The role of Schwann cells, T cells and *Mycobacterium leprae* in the immunopathogenesis of nerve damage in leprosy

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Summary Damage to peripheral nerves is the major complication of reversal (type I) reactions in leprosy. The underlying mechanism of nerve damage remains largely unresolved; however, an important role for type-1 T cells has been suggested. *Mycobacterium leprae* has a remarkable tropism for the Schwann cells that surround peripheral axons. Because reversal reactions in leprosy are often accompanied by severe and irreversible nerve destruction, and are associated with increased cellular immune reactivity against *M. leprae*, a likely immunopathogenic mechanism of damage to Schwann cells and peripheral nerves in leprosy is that infected Schwann cells process and present antigens of *M. leprae* to antigen-specific, inflammatory, type-1 T cells, and that these T cells subsequently damage and lyse infected Schwann cells. Previous animal studies with $CD8^+$ T cells revealed evidence for the existence of such a mechanism. A similar role has been suggested for $CD4^+$ T cells. These latter cells may be more important in causing nerve damage *in vivo*, given the predilection of *M. leprae* for Schwann cells, and the dominant role of $CD4^+$, serine esterase$^+$ Th1 cells in the lesions of leprosy. Antagonism of the molecular interactions among *M. leprae*, Schwann cells and inflammatory T cells may therefore provide a rational strategy for prevention of damage of Schwann cell and nerves in leprosy.

Leprosy reactions and nerve damage

Leprosy is the leading cause of all non-traumatic peripheral neuropathies worldwide.[1] Although the implementation of multidrug therapy (MDT) has caused a major decline of the prevalence of leprosy worldwide,[2] the new-case detection rate has remained stable at a level of approximately 600,000 annually.

It is well established that host immunity to *Mycobacterium leprae* dictates the clinical outcome of leprosy. It is believed that more than 99% of the population develop adequate protective immunity upon infection, and do not develop clinically detectable symptoms,[3,4] and only a minority of the infected individuals develop clinical leprosy.

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Superimposed on the leprosy spectrum, leprosy reactions, which represent acute inflammatory episodes, can occur. Two major types of reactions are distinguished: i) erythema nodosum leprosum (ENL) (type II reactions), which occur predominantly in patients at the lepromatous end of the spectrum; and ii) reversal reactions (RR) (type I reactions), which occur particularly in patients with borderline leprosy, especially during treatment.[5] Type I reactions are thought to represent episodes in which cell-mediated responses to *M. leprae* are greatly enhanced, resulting in an inflammatory response in the areas of skin and nerve affected by the disease.[6] Estimates of the prevalence of RR in leprosy range from eight to 30%.[7]

The enhanced cellular immune responses may be beneficial in terms of clearing bacteria, as they strengthen the mechanisms by which the organisms are killed. However, the accompanying inflammation in and around infected nerve tissue, or around nerves in close proximity to inflamed lesions can result in severe and irreversible damage within a matter of days, if treatment is inadequate. Clinically detectable neural involvement occurs in approximately 10% of patients with paucibacillary (PB) leprosy and 40% of those with multibacillary (MB) disease, particularly in patients with RR.[8] It has been suggested that subclinical neural involvement may occur in virtually all patients with leprosy, but that some 30% of the nerve fibres must be destroyed before sensory impairment becomes detectable.[9]

A nerve fibre consists of an axon that is almost completely enveloped in a sheath of Schwann cells. Axons are either myelinated or unmyelinated. Myelinated peripheral axons possess a myelin sheath, interposed between the Schwann cells and the axon (Figure 1a), that is derived from the Schwann cells themselves. Unmyelinated axons, which lack a myelin sheath, lie in deep grooves on the surface of the Schwann cells, with multiple axons enveloped by the same cell (Figure 1b). Externally, Schwann cells are covered by a basal lamina, which, in turn, is surrounded by endoneurial tissue. Several Schwann cell-axon units, which are embedded in endoneurium, are surrounded by the relatively impermeable perineurium, which consists of randomly oriented and highly concentrated collagen fibres. Tight junctions between the endothelial cells of the capillaries and the endothelial basement membrane separate the endoneurium from the circulation. The resulting physical 'blood-nerve barrier' is believed to be important in maintaining an appropriate physicochemical environment for the axons, and in protecting them against potentially harmful agents, including effector cells of the immune system. Nevertheless, the junctions can provide a route through which bacteria or leukocytes can ultimately enter the peripheral nervous system.

M. leprae—Schwann cell interactions

M. leprae almost exclusively infects macrophages and Schwann cells.[10] Various receptor-mediated mechanisms, similar to those exploited for invasion of macrophages, may play a role in the invasion of human Schwann cells by mycobacteria. Candidates are Fc receptors,[11] complement receptors,[11,12] the fibronectin binding protein,[13] and mannose receptors.[14] However, these mechanisms are not restricted to Schwann cells, and thus do not explain why *M. leprae* specifically homes to neural tissue. The neurotropism of *M. leprae* may be attributed to its affinity for the G-domain of the α-chain of laminin-2, an extracellular matrix protein that is present in the basal lamina of Schwann cells.[15] In turn, *M. leprae*-laminin-2 complexes bind to α/β-dystroglycan complexes expressed on the surface of the Schwann cell.[16] Recently, a 21 kDa laminin-binding receptor, a histone-like protein (HLP), has been

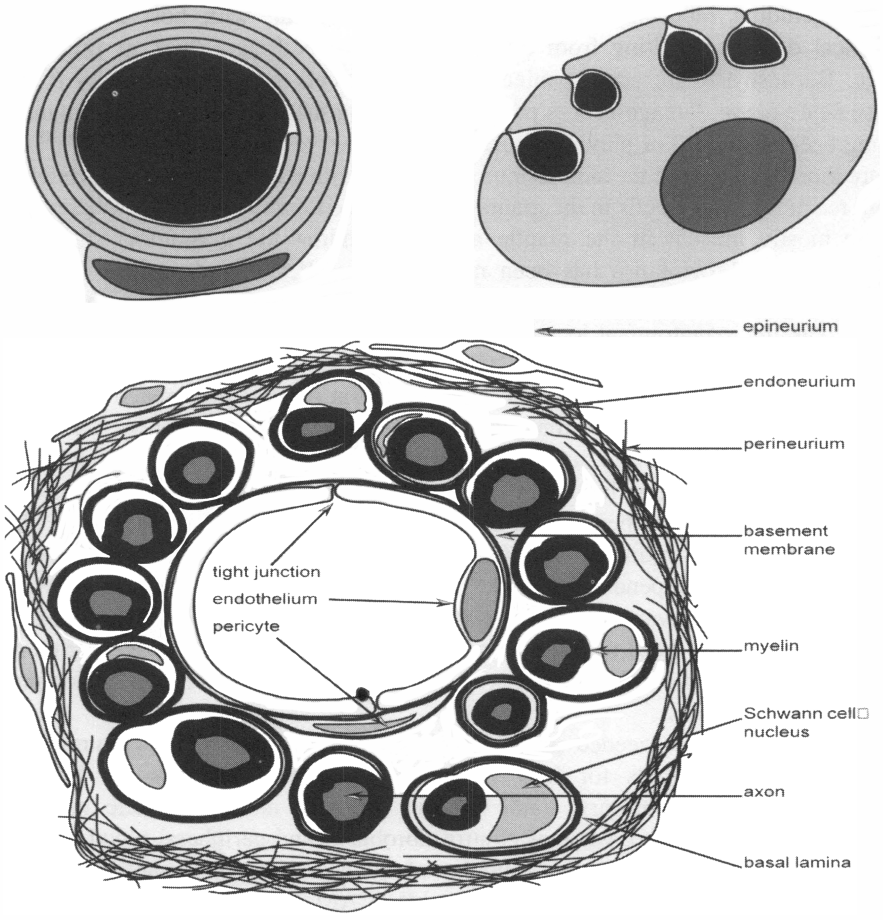

Figure 1. Myelinating (a) and non-myelinating (b) Schwann cells and the structure of peripheral nerves (c) (see text for a complete description of this figure).

identified on *M. leprae*.[17,18] This mycobacterial receptor may function as a critical surface adhesin for the G-domain of the laminin-α2 chain.

Although these interactions may play a major role in the attachment of *M. leprae* to Schwann cells, and, perhaps, in the invasion of the Schwann cells by the organisms, it is not unlikely that other receptors may also be involved. Indeed, blocking of the dystroglycan complex could not completely inhibit the adhesion of *M. leprae* to Schwann cells.[16] Other candidate (co)receptors may include integrins, which are also able to bind to laminin-2, and an as yet uncharacterized 25-kDa phosphoprotein expressed by Schwann cells, to which *M. leprae* has been reported to bind.[19] Thus, other receptor–ligand interactions may also be involved in *M. leprae*–Schwann cell attachment and invasion.

T cell-mediated and Schwann cell damage

Several pathogenetic mechanisms may be responsible for the nerve damage induced by

leprosy, including biochemical interference by *M. leprae* with host cell metabolism, mechanical damage resulting from the large influx of cells and fluid, or immunological damage. Because RR are accompanied by increased cell-mediated immunity (CMI), and because acute nerve damage occurs particularly during these reactions, the immune system has long been suspected of playing a role in causing nerve damage during RR.[6,20] $CD4^+$ T cells are more abundant in the skin lesions of patients with RR, compared to those of patients without reactions. The T cells in the granulomata are predominantly $CD4^+$, whereas $CD8^+$ T cells are mostly present in the mantle area surrounding the granuloma.[6,21,22] A strong increase of type-1 cytokines has been noted during RR,[22,24] and, indeed, T-cell clones isolated from skin biopsy specimens of patients with RR are predominantly of the Th1-type.[25,26] The microanatomical location of serine esterase$^+$ cells within tuberculoid granulomata and RR overlaps with the $CD4^+$ $CD45RO^+$ subpopulation,[22] indicating that these T cells contain cytotoxic granules. Furthermore, analysis of *M. leprae*-reactive, $CD4^+$ cytotoxic T-cell clones has confirmed that these cells are indeed highly cytotoxic for a variety of human target cells.[27] Therefore, it is likely that Th1-like cytotoxic T cells may contribute not only to protective immunity, but also to the immunopathology of leprosy neuritis, in which Schwann cells function as their target. Murine Schwann cells have already been shown to function as antigen-presenting cells for $CD8^+$ cytotoxic T cells in an MHC class I-restricted, mycobacterial antigen-dependent manner;[28] as a result of antigen-recognition, Schwann cells were killed. Rodent Schwann cells were also able to stimulate $CD4^+$ T cells via MHC class II.[29,30] To what extent these rodent studies can be extrapolated to leprosy neuritis remains unknown, largely because of the inability to culture human Schwann cells, which precludes such analyses in humans.

We have recently succeeded in establishing human Schwann cell cultures that yield sufficient numbers of cells for further studies. These cells express the Schwann-cell markers S-100β, glial fibrillary acidic protein (GFAP), and 2′,3′-cyclic nucleotide-3′-phosphohydrolase (CNPase), whereas control fibroblasts and peripheral blood mononuclear cells do not express these molecules. A number of molecules involved in antigen-presentation and T-cell stimulation, such as MHC class I and II, ICAM-1, and CD80, were also detectable (Spierings, *et al.*, unpublished data, 1997). The expression of HLA class II on human Schwann cells *in vivo* has been a point of controversy. Although a number of studies have failed to demonstrate expression,[31–33] others have reported HLA class II molecules on Schwann cells.[34,35] Furthermore, such inflammatory cytokines as interferon-γ (IFNγ) and tumour necrosis factor-α (TNFα), as well as invasion of Schwann cells by *M. leprae*, have been reported to result in up-regulation of MHC class II by rodent Schwann cells.[36–38] In leprosy patients, Schwann cells were found to express MHC class II.[39] Therefore, by presenting *M. leprae* antigens to cytotoxic T cells, Schwann cells may well be actively involved in the immunopathology of leprosy neuritis. Thus, although human Schwann cells may not express HLA class II molecules under normal conditions, infection with *M. leprae* or the subsequent local immune response probably induces expression of MHC by human Schwann cells.

These human Schwann cells indeed take up bacteria, as confirmed by confocal microscopy (data not shown). More recent results from our laboratory show that human Schwann cells can process and present intact *M. leprae* and *M. leprae*-antigens to $CD4^+$ cytotoxic T cells (Figure 2). Presentation induces proliferation of T cells and production of IFNγ (data not shown). Importantly, Schwann cells pulsed with *M. leprae* are highly susceptible to killing by type-1 $CD4^+$ T-cell clones from leprosy patients. Schwann cell killing was antigen-

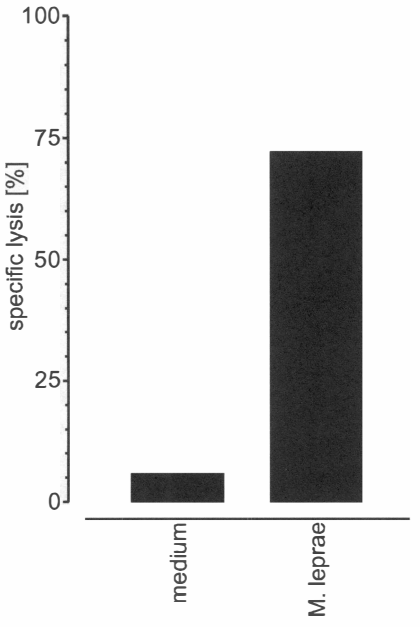

Figure 2. Presentation of mycobacterial antigens by human Schwann cells. Human Schwann cells were loaded with and without *M. leprae*, and co-incubated with $CD4^-$ cytotoxic T cells, which subsequently lysed the Schwann cells in an antigen-dependent fashion.

dependent and HLA class II-restricted (data not shown). These results show clearly that human Schwann cells can process and present *M. leprae* to *M. leprae*-specific $CD4^+$ cytotoxic T cells, and are subsequently killed. Particularly during RR mediated by inflammatory, $CD4^+$ T-cells, this mechanism may play an important role in causing local peripheral-nerve damage in leprosy. We propose that this represents a novel immunopathogenetic mechanism of nerve damage in leprosy.

Nerve destruction as collateral damage

In addition to nerve damage resulting from cognate T cell-Schwann cell interactions, non-specific bystander effects during inflammation may also be involved in triggering nerve damage. Possible mediators are TNFα, proteases, and urokinase.[40] With respect to leprosy, TNFα mRNA and protein are more abundant in lesions of patients with RR.[24,41] TNFα is predominantly produced by *M. leprae*-responsive, type-1 T cells derived from patients undergoing RR,[25] but infected and activated macrophages can also be responsible for the production. TNFα alone has little toxic effect on Schwann cells, but, in combination with transforming growth factor (TGF)-β, it has been reported to cause significant detachment and lysis of Schwann cells.[42] Little is known about the effect of TNFα-mediated target-killing on mycobacterial survival. TNFα-mediated lysis has been reported to have an effect on mycobacterial viability similar to that of FAS/FAS-L mediated lysis,[43] but this observation is not universally accepted with respect to either FAS or TNFα.

M. leprae-specific versus autoreactive T cells in leprosy lesions

At present, which antigens are recognized by lesional T cells is unknown. *M. leprae*-specific T cells can be isolated from both inflamed skin[25] and nerve tissue (Spierings *et al.*, unpublished data, 1997). It is possible that nerve damage may be caused or enhanced by autoreactive T cells, in addition to *M. leprae*-specific T cells. Such autoreactive T cells might be primed by cross-reacting mycobacterial antigens (molecular mimicry). An obvious candidate auto-antigen is HSP60. Mycobacterial HSP60-reactive murine T cells are indeed able to lyse uninfected macrophages[44,45] and IFNγ-stressed target cells.[46] Furthermore, transfer of an HSP60-specific, $CD8^+$ T cell clone into immunocompromised mice leads to severe immunopathology.[44,47] These data indicate that presentation of peptides from endogenous proteins can be responsible for cell-mediated, autoimmune-like destruction of tissue. However, it is also possible that, during inflammation, T cells are primed against autoantigens associated with non-cross-reacting Schwann cells or nerve, perhaps by cross-priming, and that such autoreactive T cells can contribute to the peripheral neuropathy of leprosy. Analysis of the antigen-specificity of the T cells that infiltrate lesions will be needed to address the role of self-antigen versus recognition of *M. leprae* antigens in the immunopathology of leprosy neuritis.

Conclusions

Human Schwann cells may play a central role in the nerve damage of leprosy. Destruction of

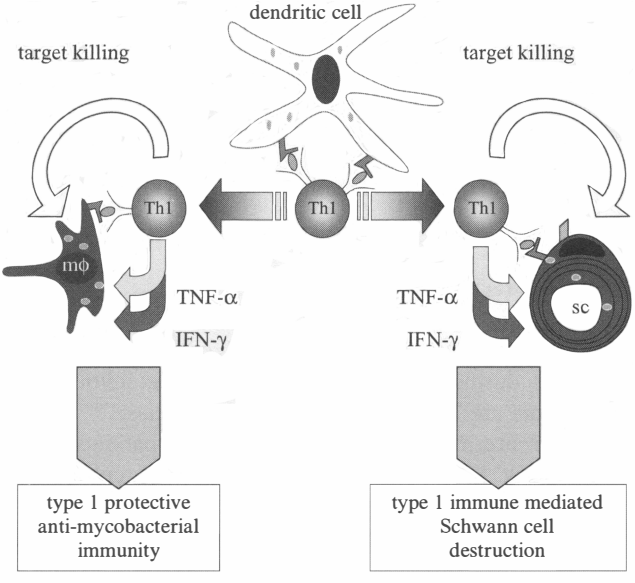

Figure 3. The potentially harmful effect of protective immunity against *M. leprae*. T-helper-1 (Th1) cells are primed by dendritic cells (ds) that are loaded with *M. leprae*. These T cells are able to attack *M. leprae*-infected macrophages (MΦ). In this way, T-helper cells may contribute to elimination of the organisms. On the other hand, the same T cells may recognise *M. leprae* presented by the Schwann cells (sc). Similar to the situation with macrophages, recognition may result in killing of infected Schwann cells, thus leading to nerve damage.

Schwann cells, and, subsequently, of the nerves they surround, probably results not only from collateral damage, but also from the direct effect of $CD4^+$, cytolytic T cells. These T cells are instrumental in controlling the multiplication of *M. leprae* in lesions by activating *M. leprae*-infected macrophages, in addition to killing the organisms. Unfortunately, however, when infected Schwann cells are killed as well, this may lead to nerve damage, which may progress to irreversible loss of peripheral nerve tissue (Figure 3). Although this process can involve both $CD8^{+28}$ and $CD4^+$ (Spierings *et al.*, unpublished data, 1999) cytotoxic T cells, the latter cells may be particularly important, because $CD4^+$ T cells are present in large numbers in the centre of the granulomata of patients with RR.

Acknowledgements

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DISCUSSION

Dr Cole: What are your controls when you study *M. leprae* in Schwann cells. If you employ a mycobacterium that is not known to invade Schwann cells, do you obtain the same results?

Dr Ottenhoff: Yes, although it is not as efficient as when *M. leprae* is employed. *M. smegmatis* also enters Schwann cells, although not as well as does *M. leprae*. We have used all manner of controls including MHC-mismatched combinations, proteins, peptides, and bacteria. The system is truly specific.

Dr Gillis: Is there any evidence that T cells attack Schwann cells *in vivo*?

Dr Ottenhoff: There is the circumstantial evidence of the co-existence of T cells and macrophages with nerve bundles in inflammatory sites. But we have never examined the lesions to learn what occurs there.

Dr Lockwood: The accumulation of T cells on the axon, suggesting a perineuritis, is very striking.

Professor Britton: Do Schwann cells express MHC Class II when they grow in culture in the absence of any stimulation? Or do you culture them in the presence of IFNγ or any other activators?

Dr Ottenhoff: We culture the Schwann cells in conditioned medium, i.e. medium to which has been added supernatant from a cell culture that is rich in IL-2, among other substances. However, if you culture the cells in medium depleted of IFNγ for 2 weeks, the Schwann cells still express MHC class II, although to a lower level. Thus, the cells express a basic, low level of MHC class II, and can be induced to express a much higher level. It has also been shown that infection by *M. leprae* can induce MHC class II expression.

Dr Sampaio: Do you believe that the neuritis of reversal reaction and that of ENL share a common mechanism, or are the mechanisms different?

Dr Ottenhoff: An increase of CMI has been reported in the lesions of ENL, so I should think that the same mechanism could operate in both types of reactions.