

Mechanisms of Nerve Injury in Leprosy

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Introduction

LOCALIZATION OF *M. LEPRAE*

Leprosy neuropathy is a slowly progressive sensorimotor polyneuropathy that can be either asymmetrical or symmetrical (1). *Mycobacterium leprae* (*M. leprae*) is the only bacterium that infects the peripheral nervous system (PNS), specifically targeting the Schwann cells (SC) of myelinated and unmyelinated axons. *M. leprae* within a nerve fiber (see Chapter 2.4) is pathognomonic of leprosy (Figure 1A). Large clusters of *M. leprae* reside in SC and macrophages, which provide a safe haven for multiplication. Bacilli are also present in smaller numbers within perineurial cells, smooth muscles, hepatocytes, and endothelial cells of blood vessels in florid lepromatous leprosy, where they do not cause toxic injury to the residing cells (2, 3, 4). *M. leprae* prefer the cooler areas of the body where they multiply, and inflammatory cells associated with the organism are observed predominantly in segments of nerve trunks that are in close proximity to the skin (Fig-

ure 1B). Consequently, the pattern of sensory loss is entirely unique in leprosy, presenting as skin lesions with varying degrees of sensory loss and a 'stocking-glove' type of anesthesia in the lepromatous type of leprosy (5, 6, 7, 8).

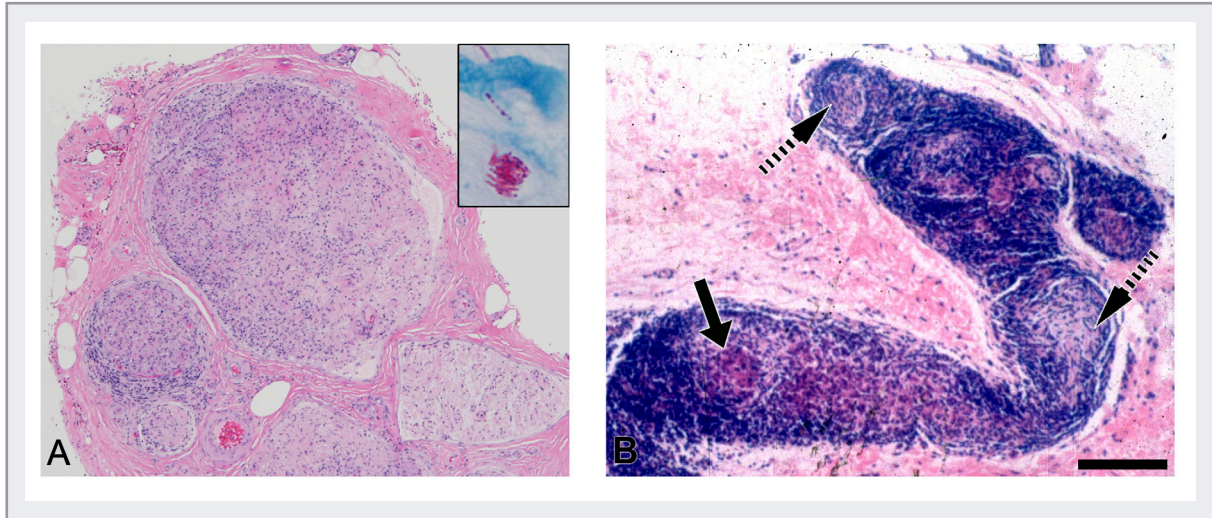


FIG 1 Microphotographs of peripheral nerve in lepromatous leprosy (A) and cutaneous nerve in skin sections of tuberculoid leprosy (B).

- A.** Hematoxylin and eosin section of peripheral nerve fascicles. Clumps of *M. leprae* were present within this nerve (inset). (HE, scale =50 microns; inset, Fite-Faraco stain, 1000x).
- B.** Skin section exhibiting a large granulomatous inflammatory cell collection consisting of small epithelioid cell clusters (arrow) and dense aggregates of lymphocytes infiltrating small dermal nerve bundles (broken arrows) (Hematoxylin & Eosin stain). Scale =100 μ m.

***M. LEPRAE* ENTRY INTO NERVES**

Though the route of entry of *M. leprae* into the peripheral nerves is still undetermined, both a direct entry to terminal nerves and a vascular route leading to intraneural infection of *M. leprae* have been proposed. Autopsy studies have shown that *M. leprae* bind to the exposed Remak Schwann cells in the dermis, probably through skin abrasions, and ascend directly from cutaneous nerves to the nerve trunks that carry both mixed sensory and motor nerve fibers trunks (Figure 2) (9, 10, 11). *M. leprae* infection of endothelial cells is well documented in human leprosy (12). In experimentally infected armadillos, studies have demonstrated that *M. leprae* collect in and affect the blood vessels of the epineurial lymphatics and infect the blood vessels of the epineurium. The endothelial cells serve as potential niches for the *M. leprae* to enter the intraneural compartment (13, 14) (FIGURE 3). In this view, the perineural inflammation characteristic of leprosy represents the 'footprints' of the perineural route of infection.

Several pathological mechanisms have been proposed for nerve damage in leprosy: the interference of *M. leprae* cell wall proteins with host cell (macrophage) metabolism, an immune-mediated inflammation triggered by T-cell/SC interactions, and a 'bystander' type of nerve injury due to the large influx of cells and edema during the course of immune and inflammatory responses to *M. leprae* (15, 16). The resultant immunopathological changes manifest clinically in the form of skin lesions, ocular changes, and peripheral nerve enlargement.

Role of Schwann Cells in Leprosy Neuritis

BINDING OF *M. LEPRAE* TO SCHWANN CELLS

Because of a lack of key functional genes, *M. leprae* relies predominantly on the host cell for survival, multiplication, and dissemination (17). The SC serves as an important habitat for *M. leprae* and as a safe niche in the center of the cellular events that orchestrate the processes of nerve injury. Though *M. leprae* cannot be cultivated in an artificial media, the availability of SC and SC-axon co-culture models have provided an opportunity for understanding the interaction

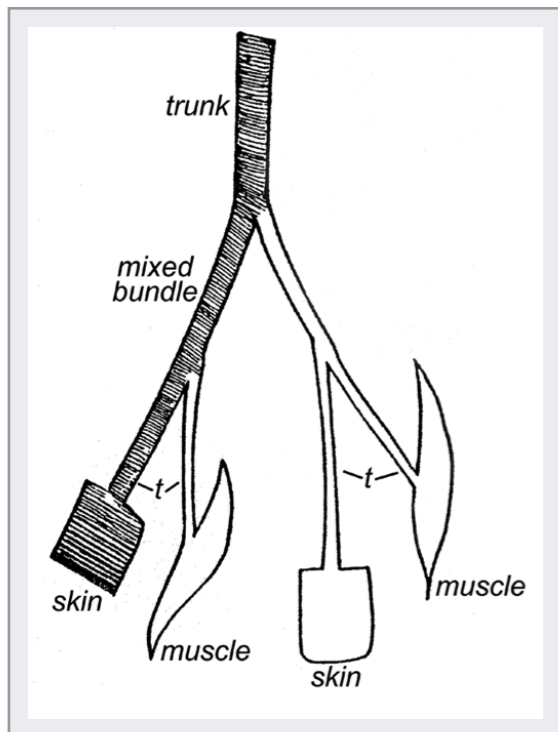


FIG 2 The initial model of ascending neuritis in leprosy. With this diagram in 1897, Dehio [9] illustrated his observations from autopsy studies of ascending neuritis involving cutaneous and muscular branches of peripheral nerves in leprosy. Inflammation (hatched areas) was observed to arise from the distal ends of cutaneous nerves in an infected skin lesion and ascend proximally along terminal branches (t) to involve larger mixed nerve bundles. As larger nerve trunks are involved, the inflammation of infected fibers also interferes with the conduction of both sensory and motor impulses in adjacent, non-infected fibers, innervating non-inflamed skin and muscle. This interference results in cutaneous anesthesia in lesions as well as in non-infected skin and in weakness and atrophy of the muscle, even if its nerve is not infected (modified from [9]).

of the bacilli with SC. Several molecules have been identified that are responsible for *M. leprae*'s adherence to and ingestion by SC (18, 19). Specifically, various receptor-mediated mechanisms, Fc receptors, complement receptors (20), the fibronectin binding protein (21), and Toll-like receptor 2 on the surface of SC (22, 23) may play a role in the invasion of human SC by *M. leprae*.

The organism binds to the G domain of the laminin alpha-2 chain, which is expressed on the surface of the SC-axon unit, through a receptor on *M. leprae* that is a 21 kDa histone-like protein (24, 25). This protein, LBP21, coded by the *ML1683* gene, is a major exposed surface antigen on *M. leprae* and serves as an adhesin for its binding with peripheral nerve laminin-2 to initiate the SC invasion. Similarly, the terminal trisaccharide of phenolic glycolipid 1 (PGL-1), which is a surface-exposed *M. leprae*-specific antigen, has been shown to bind to laminin-2, indicating that PGL-1 also is involved in the entry of *M. leprae* into SC (26, 27).

SCHWANN CELL INTERACTION AND DISSEMINATION OF *M. LEPRAE*

There are several theories on the role of infected SC in the dissemination of *M. leprae*. SC and *M. leprae* interact to establish a favorable environment for the proliferation of *M. leprae*. The viability of nude-mouse derived *M. leprae* in rat SC *in vitro* is comparable to that of the bacilli surviving within macrophages. The survival of *M. leprae* within SC *in vitro* is also conditioned by optimal temperature, as the viability of *M. leprae* is greater at 33°C than at 37°C (28). This claim is consistent with histological observations indicating that *M. leprae* appears to remain dormant and grow within SC in human nerves at superficial, cooler sites.

M. leprae also appears to alter the SC expression of a small number of genes; so far glial fibrillary acidic protein, transforming growth factor β 1, NCAM, ICAM, N-cadherin, and L1 have been studied. The functional significance of these alterations with respect to the clinical presentation of nerve injury remains to be determined (28).

SC infected *in vitro* are able to present *M. leprae* antigens (29, 30, 31). The interior milieu of the Schwann cell is also altered by this infection, producing metabolic and functional changes that trigger the immune system in recruiting cytotoxic cells T lymphocytes (32, 33) and macrophages (34, 35).

Experimental studies *in-vitro* have shown that *M. leprae* may reprogram adult SC by altering host gene expression, with the bacterially reprogrammed cells resembling progenitor/stem-like cells (pSLC) with mesenchymal traits. The pSLC acquire migratory and immuno-modulatory characteristics; release chemokines, cytokines, and growth/remodeling factors; and disseminate the bacterial infection without being detected by immune cells. These reprogrammed cells possess the ability to attract macrophages, suggesting a potential role of the innate immune response in the initiation of neuropathogenesis during early *M. leprae* infection (36, 37).

In long-standing lepromatous nerves, the SC exhibit a foamy appearance due to lipid droplets recruited to *M. leprae*-containing phagosomes, a step mediated through Toll-like receptor-6–dependent signaling (38). This lipid droplet recruitment may be an effective intracellular strategy by *M. leprae* to maintain a source for nutrition and persistence within SC.

AXONAL DEGENERATION

The precise mechanisms of axonal degeneration may differ at the lepromatous and the tubercloid portions of the leprosy spectrum (Figure 3). In the development of axonal damage, the

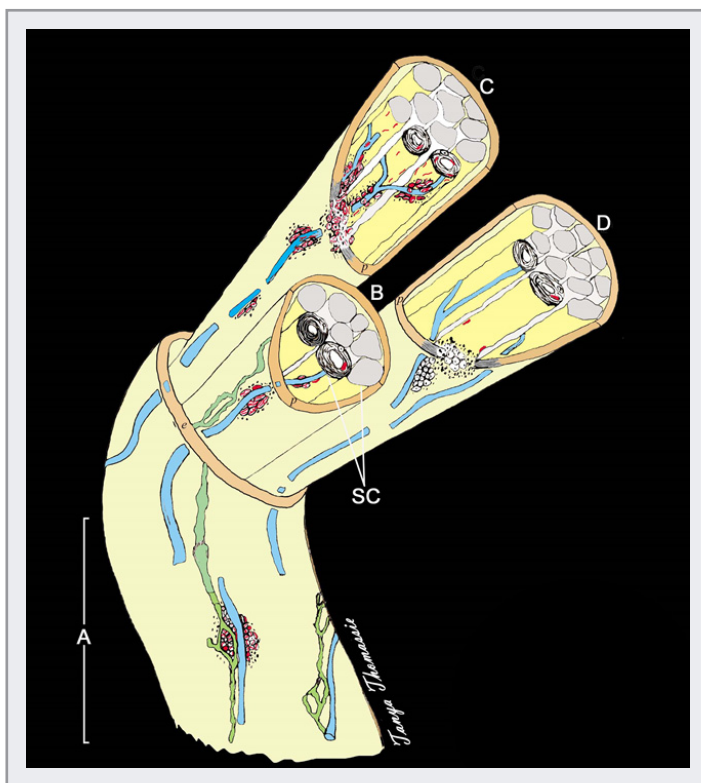


FIG 3 A proposed model of *M. leprae* infection of nerves by a vascular pathway.

The anatomic and physiological setting in which *M. leprae* infection occurs within the endoneurium of peripheral nerves is illustrated in this diagram of a small fascicle containing a blood vessel and both myelinated and non-myelinated SC. Axons are individually wrapped in SC, either with or without a myelin sheath (M). External to their cell membrane, SC also elaborate a continuous basal lamina with a biochemically distinctive composition (see text). The small blood vessels that penetrate the perineurium are lined by

endothelial cells that are anchored to their own biochemically distinctive basal lamina. No lymphatics are present beneath the perineurial wrapping, but resident macrophages (MΦ) do patrol the interstitial space between nerve fibers and blood vessels.

If *M. leprae* enter nerves via their blood supply as proposed (see text), then in order to reach the Schwann cell, bacilli must first adhere to and pass through (or between) endothelial cells and cross their basal lamina. Once in the interstitium of the fascicle, bacilli may be bound and ingested by resident macrophages, or they may bind to the basal lamina of a Schwann cell, which then ingests them. (Not illustrated is the possibility that infected circulating mononuclear phagocytes adhere to and migrate between endothelial cells, carrying *M. leprae* into close proximity to SC.) (Modified from 14)

potentially different roles of Remak SC and myelinating SC are not clear (4, 39, 40). One of the primary functions of SC is to synthesize the myelin sheath around axons, and there is evidence that *M. leprae*-induced demyelination is a result of direct bacterial ligation and activation of the ErbB2 receptor of Neuregulin-1 that regulates normal myelination (41, 42, 43). Demyelination may be induced by a variety of insults, including high levels of some pro-inflammatory cytokines (44, 45, 46). The major toxic effector molecule known to kill *M. leprae* is nitric oxide (NO), produced from L-arginine by activated macrophages expressing the inducible NO synthase (iNOS). Granuloma-associated macrophages have been observed to have iNOS reactivity. Nitrotyrosine, an end product of the metabolism of NO known to cause lipid peroxidation of myelin, has been observed in nerves in BL lesions (47, 48).

The complement system is a key component of the host defense against pathogens. Complement activation results in the cleavage of C3, followed by the cleavage of C5 and the formation of the membrane attack complex (MAC), which causes perforation of eukaryotic cell membranes, resulting in lysis of the target cell (49). A recent study has shown that in nude-mouse sciatic nerves, intraneural injections of *M. leprae* sonicate and its components—particularly lipoarabinomannan (LAM)—result in MAC deposition, myelin loss, and axonal damage (50). Human studies have reported significant serum complement consumption by *M. leprae* (51). In addition, MAC deposits have been observed on damaged nerves of lepromatous but not tuberculoid patients (50, 52), suggesting that complement activation—and specifically the MAC—may function as a disease modifier during the early events of leprosy neuritis.

DEMYELINATION

Ultimately, individual axons in nerve bundles undergo segmental demyelination in leprosy (53, 54). This process may be induced by a variety of insults, including high levels of certain pro-inflammatory cytokines (44, 45, 46) and other mediators of inflammation, along with inflammatory cells forming infiltrating intraneural granulomas (55) (Figure 4).

Whatever combination of mechanisms of injury develop during infection with *M. leprae*, the final common pathway of nerve injury is segmental demyelination. Immunohistochemical studies of human nerves have identified TNF and its receptors and TNF-converting enzymes within nerves in patients with Type 1 reactions. Coupled with studies of the induction of TNF by SC in vitro, experimental evidence suggests that SC may be made more sensitive to TNF-mediated injury and focal demyelination (56).

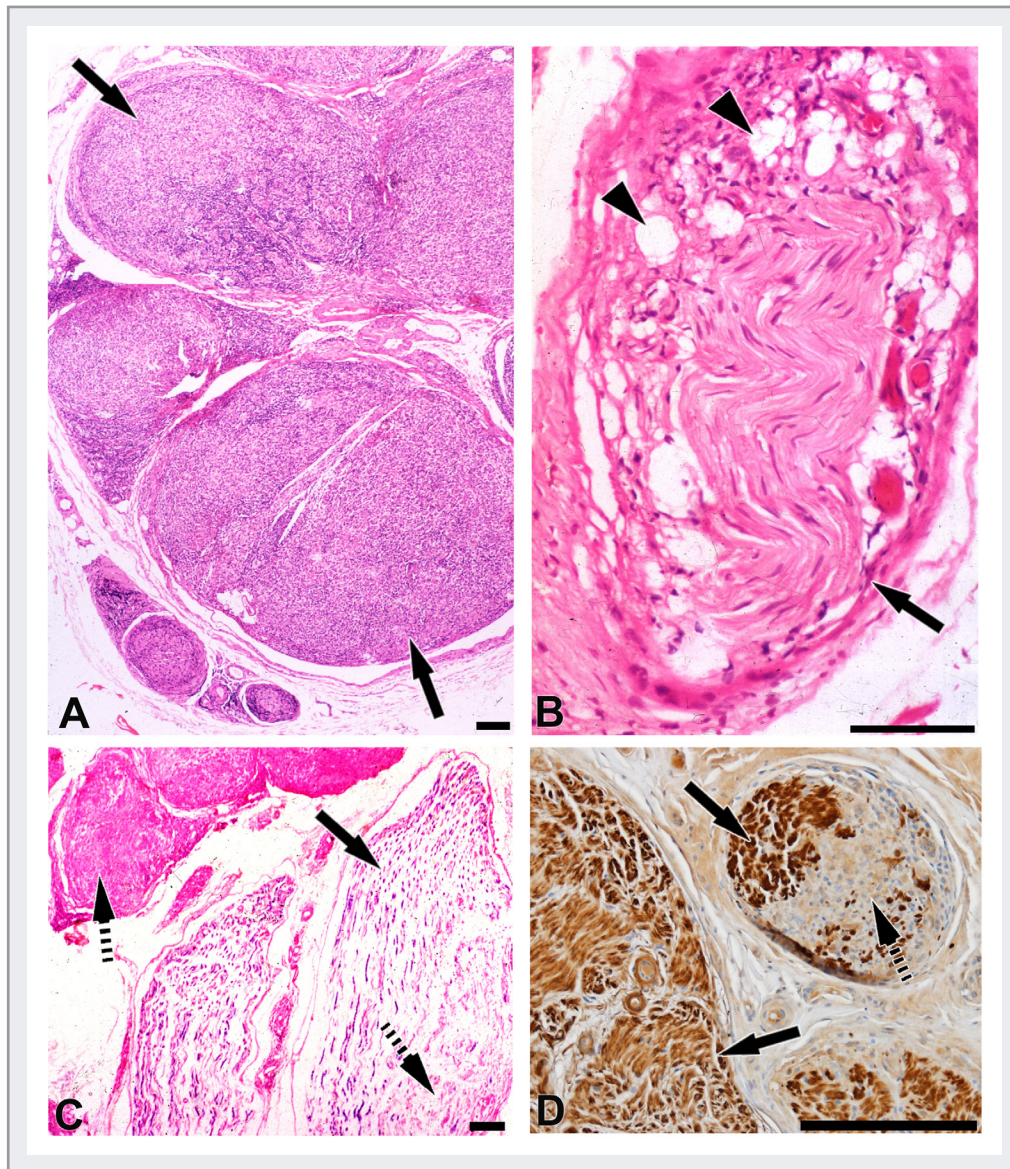


FIG 4 *M. leprae*-infected peripheral nerves exhibiting demyelination and fibrosis.

- A.** H&E stained sections from a superficial peroneal nerve with many fascicles (arrows) showing massive infiltration of inflammatory cells. Scale = 100 μ m
- B.** A nerve (arrow) from longstanding lepromatous disease with vacuolated macrophages (arrow heads) and loss of nerve fibers. Scale = 50 μ m
- C.** Sections of nerve fascicles (arrow) showing focal demyelination and fibrosis (broken arrows). Scale = 100 μ m
- D.** S100 stained nerve sections (arrows) showing focal degeneration of SC and axons (broken arrow). Scale = 100 μ m

Alteration of Immunohistochemical Markers in Distal Sensory and Autonomic Nerves

The advent of immunohistochemical markers has contributed enormously to the detection and investigation of the injury of small nerve fibers. Skin lesions across the spectrum of leprosy exhibit significant destruction of cutaneous nerves. A study in *M. leprae*-infected nude mice showed that a decrease of substances P and CGRP in central and peripheral projections of sensory neurons developed at an early stage of infection and was associated with sensory nociception (57). The levels of TNF in the skin and the nerves of leprosy lesions are similar (58), suggesting that the immunological phenomena studied extensively in leprosy skin lesions also apply to nerves.

Anhydrosis and an impaired sweat response in skin, denervation of the iris, and miotic pupil in the eye are some of the clinical manifestations of autonomic dysfunctions described in lepromatous patients (59, 60). Though extensive mechanistic studies are needed to investigate the functions of autonomic fibers in leprosy, an absence of protein gene product (PGP 9.5), a pan axonal marker, has been documented in the small nerves of the ciliary body, the scleral nerves, and the posterior ciliary nerves adjacent to the optic nerve in enucleated lepromatous eyes. This phenomenon mirrored the 'glove and stocking' type of anesthesia seen in lepromatous patients, in which there is a symmetrical loss of sensation in all four limbs due to an ascending polyneuritis of the extremities (61).

REGENERATION OF NERVES IN LEPROSY

Studies have examined the mRNA and protein levels of Ninjurin-1, an adhesion molecule involved in the response to injury and regeneration in peripheral nerves. Both mRNA upregulation and elevated levels of the protein were observed in nerve biopsy specimens from leprosy patients with neuritis (62). Soft tissue injury, wounds, and ulcers on the lower extremities are frequent complications in lepromatous patients. These findings share a common theme with other small fiber sensory neuropathies, like diabetic neuropathy. Although the regeneration of nerves is possible, regrowth is limited by the milieu of the surrounding extracellular matrix (ECM) as well as vascular insufficiency (63, 64), resulting in poor functional recovery.

FIBROSIS

Intraneural fibrosis and sclerosis are the major destructive pathological manifestations seen in chronically damaged nerves in leprosy (Figure 4). *M. leprae* are seen within collagenized lepromatous nerves in advanced stages of the disease. Attempts have been made to understand the

role of the extra cellular matrix (ECM) in leprosy nerves, and matrix metalloproteinase (MMP), transforming growth factor- β 1 (TGF- β 1), and neuro fibroblasts have been implicated. High levels of matrix metalloproteinases (MMP)-2 and MMP-9 were observed in nerves with endoneurial inflammation in leprosy patients, and these MMP are likely produced by SC and intraneural macrophages (65, 66). It has been proposed that in SC cultures, *M. leprae*-infected SC undergo phenotypical changes, induce upregulation of transforming growth factor- β 1 (TGF- β 1), and increase numbers of α -smooth muscle actin (α -SMA)-positive cells. The characteristic change to stress fibers leads them to secrete ECM that may contribute to progressive nerve fiber loss and fibrosis (67).

Neural fibroblasts (Nf) are abundant in the endoneurium and perineurium of peripheral nerves. Primary neural fibroblasts derived from peripheral nerves could serve as a susceptible recipient cell type for *M. leprae* to escape and survive when these fibroblasts come in contact with infected reprogrammed SC (pSLC) (68). It is possible that the neural collagenization in advanced leprosy may be of Nf origin (69).

NERVE INJURY IN THE ARMADILLO MODEL

The nine-banded armadillo (*Dasypus novemcinctus*; see Chapter 10.1) is the only other natural host for *M. leprae* (70), and naïve armadillos can be experimentally infected, as first described by Walsh et al. (71). The intravenous inoculation of *M. leprae* in armadillos induces peripheral neuritis and recapitulates many features of human leprosy (13, 72). Aggregates of *M. leprae* have been identified within myelinated axons, unmyelinated axons, and SC without producing any intraneural inflammatory cells, thus imitating lepromatous nerves and offering a good experimental model in which to study newer drugs and vaccines. For example, an *M. leprae* viability study using molecular markers showed that a high load of stainable *M. leprae* persists within nerves even after the completion of a full year of rifampin. The molecular viability assays (see Chapter 5.3) indicated that the bacilli were not viable, but the dead bacteria and associated antigens remained in the nerves (73). This experimental finding parallels the persistence and slow clearance of *M. leprae* in human tissues, even many years after successful anti-mycobacterial therapy (74, 75). Persisting dead *M. leprae* provide a continuing source of antigenic stimulation, and may be a major factor in the immuno-pathogenesis of long-term nerve injury in leprosy.

Armadillos respond poorly to thermal, light, or tactile nociceptive stimulants because of their thick carapace, but nerve conduction tests can be done to effectively assess the function of their motor nerves. Several investigators have used immunostaining of cutaneous nerves in 3mm skin punches for PGP9.5 to visualize the intra-epidermal nerve fibers, dermal nerves, and SC in small fiber sensory neuropathies associated with diabetes, HIV, and idiopathic small fiber sensory neuropathies (64, 76, 77, 78). Similar to humans, studies in naïve animals have shown that the distal peripheral innervation pattern is length dependent, with less nerve fibers in the distal leg in comparison to the abdomen (79, 80) (Figures 5 and 6). Preliminary studies in *M. leprae* infected armadillos have shown a slow decline of epidermal fibers but a trend towards an increasing proliferation of SC, providing indirect evidence that during early infection SC may proliferate while

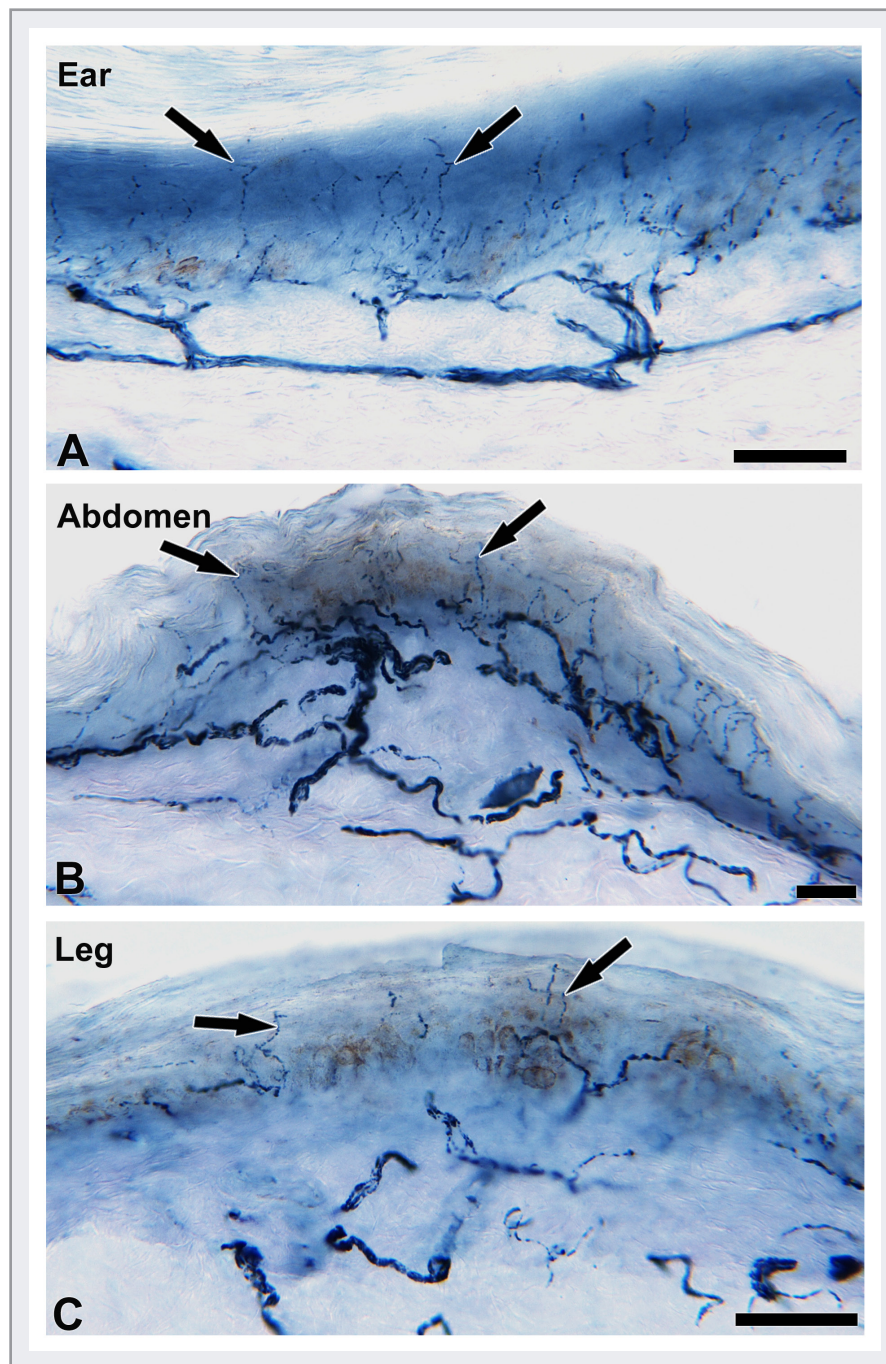


FIG 5 Epidermal Innervation in Armadillos: Skin sections immunostained with anti-PGP 9.5, a neuronal marker.

Skin sections in armadillos exhibiting length-dependent epidermal innervation, dense in the ear lobes (A) and abdomen (B) of naïve animals (black arrows), as compared to the distal leg (C). (Scale bars, A, B, C = 50 μ m) (Modified from [72]).

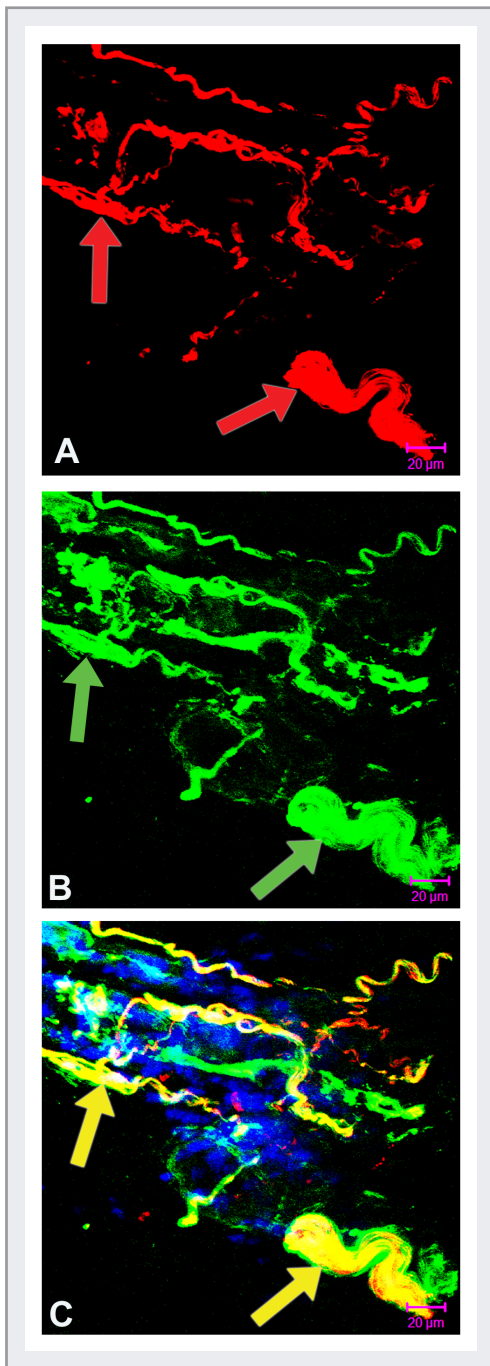


FIG 6 Skin sections of double stained confocal images (A) of dermal axons (anti-PGP 9.5) and (B) SC (anti nerve growth factor receptor, p75).

Distal leg skin section from a naïve animal showing dermal axonal bundles (red arrows) and the SC (green arrow) ensheath and co-localize (yellow arrows) on the dermal axons, exhibiting a pattern similar to human innervation. (Scale bars A, B; C = 20 μm) (Modified from [72]).

harboring *M. leprae*. These studies indicate the feasibility of studying small fibers and early infection in the armadillo using this technique, as well as the possibility of using it as a novel tool to test new drugs and therapeutic interventions.

Summary and Conclusions

In summary, the mechanisms of nerve injury in leprosy can be characterized as follows, although the natural order of the events is not certain:

- *M. leprae* prefer the cooler areas of the body for multiplication, and predominantly affect the segments of trunk nerves that are in close contact with the skin.
- *M. leprae* appears to enter the endoneurial compartment of nerves via the blood supply and endothelium.
- Several different molecules on SC bind *M. leprae* and facilitate its ingestion.
- *M. leprae* survive and proliferate within SC.
- Stimulated by *M. leprae*, SC produce several proteins that may contribute to demyelination.
- Infected nerves are physically at the center of chronic inflammation and immunological activity that may persist for many years.

- Some immune and inflammatory mediators are toxic to nerves and promote demyelination.
- Segmental demyelination occurs, and regeneration follows in some fascicles; these processes may coexist for years.
- A large number of dead bacterial cells remain within a nerve even after multi-drug therapy, and may continue to elicit immunological responses and acute and chronic neuritis.

Identifying the sequence in mechanisms of nerve injury in leprosy is challenging because of the bacteria's dormancy and long incubation period before producing any nerve damage. In addition, the major peripheral nerves affected in man can seldom be biopsied and studied. In spite of these difficulties, extraordinary progress has been made in the last decade, in large part due to new tools resulting from the sequencing of the *M. leprae* genome and that of the only animal model of leprosy neuropathy, the nine-banded armadillo.

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