.1152/physrev.00011.2002.—Chronic pain can occur after peripheral nerve injury, infection, or inflammation. Under such neuropathic pain conditions, sensory processing in the affected body region becomes grossly abnormal. Despite decades of research, currently available drugs largely fail to control such pain. This review explores the possibility that the reason for this failure lies in the fact that such drugs were designed to target neurons rather than immune or glial cells. It describes how immune cells are a natural and inextricable part of skin, peripheral nerves, dorsal root ganglia, and spinal cord. It then examines how immune and glial activation may participate in the etiology and symptomatology of diverse pathological pain states in both humans and laboratory animals. Of the variety of substances released by activated immune and glial cells, proinflammatory cytokines (tumor necrosis factor, interleukin-1, interleukin-6) appear to be of special importance in the creation of peripheral nerve and neuronal hyperexcitability. Although this review focuses on immune modulation of pain, the implications are pervasive. Indeed, all nerves and neurons regardless of modality or function are likely affected by immune and glial activation in the ways described for pain.

## I. INTRODUCTION

Pain is so simple, until it goes wrong. In healthy individuals, pain serves highly adaptive, survival-oriented purposes. The first purpose of pain is to warn of actual or impending threat of bodily harm, such as contacting sharp or dangerously hot objects. Peripheral nerves transmit this information from the body tissue to the spinal cord. Here, neurons in the spinal cord dorsal horn both relay the information to the brain and, simultaneously, trigger withdrawal reflexes to remove the endangered body part from the painful stimulus. Going hand-in-hand with this spinally mediated protective reflex is the supraspinally mediated perception that the danger arises from something in the environment that should be defended against. In contrast, the second purpose of pain is to encourage

recuperative behaviors in response to pain arising from within the body itself. Here, bodily damage has already occurred and the damaged area is now inflamed or infected. This information, in contrast to signals about environmental threats, fails to trigger spinally mediated withdrawal reflexes, as there is no external source from which to withdraw. Instead, the information is relayed to higher brain centers that organize the appropriate recuperative behaviors to protect and facilitate healing of the damaged body site. Such behaviors include disuse and protection of an injured limb and licking/cleansing the wound. In either case, pain arises when appropriate; in healthy, normal organisms pain rarely occurs in the absence of a threatening or inflammatory signal.

The chronic pain experienced following injury, infection, or inflammation of peripheral nerves, called

neuropathic pain, sharply contrasts with normal pain. Here, sensory processing for the affected body region is grossly abnormal. Environmental stimuli (e.g., thermal, touch/pressure) that would normally never create the sensation of pain now do so (allodynia), and environmental stimuli that are normally perceived as painful elicit exaggerated perceptions of pain (hyperalgesia). In addition, environmental stimuli can elicit abnormal sensations similar to electric tingling or shocks (paraesthesias) and/or sensations having unusually unpleasant qualities (dysesthesias). Lastly, pain of varying qualities and from varying perceived bodily locations is frequently spontaneous; that is, there is no known stimulus to account for the pain.

Neuropathic pain patients were not born that way. Their pain perceptions were once normal. So, how does this occur? Animal models of nerve trauma have provided insights into the neural changes that occur in response to peripheral nerve damage. They have revealed a remarkable degree of plasticity in both the sensory neurons and spinal cord (346). For example, pain-responsive peripheral nerve fibers develop spontaneous activity. This spontaneous activity can arise not only near their peripheral nerve terminals but also midaxonally from the site of nerve damage or even from the neuronal cell bodies far from the nerve injury site (133, 171). These "pain" neurons also exhibit altered peripheral terminal receptor function that increases their responsiveness to pain-inducing substances (78, 79). In addition, neurons that do not normally signal pain exhibit altered gene expression such that they now, for the first time, begin producing "pain neurotransmitters" for signaling the spinal cord (212). Furthermore, the spinal cord pain-responsive neurons show plasticity along similar lines (166, 354). On the basis of such insights of neuronal changes in response to traumatic nerve injury, a variety of drugs have been tested in hopes of controlling chronic neuropathic pain. None ends the pain. Some work partially in some patients (187, 188, 281). Even when combinations of drugs are given that target different putative causes of the pain, they fail (281).

So, why do the therapies fail? Are conclusions drawn from the animal models wrong? Alternatively, could there be another critical factor influencing the creation and maintenance of chronic pain?

One potentially critical factor that has been lacking, until very recently, has been an appreciation for the role of the immune system in pathological pain. With neuropathy as the example, it has been estimated that approximately one-half of the clinical cases are associated with infection/inflammation of peripheral nerves rather than nerve trauma (259). Yet, until just the past few years, no animal model has taken that fact into account. Furthermore, even trauma activates immune processes, yet the potential implication of this fact for pain was never explored.

Within the past few years, an explosion of research has delineated the dynamic and powerful effects of immune activation on pain. This work has explored actions of immune-derived substances at peripheral nerve terminals, along midaxonal sites, on sensory neuron cell bodies, and within the spinal cord. From this work it is now clear that each of these sites is powerfully modulated by activation of peripheral immune cells and/or immunelike glial cells and that immune activation may indeed be a critical factor in the creation and maintenance of pathological pain. The general argument will be that although immune processes are highly adaptive when directed against pathogens or cancer cells, they can also come to be directed against peripheral nerves, dorsal root ganglia, and dorsal roots, with pathological pain as the result.

The purpose of this review is to explore these issues. We focus first on sensory nerve fibers and then on sensory nerve somas as the targets of immune actions. The immune-neuronal interactions that are described have far broader implications than only for pain. The effects described occur wherever immune-derived substances come in close contact with axons or nerve cell bodies. The implications of immune-produced alterations in neural structure and function in both the peripheral nervous system and central nervous system are predicted to occur in all other sensory and sensory-related systems as well.

## II. PERIPHERAL NERVE TRUNKS AS TARGETS OF IMMUNE ACTIVATION

Peripheral nerves are the origin of almost all forms of neuropathic pain. This section is divided into two major subsections. Section II A provides an overview of peripheral nerve anatomy and immunology, by addressing issues of 1) anatomy and immune surveillance, as well as nerve damage caused by 2) antibodies, 3) complement, 4) T lymphocytes, and 5) trauma. The purpose is to provide the background for the clinically relevant discussion that follows. Section II B focuses on painful neuropathies that involve nerve trauma and/or inflammation. Within this, three clinical neuropathic pain syndromes are examined: 1) complex regional pain syndromes associated with peripheral nerve trauma and/or inflammation, 2) autoimmune neuropathies, and 3) vasculitic neuropathies. For each, an examination of the clinical findings is first discussed followed by a summary of data from relevant animal models. The argument to be developed is that immune attack of peripheral nerves, or even simply immune activation near peripheral nerves, is sufficient to create increases in peripheral nerve hyperexcitability and/or damage so as to be considered a significant contributor to the neuropathic pain observed.

**A. Overview of Peripheral Nerve Anatomy and Immunology**

*1. Anatomy and immune surveillance*

The anatomy of peripheral nerves creates a microenvironment unique from most bodily tissues. Each peripheral nerve trunk is composed of numerous nerve fascicles. Each fascicle within the nerve is surrounded by a perineurium. The connective tissue in which these perineurium-enwrapped fascicles lie is the endoneurium. Finally, the entire bundle of endoneurium-embedded fascicles is surrounded by the epineurium (226). A complex network of blood vessels penetrates the nerve, providing both nutrients as well as potential access of circulating immune factors to nerve tissue (10). This access is limited under normal conditions as the microenvironment of the nerve is protected by the blood-nerve barrier, although not as strictly limited as by the blood-brain barrier (227). Whereas the vascular supply to the epineurium includes some fenestrated capillaries and so does not exclude antibodies or other large proteins, this is not the case for the nerve interior. The endoneurium, as a general rule, is nearly impermeable to circulating immune cells, antibodies, and other plasma proteins (227, 270). However, its impenetrability does vary considerably between species, individuals, and even among fascicles (226, 227, 235). Furthermore, there is an exception to exclusion of immune cells in that peripheral nerves are under constant immune surveillance by circulating activated T lymphocytes (112).

Immune surveillance also occurs within the nerve itself (Table 1). The major nonneuronal cells in the endoneurium are fibroblasts (226). One function of these cells is removal of myelin and other cellular debris after tissue damage (268). Upon activation, these cells produce a variety of substances involved in host defense. These include chemoattractant molecules [e.g., macrophage inflammatory protein-2 and monocyte chemoattractant protein-1 (312)] that recruit immune cells (primarily neutro-

phils and macrophages) from the circulation into the nerve, proinflammatory cytokines that orchestrate the early immune response by communicating between immune cells, and nitric oxide (NO) and reactive oxygen species (ROS) which kill pathogens (e.g., viruses and bacteria) by damaging mitochondria, DNA, and other cellular machinery (189, 208, 268, 305, 332). However, the effects of proinflammatory cytokines, NO, and ROS extend beyond pathogen killing, because these fibroblast-derived substances can also directly increase nerve excitability (160, 288, 291), damage myelin (202, 249, 259, 283), and/or alter the blood-nerve barrier (83, 315). This latter effect leads to edema and infiltration of immune cells, antibodies, and other immune products (198).

Beyond fibroblasts, the endoneurium contains numerous resident macrophages, dendritic cells, mast cells, and endothelial cells (112, 226) (Table 1). Each of these cell types, upon activation, also releases proinflammatory cytokines, NO, ROS, and immune cell chemoattractants (155, 305). Activated macrophages and mast cells in addition release a variety of proteases and other degradative enzymes that evolved to destroy pathogens. In keeping with this role, release of these enzymes is stimulated by a variety of immunologic stimuli, including bacteria and parasites. However, pathogens are not the only trigger for release. To the detriment of peripheral nerves, these degradative enzymes are also released by immune cells upon detection of peripheral nerve proteins (P0 and P2). Detection of P0 and P2 occurs only after nerve damage as these peripheral nerve proteins are normally buried within the myelin sheath and thus “hidden” from immune surveillance. Their novel exposure during nerve damage leads them to be responded to by immune cells as “non-self” (130). Once released, macrophage- and mast cell-derived enzymes attack myelin and disrupt the blood-nerve barrier, allowing egress of blood-borne immune cells into the site (226).

Lastly, Schwann cells, which envelop peripheral nerves, are macrophage-like in many respects; that is,

TABLE 1. Profile of major actions exerted by immune cells resident in peripheral nerves

Cell Type	Release						Remove	
	PIC	NO	ROS	Chemoattract.	Enzy.	Acids	Damaged myelin	Cellular debris
Dendritic cells	X	X	X	X				
Endothelium	X	X	X	X				
Fibroblasts	X	X	X	X			X	X
Macrophages	X	X	X	X	X	X	X	X
Mast cells	X	X	X	X	X			
Schwann cells	X	X	X	X		X	X	X

Immune cell actions are divided into categories of substances released by these cell types and of phagocytic actions to remove damaged tissues from the site. PIC, proinflammatory cytokines (tumor necrosis factor, interleukin-1, interleukin-6); NO, nitric oxide; ROS, reactive oxygen species; Chemoattract., chemoattractant molecules that recruit immune cells to the site; Enzy., digestive and destructive enzymes.

they detect the presence of nonself substances and present them to T lymphocytes to further activate these immune cells (338). In addition, Schwann cells participate in the removal of damaged myelin and cellular debris. Upon activation, these cells release chemoattractants, proinflammatory cytokines, ROS, and NO (13, 80) (Table 1). Monocyte chemoattractant protein-1 is notable among the chemoattractants released by Schwann cells. This protein is rapidly produced by Schwann cells upon nerve damage and serves to selectively recruit monocytes (circulating macrophages) from the systemic circulation to the site of nerve degeneration (112).

Taken together, it is clear that there are numerous intraneurial and circulating immune cell types that can potentially effect peripheral nerves. The following sections briefly review immune responses relevant to understanding immune-related neuropathies.

## 2. Antibody-mediated nerve damage

Most humans do not have antibodies in their bloodstream that attack peripheral nerves. Even when this occurs it is often without clinical consequence, likely due in large part to the integrity of the blood-nerve barrier (243). Antibodies that attack peripheral nerves often arise by "molecular mimicry" (Table 2). Molecular mimicry refers to similarities between the three-dimensional structures (epitopes) expressed on the external surface of normal nerve versus those expressed by either pathogens such as viruses and bacteria (243) or cancer cells (e.g., small cell lung carcinoma, melanoma, neuroblastoma; Ref. 325). Epitopes of pathogens and cancer cells that are recognized as nonself stimulate the formation of antibodies that specifically bind to them. If these nonself epitopes are sufficiently similar to epitopes expressed by peripheral nerve, the antibodies may cross-react and attack peripheral nerves as well. As the antibodies are now attacking "self," they are referred to as "autoantibodies" which create autoimmune neuropathies. Under such circumstances, killing the pathogen does not relieve the

neuropathic symptoms since the autoantibodies have already formed and the immune cells that generate them are long-lived.

Antiperipheral nerve antibodies can also arise as a result of nerve trauma which exposes P0 and P2 to immune surveillance (see sect. 1A5). As noted in section 1A1, these peripheral nerve proteins are not normally encountered by the immune system and so are responded to as nonself when they are exposed by nerve damage, hence generating an immune response (150). Indeed, P0 and P2 are the peripheral nerve proteins that are injected into laboratory animals to create the autoimmune neuropathy model called "experimental allergic neuritis" (EAN) (88). EAN is discussed further in section 1C2A.

Finally, antibodies may be directed against pathogens that have invaded the nerve (Table 2). In this case, the immune response triggered by antibodies is not directed at the nerve, but rather by the recognition of nonself within the nerve bundle. In this case, peripheral nerves can suffer "innocent-bystander" damage; that is, substances released during the ensuing immune response to the pathogen (e.g., proinflammatory cytokines, NO, ROS, degradative enzymes, etc.) can alter the structure and function of nearby nerve fibers as well.

As noted in section 1A1, antibodies do not readily access the microenvironment of peripheral nerves under normal conditions; rather, they primarily gain entry to the nerve interior upon breakdown of the blood-nerve barrier. Upon entry, antibodies that recognize epitopes within the nerve bundle bind to them. Being bound to an epitope allows these antibodies to be recognized by specific receptors expressed on macrophages and Schwann cells (Table 2). Binding of these immune cells to bound antibody triggers the extracellular release of a variety of highly toxic substances. These macrophage and Schwann cell-derived substances include acids, ROS (superoxide, hydrogen peroxide, singlet oxygen, hydroxyl radicals, hypohalite), NO, and so forth (69, 165). In addition, macrophages and Schwann cells are phagocytic cells; that is, they engulf nonself to destroy it. Binding of these immune cells to bound antibody triggers phagocytosis of the antibody-bound site (123, 322). If the antibody-bound entity is too large to be engulfed (a myelinated axon, for example), then the bound phagocyte releases digestive enzymes into the extracellular space toward the antibody-bound site. While the purpose of these digestive enzymes and toxic substances is to kill pathogens, these immune cell products will also cause innocent-bystander damage to all nearby cells, including nerves (95).

Of the destructive weaponry wielded by immune cells following their detection of bound antibody, NO and ROS are especially relevant to neuropathies as they damage subcellular organelles, membranes, and enzymes through actions on proteins, lipids, and DNA (283) (Table 3). Myelin is a preferential target due to its high lipid-to-

TABLE 2. Profile of antibody generation and actions as they relate to neuropathies

Antibodies
Generated in response to
Molecular mimicry
PO exposure
Pathogens (bacteria, viruses)
Bound antibodies stimulate
Engulfment by macrophages/Schwann cells
Release of degradative substances by macrophages/Schwann cells
Complement activation

PO, a major peripheral nerve protein that is normally "hidden" from immune detection and so is responded to as "nonself" when exposed by nerve damage.

TABLE 3. *Profile of nitric oxide, reactive oxygen species, and proinflammatory cytokine actions as they relate to neuropathies*

NO and ROS
Chemically alter the structure of proteins, lipids, and DNA
Damage DNA, mitochondria, membranes, enzymes, ion channels, transporters, etc.
Preferentially attack myelin due to its chemical structure
Proinflammatory cytokines
Recruit immune cells to site of damage/infection
Activate the recruited immune cells, causing further release of immune substances at the site
Upregulate complement production
Stimulate osteoclast proliferation and activation
Inhibit osteoblast proliferation and activation
Inhibit hair growth
Stimulate fibrotic changes in skin
Increase substance P transport to peripheral terminals

NO, nitric oxide; ROS, reactive oxygen species.

protein ratio (21). Moreover, antioxidant levels and activities are lower in nerve than in other tissues, making nerves an “at risk” site for NO- and ROS-mediated effects (253, 254). NO- and ROS-induced damage causes decompaction of myelin lamellae (21); that is, while such axons are normally enwrapped by multiple tightly packed layers of myelin, NO- and ROS-induced damage causes these myelin layers to split apart and physically separate. This renders the myelin susceptible to degradation by extracellular proteases liberated by macrophages. Lipid peroxidation and protein nitration are the most destructive result of ROS and NO, as these damage a variety of peripheral nerve structures, including ion channels, ATPases, ion transporters, ion exchangers, glucose transporters, membrane-bound enzymes, and mitochondria (149, 154, 184). Damaged ion channels and transport systems increase neuronal excitability (154, 184, 283) and alter a variety of second messenger systems via increases in intracellular calcium (154). Increases in inducible immune-derived NO synthase (iNOS) and ROS-generating enzyme activity are correlated with the expression of neuropathic pain behaviors in animals (145, 165). Indeed, disrupting NO and ROS production reduces peripheral nerve damage and pain associated with animal models of neuropathy (95, 145, 154, 283, 304, 307, 326).

3. *Complement-mediated nerve damage*

Complement is a family of proteins each of which is called a complement component. These proteins are normally present in serum and extracellular fluid, and under basal conditions are largely synthesized by the liver to provide a background readiness in case of immune challenge. In addition, complement components are released by a variety of activated immune cells (67) and Schwann cells (151) so that levels at sites of infection can be immediately elevated. In fact, the major source of complement at inflammatory foci is local production (95).

There is more than one way to activate complement (Table 4). Several types of antibody associated with neuropathies (IgM, IgG1, and IgG3) are termed “complement fixing” as, once bound, they activate the complement cascade. The complement cascade can also be activated, independently of antibody, by the presence of various viruses, yeasts, and bacteria (200) and by contact with peripheral nerve protein P0 (153).

Activation of the complement cascade creates wide-ranging effects on nerves (Table 4). Complement components recruit macrophages and neutrophils from the general circulation into nerve (353), “coat” bound antibody to enhance its destruction by phagocytes (67), and disrupt Schwann cell function (50). Although complement, by and large, does not kill Schwann cells, it inhibits the expression of Schwann cell genes important in myelin formation and compaction. For example, complement disrupts transcription of P0 as well as enhancing degradation of P0 mRNA (50).

In addition, complement activation causes the formation of membrane attack complexes (MACs) (150). MACs insert into lipid membranes, forming cation pores. Their insertion helps kill pathogens. However, MACs are indiscriminate, as they insert into the host’s own tissues as well. Many of the hosts’ cells are fairly well protected from MAC-induced cell death by specific cytoplasmic factors that rapidly expel the MACs (150, 169). However, myelin sheaths have no such protection against MAC attack (28, 150). MAC insertion into the myelin sheath is associated with morphological changes of the myelin, wherein the sheath’s lamellae are split, showing signs of decompaction (Table 4). In the course of these changes in myelin morphology, the major peripheral nerve proteins (e.g., P0, P2) are exposed. These activate the complement cascade (95, 150), leading to further myelin damage, further exposure of P0 and P2, and so on (95, 150). This provides a local means of perseveration of peripheral nerve damage and excitability beyond the presence of the

TABLE 4. *Profile of complement activation and effects as regards neuropathies*

Complement Activation
Occurs in response to
Bound antibody
Pathogens
PO exposure
Effects
Recruits immune cells
Enhances phagocytosis
Inhibits Schwann cell myelin production/compaction
MAC-induced myelin destruction
MAC-induced calcium entry and calpain activation

P0, a major peripheral nerve protein that is normally “hidden” from immune detection and so is responded to as “nonself” when exposed by nerve damage; MAC, membrane attack complexes which are an end product of activation of the complement cascade.

original immune activator (95). Indeed, complement activation in close association with peripheral nerves has been linked to the development of neuropathic pain (267).

Finally, MAC attack on myelin, axons, and Schwann cells leads to calcium entry and activation of calcium-sensitive enzymes such as phospholipase A<sub>2</sub> and calpain (Table 4). Calpain, a calcium-activated neutral protease, is found in myelin of peripheral nerves (168), in Schwann cells (183), and in both myelinated and unmyelinated peripheral nerve axons (6). As noted above, myelin is highly susceptible to MAC-associated damage. Indeed, intraneurial injection of calcium ionophore is sufficient to cause demyelination *in vivo* as calpain activation causes the myelin to self-destruct (90). Calpain has been implicated in Wallerian degeneration (112) and has been found to be necessary and sufficient for axonal degeneration (77).

#### 4. *T lymphocyte-mediated nerve damage*

T lymphocytes have been implicated in animal models of neuropathy such as EAN (see above). Each T lymphocyte can bind to and become activated by a single epitope. As activated T lymphocytes move into and through peripheral nerves in the normal course of immune surveillance, they exit if they do not identify their epitope. On the other hand, if the epitope is detected, the T lymphocytes remain at the site and begin to both proliferate and release a variety of substances. Some of these substances disrupt the blood-nerve barrier, allowing entry of antibodies and immune cells (290). Others, such as interleukin (IL)-2, tumor necrosis factor (TNF), and interferon- $\gamma$ , stimulate Schwann cells and macrophages to enhance their antigen-presenting capabilities and to begin releasing substances such as proinflammatory cytokines and ROS (19, 209). In addition, these T-cell products stimulate a subset of T lymphocytes, called "cytotoxic T cells." Upon binding to the epitope expressed on a cell, the activated cytotoxic T cells release specialized lytic granules directly at the cell. These lytic granules contain the cytotoxic protein perforin and a family of destructive proteases called granzymes. Perforin creates transmembrane pores in the target cell membrane, disrupting ion balances and allowing granzymes to enter. Once inside, granzymes trigger programmed cell death (apoptosis) of the target cell (89). The killed cells are then engulfed and destroyed by phagocytic immune cells, including macrophages and Schwann cells. As with other immune processes, this cytotoxic T-cell response is adaptive when directed against threats, such as bacteria, viruses, or cancer cells. However, nearby nerves can also be destroyed.

#### 5. *Trauma-induced nerve damage*

Traumatic nerve injury leads in many cases to post-traumatic neuropathic pain. In traumatic injury, there is not only trauma-induced tissue destruction, but likely

bacterial contamination of the injury site as well. Many bacteria activate the complement cascade, as noted above. In addition, the presence of bacteria causes release of chemoattractants that recruit and activate phagocytic cells (neutrophils and macrophages). These phagocytes express surface receptors that recognize and bind to evolutionarily conserved epitopes on bacterial surfaces. Binding triggers phagocytosis as well as release of NO, ROS, and proinflammatory cytokines (IL-1, IL-6, and TNF). NO and ROS are key antibacterial products as they damage DNA, mitochondria, and other cellular machinery leading to bacterial demise (Table 3).

On the other hand, IL-1, IL-6, and TNF orchestrate the early immune response to infection and damage by serving as chemoattractants to recruit immune cells to the site from the general circulation (229) (Table 3). These immune-derived proteins also activate the recruited immune cells to release a variety of substances that enhance host defense. Thus, in response to these proinflammatory cytokines, there is further release of NO and ROS (169, 249) and upregulation of the production of complement components by recruited immune cells and Schwann cells (169). In turn, engulfment of damaged myelin by recruited and resident phagocytes further increases their production of proinflammatory cytokines long after the original immune stimulus (169). As reviewed in section  $\parallel B2$ , proinflammatory cytokines have been repeatedly implicated in demyelination and degeneration of peripheral nerves, increases in sensory afferent excitability, and creation of neuropathic pain.

Beyond introducing bacteria, traumatic injury stimulates immune responses in two ways. First, nerve damage exposes the peripheral nerve proteins P0 and P2. As described in sect.  $\parallel A1$ , these are responded to as nonself by the immune system, so this initiates an immune response similar to that triggered by pathogens. Second, physical injury and ischemic injury that occur with trauma lead to cell disintegration (necrosis). In peripheral nerve, this is associated with Wallerian degeneration characterized by demyelination and denervation followed by remyelination and reinnervation (296, 326). This process has been extensively studied and found to involve edema-associated disruption of the blood-nerve barrier and the activation of recruited and resident macrophages, fibroblasts, and Schwann cells (296). All of these cell types are active in phagocytosing necrotic peripheral nerve tissue (268). Locally produced proinflammatory cytokines are intimately involved in the Wallerian degeneration process as well (279, 280).

### **B. Painful Neuropathies Involving Nerve Trauma and Inflammation**

As documented in section  $\parallel A$ , multiple immunologic mechanisms exist in peripheral nerves that, upon activa-

tion, can extensively damage or destroy its function. Below we discuss how these may relate to clinical peripheral nerve neuropathies and their associated animal models. What will be presented are examples of immunologically related neuropathies. The discussion is not intended to be inclusive. However, the examples were chosen to illustrate the types of immunological changes that occur under a number of very different precipitating events.

### 1. *Clinical correlations: complex regional pain syndromes (causalgia and reflex sympathetic dystrophy)*

A) ETIOLOGY AND GENERAL SYMPTOMOLOGY. Reflex sympathetic dystrophy (RSD) and causalgia have recently been controversially reclassified as complex regional pain syndrome (CRPS) I and II, respectively (8). While the CRPS I and II terminology will be followed here, the reader should be clear that RSD and causalgia are the syndromes discussed. CRPS I and II are painful conditions that appear to regionally and typically affect limbs rather than the body trunk. There is no consensus on the pathophysiological mechanisms underlying these pain syndromes (8, 293). Not surprisingly, given the mysteries surrounding CRPS I and II, current drug therapies targeting neurons as the basis of these syndromes fail to control the neuropathic pains (293, 329). The symptomology to be described below raises the possibility of an immunological basis of these syndromes.

CRPS I and II have many features in common. The principal feature that distinguishes them is that CRPS II (causalgia) develops after partial injury of a peripheral nerve trunk. In contrast, CRPS I (RSD) occurs in the apparent absence of known injury to nerve trunks. Minor injuries to the limb, injuries to remote body regions, low-grade infection, frostbite, burns, myocardial infarction, stroke, neurologic and rheumatologic diseases, fractures, surgery, or even a minor sprain or contusion can precede the onset of CRPS I symptoms (8, 228). Indeed, no identifiable precipitating event can be identified in 35% of CRPS I cases (320). It is not known whether minor injuries or unidentified events also contribute to the CRPS II pain assumed to arise from nerve trauma. This possibility exists as CRPS II pain can arise from body regions outside of known nerve trauma. For both CRPS I and II, the magnitude and duration of pain greatly exceeds that predicted by the inciting injury, and there is variable progression of pain over time (8). Also, in both syndromes, the affected region is characterized by abnormalities in blood flow and sweating, swelling, trophic skin changes (e.g., thinning, shiny), fibrosis, either decreased or increased hair growth, and patchy bone demineralization (osteoporosis) (8). Many but not all patients exhibit altered sympathetic function as well (see sect. *1B1c*) (228).

The pain associated with CRPS includes both a spontaneous burning sensation as well as allodynia to both touch/pressure and cold stimuli; heat hyperalgesia is also observed in some patients (8, 239, 240). A peripheral trigger for the pain of at least CRPS II is supported by the report that local anesthetic block of the site of prior trauma blocks mechanical allodynia, cold allodynia, and spontaneous pain perceived from sites beyond the area of anesthesia (82). Pain returns upon loss of anesthesia at the trauma site. Based on these findings, it has been proposed that ongoing sensory information arising from such pain-triggering foci create and maintain pathological pain of CRPS by actions on the spinal cord (82).

One striking feature of the pain of CRPS I and II is that it changes with time. Typically, pain begins at a relatively focal site. A hallmark of CRPS is that the painful area does not follow either neural, vascular, or muscular patterns (23). The pain expands along the limb and/or migrates to other body parts in nearly 70% of patients, and bilateral pain occurs in ~50% of cases (137). Indeed, the pain may expand to encompass a body quadrant or even the entire body (158, 323). Such "anatomically impossible" patterns of pain led to the hypothesis that CRPS was of psychological rather than physical origin, but this explanation has been dismissed (363). An alternative hypothesis is that the anatomically impossible pain distributions and expansions of the painful region with time are created by spinal cord sensitization, likely involving immunelike glial cells (335, 336a). Here, spinal sensitization refers to dynamic changes that occur in the spinal cord dorsal horn in response to intense and/or prolonged pain signals received from peripheral nerves. These intense/prolonged signals arriving at dorsal horn pain responsive neurons cause these spinal neurons to become hyperexcitable; that is, these neurons now respond to stimuli that are not normally perceived as painful as if they were painful, and overreact to stimuli that are normally perceived as painful. The possibility that glial cells drive this spinal hyperexcitable state will be addressed in section *1B2D*.

B) EVIDENCE AND IMPLICATIONS OF A PERSEVERATIVE INFLAMMATORY STATE. The central sensitization of CRPS may, at least in part, be sustained by the presence of a chronic inflammatory state in the affected body region. There are many features of CRPS that suggest that the pathological region is exhibiting an excessive inflammatory response for at least the first several months of the disease process (Table 5). As typical for an inflammatory event, the affected region exhibits increased blood flow, increased vascular permeability, edema of soft tissues and bone, hypervascularity in synovium and skeletal muscle, impaired local oxygen utilization leading to ischemic oxidative stress of the involved tissues, tissue accumulation of antibodies and immune cells (neutrophils), and degenerative tissue changes due to localized ROS-induced lipid peroxidation

TABLE 5. *Summary of the signs of perseverative, exaggerated inflammation of body regions affected by complex regional pain syndromes I and II*

Signs of Inflammation in CRPS
Increased blood flow
Increased vascular permeability
Edema of soft tissues and bone
Hypervascularity of synovium and muscle
Oxidative stress
Accumulation of immune cells
ROS production and consequent lipid peroxidation
Patchy osteoporosis
Proliferation of epidermal immune cells
Altered skin, nail, and hair growth

ROS, reactive oxygen species; CRPS, complex regional pain syndromes I and II (reflex sympathetic dystrophy and causalgia, respectively).

(158, 161, 228, 251, 318, 361). Patients may exhibit increased circulating levels of bradykinin, which has been associated with inflammatory pain (18). Furthermore, shifts in acute phase protein concentrations in blood and blood cell counts are consistent with a subacute inflammatory process (161). Supportive of inflammatory mediation of CRPS, scavengers of ROS decrease the symptoms (81). The clinical finding that treatment with immunosuppressive doses of corticosteroid decreases CRPS complaints is also supportive of an inflammatory basis of CRPS (318).

The patchy osteoporosis, proliferation of epidermal immune cells, and alterations in skin and hair growth observed in CRPS patients are also consistent with a regional inflammatory process (362). Both IL-1 and IL-6 cause proliferation and activation of osteoclasts (the cells that mobilize calcium via bone destruction) and suppress the activity of osteoblasts (the cells that create new bone) (179, 297). Furthermore, skin biopsies of CRPS patients show striking increases in the numbers of epidermal Langerhans cells (31) which, like keratinocytes and several other cell types in epidermis, can release immune cell chemoattractants and proinflammatory cytokines (44, 109). Indeed, denervation of the skin causes rapid activation and proliferation of Langerhans cells and keratinocytes that continues until reinnervation occurs (117, 292). This suggests that such cell proliferation in CRPS may reflect partial denervation of the affected region. Lastly, skin and hair changes may be proinflammatory cytokine related. Recall that CRPS is characterized by the seemingly strange symptom of both decreased and increased hair growth. But, in actuality, both decreased and increased hair growth can be created by proinflammatory cytokines by direct and indirect pathways, respectively. TNF and IL-1 directly inhibit hair growth in that they are highly potent inhibitors of both the growth of human hair follicles and elongation of the hair shaft (284, 351). Kera-

tinocyte-derived TNF and IL-6 cause retarded hair growth, signs of fibrosis, and immune infiltration of the dermis (34, 314), as observed in CRPS patients. Proinflammatory cytokines can also exert the counterintuitive effect of indirectly stimulating hair growth. In humans, proinflammatory cytokines can stimulate the release of hepatocyte growth factor/scatter factor from hair follicle cells, resulting in enhanced hair growth (276).

Given the inflammatory profile of CRPS, it was natural to consider whether an infective or autoimmune process underlies the disease. Thus attempts have been made to link CRPS to specific preceding infections. Although a few cases of CRPS have been noted to follow *Borrelia* infections (23) and spirochetal infections (211), no links to other pathogens have been reported, nor have antiperipheral nerve antibodies been identified in these patients.

Because autoimmunity and infection do not account for CRPS, exaggerated neurogenic inflammation has been proposed (14). Neurogenic inflammation refers to the fact that painful stimulation of the receptive fields of certain pain responsive fibers (termed C fibers) causes two results. The first is sending a pain message to the spinal cord, leading to sensation/perception. The second is release of substances by these same nerve terminals into their own receptive fields. These neurally released substances (e.g., substance P) trigger all of the cardinal signs of inflammation: reddening of the area, swelling, and pain. Because this inflammatory response is created by a local nerve "reflex," this has been called neurogenic inflammation. Exaggerated neurogenic inflammation would exaggerate the release of substance P from peripheral nerve terminals. In turn, substance P would cause local swelling, redness, and pain, consistent with symptoms of CRPS (14). Such an exaggerated release of substance P is intriguing from an immunological viewpoint, as this would potentially provide an additional mechanism for creating pain. Substance P induces proinflammatory cytokine release from a variety of immune cells (175, 223, 360) and has been shown to induce at least TNF and IL-1 release from human skin (26, 222). In turn, proinflammatory cytokines induce pain by activating pain-responsive sensory nerve terminals (46, 68, 345). Indeed, a substance P-proinflammatory cytokine positive-feedback loop would be predicted in CRPS, given that even a single intraplantar injection of IL-1 produces a long-term increase in axonal transport of substance P to cutaneous nerve terminals (128). Such a hypothesized positive feedback loop would be predicted to provide a perseverative "drive" to create and maintain spinal cord sensitization.

If true, such a substance P-proinflammatory cytokine positive feedback loop would also have implications for the elevated bradykinin levels observed in CRPS patients (18). This CRPS-related elevated systemic bradykinin may potentially interact with the proposed substance P-proin-

flammatory cytokine positive-feedback loop because proinflammatory cytokines upregulate the expression of bradykinin receptors in a variety of tissues (9, 86), and bradykinin receptors contribute to pain hypersensitivity (52). Bradykinin may also further stimulate the proposed substance P-proinflammatory cytokine loop, as bradykinin increases IL-1, TNF, and IL-6 (199, 310).

Taken together, numerous lines of evidence suggest that prolonged localized release of proinflammatory cytokines may occur in body regions affected by CRPS. Although clearly speculative, if this does occur, it suggests that such perseverative proinflammatory cytokine release could, by stimulation of sensory nerves, be a contributing factor to the maintenance of central sensitization observed in CRPS patients.

C) IMPLICATIONS OF SYMPATHETIC NERVOUS SYSTEM INVOLVEMENT FOR IMMUNE RESPONSES. Although controversial (215, 294), CRPS is often reported to be a sympathetically maintained pain syndrome (8, 239). Sympathetic involvement in CRPS is supported by the facts that 1) there is overlap of body regions exhibiting pain and autonomic dysfunction (sweating, temperature, and blood flow abnormalities) and 2) blocking sympathetic function relieves pain (293, 329). While an increase in sympathetic activity was originally thought to occur, more recent evidence suggests instead that there is reduced sympathetic activity in the affected region that, with time, develops supersensitivity to catecholamines (60). While catecholamines and sympathetic activation do not cause pain in normal humans, they do create pain in CRPS patients (2). This pain response is thought to be due, at least in part, to novel expression of  $\alpha_1$ -adrenergic receptors on pain-responsive sensory fibers (8). Blocking sympathetic function, whether by surgical sympathectomy, systemic phentolamine, or systemic guanethidine, relieves partial nerve injury-induced neuropathic pain in laboratory animal models as well as humans (8, 35, 146, 239, 278). Indeed, sympathectomy does not just relieve pathological pain in the body region ipsilateral to the CRPS-initiating event; rather, it also relieves pain arising from anatomically impossible mirror-image sites, that is, the identical body region contralateral to the initiating event (278). Thus sympathectomy must somehow quiet the contralateral spread of spinal cord hyperexcitability underlying mirror-image pain.

Alterations in sympathetic fibers rapidly follow peripheral nerve injury. This occurs as sprouting of sympathetic fibers, creating aberrant communication pathways from the new sympathetic terminals to sensory neurons (35). Sympathetic sprouting has been documented in the region of peripheral terminal fields of sensory neurons (262), at the site of nerve trauma (57), and within the dorsal root ganglia (DRG) containing cell bodies of sensory neurons (248, 343). Each of these sites develops

spontaneous activity and sensitivity for catecholamines and sympathetic activation (8, 53).

The clearest evidence that immune activation participates in sympathetic sprouting comes from studies of the DRG. DRG cells receive signals that peripheral nerve injury has occurred via retrograde axonal transport from the trauma site. These retrogradely transported signals trigger sympathetic nerve sprouting into DRG (205, 308). As a result of nerve damage-induced retrogradely transported signals, glial cells within the DRG (called satellite cells) proliferate (248) and become activated (343); macrophages are recruited to the DRG as well (63, 176). In turn, the activated satellite glial cells (and, presumably, the macrophages) release proinflammatory cytokines and a variety of growth factors into the extracellular fluid of the DRG (206, 246–248, 258, 277, 308, 358). These substances stimulate and direct the growth of sympathetic fibers, which form basket-like terminals around the satellite cells that, in turn, surround neuronal cell bodies (247, 248, 343). For discussion of satellite cell functions, see section IIIA.

Until recently, the sympathetic sprouting, rather than the glial (satellite cell) activation, has attracted the attention of pain researchers. The satellite cells were ignored as they were thought to be irrelevant to the creation of exaggerated pain states. However, it may be speculated that the satellite cells, rather than the sympathetic sprouts, have the most impact on pain. Although electrical stimulation of these DRG sympathetic sprouts does excite DRG neurons (8), other observations cast doubt on the relevance of these sprouts for pathological pain. First, these sympathetic sprouts predominantly form terminal fields around large-diameter neurons that, as a class, do not transmit pain information (247, 358). Second, the density of sympathetic sprouts in the DRG does not correlate with neuropathic pain intensity (8). Given that 1) DRG neurons express receptors for satellite cell-derived proinflammatory cytokines and growth factors (204, 216) and 2) these proinflammatory cytokines and growth factors act in a paracrine fashion to influence large numbers of cells, perhaps it may be that these satellite cell-derived substances are really the basis for altered pain, rather than the sympathetic sprouts. From this perspective, sympathetic sprouting into DRGs may simply be a “side effect” of glial activation.

If this is true, then satellite cell-derived substances should have demonstrable effects consistent with enhanced pain. Satellite cell-derived substances include, for example, nerve growth factor (NGF) (159, 358), glially derived neurotrophic factor (GDNF) (91), brain-derived neurotrophic factor (BDNF) (339), neurotrophin-3 (NT-3) (244, 358), and proinflammatory cytokines (42). Each of these does indeed exert effects consistent with enhanced pain. 1) GDNF upregulates the expression of pain-relevant sodium receptor subtypes in DRG neurons (45); 2)

intra-DRG injection of NGF and BDNF each induces mechanical allodynia in the absence of nerve damage (359); 3) antibodies to NGF, NT-3, and BDNF each reduces mechanical allodynia induced by nerve damage (359); 4) IL-1 induces the release of substance P from DRG neurons in cell culture (125); 5) neuropathic pain is reduced in IL-6 knock-out mice (247); 6) almost all DRG neurons express IL-6 receptors (204); and 7) TNF induces abnormal spontaneous activity in DRG neurons (170, 357). Thus the actions of satellite cell-derived substances strongly suggest that immune activation in the DRG can facilitate pain.

Beyond the DRG, peripheral nerve injury induces sympathetic nerve sprouting into the upper dermis as well (256). This aberrant pattern of sympathetic innervation of the skin has been proposed to have important implications for sympathetic interactions with pain-responsive sensory terminals (256). In support of this perspective, intradermal injection of norepinephrine, while having no effect on normals, produces pain in CRPS patients (2), and subcutaneous norepinephrine excites pain-responsive C-fiber terminals in the skin of rodents after, but not before, peripheral nerve damage (262). As noted above,  $\alpha_1$ -adrenoceptors have been implicated in sympathetically maintained pain (2).

This remarkable change in catecholaminergic sensitivity of the skin in CRPS and nerve damage is potentially intriguing from an immunologic point of view. Under normal conditions, catecholamines act via  $\beta_2$ -adrenergic receptors on immune cells to inhibit the production and release of proinflammatory cytokines (106). These cells do not express  $\alpha_1$ -adrenergic receptors under basal conditions (138). However, the situation can dramatically change in chronic inflammation. Now, immune cells downregulate their expression of  $\beta_2$ -adrenergic receptors and upregulate their expression of  $\alpha_1$ -adrenergic receptors over time (106). Such a shift to a predominant  $\alpha_1$ -expression may potentially have implications for inflammation and pain associated with CRPS and nerve damage. This is because  $\alpha_1$ -receptors stimulate the production and release of proinflammatory cytokines (106, 138). Clearly, if  $\alpha_1$ -adrenoceptors were to become expressed by the resident and/or recruited immune or immunocompetent cells of the affected CRPS sites (synoviocytes, endothelial cells, Langerhans cells, keratinocytes, fibroblasts, classical immune cells, etc.), then sympathetic activation would be predicted to cause pain, at least in part, via proinflammatory cytokine release (237). Indeed, while the density of  $\alpha_1$ -adrenoceptors is known to increase in hyperalgesic skin of CRPS patients, these studies have either used tissue homogenates or low-resolution autoradiography that preclude the authors from identifying the cell type(s) expressing the receptors (60, 252). Although the autoradiographic study shows marked increases in  $\alpha_1$ -adrenergic expression in skin regions not accounted for by peripheral nerves (60), this finding has never been explored

to define whether  $\alpha_1$ -expressing immune cells may indeed account for this upregulated receptor expression.

Thus, from a variety of angles, immune activation within skin, peripheral nerves, DRG, and spinal cord may represent under-appreciated sources of pain in CRPS. Although highly speculative, the evidence suggests that investigations into the potential involvement of immune activation in CRPS are warranted. As is reviewed in the following sections, evidence from animal models provides further support for the plausibility of this proposal.

## 2. Animal models

A) IMMUNE INVOLVEMENT IN PAIN FROM NERVE TRAUMA: FROM *APLYSIA* TO RATS. Evidence for altered pain processing due to immune activation near peripheral nerve trunks has arisen primarily from two animals: rats and the simpler *Aplysia*. Regarding *Aplysia*, it has been known since the mid 1980s that sensory nerve damage creates prolonged enhanced pain responses (for review, see Ref. 331) (Table 6). These studies led to the discovery that large numbers of immunocytes (immune-like cells of *Aplysia*) are attracted to the site of nerve damage (36). This is intriguing since immunocytes are strikingly similar in function to mammalian macrophages in that they are phagocytes that release proinflammatory cytokine-like molecules (IL-1-like and TNF-like) upon activation by foreign (nonself) substances (37). The link to IL-1 and TNF is interesting since these proinflammatory cytokines alter ion channels in *Aplysia* neurons, causing hyperexcitability (264, 301) (Table 7). These findings led to the discovery that 1) hyperexcitability of injured nerves is significantly greater in the

TABLE 6. Summary of evidence that sensory nerve damage in *Aplysia* and rat is associated with exaggerated pain responses, which are linked with recruitment of immune cells to the site

Sensory nerve damage in <i>Aplysia</i>
Exaggerates pain responses
Exaggerates electrical responses of pain-responsive neurons
Associated with macrophage-like immune cell (immunocyte) invasion of the injury site
Associated with release of interleukin-1-like and tumor necrosis factor-like substances from immunocytes
Nerve hyperexcitability is enhanced by the presence of activated immunocytes
Sensory nerve damage in rat
Exaggerates pain responses
Exaggerates electrical responses of pain-responsive neurons
Associated with macrophage invasion of the injury site
Associated with activation of resident immune cells
Associated with release of interleukin-1, tumor necrosis factor, and interleukin-6 from immune cells
Nerve hyperexcitability is enhanced by the presence of activated immune cells

These immune cells appear to contribute to the exaggerated pain responses that ensue.

TABLE 7. *Summary of evidence that Aplysia and rat proinflammatory cytokines enhance responses to pain stimuli*

Proinflammatory cytokine-like substances in <i>Aplysia</i>
Exaggerate pain responses
Create neuronal hyperexcitability via alterations in ion channels
Enhance injury-induced hyperexcitability, just as activated immunocytes do
Proinflammatory cytokines in rat
Exaggerate pain responses
Create neuronal hyperexcitability, likely via alterations in ion channels
Implicated in neuropathy-induced pain

presence of activated immunocytes (39) and 2) IL-1 application enhances nerve injury hyperexcitability (38).

During this same time period, parallel findings began to appear with rodents. A number of partial nerve injury models were developed in rodents (12, 147, 271), because causalgia (CRPS II) and other posttraumatic neuropathies are associated with partial nerve injuries. Similarities have been noted between such models and CRPS symptoms. For example, partial nerve injury in rodents causes vasodilation in the limb that is associated with increased vascular permeability and edema (47, 48, 158), accumulation of immune cells (neutrophils) in the nerve-injured hindpaw and muscle (47, 48), spinal sensitization (180), enhanced release of substance P from peripheral endings of activated C fibers (158), sympathetic dysfunction with denervation-induced supersensitivity to catecholamines (157), and increased local production of cytokines (76, 328). In parallel with human studies implicating ROS in the generation of CRPS (81), ROS are implicated in CRPS-like pain in rodents since 1) the generation of ROS in healthy rat hindlimbs elicits pain and other symptoms of CRPS (317) and 2) blockade of ROS attenuates neuropathic pain from partial nerve injury (173).

By the mid-1980s it was known that partial nerve injury causes exaggerated pain states and axonal hyperexcitability as well as Wallerian degeneration (231) (Table 6). Both trauma-induced Wallerian degeneration (15) and enhanced pain (174) have been linked to the activity of macrophages recruited to the site of injury. Indeed, simply delaying the recruitment of macrophages to the area of damage delays the development of both neuropathic pain and Wallerian degeneration (210, 245). In contrast, neuropathic pain behaviors are enhanced by attracting activated immune cells to the injury (40, 186). Of the various neuroactive substances released by activated macrophages, most evidence implicates the proinflammatory cytokines IL-1, TNF, and IL-6 (Table 7). These increase at sites of nerve trauma, via production by Schwann cells, endothelial cells, and resident and recruited macrophages (19, 164, 255, 279). Blockade of IL-1 (286) or TNF (122, 285, 287) activity after sciatic injury reduces thermal hyperalgesia and mechanical allodynia.

Blockade of IL-1 and TNF at the level of the sciatic nerve also prevents and reverses pain changes induced by sciatic inflammatory neuropathy (267). Furthermore, neuropathic pain is prevented in IL-6 knock-out mice (247), and the magnitude of mechanical allodynia that develops after peripheral nerve injury directly correlates with both the number of activated macrophages and the number of IL-6-producing cells at the injury site (43). In addition to proinflammatory cytokines, ROS (peroxynitrite) have also been implicated in creating nerve trauma-induced exaggerated pain states through their actions at the site of nerve injury (173).

B) IMMUNE INVOLVEMENT IN PAIN FROM NERVE INFLAMMATION IN THE ABSENCE OF TRAUMA. Pain facilitation can occur even in the absence of apparent physical trauma to peripheral nerves. The simple placement of immunologically activated immunocytes near healthy *Aplysia* sensory nerves increases their excitability (36). Furthermore, exposing healthy rat sciatic nerve to gut suture (186), killed bacteria (64), algae protein (carrageenan) (64), yeast cell walls (zymosan) (33, 75), or the HIV-1 envelope glycoprotein gp120 (108) increases behavioral responsiveness to touch/pressure and/or heat stimuli; that is, these manipulations induce mechanical allodynia and thermal hyperalgesia. Such changes are mimicked by proinflammatory cytokines. TNF injected into the sciatic produces thermal hyperalgesia and mechanical allodynia (327) as well as endoneurial inflammation, demyelination, and axonal degeneration (249). Furthermore, TNF applied to the sciatic induces ectopic activity in single primary afferent nociceptive fibers (288). TNF is not alone in this regard as ATP also ectopically activates peripheral nerves, including fibers associated with pain transmission (126). ROS also appear sufficient to drive exaggerated pain states in the absence of physical trauma to nerves as intra-arterial infusion of free radical donors into one hindlimb of rats causes increased sensitivity to mechanical and thermal stimuli as well as spontaneous pain (317).

Although it is clear that proinflammatory cytokines induce pain, how they do this from midaxonal sites is controversial. While proinflammatory cytokine receptors are known to be expressed on DRG cell bodies (236), whether these receptors are expressed along the course of their peripheral nerve fibers has never been investigated. Of the proinflammatory cytokines, TNF has received the most study to date and is known to rapidly alter neural activity, suggesting that it may directly affect axonal excitability (160, 288). Indeed, studies of the structure of TNF indicate that it can insert into lipid membranes to form a central porelike region due to its three-dimensional conformation (131). Insertion is facilitated by a physiologically relevant lowering of pH, which occurs at sites of inflammation (131). The inserted TNF molecules form voltage-dependent sodium channels (131). Other evidence suggests that TNF interacts with

endogenous sodium and calcium channels to increase membrane conductance (316, 341). Like TNF, IL-1 rapidly increases neuronal excitation (219) and produces long-lasting increases in conductance of voltage-sensitive sodium and calcium channels (266, 341). IL-6 enhances conductance of these ion channels as well (242). Whether IL-1 or IL-6 can insert into lipid membranes has not been reported.

Of the inflammatory paradigms described above, perhaps the one that has been the most fully developed to date is exposure of a healthy sciatic nerve to yeast cell walls (zymosan). This manipulation has been termed “sciatic inflammatory neuropathy” (SIN) (33, 75). To create SIN, immune activation is initiated in unanesthetized rats by unilateral injection of zymosan (yeast cell walls) into preimplanted gelfoam enwrapping one healthy sciatic nerve at midthigh level (33, 75). SIN creates a rapid (within 1 h) mechanical allodynia at both territorial (sciatic) and extraterritorial (saphenous) skin innervation sites. With a single zymosan injection, the allodynia lasts sev-

eral days; with repeated zymosan injections, maximal allodynia can be maintained at least several weeks (336, 336a). No thermal hyperalgesia develops (33). This contrasts with the combined unilateral allodynia and hyperalgesia and much slower onset of effect, observed after acute delivery of immune activators delivered during sciatic surgery (11, 64, 108).

One of the striking aspects of SIN-induced allodynia is the pattern of pain changes (Fig. 1). Unilateral low-dose zymosan injection ( $4 \mu\text{g}$ ) induces an ipsilateral hindpaw allodynia. Unilateral higher doses induce bilateral effects (33, 336); that is, allodynia develops in both the limb that was injected with perisciatic zymosan as well as the “mirror-image” limb. The mirror-image allodynic effect cannot be accounted for by systemic spread of the immune activator (33, 75, 336); rather, its appearance is correlated with well-defined immunologic and anatomic changes in and around the sciatic nerve. Injection of low-dose zymosan (which creates only ipsilateral allodynia) is characterized by release of high levels of TNF from the immune

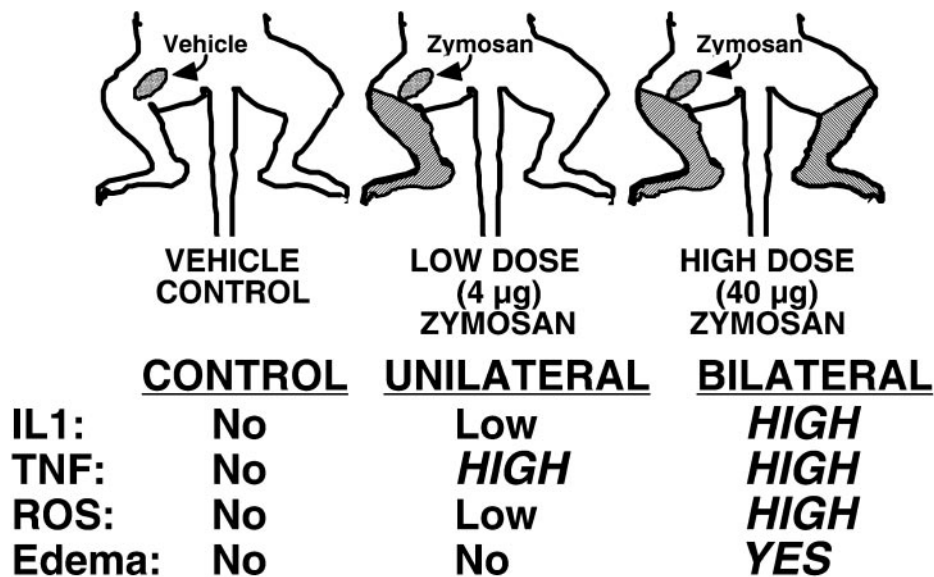


FIG. 1. Schematic of the dose-dependent patterns of low-threshold allodynia (*top*) and immune responses (*bottom*) produced by sciatic inflammatory neuropathy (SIN). *Top*: to produce SIN, an immune activator [here zymosan (yeast cell walls) is shown as the example] or vehicle is injected into unanesthetized, unrestrained rats via an indwelling catheter. This catheter leads to a spongelike material enwrapping a single healthy sciatic nerve at mid-thigh level. When vehicle is injected unilaterally around the sciatic, no behavioral change is observed (*top left*). When low-dose zymosan is injected unilaterally around the sciatic, territorial and extraterritorial mechanical allodynia develops only in the injected leg; that is, ipsilaterally (*top middle*). When higher doses of zymosan are injected unilaterally around the sciatic, territorial and extraterritorial ipsilateral allodynia again occurs. However, now a “mirror image” allodynia appears as well in the contralateral hindpaw (*top right*). The mirror-image pain changes and extraterritorial pain changes cannot be accounted for by spread of the immune activator beyond the injection site. *Bottom*: this chart summarizes the immune changes occurring around the sciatic nerve in response to perisciatic injections. After control (vehicle) injections, no immune response is produced. After low-dose zymosan that produces ipsilateral allodynia, the predominant immune response is high levels of tumor necrosis factor (TNF) release. Only small responses of interleukin-1 (IL-1) and reactive oxygen species (ROS) occur. In contrast, the high-dose zymosan that produces both ipsilateral and mirror-image allodynia stimulates not only TNF release, but IL-1 and ROS release as well. Given that zymosan is a classic complement activator, formation of membrane attack complexes is assumed to occur as well. These dose-dependent changes in immune responses to zymosan are reflected in the anatomy of the sciatic nerve. After low doses of zymosan, no change in sciatic anatomy is observed compared with control. After higher doses of zymosan, edema occurs along the outer rim of the sciatic nerve where the nerve contacts the immune activation occurring around it.

cells around the sciatic nerve (macrophages and neutrophils), and no morphological change in the sciatic nerve is detected (75) (Fig. 1). In contrast, response to higher zymosan doses (which create ipsilateral plus mirror-image contralateral allodynia) is characterized not only by high levels of TNF but also high levels of IL-1 and ROS around the nerve (75). In addition, complement-derived MACs assumed to be produced as zymosan are well established to potently trigger MAC production (49). At least perisciatic complement and proinflammatory cytokines are involved in producing SIN-induced allodynia, since perisciatic delivery of antagonists against these immune-derived substances blocks the pain changes (267). The immunological profile for the higher zymosan dose is associated with distinctive pathology of the sciatic nerve. Edema is observed 24 h after higher dose zymosan perisciatic injection. Strikingly, this edema occurs along the outer rim of the nerve where the nerve is in contact with substances released by zymosan-activated immune cells (75) (Fig. 2). Thus the appearance of mirror-image pain co-occurs with distinctive immunological and anatomic changes at the level of the sciatic nerve.

C) IMPACT OF TRAUMA AND INFLAMMATION ON NEIGHBORING INTACT NERVE FIBERS. One aspect of partial nerve injuries that has only recently received attention is the fascinating question of whether neuropathic pain is being generated solely by the damaged nerves; that is, could pathological pain be created because the function of the remaining intact neurons is being altered by immune-derived substances released during demyelination and degeneration of the intermingled damaged peripheral nerves and/or associated changes in the damaged cell bodies in the DRG? While a number of widely used partial nerve injury models exist for rats, many cause nerve injury in such a way that there

is intermingling of damaged and intact peripheral nerves as well as intermingling of damaged and intact DRG somas (12, 271). Thus the relative contributions of signals from damaged peripheral nerves versus DRG somas of damaged nerves cannot be dissociated.

In response, new models have recently developed that either have 1) damaged peripheral nerves intermingled with intact peripheral nerves, yet maintain complete segregation of damaged versus intact sensory nerve somas (73, 347), or 2) damaged DRG somas intermingled with intact DRG somas, yet maintain almost complete segregation of damaged versus intact peripheral nerves (54). Thus the influence of damaged nerves and the influence of damaged somas can be studied independently.

The effect that intermingled damaged axons have on intact neuronal function has been examined by selectively damaging the fifth lumbar ( $L_5$ ) spinal nerve (167, 347). This creates intermingling of damaged  $L_5$  and undamaged  $L_4$  fibers within the sciatic nerve. At the same time, separate DRG locations of the injured ( $L_5$ ) and intact ( $L_4$ ) DRG somas are maintained (147, 347). Thus peripheral nerves arising from  $L_4$  somas are exposed to immune-derived substances released in their vicinity as a result of the demyelination and degeneration of sciatic  $L_5$ -derived nerves and from the intermingling of their receptive fields in the skin (347). Intriguing changes in the intact  $L_4$  nerves result. Within 1 day after  $L_5$  spinal nerve damage (the earliest time tested),  $L_4$  spinal nerve fibers develop spontaneous activity (3). Mechanical allodynia develops correlated with this change and is abolished by transection of  $L_4$  spinal nerves, indicating that intact  $L_4$  has become the driving force for creating allodynia (167). Spontaneous activity also develops in monkey uninjured nerve fibers follow-

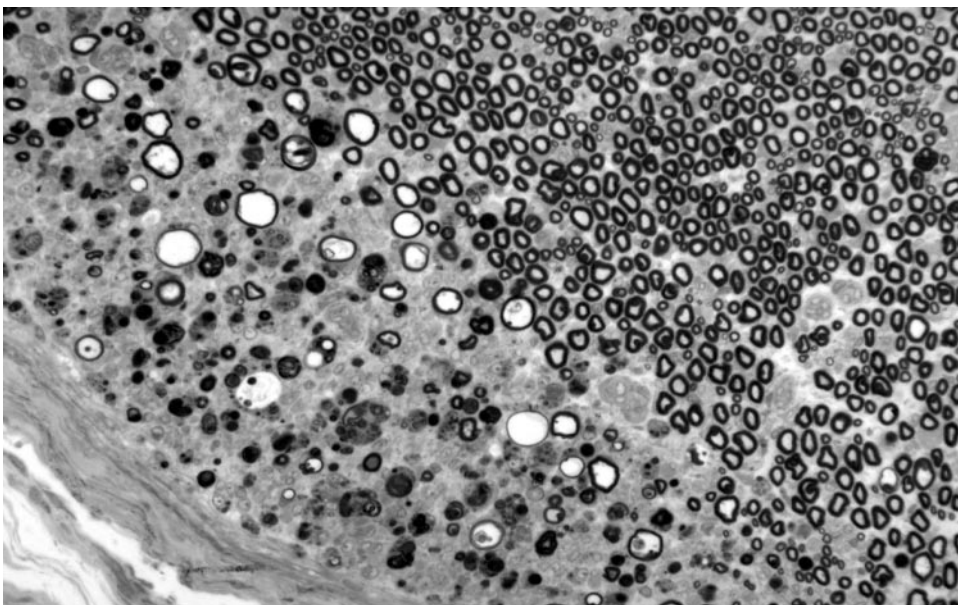


FIG. 2. Example of the edema pattern produced 24 h after perisciatic administration of high-dose zymosan. Recall that this is the intensity of immune activation that creates mirror-image pain changes. Under normal conditions, the edge of the nerve (*left side* of image) would look identical to deeper portions of the nerve (*right side* of image). Edema causes the fascicles in the nerve rim to appear to be spaced further apart than are deeper fascicles. [From Gazda et al. (75). Reprinted with permission from *Journal of the Peripheral Nervous System*.]

ing a similar procedure (3). This nerve fiber activity develops in the cutaneous receptive field region and along the peripheral nerve, rather than within the DRG (3).

As discussed in section II A 5, nerve damage results in immune activation and the release of a host of neuroactive substances along the length of the degenerating fibers. These could have direct influences on the electrical activity of intermingled uninjured axons. Alternatively, such substances may serve as retrogradely transported signals to influence gene activation in intact DRG somas. Indeed, a number of changes have been detected in the L<sub>4</sub> DRG somas of spared axons in partial nerve injury paradigms. First, immune-derived substances such as leukemia inhibitory factor (LIF) (306), IL-6 (127), and NGF (163, 172, 261) are released at the injury site and are retrogradely transported by both intact as well as injured axons (72, 204, 248, 308). These retrogradely transported signals in intact L<sub>4</sub> nerves result in increased DRG neuronal expression of BDNF mRNA and protein, vanilloid receptors type 1 (VR1) mRNA and protein, calcitonin gene-related peptide (CGRP) mRNA, preprotachykinin (PPT) mRNA and its protein product substance P, and galanin (72, 73, 118, 177, 308, 309), as well as increased expression of PN3, a tetrodotoxin-resistant sodium channel subunit (238). This pattern contrasts what is observed after transection of all nerves in a bundle (axotomy), namely, downregulation of DRG expression of VR1 (191), substance P (272), CGRP (272), and PN3 (22). The increases in expression of these factors after partial (rather than complete) nerve injury may be relevant to pathological pain as increased VR1 expression suggests increased responsiveness of the peripheral nerves to heat stimuli, PN3 could increase neuronal excitability, and substance P and CGRP are pain transmitters of sensory neurons. Furthermore, BDNF administered to intact DRG causes mechanical allodynia (359), and increased DRG neuronal expression of BDNF has been linked to neuropathic pain as intrathecal delivery of anti-BDNF antibodies attenuates thermal hyperalgesia in a spinal nerve injury model (72). Indeed, BDNF release in spinal cord phosphorylates spinal *N*-methyl-D-aspartate (NMDA) receptors, which is one of the mechanisms known to create and maintain central sensitization (72).

In the second type of spared nerve injury model, the tibial and common peroneal terminal branches of the sciatic are lesioned, leaving the sural nerve intact (54). In doing so, there is minimal comingling of injured and noninjured peripheral nerves, yet considerable DRG comingling of injured tibial and common peroneal somas with noninjured sural nerve somas (54). Thus this allows examination of possible paracrine signals arising from injured somas acting on nearby noninjured ones to alter their function and/or excitability. With this procedure, mechanical allodynia without thermal hyperalgesia develops from the uninjured sural sensory neurons within 24 h,

an effect that lasts for over 6 mo (54). While satellite cell products are potentially involved, such studies have not yet been done.

D) SPINAL IMMUNELIKE GLIAL CELL INVOLVEMENT IN TERRITORIAL, EXTRATERRITORIAL, AND MIRROR-IMAGE NEUROPATHIC PAIN. There is growing recognition that immune involvement in pathological pain states occurs within the spinal cord as well as in the periphery (335). In spinal cord, immunelike glial cells (astrocytes and microglia) are activated in response to diverse conditions that create exaggerated pain states: subcutaneous inflammation, peripheral nerve trauma, peripheral nerve inflammation, spinal nerve trauma, and spinal nerve inflammation (41, 71, 74, 99, 190, 192, 298, 334, 342). Activation of glia after nerve trauma can occur as a consequence of degeneration of central terminals of the dying sensory neurons. In addition, glia express receptors for a host of substances released by incoming pain-responsive sensory afferents: substance P, excitatory amino acids, CGRP, and ATP (111, 148, 181, 182, 230, 274, 348) (Fig. 3). In addition, they may be activated in response to substances released by pain-responsive neurons in the dorsal horn, such as prostaglandins, NO, and fractalkine (114, 194, 233).

These glia are not only activated but involved in the creation and maintenance of pathological pain states. Drugs that disrupt glial activation or block the actions of glially released proinflammatory cytokines (TNF, IL-1, IL-6) prevent and/or reverse exaggerated pain states produced by subcutaneous inflammation, peripheral nerve trauma, peripheral nerve inflammation, spinal nerve trauma, and spinal nerve inflammation (5, 100, 192, 299, 300, 334, 336a). Indeed, proinflammatory cytokines administered perispinally (intrathecally) can create exaggerated pain responses (56, 250, 302) as well as create hyperexcitability of pain-responsive neurons in spinal cord dorsal horns (250). Activated glia also release (or increase the extracellular concentration of) a number of other substances implicated in the creation and maintenance of pathological pain states, including NO, ROS, prostaglandins, and excitatory amino acids (134, 195, 214). Lastly, glial products can enhance the release of pain transmitters from primary afferent terminals (125). Thus glially derived, as well as neuronally derived, sources of these substances likely contribute to the pathological pain states that ensue.

While it is easy to understand how peripheral nerve injury and inflammation lead to hyperexcitability in the dorsal horn termination area of the involved sensory nerves, the phenomena of extraterritorial pain and mirror-image pain have proven enigmatic. These are important phenomena to understand as both extraterritorial and mirror-image pain changes are reported by neuropathic pain patients, including those with CRPS I and II (178, 323, 324). The recent discovery of the importance of spinal cord glia to neuropathic pain has led to new in-

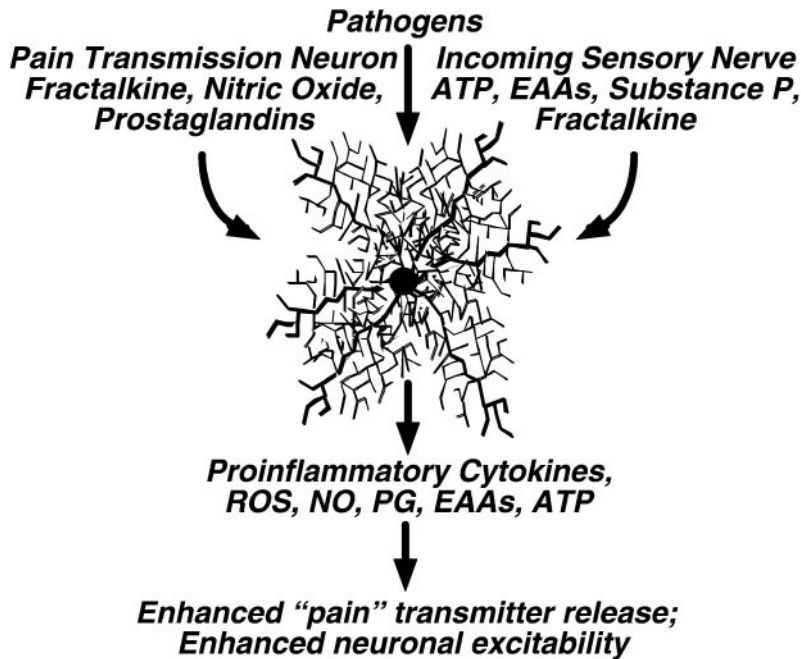


FIG. 3. Schematic of spinal cord glial regulation of pain. Glia (microglia and astrocytes) can be activated by either 1) pathogens (viruses; bacteria) in their role as immunelike cells; 2) substances released by incoming primary afferents that relay "pain" information [ATP, excitatory amino acids (EAAs), substance P], or 3) substances released by spinal cord dorsal horn neurons that relay pain information [pain transmission neurons, fractalkine, nitric oxide (NO), prostaglandins (PG)]. Once activated, glia release a host of substances, including proinflammatory cytokines, reactive oxygen species (ROS), NO, PG, EAAs, and ATP. Of these, the proinflammatory cytokines act in a paracrine fashion to affect cells far beyond their site of release. Glia form positive-feedback circuits whereby substances they release further activate these cells, creating perseverative responses. In turn, glially derived substances increase pain by 1) increasing the release of "pain" transmitters from incoming primary afferents and 2) increasing the excitability of pain transmission neurons. It should be noted that glia are interconnected into large networks via gap junctions and propagated calcium waves as well (not shown). Taken together, glia are perfectly positioned to create perseverative and widespread pain changes in spinal cord.

sights into extraterritorial and mirror-image pain as well. As noted in section 11B2B, the SIN model creates both extraterritorial (saphenous nerve innervation sites) and mirror-image (contralateral) pain (33). Glia appear to be involved, as drugs that disrupt glial activation abolish not only the sciatic territorial pain changes, but extraterritorial and mirror-image pain changes as well (336, 336a). Furthermore, blocking the activity of glially released proinflammatory cytokines blocks extraterritorial and mirror-image pain changes, as well as territorial pain (336, 336a). Notably, even well-established chronic SIN pains are reversed by intrathecal proinflammatory cytokine antagonists, supporting the idea that these glially derived substances are important for maintenance as well as creation of neuropathic pain (335, 336, 336a).

Glia are especially well suited for creating extraterritorial and mirror-image pain for two reasons. First, proinflammatory cytokines classically act in a paracrine, rather than synaptic, fashion; that is, these immune-derived substances are released into the extracellular fluid so as to affect surrounding populations of cells well beyond their site of release (16, 333). Thus they could readily influence neurons in neighboring spinal termination regions, creating extraterritorial pain changes. Second, glia are organized into widespread networks via gap junctions and propagated calcium waves. Thus excitation of glia at one site can lead to the activation of distant glia, consistent with the creation of both extraterritorial and mirror-image effects. Activation in such a manner can lead, in turn, to release of pain-enhancing glial products (101, 124, 232).

### 3. Summary

Neuropathic pain can occur as a consequence of frank nerve trauma, and almost all animal models developed to date have focused on such peripheral nerve injuries. Certainly, the physical damage to nerves, in its own right, alters pain perception and the functioning of pain transmission pathways. However, neuropathic pain also develops in the absence of detectable physical injury to nerves. Here pathological pain follows peripheral immune activation and inflammation. New animal models of such inflammatory neuropathic conditions are providing insights into how immune activation may create such pain changes. Recognition that immune activation can modulate peripheral nerve function has implications for neuropathic pain arising from nerve trauma as well. This is because immune activation will by necessity occur whenever there is damage to peripheral nerves or associated tissues. Hence, immune activation is a natural component of all forms of traumatic and inflammatory neuropathic pain conditions.

From animal models of both traumatic and inflammatory neuropathies, a consistent picture is beginning to emerge for immune involvement in pain. In both traumatic and inflammatory models, the key immune cells involved at the level of the peripheral nerve are likely neutrophils and macrophages recruited into the affected area from the general circulation, plus a host of resident immune cells. These include fibroblasts, endothelial cells, Schwann cells, mast cells, resident macrophages, and resident dendritic cells. The importance of proinflammatory cytokines (TNF, IL-1, IL-6) in the creation and main-

tenance of pathological pain is the most consistent finding across models. NO, ROS, and complement have also been implicated, at least in the few models in which they have been tested to date. The potential involvement of other substances released under traumatic and inflammatory conditions (acids, proteases, digestive enzymes, chemoattractant molecules) has not yet been assessed, other than noting that decreasing pH (increasing acidity) near peripheral nerves enhances pain (185).

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. Here, immunelike astrocytes and microglia are key players in the creation and maintenance of diverse neuropathic pain states. Once again, proinflammatory cytokines have been identified as key mediators, this time released by glia. In addition, these glia enhance neurotransmitter release from primary afferent terminals and release a host of substances classically implicated in pain, including ATP, numerous excitatory amino acids, NO, prostaglandins, and ROS. Indeed, glia may well provide an answer to the mystery of extraterritorial and mirror-image pains reported by patients with traumatic and inflammatory neuropathies. Glia are perfectly suited to create such anatomically impossible pain phenomena as their activation leads both to the release of proinflammatory cytokines that act in a paracrine fashion and to widespread glial activation via gap junctions and propagated calcium waves connecting widespread glial networks (335).

### C. Painful Neuropathies Involving Antibody Attack of Peripheral Nerves

#### 1. Clinical correlation: Guillain-Barre syndrome

A) GENERAL SYMPTOMOLOGY. Guillain-Barre syndrome (GBS) is an acute inflammatory neuropathy that can simultaneously attack peripheral nerves throughout the body. GBS is made life-threatening by the fact that motor and autonomic, as well as sensory, nerves are affected. In addition, neuropathic pain is a common symptom. Pain, including abnormal sensations such as electric shocklike sensations (paraesthesias), sensations having unusually unpleasant qualities (dysesthesias), muscle pain, joint pain, and visceral pain, occurs in 70–90% of adult cases (201, 234). In children, pain is often the major symptom, with 80% of children reporting pain on admission and 100% of children reporting pain during the course of the disease (213).

B) EVIDENCE FOR INVOLVEMENT OF AUTOIMMUNE ANTIBODIES. Which immune factor(s) are critical in GBS remains a matter of some debate. T lymphocytes (see sect. 11A4) have long been posited as playing a major role, based both on GBS nerve autopsy and biopsy studies indicating the

presence of large numbers of T lymphocytes and, more specifically, on the presence of T lymphocytes sensitized to myelin epitopes (98). T-lymphocyte products such as IL-2 are elevated in the serum of GBS patients, supporting activation of this immune cell type (98). However, other autopsy/biopsy cases fail to reveal T-lymphocytic infiltration, especially at the initial stages of nerve lesions (241). The absence of T lymphocytes at the stage of nerve lesion formation led to the idea that T-lymphocyte invasion occurs after the initial peripheral nerve damage has already occurred. In support, some GBS patients have activated T cells that respond to the major peripheral nerve proteins P0 and P2 (119, 243). This is noteworthy since, as discussed in section 11A1, P0 and P2 are enwrapped within the myelin sheath of intact nerves and so are not normally detected by T lymphocytes. Hence, nerve damage must have occurred before T lymphocytes could become sensitized to these peripheral nerve proteins. The role of T lymphocytes may be to further the damage that has already begun, by releasing substances that destroy their targets, attract immune cells to the site, and facilitate further entry of other immune cells and products by disrupting the blood-nerve barrier.

Given the efficacy of exchanging patients' plasma with artificial plasma (i.e., plasmapheresis) for relieving GBS symptoms, some soluble factor in the general circulation does appear important to the etiology of at least some forms of GBS (112). Circulating antibodies are the most likely candidates (see sect. 11A2). Indeed, numerous examples of autoimmune attack of peripheral nerves exist in the clinical literature. Clinically, high titers of serum antibodies directed against peripheral nerve and/or sensory nerve somas occur in a variety of neuropathies, including monoclonal gammopathy, inflammatory polyneuropathies (including, but not limited to, GBS), and paraneoplastic neuropathies (e.g., anti-Hu neuropathy associated with small cell lung carcinoma). The presence of such autoantibodies is a useful diagnostic. These antibodies typically bind to glycolipids, glycoproteins, and glycosaminoglycans, but some (e.g., anti-Hu) are directed against intracellular proteins as well (243). Antibody interactions with nerves can cause a variety of dysfunctions. For example, antibodies against GM1 ganglioside (a component of peripheral nerve myelin sheaths) are associated with motor neuropathies, anti-MAG antibodies are associated with sensory or sensorimotor neuropathies, and antibodies directed against GD3, GD2, GD1a, and GQ1b gangliosides, chondroitin sulfate, and sulfatides are all associated with sensory neuropathies (243, 295). In inflammatory polyneuropathies such as GBS, it is not uncommon to find multiple types of autoimmune antibodies in the general circulation of individual patients, each directed against different nerve targets (243, 295).

"Molecular mimicry" is posited as the underlying cause of antibody-directed attack of peripheral nerves in

several forms of GBS (355). Molecular mimicry has been identified between peripheral motor nerve ganglioside GM1 and the lipopolysaccharide component of *Campylobacter jejuni*, between the sensorimotor nerve ganglioside GD2 and cytomegalovirus, and between the peripheral nerve major protein P0 and several viruses linked to GBS including cytomegalovirus, Epstein-Barr virus, varicella zoster, and HIV-1 (119, 319).

In support of a role of autoimmune antibodies in the etiology of this disorder, GBS sera with high antiperipheral nerve myelin antibody activity have been found to cause 50–80% demyelination in rat DRG cultures, and the degree of demyelination correlates with antiperipheral nerve myelin activity and damage (265). Indeed, even when sera are not screened for high antiperipheral nerve myelin antibody activity, one-third of GBS patient sera samples cause myelin breakdown in rat DRG neuronal cultures and rat Schwann cell cultures (196).

While autoimmune antibodies are most commonly thought of in terms of their direct peripheral nerve damaging effects, they can also disrupt nerve function in other ways. For example, antibodies in the sera of GBS patients alter presynaptic neurotransmitter release and disrupt activation of postsynaptic ion channels in mouse hemidiaphragm preparations (29). Some forms of GBS antibodies can also bind to nodes of Ranvier, altering sodium channel function, and resulting in conduction block independent of anatomic damage to the axons (112). The fact that plasma exchange can sometimes produce spectacular results in GBS patients, with a bed-ridden patient returning to near-normal walking within a few hours, clearly demonstrates that at least some GBS-associated antibodies (or other serum components) can cause peripheral nerve dysfunction rather than demyelination/damage that could not be so rapidly reversed (112).

C) EVIDENCE FOR INVOLVEMENT OF OTHER IMMUNE PRODUCTS. Soluble immune substances other than antibodies likely also contribute to GBS. Substances released from mast cells have only recently been proposed to exert a major role in GBS, predominantly in nerve demyelination (58). Also, in the acute stages of GBS, there is upregulation of immune cell adhesion molecules involved in migration of immune cells out of the blood and into sites of inflammation (96, 217, 313). Circulating levels of TNF and IL-6 are likewise elevated in GBS patients (94, 275). However, their potential involvement in pain or other symptomology of the disease is not yet clear. What has been reported is that plasma TNF levels correlate with severity of clinical and nerve conduction deficits (66, 273). Interest in the potential role of proinflammatory cytokines in GBS originally arose due to their known neurotoxic and neuroexcitatory effects (249, 259). However, given that these same proinflammatory cytokines are upregulated locally in Schwann cells, fibroblasts and macrophages of GBS nerve biopsies (208), it may be that proinflammatory cytokines in nerves

rather than in the general circulation are linked to GBS symptomology.

In contrast, involvement of the complement cascade in GBS is indicated by multiple findings. 1) The antiperipheral nerve myelin antibodies of GBS patients are complement-fixing; that is, they activate the complement cascade and bind products formed by complement activation (265). As noted in section II A 3, this facilitates macrophage attack of the antibody-bound myelin (241), creates chemoattractants that recruit and activate immune cells, and disrupts the blood-nerve barrier as is known to occur in GBS patients (86). 2) MACs, the destructive end product of complement activation, are found in 100% of GBS sera while being nonexistent in controls (152). This sign of complement activation is linked to the expression of antiperipheral nerve antibody in these patients, and MACs are bound to peripheral nerves of GBS patient biopsies (152). 3) Blockade of MAC formation prevents GBS sera from inducing myelin disruption in rat DRG cultures (265). 4) Autopsy of GBS patients who died 3–9 days after disease onset reveals products of complement activation (complement activation marker C3d and MACs) bound to the outer rim of many peripheral nerve fibers and Schwann cell membranes (87). In these patients, disruption of myelin occurs after complement activation but before the invasion of the region by macrophages (87). This indicates that the initial nerve damage is complement mediated and associated with MAC, rather than macrophage, attack (87). This damage is then amplified by macrophages which, in addition to engulfing myelin, may further the damage by releasing injurious molecules such as ROS, NO, proteases, acids, eicosanoids, and proinflammatory cytokines (97).

Based on the evidence supporting a role for both autoimmune antibodies and complement in GBS, it has been proposed that GBS-associated nerve damage is initiated by autoimmune antibody binding to the outer surface of Schwann cells (112). The antibody, in turn, activates complement. Formation of MACs creates transmembrane pores. This leads to calcium entry, activating calcium-sensitive enzymes, such as phospholipase A<sub>2</sub>, calpain, and proteases that degrade myelin proteins. Macrophages are recruited by the complement-bound antibody and complement-driven myelin damage, leading to further myelin damage and phagocytosis (112). What is clearly missing from this schema is an answer to what causes the initial breakdown of the blood-nerve barrier to allow the entry of autoimmune antibody. Given that nerve fascicles vary in the “tightness” of their blood-nerve barriers (see sect. II A 1), it may simply be that the pathological process begins in fascicles where the blood-nerve barrier is weak, and the ensuing immune cascade then leads to disruption of the blood-nerve barrier more broadly.

## 2. *Animal models*

A) EAN: A MODEL OF GBS PATHOLOGY. Interest in developing animal models of antibody-driven attack of peripheral neurons arose upon recognition of numerous clinical syndromes associated with circulating levels of neurotoxic antibodies. EAN has provided an animal model for the motoric and axonal damage associated with GBS. However, it is quite striking from a review of this literature that altered pain processing has not been a focus of study. One reason may be that EAN can be so severe as to create complete nerve conduction blocks (107, 113, 290), making examination of sensory changes moot. Despite the lack of information about potential pain changes in this model, EAN is still instructive from an immunological point of view, and so is included here.

EAN develops as a consequence of immunizing rodents with either P0 or P2, the two major peripheral nerve proteins that, under normal circumstances, are sequestered within the nerve and hence functionally invisible to immune surveillance (119). Development of anti-P0 and anti-P2 antibodies leads to destructive attack of peripheral nerve myelin, conduction block, and paralysis similar to GBS. Indeed, T-cell responses to myelin proteins alone are sufficient to produce EAN, since T-cell infiltration and nerve demyelination begin rapidly after intravenous transfer of activated anti-P0 T lymphocytes to immunologically naive animals (119). However, the combination of auto-antibodies plus adoptive transfer of activated peripheral nerve-specific T cells elicits a more severe disease with more rampant demyelination than that produced by adoptive transfer of the T cells alone (119). Thus, in keeping with the classic but disputed view that GBS is a T cell-mediated neuropathy, activated anti-P0/P2 T cells have been proposed to cross the blood-nerve barrier in the normal course of immunologic surveillance of the nerve. These cells then become further activated upon recognition of their epitope, releasing substances that open the blood-nerve barrier and allow egress of antibodies. Antibodies that recognize cell surface molecules then bind, and complement activation ensues. Also similar to GBS, vascular permeability increases in EAN as the disease progresses, allowing additional damage by circulating factors that normally have no to limited access to peripheral nerve (119).

One point that has been clarified by the rodent EAN model is the importance of the blood-nerve barrier in disease progression. The endoneurium is critical for the protection of peripheral nerves from substances in the systemic circulation. The strength of this blood-nerve barrier is species dependent. EAN damage is greater in guinea pigs than rabbits, for example, due to species differences in blood-nerve barrier (88). The integrity of the blood-nerve barrier has led to the direct intraneurial

injection of purified antibody and GBS patient sera to test their effects on laboratory animal nerves. This is required in immunologically naive animals since they do not possess the nerve-specific activated T cells present in EAN immunized subjects. Activated T cells, but not nonactivated T cells, are allowed to cross the blood-nerve barrier during normal immune surveillance and release blood-nerve disrupting factors upon detection of their epitopes (e.g., P0, P2) (112).

B) ANTI-GD2-INDUCED SENSORY NEUROPATHY: FROM CLINICAL SIDE EFFECT TO ANIMAL MODEL OF NEUROPATHIC PAIN. An animal model has recently been developed in which antiperipheral nerve antibody causes pain characteristic of neuropathy. This rat model was created based on two clinical observations. First, gangliosides had been administered to humans in an attempt to aid their recuperation from various neurological disorders. However, some of these patients instead developed antibodies against gangliosides that attacked their peripheral nerves, causing GBS symptomatology, including pathological pain (355). 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 (325). In response, patients developed a demyelinating neuropathy affecting sensory and motor nerves throughout the body (i.e., sensorimotor demyelinating polyneuropathy). This was associated with aching/burning pain (207), severe shooting pain (207), intense mechanical allodynia (330), moderate-to-severe abdominal pain (325), joint pain (92), moderate-to-severe extremity pain, headache, sensations having unusually unpleasant qualities (dysesthesia), and muscle pain (myalgia) (207). Studies that followed revealed that anti-GD2 reacted with peripheral nerve myelin sheaths and DRG neuronal cell bodies (325, 356).

Administering anti-GD2 intravenously to rats rapidly creates mechanical allodynia, and whole body touch evoked agitation (282, 350). Single sensory nerve fiber recordings reveal that anti-GD2 induces high-frequency spontaneous activity and hyperexcitability of pain-responsive peripheral nerves (350). Although similar effects on DRG somas would be expected, this has not yet been tested. Because this antibody activates the complement cascade (7), complement has a potential role as well.

## 3. *Summary*

Neuropathic pain can occur as a consequence of antibody attack on peripheral nerves. Antibodies exert two distinct types of effects on nerves. First is alteration of nerve function via antibody binding-induced changes in ion channel function. This effect of antibodies has not yet been assessed for its potential involvement in pain modulation. Second is immune-driven nerve damage that occurs secondary to complement activation and macro-

phage attack of the antibody-bound sites. Complement activation disrupts the blood-nerve barrier, creates MACs that punch holes in nerves, and facilitates macrophage destruction of antibody-bound sites. Macrophages further amplify the damage begun by complement activation by releasing injurious molecules such as ROS, NO, proteases, acids, eicosanoids, and proinflammatory cytokines. Which aspect(s) of this antibody-induced cascade of events is directly involved in the creation of neuropathic pain is unknown, but several are excellent candidates.

To date, EAN has been the most widely studied model of pathology associated with GBS. Unfortunately, the model is so severe as to create complete conduction blocks 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 GBS, one should not overlook the fact that 80–100% of GBS patients suffer from neuropathic pain. Models are needed to understand this aspect of GBS as well. Toward that end, a new animal model appears appropriate for this goal. Here, antibody against the peripheral nerve ganglioside GD2 is administered intravenously, producing allodynia and electrophysiological signs of peripheral nerve hyperexcitability. Hopefully, future studies of this model will clarify which immunologic changes are responsible for creating the neuropathic pains observed.

#### **D. Painful Neuropathies Involving Immune Attack of Peripheral Nerve Blood Vessels**

##### *1. Clinical correlation: vasculitic neuropathy*

A) GENERAL SYMPTOMOLOGY. Vasculitic neuropathies (VN) result from vasculitis, an immune attack of peripheral nerve blood vessels. Vasculitis refers to a broad group of syndromes characterized by inflammation and necrosis of blood vessel walls, causing narrowing and occlusion of the vessel lumen (120). Although some forms of vasculitis selectively target the blood vessels of nerves (51, 62), the more typical syndrome includes widespread attack of vessels throughout the body (120). Pain is a frequent symptom in these neuropathies (103).

B) EVIDENCE FOR IMMUNE INVOLVEMENT. Immune involvement in vasculitis is complex. Some forms of vasculitis are thought to result from viral infections, including HIV-1 and hepatitis C (85). While cytotoxic T lymphocytes have been proposed as the mediator of some forms of vasculitis (102), several studies fail to find evidence of T-lymphocyte involvement (24, 110). Other forms of vasculitis may derive from the expression of autoantibodies. Antineutrophil cytoplasmic antibodies (ANCA) are antibodies directed against any of several neutrophil constituents, monocytes, and endothelial cells (84). Molecular mimicry of these antibodies to viruses or bacteria has

never been shown; hence, how these autoantibodies form is unknown (84). However, their presence is diagnostic of several forms of vasculitis including polyarteritis nodosa, microscopic polyarteritis, necrotizing glomerulonephritis, Churg-Straus syndrome, systemic lupus erythematosus, and cryoglobulinemic vasculitis (120, 129, 269). These autoantibodies are suspected to be important in the pathogenesis of vasculitis (84). ANCA, for example, have been proposed to induce vasculitis by causing proinflammatory cytokine release from neutrophils. This in turn causes the upregulation of adhesion factors [intercellular adhesion molecules (ICAMs)]. ICAMs cause attachment of immune cells to vessel lumen walls. They then damage these vessel walls via release of neutrophil-derived ROS and degranulation products, including degradative enzymes (269). Furthermore, attachment of IgG or IgM ANCA to vessel walls causes the activation of the complement cascade. This recruits more immune cells to the area and directly damages the endothelium (20).

C) EVIDENCE FOR ISCHEMIA-INDUCED NERVE AND IMMUNE CHANGES. Whatever the underlying etiology, induction of vasculitis in the peripheral nerve blood vessels results in an environment characterized by widespread occlusion of blood vessels due to clot formation within the lumen of these vessels (i.e., disseminated intravascular coagulation), increased attachment of immune cells to vessel walls, and disruption of the blood-nerve barrier leading to edema and endoneurial immune cell migration (263, 311). VN typically occurs at multiple sites throughout the body, scattered randomly across nerves (i.e., an asymmetrical polyneuropathy) (102, 337). It is caused primarily as a result of the ischemia that follows blood vessel injury, coagulation, and necrosis (110). VN is characterized by acute pain as well as edema in the affected portion of the limb. There is a partial loss of both myelinated and unmyelinated nerve fibers (102). Macrophage infiltrates are found in nerve biopsies of VN patients (65). These cells are likely there as a result of ischemic injury and death of nerves and resident cells of the nerve. As demyelination and cell death ensue, immune complexes and complement products may be deposited in VN nerves (51, 105), leading to more efficient clearing of the debris by activated macrophages. Whether there is an increase in T lymphocytes relative to normal controls is controversial (24, 321). If present, T cells may have been attracted to the region primarily as the result of the underlying vasculitis and appear to be infiltrating into the nerve due to ischemic-induced blood-nerve barrier breakdown. If antigen-specific T cells were being actively recruited into the nerve by an immune stimulus and/or proliferating there in response to a local immune stimulus (i.e., clonal expansion), one would expect that many of the T cells present in the biopsy would express receptors for the same epitopes. Since they do not (25), the more likely explana-

tion of increased T lymphocytes in the region rests simply on blood-nerve barrier breakdown.

## 2. *Animal models*

An animal model relevant to necrotizing VN pain has recently been developed. This is a photochemical ischemia model in which the sciatic nerve is unilaterally irradiated with an argon laser after intravenous administration of a photosensitizing dye, erythrosin B (93). Thus, although there is not an immune attack on the nerve blood vessels per se, vessel coagulation with resultant ischemia-induced nerve damage does result. Earlier anatomic work had shown that this type of ischemic lesion produced axonal neurofilament disintegration within 4 h, followed by Wallerian degeneration-induced macrophage infiltration and phagocytosis of the necrotic debris (340). Although no studies have yet been done to examine potential immune mediators at either peripheral or central sites, what is known is that damage occurs to both myelinated and unmyelinated fibers and this ischemic nerve lesion indeed produces both mechanical allodynia and thermal hyperalgesia (93, 156). In this model, these pain changes can be observed in the mirror-image (contralateral) limb as well (93). Furthermore, enhanced pain is correlated with the partial demyelination and axon degeneration (93, 156). Thus, although not yet studied, involvement of immune-derived substances in the neuropathic pain changes would be predicted at both peripheral and spinal sites.

## 3. *Summary*

Neuropathic pain can arise as a result of immune attack of peripheral blood vessels. While blood vessels of nerves can occasionally be specifically targeted, it is far more common that vessels throughout the body are attacked. Diverse immune cells have been implicated in VN, and diverse immune cell products are released as a result. Which of these underlies the associated neuropathic pain has not yet been determined. Clearly, though, there are multiple candidate immune mediators with known pain-enhancing effects.

In terms of animal models, no model yet exists for immune attack on blood vessels. However, if the assumption is true that localized ischemic damage of the nerve and consequent immune activation may be proximate causes of pain, one recently developed animal model may be relevant. This model causes ischemic changes in peripheral nerves with associated immune activation in the affected region. It is hoped that future studies of this model will reveal whether immune-derived substances are involved in the resultant pain state.

## III. DORSAL ROOT GANGLIA AND DORSAL ROOTS AS TARGETS OF IMMUNE ACTIVATION

DRG and dorsal roots can be the origin of pain for herniated disks and related back pathologies. This section is divided into two major subsections. Section IIIA provides an overview of DRG and dorsal root anatomy and immunology. This will provide the background for the clinically relevant discussion that follows. Section IIIB focuses on pain arising from herniated discs. Within this, an examination of the clinical findings will first be discussed followed by a summary of data from relevant animal models. The argument to be developed is that immune-derived substances created and released as a result of disc herniation are sufficient to create increases in peripheral nerve hyperexcitability and/or damage so as to be considered a significant contributor to the pathological pain observed.

### A. Overview of DRG and Dorsal Root Anatomy and Immunology

DRG consist of cell bodies of sensory neurons, glially derived satellite cells, dendritic cells, macrophages, and endothelial cells (226). The dorsal roots contain the proximal axons of DRG neurons, and the anatomy of these axon bundles is consistent with that described above for peripheral nerves. Each neuronal cell body in the DRG is encapsulated by a layer of satellite cells, with a basement lamina separating neighboring glially encapsulated neuronal soma (226). Satellite cells are glial cells and as such share many of their regulatory and immunelike functions. For example, satellite cells regulate uptake of extracellular glutamate and aspartate as well as the availability of the glutamate precursor glutamine to DRG neurons (61, 132), are a reservoir of the nitric oxide precursor L-arginine and thought to regulate L-arginine availability to DRG neurons (4), upregulate their expression of the astrocyte-associated activation marker glial fibrillary acidic protein (GFAP) in response to peripheral nerve damage (344), and communicate, in part, via gap junctions (260). In addition, as noted previously, peripheral nerve injury stimulates activated satellite cells (and presumably macrophages) to release proinflammatory cytokines and a variety of growth factors in the DRG (206, 246–248, 258, 277, 308, 358). Thus these satellite cells are well positioned to regulate neuronal activity in the DRG.

In normal DRG, the great majority of resident macrophages contact the neuron/satellite cell complexes; the remaining few are mostly perivascular (226). An impermeable capsule surrounds the entire DRG. This capsule is composed of connective tissue and a perineurium similar to that of peripheral nerves (226). This capsule keeps the

DRG microenvironment separate from surrounding extracellular fluid under normal circumstances (226).

DRG blood vessels do not share the properties of the vessels in the central nervous system; that is, they are outside of the blood-brain barrier and so allow large-molecular-weight molecules to pass easily (226). In both human and rat DRG, almost every neuron soma is bordered by a rich network of capillaries (226). Indeed, the DRG has a far denser blood supply than does either peripheral nerve or dorsal root (226).

As the dorsal roots begin to course toward the spinal cord via the intervertebral space, they pierce the meninges and enter the cerebrospinal fluid. Thus the dorsal roots, but not DRG somas, are in direct contact with cerebrospinal fluid constituents. The dorsal roots also become governed by the blood-brain barrier, which severely limits what blood-borne substances can contact dorsal root fibers (226).

## B. Painful Conditions Involving Immune Effects on DRG and Dorsal Roots

### 1. Clinical correlation: herniated discs

A) GENERAL SYMPTOMOLOGY. Back pain remains an elusive clinical problem in that ~80% of all back pain episodes are of unknown etiology (55, 203). Dislocation of intervertebral disc tissue (nucleus pulposus: the distensible material that serves as “shock absorbers” of the spine) by protrusion or extrusion (i.e., herniated disc) is a common source of severe pain, occurring in 1–3% of the general population (32). This condition results in a surgery rate of 450–900 per 100,000 in the United States (32). Herniation of the nucleus pulposus causes it to contact and compress the DRG and spinal root that enters at that vertebral level (32). Acute mechanical compression is sufficient to produce spontaneous activity in the sensory afferents of laboratory animals (116), supporting the classic assumption that mechanical compression is the cause of pain and other neurological symptoms. This mechanical compression has been posited to account for the ischemia, edema, and demyelination that subsequently occur in DRG and dorsal root (257). In addition, pain may arise from nerve endings in the outer annulus fibrosus, longitudinal ligaments, facet capsules, and paraspinal muscles (135).

B) EVIDENCE FOR IMMUNE INVOLVEMENT. Extending the classical views of disc pain, there is mounting evidence that immune factors may be involved as well. Herniated discs are reacted to as “foreign” and so produce an autoimmune inflammatory response (59). Herniated discs spontaneously produce a variety of pain-producing substances including NO, proinflammatory cytokines (TNF, IL-1, IL-6, IL-8), as well as cyclooxygenase-2 (COX-2), phospholipase A<sub>2</sub>, thromboxanes, leukotrienes, and PGE<sub>2</sub> (1, 70, 136, 140, 197, 303). These are produced not only by infil-

trating macrophages (59), but also by histiocytes, fibroblasts, endothelial cells, and chondrocytes of the disc itself (135, 303). In addition, herniated discs produce elevated levels of matrix metalloproteinases (MMPs), which are important in the release of bioactive TNF into the extracellular fluid (136). These MMPs, released by both TNF and IL-1, are also implicated in the breakdown of cartilage and inhibiting the biosynthesis of proteoglycans (135). Furthermore, herniated discs become hyperresponsive to inflammatory stimuli. For example, they show exaggerated release of NO, IL-6, and PGE<sub>2</sub> upon stimulation with IL-1 (136). Such findings have led to the suggestion that such disc-associated proinflammatory substances may be a major factor in the creation of back pain (1, 30).

If proinflammatory cytokines indeed contribute to pain and neuropathological changes in the sensory neurons, this is important as this may provide a much-needed alternative approach to treatment. Surgery for herniated discs is not without cost. The surgical failure rate ranges from 5 to 50% (289). In addition to failure to relieve the pain, postoperative complications include death (0.3%), surgical damage to nerve roots (0.5–3%), motor weakness (>5%), disc inflammation experienced as violent spasmodic back pain induced by body movement, (1–2%), and inflammation of spinal meninges, which is experienced as constant burning or cramping back pain radiating to one or both legs (289, 349). As a result, except in emergencies (e.g., rupture of the disc into the spinal canal), surgical treatment of disc herniation is advised only if nonsurgical treatments fail after being attempted for several months (289). Thus patients are often in severe pain for prolonged periods.

### 2. Animal models

There is also a growing laboratory animal literature that DRG and nerve roots are vulnerable to immune-derived substances released from herniated discs. The disc nucleus pulposus is implicated as a mediator of neuropathological changes as its application to L<sub>5</sub> nerve root causes edema and altered conduction velocity (225, 352) as well as spontaneous electrical activity in sensory afferents (32). Exposure of nerve roots to autologous nucleus pulposus (that is, collected from genetically identical organisms) leads to altered gene expression in DRG, with increases in mRNA for IL-1, IL-6, iNOS, and phospholipase A<sub>2</sub> (139). Furthermore, autologous nucleus pulposus applied to nerve roots produces mechanical allodynia (142). Combining nucleus pulposus exposure with either compression of the nerve root (142) or disc annulus fibrosis application (144) results in both mechanical allodynia and thermal hyperalgesia. Indeed, mechanical allodynia correlates with DRG mRNA changes noted above (139).

Given that TNF is a major inflammatory mediator expressed by human herniated nucleus pulposus (224), it was natural to examine the effect of this proinflammatory cytokine in animal models. Disrupting TNF function prevents edema and altered conduction velocity produced by autologous nucleus pulposus application (225, 352). Topical application of TNF to DRG and proximal nerve roots elicits abnormal spontaneous activity (170, 357), presumably via binding to TNF receptors known to be expressed by DRG neurons (236). Indeed, applying TNF *in vivo* to rat nerve roots produces the same neuropathological changes as does autologous nucleus pulposus (121); that is, TNF produces Schwann cell activation, edema, and macrophage recruitment as well as triggering further production of endoneurial TNF (121). TNF applied to spinal nerve produces mechanical allodynia; combining TNF with compression of the nerve root results in both mechanical allodynia and thermal hyperalgesia (115, 121). Blocking endogenous TNF activity delays the development of this mechanical allodynia (115).

Little is known regarding the impact of immune factors other than TNF on pain from herniated discs. To date, all that is known is that inhibitors of thromboxane, leukotrine, and phospholipase A<sub>2</sub> reduce mechanical allodynia produced by applying nucleus pulposus to spinal roots (140, 141, 143). Although not yet tested for its potential pain-reducing effects, an iNOS inhibitor has recently been reported to decrease autologous nucleus pulposus-induced spinal nerve edema and conduction velocity changes (27), so effects on pain would not be unexpected. The only study to date on IL-1 relevant to herniated discs relates to the finding (above) that exposure of spinal roots to autologous nucleus pulposus increases IL-1 mRNA in DRG, likely in satellite glial cells. This is intriguing since *in vitro* exposure of DRG to IL-1 causes release of the pain transmitter substance P (125), suggesting that IL-1 induction in DRG may contribute to pain. No studies have yet examined the potential effects of IL-6 or other immune-derived substances. However, it is notable that these proinflammatory cytokines have been implicated in exaggerated pain states created by innocent bystander effects of immune activation near healthy peripheral nerves (see sect. III B2B). Hence, it would not be surprising if these factors exert the same pain-inducing effects on nerve roots that they do in peripheral nerve.

### 3. Summary

Pathological pain can arise as a result of protrusion of the nucleus pulposus so as to contact the DRG and dorsal root. While pressure *per se* has classically been considered as a major basis of pain, there is growing evidence that immune-derived substances may be involved as well. Diverse immune cells and equally diverse

immune cell products are potential mediators. Of these, proinflammatory cytokines have received by far the most attention. Data to date suggest a strong case in support of proinflammatory cytokine involvement in the pain of herniated discs. How the proinflammatory cytokines do this may well be related to two factors. First is expression of proinflammatory cytokine receptors within DRGs. Within DRGs, although given the anatomy of the DRG capsule, it is not clear that proinflammatory cytokines induced extrinsic to the DRG could penetrate. Second, is axonal interactions of proinflammatory cytokines with the sensory nerve fibers as innocent bystanders. Here, proinflammatory cytokines could form novel cation channels and/or increase the conductivity of endogenously expressed cation channels as described previously.

## IV. CONCLUSIONS AND IMPLICATIONS

Although the present review has focused on immune modulation of pain, it should be clear that there is nothing *a priori* unique about the nerves or neurons involved. All peripheral nerves, regardless of modality or function, would be expected to be affected by immune activation in the ways described for pain. Glial modulation of central nervous system function likewise is not the purview of pain. Indeed, there is growing recognition of the powerful influences glia wield over neurons at all levels of the neuraxis (17, 104, 162).

This review has approached immune interactions with pain systems at multiple levels. It described how immune cells are a natural and inextricable part of 1) skin, where nerves form their terminal receptive fields and express receptors for immune products; 2) peripheral nerves, where multiple types of immune cells are in constant intimate contact with the nerve fibers and can mid-axonally alter nerve anatomy and physiology; 3) dorsal root ganglia, where they ensheath and modulate every neuronal cell body; and 4) spinal cord, where they form dynamic networks well suited for maintaining and spreading excitation. This review has also attempted to define the varied ways that immune activation can both damage peripheral nerves and enhance their excitability. Finally, it illustrated how such immune-derived changes might participate in the etiology and symptomatology of various pathological pain states.

There are several major points covered during the course of this review that bear reemphasis. First and foremost is that immune activation is important for neuropathic pain. Classical immune cells (e.g., macrophages, T lymphocytes, mast cells, dendritic cells, etc.), immunocompetent cells that can release factors classically thought of as immune-cell products (e.g., endothelial cells, fibroblasts, keratinocytes, Schwann cells, etc.), and immunelike spinal cord glial cells (astrocytes and micro-

glia) must all be considered when one examines the potential role of immune activation in neuropathic pain. The recent recognition of peripheral and spinal immune involvement in neuropathic pain potentially has profound implications for the understanding of how such pain states occur. Equally profound are the implications that immune involvement pose for the future development of drug therapies aimed at controlling neuropathic pain. Given that all available pain therapies exclusively target neurons, the recognition of peripheral and central immune cell involvement in neuropathic pain of diverse etiologies offers hope for whole new approaches for pain control.

The second major point is the pervasiveness of proinflammatory cytokine involvement in diverse neuropathic pain conditions. There are multiple sites and situations where these immune-derived proteins (TNF, IL-1, IL-6) are correlated with and likely causal to neuropathic pain conditions. Some "sites and situations" currently enjoy substantial experimental support, such as the pain-enhancing effects of proinflammatory cytokines at sites of peripheral nerve trauma and herniated discs. Some are unexpected and as yet unstudied with regard to neuropathic pain. An example is the immune cell phenotypic shift from  $\beta_2$ - to  $\alpha_1$ -adrenergic receptors that can occur in some chronic inflammatory conditions. If such a change were to occur in association with neuropathic pain conditions, it may provide a novel source for proinflammatory cytokine production via  $\alpha_1$ -adrenergic stimulation. If such a shift occurs in "sympathetically maintained" pain states such as CRPS, this predicts that proinflammatory cytokines may contribute to such pain. Finally, some sites and situations are only recently recognized but striking in their implications. An example is pain enhancement by immunelike glial cells in the spinal cord. Indeed, spinal cord may well not be alone in the importance of glia in pain modulation. There certainly is no a priori reason to assume a preeminence for spinal cord glia over glia in higher centers. While beyond the scope of the present review, there is growing evidence that supraspinal glia and glially derived proinflammatory cytokines can have major influences on pain modulation as well (218, 220, 221). Taken together, the pervasive and potentially key involvement of proinflammatory cytokines from periphery to higher centers suggests that targeting these immune products either locally within an affected body region or within the spinal cord may be a desirable strategy for drug development (for discussion of drug therapy approaches, see Ref. 336).

The last major point is that investigation of immune involvement in neuropathic pain is in its infancy. Many more immune cells and immune-derived substances are implicated in the etiology of pathological pain syndromes than have been studied for their potential involvement in the ensuing long-lasting neuropathic pains. Furthermore,

precious little is understood about the immunelike glial cells within the pain modulatory laminae of the spinal cord dorsal horn. This is because it has only very recently been recognized that glia residing in various sites in the central nervous system are not all the same; rather, their receptor expression and function reflect their microenvironment and so must be understood in this context. Given that studies of glial function have almost exclusively used glia isolated from supraspinal sites, much remains to be learned about the dynamics of dorsal spinal cord glial function as regards pain modulation and neural function more generally.

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