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Editorial

IMMUNOPATHOLOGY OF NERVE INVOLVEMENT IN LEPROSY

Nerve granulomas may be found at all points across the leprosy spectrum. At the lepromatous end the granuloma is of the typical macrophage type, whereas at the tuberculoid end the granuloma is more like that associated with delayed hypersensitivity. In between one-third and one-half of the tuberculoid cases there may be discordance as to the type of granuloma seen in nerves and skin.[1]

The nature of the cellular infiltrate, particularly cells of the macrophage–monocyte system in neural granulomas in leprosy, has been studied using electron microscopy.[2] At the tuberculoid end of the spectrum, large mononuclear cells with abundant cytoplasm with vacuoles containing finely granular material and pale indented nuclei were seen and identified as epithelioid cells. At the lepromatous end of the spectrum the Schwann cells were often heavily bacillated, as were the macrophages and perineural cells. The end result of inflammatory changes caused by leprosy bacilli in the nerves is the total destruction of the nerve. At the tuberculoid end of the spectrum, and especially in reversal reactions (Type I), it is clear that the inflammatory reaction is related to hypersensitivity and is an immunological phenomenon. However, it is not clear what causes the inflammatory reaction in lepromatous cases. In erythema nodosum leprosum (Type II reaction), which occurs at the lepromatous end of the spectrum, the inflammation of nerves is probably immune complex mediated since it is associated with other aspects of immune complex disease in which there is evidence of circulating immune complexes.[3] Immune complexes in the nerve could be formed directly with mycobacterial antigen released locally, or may be the result of the deposition of circulating immune complexes. A further cause of granuloma formation in nerves in lepromatous leprosy in the absence of immune complexes is likely to be the direct activation of the alternative pathway of complement by *Mycobacterium leprae* or its breakdown products.[4] The activation of the complement cascade by the classical or alternative pathways will result in the local release of the inflammatory components C3a, C3b, C5a and C5b which could lead to macrophage accumulation at the site. Immunohistology revealed deposits of C3 and C9 on the Schwann cells of affected nerves in patients with lepromatous leprosy. There was also moderate staining for C9 in tuberculoid leprosy patients.[5] The role of the C9 in nerve damage is still open to interpretation.

Lymphocyte subtyping in leprosy neuritis has been studied in small numbers of patients across the leprosy spectrum, using monoclonal antibodies to identify T cells and anti-IgG antibodies to identify B cells.[5] Between 40% and 50% of the mononuclear cells were T cells and between 20% and 25% were B cells. The CD4/CD8 ratio in paucibacillary

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nerves was 1·8:1 while in multibacillary nerves it was 0·5:1. Narayanan *et al.*[6] found an average of 85% of lymphocytes in the nerve granulomas of patients at the tuberculoid pole to be CD3 positive, 80% CD4 positive and 30% CD8 positive. CD4+ cells were diffusely scattered within the epithelioid cell granulomas, while CD8+ cells were localized in the peripheral mantle of the granuloma. At the lepromatous end of the spectrum, very few T cells were found in nerve granulomas.

Thus, the cellular infiltration of granulomas within the peripheral nerve mirrored the type of cellular infiltration seen in granulomas of the skin. Where the lesion is paucibacillary, there is a typical epithelioid cell granuloma with the presence of a high proportion of CD4+ lymphocytes. Where the lesion is multibacillary, cells of the macrophage/monocyte series are phagocytosing cells and T cells are less evident, with a lower CD4/CD8 ratio. An interesting group of nerves was studied, in addition, by Nilsen *et al.*[5] This consisted of untreated patients with a histology of BT leprosy in the skin and multibacillary leprosy (BI > 3) of the nerve. These nerves contained similar proportions of T cells to those found in patients in both the tuberculoid and lepromatous groups (40–50%). However, the CD4/CD8 ratio (0·6:1) was similar to that found in the lepromatous group (0·5:1) and considerably lower than that found in the tuberculoid group (1·8:1). Thus, the granuloma of the nerve in all cases resembled that found in lepromatous leprosy rather than that found in the skin.

Nerve axons are enclosed by the cytoplasm of Schwann cells, which are actively phagocytic and can engulf mycobacteria. Dastur *et al.*[2] showed intact acid-fast bacilli within swollen Schwann cells of nerves from patients with lepromatous leprosy. Shetty *et al.*[7] found bacilli within the Schwann cells of unmyelinated fibres from lepromatous nerves. There has been much interest in recent years as to whether or not Schwann cells are capable of presenting *M. leprae* antigens to T lymphocytes in leprosy. Samuel *et al.*[8] cultured human foetal Schwann cells. These normally express Class I MHC antigens, but not Class II. Schwann cells in culture phagocytose *M. leprae*. Treatment of normal and *M. leprae* infected cultures with gamma-interferon induces Class II expression on Schwann cells, but not fibroblasts. This suggested that the Schwann cells might be able to present *M. leprae* antigens to T lymphocytes *in vivo*. Class II staining cells presumed by their morphology to be Schwann cells were found in leprosy nerves in frozen tissue sections by light microscopy.[5] However, resolution at the light microscopic level is not sufficient to distinguish Schwann cells from fibroblasts and infiltrating cells. Schwann cells can only be clearly identified and their intercellular relationships observed, at the electron microscopic (EM) level. Using immunoEM techniques it was possible to label cell surface proteins such as the MHC antigens whilst preserving the morphology of the cellular ultrastructure sufficiently well to distinguish Schwann cells from other cells in the granuloma.[9] Class II positive macrophages, lymphocytes and fibroblasts were found in paucibacillary and multibacillary leprosy nerves. However, Schwann cells were consistently negative. These observations indicate that it is unlikely that Schwann cells are involved in presenting *M. leprae* antigens to T cells *in vivo* in leprosy patients, despite the suggestion that they might be able to do so *in vitro*.[8]

Axonal changes in leprosy neuritis

The changes that occur in the axons in early leprosy neuritis have been studied.[7]

Quantitative measurement of the frequency distribution of myelinated fibres, comparing leprosy nerves (BT and BL) with controls, showed a shift to the left in the histogram with an increased proportion of small fibres. There was evidence of 'inappropriately' thin myelin sheaths and of some loss of unmyelinated axons. Oedema was the most consistent finding in these nerves. This included endoneurial oedema. There was some evidence of axonal atrophy in nearly all the nerves examined. These authors emphasize the increased numbers of macrophages and fibroblasts to be seen in the lesions.

Another way of looking at nerve function in leprosy is to study the change in neuropeptides in cutaneous nerves.[10] The peptides visualized by immunocytochemistry were substance P (SP), calcitonin gene-related protein (CGRP), vasoactive intestinal protein (VIP), neuropeptide tyrosine (NPY) and its C-terminal flanking peptide (CPON). Cutaneous nerves in all types of leprosy showed a reduction or complete absence of staining for these neuropeptides. Substance P and CGRP are found in unmyelinated sensory C fibres which are responsible for initiating pain and temperature reactions. These were completely absent from leprosy nerves. VIP and NPY are found in the nerves supplying sweat glands. The absence of these neuropeptides indicates the involvement of autonomic nerves and underlines the absence of sweating that occurs in areas of the skin supplied by leprosy nerves. This study demonstrates an almost total absence of sensory and autonomic neuropeptide immunoreactivity in nerve fibres across the leprosy spectrum.

Experimental models of leprosy granulomas in nerves

The intradermal injection of mycobacteria into the guinea-pig ear results in the development of mycobacterial granulomas in the draining post auricular, lymph node.[11] The injection of *Mycobacterium bovis* BCG induced the development of secretory epithelioid cell granulomas similar to those found in the skin at the tuberculoid pole of the leprosy spectrum. *M. leprae*, on the other hand, induced the development of a granuloma that consisted exclusively of phagocytosing macrophages as found at the lepromatous pole.

The establishment of similar mycobacterial granulomas in guinea-pig sciatic nerves[12] enabled a deeper study of the relationship between such granulomas and nerve damage. As with the lymph node granulomas the intraneural injection of BCG induced a mainly epithelioid cell granuloma maximal 2 weeks after injection, whilst the intraneural injection of *M. leprae* induced a granuloma consisting mainly of phagocytosing macrophages maximal 5 weeks after injection. Nerve damage correlated with the degree of endoneurial infiltration. Axonal degeneration was preceded by demyelination. Electrophysiological studies showed that both types of granulomas produced a functional impairment of the ability of nerve fibres to conduct action potentials. It was generally impossible to elicit a peak relating to the fastest conduction velocity. Intraneural *M. leprae* granulomas appeared to cause more chronic functional impairment, up to 150 days more, than intraneural BCG granulomas where the nerve had functionally recovered by 150 days.

Morphometric analysis of the myelinated fibres in the granuloma or distal to the granuloma showed a considerable decrease in fibre numbers 5 weeks after the intraneural injection of *M. leprae*. By 150 days, the number of fibres had recovered slightly, but was

still significantly different from normal values in the nerve distal to the granuloma. BCG injection leads to significantly reduced numbers of myelinated fibres after 2 weeks, again with fewer fibres in the distal part of the nerve. However, by 150 days the number of fibres had nearly regained normal levels.

In addition, there was a marked reduction in mean fibre diameter in *M. leprae* injected nerves at 5 weeks which persisted distal to the granuloma beyond 150 days. In BCG injected nerves mean fibre diameters were significantly lower than normal after 2 weeks, but recovered by 150 days. Overall, the morphometric findings indicated that *M. leprae* injected nerves were still chronically damaged at 150 days after injection, whereas BCG injected nerves had recovered by that time.[13]

The experimental model of mycobacterial induced nerve granulomas can also be studied by immunocytochemical techniques for MHC Class II and CD markers.[14] As in human leprosy nerves, by using electron microscopy it was shown that in *M. leprae* induced granulomas in guinea-pig nerves, macrophages and fibroblasts were Class II positive. However, no evidence of Class II expression could be detected on the cell membrane of Schwann cells. This again suggested that these cells played little role in antigen presentation *in vivo*.

Mechanism of axonal damage in leprosy neuritis

The functional deficit in leprosy neuritis is mainly due to axonal damage occurring as a result of granuloma formation within the nerve. There is no doubt that some damage is caused by pressure on the nerves resulting from perineurial oedema, as nerve decompression can have a certain beneficial effect.[15]

The mechanism of axonal damage at the tuberculoid end of the spectrum may differ from that at the lepromatous end. In lepromatous leprosy there is likely to be complement activation. This might be by the classical pathway in Type 2 reactions where there is evidence of immune complex formation. The alternative pathway can be activated by the high concentration of *M. leprae* antigens.[4] Activation of the terminal components of complement[5] can produce a membrane attack complex that is well known to be active in cell membrane damage.

At the tuberculoid end of the leprosy spectrum, the epithelioid cell granuloma that is formed has certain analogies with delayed type hypersensitivity reactions. It is, therefore, likely to be CD4 T lymphocyte mediated. CD4 cells produce cytokines that activate macrophages, such as interferon gamma. The macrophages will then produce other cytokines such as TNFα (tumour necrosis factor). TNFα may also be produced by macrophages in lepromatous leprosy. Lipoarabinomannan, a major component of mycobacterial cell walls, can induce macrophages to produce TNFα.[16] The role of TNFα in axonal damage is yet to be determined, although treatment with antibodies against TNFα can protect experimental animals from immune mediated demyelination.[17,18]

CD8 lymphocytes may also be involved. Murine Schwann cell lysis by CD8 cells occurs when they present *M. leprae* antigens in association with MHC Class I.[19] Murine CD8 lymphocytes against mycobacterial heat shock protein 65 recognize and lyse stressed Schwann cells even in the absence of *M. leprae*[20] and could explain nerve damage when there is a low bacterial load.

The role of lymphocyte factors in inducing axonal damage must also be considered.

The cytokine TNFβ (lymphotoxin) is produced by CD4 lymphocytes. However, it is proposed that T cells, both CD4 and CD8, might produce cytotoxicity more directly by secreting a 'killer' protein, perforin/cytolysin.[21] Perforins may exhibit considerable structural homology with complement components. It has been proposed that perforins, as well as complement, damage biological membranes through the formation of 10–20 nm transmembrane pores. Both perforins and complement seem to need Ca^{++} ions for their action *in vitro*.

In conclusion, *M. leprae* is the only bacterium that penetrates the epineurium. In leprosy, axonal damage occurs as a result of the release of pharmacological mediators produced by infiltrating inflammatory cells. These cells come in as a result of either an immunological reaction or a paraimmunological reaction such as the activation of the alternative pathway of complement.

Department of Pathology
The Royal College of Surgeons of England
Lincoln's Inn Fields
London WC2 3PN

J. L. TURK
JILL CURTIS &
GAIL DE BLAQUIÈRE

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