Mechanisms Involved in Peripheral Nerve Damage in Leprosy with Special Reference to Insights Obtained from In Vitro Studies and the Experimental Mouse Model

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ABSTRACT
The histopathological observations of Khanolkar and Iyer, that M. leprae has a predilection for nerves, first highlighted the central role of peripheral nerves in the pathology of leprosy. It is now well recognized that nerve damage in leprosy will still continue to be an important problem in control and rehabilitation despite the presence of more efficient therapy. The multiplicity of mechanisms postulated, identified, and demonstrated in the last three decades has received little recognition from the scientific community at large. This review is therefore an attempt to collate these multiple studies on mechanisms of nerve damage into a cohesive analysis, which would facilitate the design of future studies. The objective of this review is to focus therefore only on studies which serve to illustrate mechanisms of nerve damage.

RESUMÉ
Les observations histopathologiques de Khanolkar et de Iyer, montrant que les M. leprae présentent une prédilection pour les nerfs, a permis de mettre en lumière le rôle central des nerfs périphériques dans la pathogénie de la lépre. Il est maintenant bien établi que les altérations nerveuses de la lépre vont continuer à être un problème majeur dans le contrôle et la réhabilitation, malgré la présence de thérapies de plus en plus efficaces. Le grand nombre des mécanismes postulés, identifiés et démontrés depuis les 3 dernières décennies, n’a reçu que peu de reconnaissance de la part de la communauté scientifique en général. Cette revue est donc une tentative de rassembler ces nombreuses études sur les mécanismes de l’altération nerveuse au sein d’une analyse cohérente, dont le but est d’aider à l’élaboration de futures études. Cette revue a pour mission de ne se focaliser que sur les études qui permettent d’illustrer les mécanismes d’altération nerveuse.

RESUMEN
Las observaciones histopatológicas de Khanolkar e Iyer que indicaron que M. leprae tiene una predilección por nervios, subrayaron el papel central de los nervios periféricos en la patología de la lepra. Ahora está bien reconocido que el daño a nervios en la lepra continuará siendo un importante problema para el control y la rehabilitación de la lepra, no obstante la existencia de nuevas y más eficientes formas de terapia. La multiplicidad de los mecanismos postulados, identificados, y demostrados en las últimas tres décadas ha recibido, sin embargo, poco reconocimiento por parte de la comunidad científica. Esta revisión es por lo tanto, un intento de unir, de alguna manera, estos múltiples estudios sobre los mecanismos de daño a nervios, en un análisis cohesivo que pudiera facilitar el diseño de futuros estudios. Por lo tanto, esta revisión se enfoca sólo a los estudios orientados a ilustrar los mecanismos de daño a los nervios.
Anatomical and mechanical factors involved in leprous neuropathy have been reviewed extensively by Wadia (103). On the other hand, Dastur (16, 18), Palande (61), and Antia (1) concluded that raised intraneural tension was an important contributing factor, and that fibrous tunnels at the joints may further exacerbate the nerve damage which results in sites of predilection usually being located at the entrapment site.

Maintenance of the peripheral nervous system is the result of a complex balance between the two functions of the immune system viz. assistance in maintenance of nerve physiology/ regeneration (64) and defense against infections. Leprosy serves as a unique model where, due to the insidious nature of the infecting organism, *Mycobacterium leprae* and its predilection for peripheral nerve Schwann cells, the two functions of the immune system co-exist at least during the initial stages.

The nerve damage following *M. leprae* infection of the peripheral nerve can be divided into two stages. The initial phase is common to both lepromatous and tuberculoid patients and occurs despite the absence of inflammatory cells (80, 81, 85). In the experimental mouse model, the onset and initial progress of *M. leprae*-induced nerve damage remained unchanged in thymectomized irradiated mice and mice treated with anti-Thy 1.2 or Cyclosporin A, further emphasizing that the early events are not immunologically mediated (82). A recent study by Rambukkana, et al. using Rag 1-knockout mice also demonstrated that nerve damage can be initiated by *M. leprae* infection in the absence of an immune response (67). The studies of Brand, et al. (9) suggested that *M. leprae* survived better within the cooler regions of the body. Therefore, the metabolic alterations in Schwann cells and/or axon as a consequence *M. leprae* infection have been implicated as the major factor in this phase of nerve damage (51, 56).

In the later phase, there is an influx of mononuclear cells, which is predominantly lymphocytic in tuberculoid patients and macrophagic in the lepromatous (62).

**Initial phase of nerve damage.** The initial phase of nerve damage observed in patients across the leprosy spectrum and in a proportion of contacts is characterized by an absence of inflammatory cells (80, 81). The predominant histopathological features common in lepromatous and tuberculoid patients (78, 80) include: i) sub-perineural oedema consisting of a proteinaceous granular matrix, interspersed with small pockets of collagen; ii) axonal atrophy and secondary demyelination; iii) loss of unmyelinated fibers; iv) activation of resident macrophages and fibroblast within the endoneurial space.

In lepromatous patients, *M. leprae* were detected mainly in the Schwann cells of the unmyelinated fibers (80, 81). Though no acid-fast bacilli (AFB) are seen in nerves of tuberculoid patients, osmiophilic structures suggestive of bacteria are observed in the electron micrographs (2). In addition, viable *M. leprae* were recovered from homogenates of skin biopsies from tuberculoid patients inoculated into the mouse footpad (86).

Accompanying these degenerative changes listed above, regenerative activity was also observed across the spectrum (78), thus implying that there is a balance between regeneration and degeneration with the outcome depending on the predominating activity.

These features in the initial phase of nerve damage have also been studied in the mouse model (85). The major limitation in the mouse model has been the absence of inflammatory cells and granulomatous reactions mimicking the spectral disease of the human, as well as the inability to demonstrate bacilli in Schwann cells of the affected nerves. However, the presence of nerve abnormalities in the absence of inflammatory cells and granulomatous reactions mimicking the spectral disease of the human, as well as the inability to demonstrate bacilli in Schwann cells of the affected nerves. However, the presence of nerve abnormalities in the absence of inflammatory cells and granulomatous reactions mimicking the spectral disease of the human, as well as the inability to demonstrate bacilli in Schwann cells of the affected nerves.

The Swiss White (SW) mouse is a strain in which the response of host cells to *M. leprae* infection parallels those observed in lepromatous patients as opposed to the C57BL/6 strain in which the response to *M. leprae* infection parallels those observed in lepromatous patients as opposed to the C57BL/6 strain in which the response to *M. leprae* is similar to that observed in tuberculoid patients or normal individuals (2). The histopathological features of early nerve damage parallel the observation in patients and are similar in experimentally infected SW and C57 mice (2).
Entry of *M. leprae* into the nerve, mediated by the endoneurial endothelial cells, has been suggested by Mehta (46); Dastur, et al. (19, 20); Boddingues, et al. (1-8), and Scol-

lard (75) who have documented the presence of *M. leprae* in the endoneurial cells. Scol-

lard (75) has proposed that no difference ex-

ists in the mechanism of initial infection across the leprosy spectrum. Bacteremia has also been reported in leprosy patients across the spectrum (12, 30). This is in keep-

ing with the histopathological features of early nerve damage.

*M. leprae* entry into Schwann cells is the most important event in the induction of nerve damage. Based on studies utilizing murine dissociated Schwann cell cultures, early entry of *M. leprae* (within 6 hours) is observed only with viable bacteria (15). Such forced entry into macrophages has also been reported for *M. leprae* as well as (49) Leish-

mania donovani (11). Recent studies by Rambukkana, et al. (66) have suggested that α2 laminin and a dystroglycan are responsi-

ble for the specific predilection of *M. leprae* for Schwann cells. However, since both components are present in the basal lamina of myelinating and non-myelinating fibers, this hypothesis fails to explain the ultra-

structural observations that, at least in the initial stages the *M. leprae*, are predomi-

nantly observed in Schwann cells of non-

myelinating fibers (74). The studies by Choudhary, et al. (13) did not find evidence for a unique bacterial surface component for bacterial entry.

An important feature of infection of host cells by *M. leprae* is the down regulation cell-cell communication channels of the host cell from lepromatous patients (3, 88, 89). Once *M. leprae* have colonized the Schwann cells, expression of nerve growth factor (NGF) receptor and fibronectin secretion are down regulated (89, 90). This has been demonstrated *in vitro* in Schwann cells from SW and C57BL/6 mice. Receptor down regu-

lation would result in decreased utilization of NGF resulting in axonal atrophy, and the decrease in fibronectin secretion would hamper regeneration. Both of these features are characteristic of the initial phase of nerve damage and are seen in patients across the spectrum (78, 80) and in the nerves of experimentally infected SW and C57BL/6 mice (5).

However, while in SW mice the damage progresses and increased demyelination is observed, in C57BL/6 the damage is arrested or delayed (18). A possible ex-

planation is provided by the effect of *M. leprae* infection of Schwann cells *in vitro* on neural glia cell adhesion molecule (NgCAM) expression (88), Schwann cell proliferation (87), and production of colla-

gen (90). Since none of the three parameters were affected by *M. leprae* infection of Schwann cells from C57BL/6 mice, the re-
generative capacity in this strain is not ham-

pered. In contrast, *M. leprae* infection of SW Schwann cell cultures resulted in di-

minished expression of NgCAM (88), which is required for efficient Schwann cell-axon interac-

tion and is normally enhanced during re-

generation. The aberrant myelination ob-

served in infected nerves (76, 77) may be a re-

flection of a decrease in NgCAM expression.

Schwann cell proliferation, an important pre-

requisite for peripheral nerve regeneration, was also decreased. In addition, SW Schwann cells secreted increased levels of collagen *in vitro* (86), which correlated with observa-

tions in sciatic nerves from experimentally infected SW (5, 85) mice and nerves of lep-

rosy patients (78).

In summary, the initial nerve pathology observed in the sciatic nerves of SW and C57BL/6 mice infected with *M. leprae* in the footpad are similar and resemble the early changes in the nerves of lepromatous and tuberculoid patients. Therefore, it was hypothesized that the response of Schwann cells to the presence of *M. leprae* would be similar. In keeping with this hypothesis, some common aberrations were noted in the two strains in their Schwann cell response to *M. leprae* infection, which included down regulation of NGF-R expression and pro-

duction of fibronectin. Nevertheless, a num-

ber of Schwann cell responses to *M. leprae* infection were also diverse in the two strains. SW Schwann cells showed decrease in proliferation, production of fibronectin, and expression of NGF-R and NgCAM, while an increase was noted in the produc-

tion of collagens and laminin. Such differ-

ing responses of Schwann cells to *M. leprae* infection in the two strains in the early stages may have important implications for the later stages of nerve damage. First, it may result in differing degrees of Schwann
cell mediated regenerative activities in the two strains and this may explain the progression of early pathology to extensive damage only in SW strain (5). Secondly, such a response in patients’ nerves would probably provide signals to different populations of inflammatory cells and consequently contribute to the differing composition of the infiltrating cell population in lepromatous and tuberculoid nerves (44, 104).

Concurrent with the metabolic damage induced, the bacteria continue to multiply intracellularly within the Schwann cells. It appears that M. leprae growth is equally supported across the leprosy spectrum and in Schwann cells of SW and C57BL/6 mice (unpublished observations). However, the immune response generated by the host is decisive in determining the later sequence of events.

Later phase of nerve damage. The hallmark of the later phase of nerve damage is the presence of inflammatory cells. Studies by Dastur, et al. (18, 19, 20); Job, et al. (31, 32); and Ridley (68), among others, have attempted to elucidate the pathology of the later phase of nerve damage across the spectrum. The central question that arises is, “What is the signal for the inflammatory cell influx and how is it regulated?”

One of the earliest theories put forward was that the inflammatory response especially in tuberculoid patients was the result of autoimmunity (42). Auto-antibodies in leprosy have been reported against several nerve components (24, 105). In addition, human nerves and skin have a number of antigenic determinants in common with M. leprae (58, 65, 99). Many of these epitopes are heat-shock proteins (hsp) (36, 60). In animal models, it has been shown that M. leprae-primed macrophages attack the Schwann cells, not only in the presence, but also in the absence of detectable M. leprae (94), and M. leprae sensitized T cells also react with Schwann cell components (92). However, studies by Mshana, et al. (54) and Ghaswalla, et al. (27) failed to consistently find antibodies against neural antigens. Lymphocyte proliferation in response to neural antigens was also absent (25, 55). Thus, it is suggested that autoimmunity may only contribute to the exacerbation of the lesion.

On the basis of the studies cited in the previous section, Schwann cells can be viewed as more than just supportive cells for M. leprae multiplication, or as mere antigen depots. Instead, the initial response of Schwann cells to M. leprae infection may have important and divergent influences on the immunological profile of lepromatous and tuberculoid nerves.

An alternative hypothesis is that the mononuclear cells crossing the barrier in the course of normal surveillance (38) encounter antigen presented by antigen-presenting cells (APC) leading to their activation, which in turn signals the influx of fresh cells.

Though the Schwann cell is capable of presentation of mycobacterial antigens on its cell membrane, this expression on the membrane is probably a result of integration of bacterial antigen with host membrane components during bacterial entry rather than active processing by Schwann cells (6).

Nevertheless, M. leprae-infected Schwann cells from both strains of mice are capable of sensitizing lymphoid cells in the murine dissociated Schwann cell culture system (50). However, this ability was dependant on the sensitization level of the lymphocytes prior to co-culture with Schwann cells, the antigen used, and the requirement of accessory cells (50). In addition, an increase in the fibroblast population in culture enabled dissociated Schwann cells to induce lymphoproliferation to M. leprae and 65Kd antigen even in the absence of adherent cells, demonstrating that fibroblasts could undertake the role of accessory cells, and in conjunction with infected Schwann cells, precipitate an immunological attack (50). This is important since macrophages at the site of old lesions are paralyzed/senescent.

The role of fibroblasts, as well as Schwann cells, in leprous neuropathy was also indicated in studies using immune modulated mice (82). Progressive, late nerve damage associated with demyelination was significantly reduced in mice treated with anti-thy 1.2 antibody where, along with the depletion of T-helper cells, fibroblast—which express thy 1.2 marker—would also be affected. In contrast, thymectomized and irradiated mice, where both T and B cell populations would be affected leaving the fibroblast population intact, showed similar intensity of nerve damage as the untreated
animals. This indicates an important role for fibroblasts in leprosy neuropathy.

The presence of MHC class I products and mycobacterial antigens on the surface of the Schwann cell (6, 94) may result in bystander damage due to direct cellular cytotoxicity. Studies have demonstrated the lysis of M. leprae-infected Schwann cells by sensitized T cells in vitro (95).

In tuberculoid patients, the influx of inflammatory cells functions as a double-edged sword. The influx of macrophages enhances the regenerative process (63). In addition, the lymphocytes stimulated by M. leprae-antigens presented on Schwann cells are able to activate macrophages to kill intracellular M. leprae (59). Simultaneously with these beneficial effects, the release of protease results in collagen breakdown, which exacerbates granuloma formation (66). Reactive oxygen intermediates (ROI), produced by both Schwann cells (51) and macrophages as a consequence of the inflammatory process, results in further nerve damage at the site of the granuloma.

In lepromatous patients, the invading macrophages further down regulate the NGF-R (69), thus depriving the cell from utilizing the available NGF, leading to slow nerve damage. In addition, the NGF levels have also been reported by Facer, et al. (25, 26) to be decreased in lepromatous patients. Macrophages simultaneously enhance Schwann cell proliferation and NgCAM expression, thus aiding in the regenerative process (71, 72). The transforming growth factor-β (TGF-β) is important in the development and repair of the peripheral nerve (41). Conversely, it is also implicated in peripheral neuropathies, such as experimental allergic neuritis (EAN) and experimental Wallerian degeneration (71). TGF-β has also been reported to enhance Schwann cell proliferation, up regulate NgCAM, and enhance collagen synthesis (66). These functions of TGF-β, along with its immunosuppressive action, allow us to propose that TGF-β secreted by M. leprae-infected macrophages from lepromatous patients/SW mice may be responsible for the decreased regenerative activity in these nerves. This is supported by the observation of Khanolkar, et al. (35) that maximum TGF-β was observed within lepromatous lesions.

In addition, further invasion by lymphocytes is probably curtailed by the production of suppressor factors by the infected Schwann cells (47) and the invading M. leprae-infected macrophages (4, 72). The data of Mehta, et al. (47), indicate that M. leprae infected murine Schwann cells, though initially stimulating lymphocytes (96), release a factor that results in cell death probably due to apoptosis. These lymphoid cells are then engulfed by Schwann cells and slowly degraded in culture (84). Apoptosis of antigen-specific T cells in lesions of experimental allergic encephalomyelitis (EAE) and EAN has been identified as an effective mechanism in stopping neural inflammation. It may be the consequence of production of a glucocorticoid by M. leprae infected Schwann cells (108, 109). The possibility also exists that M. leprae infected Schwann cells like astrocytes (99) may be secreting a tumor necrosis factor (TNF)-like factor. The refractoriness of factor production to cycloheximide treatment of M. leprae-infected Schwann cells (47) implicates the release of glucocorticoid resembling substances (97), which may explain the predominantly macrophage infiltration in nerve granulomas of lepromatous patients.

Silent nerve damage. The studies of Shetty, et al. (29, 73, 84) and Srinivisan and Gupte, et al. (93) on silent nerve damage, which is a major concern in patient management, have extensively documented that neuropathy progresses in patients after the infection is cured and even in the absence of inflammatory cells. Much less is known about the mechanism by which this occurs.

Nevertheless, there is evidence that the demyelination observed is a consequence of atrophic changes in the axonal compartment (78). It has been demonstrated that this axonal atrophy is due to hypophosphorylation of the axonal neurofilaments (87). It is of interest to note that the degenerative disorders of the central nervous system are characterized by the presence of hyperphosphorylation, whereas in leprosy nerves there is decreased phosphorylation of the neurofilaments resulting in their degradation. The observation that mycobacterial antigens persist in nerves long after clinical cure (84) suggests that this persisting antigen may be important for the continued progression of nerve damage.

Preliminary findings by Shetty, et al. (93)
demonstrated that the decreased phosphorylation of neurofilaments was also seen in mice inoculated in the footpads with either viable or heat-killed \textit{M. leprae}. Treatment with the TGF-\(\beta\) and heat shock proteins has been shown to inhibit mammalian cyclin dependent kinase (CDK) (\textsuperscript{37}).

In a culture system using macrophages Chan, \textit{et al.} (\textsuperscript{10}) have shown that lipoarabinomannan (LAM) from \textit{M. tuberculosis} and \textit{M. leprae} inhibited the enzyme protein kinase C (PKC). The neuron specific kinase, responsible for phosphorylation of neurofilament proteins, CDK5 and PKC are known to share common inhibitors (\textsuperscript{37}). CDK5 may therefore follow the same pattern of inhibition (\textsuperscript{40, 79}). Increase in phosphatase activity, an indicator of dephosphorylation of NF, has been reported in leprous nerves and skin lesions by Dastur and Dabholkar (\textsuperscript{17}). Thus, it is possible that the persisting antigens may interfere with the functions of the regulatory enzymes leading to hypophosphorylation.

Further evidence for the role of \textit{M. leprae} antigens being involved directly in silent neuritis is obtained from the study of Croft who reported that in 32\% of all impaired nerves studied, nerve function did not improve despite prednisolone therapy (\textsuperscript{15}). Since prednisolone would only decrease inflammation and has no effect on the bacterial antigenic load, this further emphasizes the importance of \textit{M. leprae} antigens in mediating silent neuritis.

**Reactions in leprosy.** The acute inflammatory response in Type I reactions is of special concern since it significantly exacerbates nerve damage. Histopathologically, the lesions show all the characteristics of a delayed-type hypersensitivity reaction (\textsuperscript{57, 68}) with an influx of mainly CD4 lymphocytes, especially of the Th1 class (\textsuperscript{52, 53, 59, 102, 106, 107}). There is an increase in IL-2 and TNF-\(\alpha\), which confirms a shift to the Th1 subtype during a reaction (\textsuperscript{34, 100, 101}). Possibly due to this shift, the humoral immunity during Type I reaction seems to be diminished (\textsuperscript{100}). However, a shift to Th2 may also occur since there is an increase in mRNA for IL-4 in some of the lesions (\textsuperscript{53, 102, 107}).

While reactional episodes can occur at anytime during the course of the disease, recent studies suggest that their incidence has increased especially among lepromatous patients in the first year of multidrug therapy (MDT) (\textsuperscript{78}). This may be due to the cidal drugs in the MDT regimen which result in the suppressor factors which are dependent on viability of \textit{M. leprae} and are therefore no longer being produced by infected macrophages (\textsuperscript{4, 72}) and Schwann cells (\textsuperscript{47}), while the killed bacilli act as antigen depots, which serve to stimulate an inflammatory response. The reduced levels of TGF-\(\beta\) reported in reactional lesions further supports this hypothesis (\textsuperscript{15}).

An alternative mechanism has been postulated by Rook, \textit{et al.} (\textsuperscript{70}) who suggests a neuroendocrine control of inflammation, wherein destruction of C fibers would diminish the activity of neural feedback pathways due to decreased secretion of active peptides. This in turn would prevent activation of the hypothalamus pituitary axis, resulting in local deactivation of cortisol, thereby precipitating an inflammatory response. While the first hypothesis is restricted to lepromatous patients where suppressor factors play a dominant role, destruction of C fibers, which is common to lepromatous and tuberculoid patients (\textsuperscript{78, 80}) suggests that the mechanism postulated by Rook, \textit{et al.} may explain reactions at both ends of the spectrum. The involvement of the Cortisol-Cortisone shuttle may also explain the clinical observations that reactional episodes are more frequent during pregnancy (\textsuperscript{23}) since the enzymes of the shuttle are affected by progesterone and pregnancy (\textsuperscript{96}).

While specificity of lymphoid cells isolated from nerves of non-reactional tuberculoid patients is against mycobacterial antigens and not neural antigens (\textsuperscript{22, 54}), studies on antibody responses to neural antigens have resulted in conflicting results [reviewed by Desikan (\textsuperscript{21})]. The observation that sera from all patients with erythema nodosum leprosum (ENL) contain demyelinating antibodies is of particular interest (N. F. Mistry, personal communication). Nevertheless, since systemic manifestations are rare and there is no evidence of a specific reaction precipitating mycobacterial antigen (\textsuperscript{50}), the difference may lie in local factors such as differential activation and tissue distribution, as well as the status of the host immune response and genetic restriction.
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