Challenges presented by nerve damage in leprosy

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Summary The basis of nerve damage in leprosy is the unique tendency of Mycobacterium leprae to invade Schwann cells. αβ-Dystroglycan on the basement membrane of Schwann cells binds to laminin α2, in turn binding to receptors on the M. leprae surface, comprising a histone-like protein and phenoglycolipid-1. When nerve damage during reversal reactions was found to be associated with an abrupt increase in delayed type hypersensitivity against M. leprae antigenic determinants released from Schwann cells, it suggested that the nerve is damaged as an innocent bystander during the immune response. This strongly influenced the introduction of therapy based on immunosuppression combined with continued anti-mycobacterial medication. Lysis of Schwann cells presenting M. leprae antigenic determinants by activated CD4+ T cells and interaction of M. leprae with Toll-like receptors on Schwann cells are additional mechanisms implicated in nerve damage. Persistence of M. leprae antigen in local lesions after regular multiple drug therapy (MDT) is an important risk factor for late reactions. In spite of significant advances in the provision of MDT globally, early diagnosis, together with effective treatment of the disease and associated nerve damage at initial presentation remains a major challenge for the health services. Reduced prevalence as a result of MDT should not be taken to indicate that the challenges of leprosy control are diminished as long as nerve damage is not controlled and new case detection rates are not declining.

Introduction

Nerve damage, the hallmark and most serious consequence of leprosy, usually develops in connection with immunological reactions of two distinct types. In type 1 reversal reactions in borderline leprosy, delayed type hypersensitivity (DTH) is a central feature. In erythema nodosum lepromatous leprosy (ENL) towards the lepromatous end of the spectrum, type 2 reaction
is considered as a prototype of immune complex disease, but DTH also plays an essential role.

This review focuses on the importance of immune reactions and their molecular mechanisms in reactions and nerve damage. Increased understanding of pathogenetic mechanisms has been essential for development of new principles in therapy and prophylaxis. This reinforces the requirement and advantage of basic laboratory studies closely associated with regular patient care, as illustrated by joint studies at the Armauer Hansen Research Institute (AHRI) and the All Africa Leprosy, Tuberculosis, Rehabilitation, Research and Training Centre (ALERT) in Addis Ababa, Ethiopia.

Affinity of *Mycobacterium leprae* for nerves

The central feature behind development of nerve damage in leprosy is the unique ability of *M. leprae* to invade the peripheral nervous system. The basic mechanisms of this interaction remained a mystery for decades even though the clinical features of leprous neuropathy were well known. Essential features of the interaction were revealed stepwise during the last decade defining a link between the bacillus and its main habitat, the Schwann cells of peripheral nerves.

The first step in revealing the mechanism was to show that *M. leprae* binds to the G domain of the laminin α2-chain (LN-α2), which is expressed on the surface of the Schwann cell-axon unit. In contrast to other isoforms, the LN-α2 chain has a limited tissue distribution on Schwann cells, striated muscle and the trophoblast of the placenta, correlating with sites of natural *M. leprae* infection.

It was then shown that αβ-dystroglycan (DG) in the basal lamina acts as a Schwann cell receptor for laminin-α2/M. leprae complexes. Other receptors may also be involved in the *M. leprae*-Schwann cell interaction, since blocking of the DG complex with purified αDG in competition assays did not completely inhibit adhesion of *M. leprae*.

The third step was to define the receptor on *M. leprae* accounting for its binding to laminin-2, being primarily shown to be a 21 kDa histone-like protein. This protein, LBP21, coded by the ML1683 gene, is a major surface-exposed antigen on *M. leprae*, probably serving as an adhesin for its interaction with peripheral nerves. Details of this interaction remain to be further elucidated since the LBP21 protein of *M. leprae* shows extensive homology with a histone-like protein in other mycobacteria. Minor amino acid differences in the C-terminal part of the polypeptide chain probably account for the high specificity of *M. leprae* binding to the LN-α2 chain.

In addition, the terminal trisaccharide of the abundant surface-exposed *M. leprae* specific phenoglycolipid 1 (PGL-1) has been shown to bind to laminin-2. Thus PGL-1 is also involved in the invasion of Schwann cells through the basal lamina in a laminin-2-dependent pathway. It is thought to act as a second receptor on *M. leprae* in which the combined action of LBP21 and PGL-1 appears to provide sufficient energy of binding to ensure safe entry of *M. leprae* into the Schwann cell.

Mechanisms of nerve damage during reactions

Reversal reactions are associated with an abrupt increase in cell mediated immune reactions to mycobacterial antigenic determinants, initially demonstrated by lymphocyte culture in
vitro by Godal et al. and Bjune et al. Histologically, the lesions are invaded by mononuclear cells associated with edema and hyperemia. These observations led to the view that DTH reactions were induced against M. leprae antigenic determinants released from Schwann cells, leading to damage of the nerve as an innocent bystander. At the time, these combined laboratory and clinical observations strongly influenced introduction of new therapeutic principles based on immunosuppression combined with continued anti-mycobacterial chemotherapy.

These views remain valid today. In further experiments, rabbits were immunized with M. leprae followed by injection of M. leprae sonicate into the sciatic nerves at the peak of hypersensitivity. Three days later histological examination showed that mononuclear cell infiltration and axonal degeneration occurred as a consequence of hypersensitivity to intraneural M. leprae antigenic determinants.

Later, additional readouts further emphasized a marked increase of type 1 cytokines and DTH reactivity in skin lesions during reversal reactions. TNF-α mRNA and TNF-α protein have been demonstrated in nerves in reversal reactions by in situ hybridization and monoclonal antibody techniques. TNF-α mRNA is more abundant than TNF-α protein, which is thought to reflect the rapid turnover of TNF-α protein in this ‘immunologically dynamic situation’. The M. leprae antigenic determinants involved in reversal reactions have later been studied by immunohistochemistry. The 28 kDa antigen, ML0091, was present most strongly in the lesions. Lipoarabinomannan (LAM) also stained strongly and persisted after treatment. Additional information on the specific antigenic determinants involved is urgently needed. Cell mediated autoimmunity against similar antigenic determinants in host cells and M. leprae is also a distinct possibility needing further study.

Contact-dependent demyelination induced by M. leprae in nerve tissue culture in the absence of immune cells has been described by Rambukkana et al. suggesting a role of nonimmune mechanisms during the initial stage of infection and nerve involvement in leprosy. In a recent review this view is further expanded. Two experimental systems were used, a myelinating Schwann cell-dorsal root ganglion (DRG) neuron coculture system, and immunodeficient Rag1 knockout (Rag1 -/-) mice which lack mature B and T lymphocytes, as in vitro and in vivo models, respectively. It remains uncertain, however, whether or to which extent these models are good correlates for leprosy. On the other hand, combined clinical and laboratory studies point strongly to a predominant role of delayed type hypersensitivity, and thus of adaptive immunity in further development of the infection with induction of clinical symptoms of nerve damage during reactions. The latter view is further substantiated by the pronounced effect of immunosuppressive therapy during reactions with neuritis.

Type 2 reactions in ENL occur against a background of the lepromatous end of the spectrum, and they have been considered as a prototype of immune complex disease. During development of acute ENL with a type 2 cytokine pattern in eight patients a selective dynamic increase in IL-6, IL-8 and IL-10 mRNA and persistent IL-4 and IL-5 mRNA expression was found in the lesions. Chronic ENL often involves nerve damage, probably induced by local immune complex deposition with granulocyte attraction leading to tissue damage, and complement activation. The role of autoimmunity is still discussed. Antibodies to neural antigens occur with increased frequency in ENL, but may be an epiphenomenon.
Schwann cells as antigen presenting cells

The presence of MHC class II protein on the cell surface determines whether a particular cell type can induce immune responses in naïve T cells. Initially, HLA class II antigen was not found on Schwann cells in tissues of normal controls but was demonstrated in nerve biopsies from patients with peripheral neuropathies. In rats, INF-γ and TNF-α induce class II expression on Schwann cells. Early studies in leprosy reported both negative and positive findings.

Another hypothesis for an ‘immunopathogenic mechanism of damage to Schwann cells and peripheral nerves in leprosy’ is that infected Schwann cells process and present antigenic determinants of  M. leprae  to antigen-specific, inflammatory type-1 T cells, and that these T cells subsequently damage and lyse infected Schwann cells.

In a murine model, CD8+ T cells were shown to recognize and lyse Schwann cells presenting  M. leprae  antigen in the context of class I gene products. New experimental findings were made by Spierings et al. regarding CD4+ T cell mediated lysis of human Schwann cells presenting  M. leprae  antigenic determinants. In long term cultures human Schwann cells were shown to express MHC class I and II, ICAM-1, and CD80 surface molecules involved in antigen presentation. Human Schwann cells processed and presented  M. leprae  as well as recombinant  M. leprae  proteins and peptides to MHC class II-restricted CD4+ T cells, and were efficiently killed by these activated T cells. These findings elucidate another mechanism probably involved in killing of Schwann cells, resulting in nerve damage during reversal reactions.

Influence of innate immunity and Toll like receptors

The Toll-like receptor (TLR) family has been extensively conserved throughout evolution and is essential in innate immune responses and protection against infection from insects to humans. In turn it is also a major determinant of adaptive immune responses in vertebrates. TLRs function as ‘pattern recognition receptors’ that recognize various broad classes of microbial ligands. TLR-2 has been shown to recognize mycobacterial lipoproteins. TLR-2 and TLR-1 are more strongly expressed in lesions from tuberculoid than lepromatous leprosy.

A mutation substituting arginine with tryptophan at residue 677 in one of the conserved regions of TLR-2 was shown to have a role in susceptibility to lepromatous, but not tuberculoid leprosy. Later, this mutation was shown to abolish intracellular signaling and activation of NF-κB after exposure of different cell types to  M. leprae  and  M. tuberculosis. Oliveira et al. demonstrated TLR-2 on the human Schwann cell line ST88-14 and on Schwann cells in skin biopsy specimens from leprosy patients. Activation of Schwann cells in vitro with a synthetic lipopeptide of the putative  M. leprae  19 kDa lipoprotein LpqH, ML1966, triggered nuclear apoptosis, and Schwann cells in leprosy lesions were also demonstrated to have undergone apoptosis. These observations indicate that activation of TLR-2 on Schwann cells contributes to nerve damage in leprosy and are of great interest when comparing the significance of innate and adaptive immunity for development of nerve damage.

Since  M. leprae  is an obligate intracellular parasite, T cell mediated immune reactions are essential for protection after infection. Depending on the kind and quantity of cytokine
release during these reactions, T cells may also induce tissue damage, and T cell mediated immune responses may therefore be considered as a double-edged sword. Innate immunity is also involved in protection as well as tissue damage. Improved understanding of this dichotomy is a main challenge for current work on mycobacterial infection, essential questions being very similar in leprosy and tuberculosis.42,43

Nerve damage after cessation of regular multiple drug therapy (MDT)

The ALERT MDT Field Evaluation Study (AMFES) in Ethiopia began in 1988 and has involved follow-up of 594 new patients for 6–11 years after start of MDT, including 6-monthly assessments of nerve function. Compared with similar studies in India and Bangladesh, the Ethiopian cohort presented late, had a very high rate of disability at diagnosis (55% for grades 1 and 2 combined), a high rate of multibacillary disease (51%), and a high rate of subsequent neuropathy (43%). Acute neuropathy had a very good prognosis when treated with a standard course of steroids; full recovery was observed in 88% of nerves.44 This striking response to steroids may be explained by being a select group with high frequency of disability already at diagnosis compared with the usual response rates to steroids of 44–60%.45,46 In the Ethiopian cohort, chronic and recurrent neuropathy had a worse prognosis, requiring early identification and careful management with new steroid regimens or new drugs.44

The incidence of reversal reactions was highest during year 1 and declined steadily after start of treatment, but the first episode occurred as long as 5 years after diagnosis in both paucibacillary (PB) and multibacillary (MB) patients.47 Reversal reaction may occur as late as 16 years after beginning antibacterial treatment with dapsone and rifampin in BL leprosy.48

In the AMFES study, 16 patients (5·3% of 300 new MB patients) had ENL reactions. In general ENL reactions appeared later, the incidence being highest in the 2nd and 3rd year after start of treatment. Ten (3·3%) of the 16 cases had recurrent episodes, and five (1·6%) had at least five episodes occurring over a period of more than 2 years.49

Late reactions occurring after termination of MDT according to standard regimens merit increased attention, and they should be studied in depth clinically and with up-to-date technologies in immunology and molecular biology. In PB leprosy, differentiation between reaction and relapse is difficult regarding diagnosis, and further studies are clearly needed to better understand the underlying pathogenesis.

After completion of MDT according to standard regimens, WHO recommends that the term ‘leprosy patient’ should no longer be used for recording of individual cases.50 The patients are considered cured of the infection and are referred to as ‘people affected by leprosy’. This is done to decrease the stigma of leprosy emphasizing that leprosy is a curable infectious disease, but implies a risk of decreased attention to late occurring reactions ending in deformity. Care after cure needs to be promoted equally. A consistent strategy should be sought to treat cases of neuritis, reversal reaction and ENL after release from treatment at completion of MDT. It is especially important to teach health professionals how to recognize neuritis and to uphold prevention of disability work.

During effective treatment *M. leprae* disappears from the lesions indicated by decreasing numbers of acid fast stained bacilli. When no acid fast stained material can be demonstrated, immunohistochemical examination still reveals *M. leprae* antigen, lipoarabinomannan
Information on other defined antigenic constituents is still limited, but *M. leprae* antigenic determinants persist in the lesions for a long time with a risk of renewed stimulation of the immune system and corresponding clinical symptoms of reaction even after most of the bacilli are killed.\(^{51}\)

**Current leprosy epidemiology**

After application of MDT for more than 20 years the number of registered leprosy patients, i.e. the prevalence, has decreased dramatically.\(^{52}\) This has in part been influenced by a change in definitions used for classification, registration, and length of treatment of leprosy patients. In Ethiopia, a marked decrease in prevalence was seen from 1983 to 1996, with the curve then leveling off,\(^{53}\) and similar curves have been documented in many countries.\(^{54}\)

The incidence of infection with *M. leprae* is difficult to assess since *M. leprae* has a long generation time and the infection develops slowly, often with an insidious onset of clinical symptoms. Indirect methods have to be used, and the number of new cases is often used as a main indicator.\(^{55}\) Recently Meima et al. constructed complete time series of leprosy case detection rates for 1985–1998 for a group of 33 high-endemic countries contributing 99% and 98% to global case detection in 1994 and 1998, respectively. The contribution of the 33 high-endemic countries to leprosy case detection rates hardly changed over the 1985–1998 period studied, and their conclusion is: ‘There is no general decline in case detection to date, and several important countries still have high case detection rates. Prevalence is an irrelevant indicator for monitoring epidemiological changes in leprosy’.\(^{56}\) For a communicable disease like leprosy, a decline in prevalence while the new case detection curves are flat indicates that the infection is still transmitted at community level which is a matter of grave concern.\(^{53,54,57,58}\)

In Ethiopia, grade 2 disability is found in about 15% of new leprosy cases, which shows that patients are diagnosed late.\(^{59}\) Thus, early recognition with adequate treatment of the disease and associated nerve damage at initial presentation remains a major challenge of the health services in our time. A skewed emphasis on decreased prevalence rates alone may underestimate the challenges of early case detection, care after completion of MDT with appropriate and early management of neuritis, and rehabilitation in leprosy control work.

**Emerging opportunities**

Because of its significance, validation of the number of new cases is extensively discussed, and it has been claimed that the numbers may be inflated due to wrong diagnosis, re-registration of old cases, and non-existent cases being defined as patients.\(^{60}\) A new generation of *M. leprae* related reagents is now being developed based on comparison between the *M. tuberculosis*, *M. leprae* and other mycobacterial genomes,\(^{61}\) and they are expected to provide an unprecedented level of specificity for diagnosis of infection.

Application of these in different epidemiological settings should have high priority, e.g. in Brazil and India which are two high endemic countries, and in Indonesia where the goal of a prevalence <10/10,000 has been reached at national level although pockets of high endemicity remain.\(^{62}\) Polymorphic short tandem repeat (STR) loci now also permit molecular discrimination between isolates of *M. leprae* to track the transmission.\(^{63}\) More sensitive and specific diagnosis of infection will provide a new basis for our understanding of current
leprosy epidemiology with development and application of novel strategies to break transmission, and as a result, better prevention of nerve damage and deformity.

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