chapter 1
general introduction

For other illnesses found here
Wise doctors on the scene appear,
Who understand disease.
To hospital those sick are brought,
And for their plight a cure is sought.
Thus their ills are relieved,
And all their wounds are dressed.

We lepers can no doctors get:
Here must we stay and wait and fret,
Until our time is up.
Peter from prison did escape
Because on God's grace he did wait.
O God, break now the chains
Which bind our limbs with pains.

excerpt from “En Klagesang”, by Peder Olsen Feidie,
patient in St. George's hospital for lepers in Bergen from 1832 to 1849.

leprosy

a major health care problem

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. Although leprosy was found almost worldwide in the past, the disease currently occurs mainly in tropical and subtropical countries. In endemic regions, the prevalence rates can exceed 5 per 10,000. The most prominent feature of leprosy is involvement of the skin and nerves, which can lead to severe physical deformities of hand, feet, face and eyes, and permanent disabilities. Overall, 2 to 3 million individuals are disabled as a result of the disease (Noordeen 1994). This is the main reason that leprosy represents a major social stigma. The introduction of multiple drug therapy (MDT) in 1982 has caused a major decline in the prevalence of leprosy (World Health Organization 1997). The estimated number of leprosy patients has declined from 10-12 million in 1985 to less than 2 million in 1995 as a result of this (World Health Organization 1995; Noordeen 1995). Despite the drop in prevalence, the number of newly detected cases of leprosy worldwide has remained stable at a level of approximately 600,000 new cases annually, thus creating a large population at risk of developing nerve damage (Smith 1997).

the bacillus

The causative agent of leprosy, *M. leprae*, is a virtually non-toxic, obligatory intracellular bacterium, which particularly infects macrophages and Schwann cells. G.A. Hansen discovered the bacterium as early as 1874 (Hansen 1874), but it is still impossible to culture the bacterium *in vitro*. The genus 'Mycobacterium' contains gram-positive bacteria, with a characteristic cell wall composition. The most characteristic feature is the presence of mycolic acids that reduce the permeability of the mycobacterial cell wall and are also responsible for the acid fast staining of the bacilli (Bishop and Neumann 1970; Jarlier and Nikaido 1994). Although mycobacteria share a number characteristics, *M. leprae* has some unique features, including the presence of phenolic glycolipid I (PGL-1) (Hunter and Brennan 1981; Hunter *et al.* 1982). These unique components may have the potential to detect *M. leprae* infected patients specifically (Lal *et al.* 1993; Luna-Herrera *et al.* 1996; Buhrer *et al.* 1998).

an immunological disease

Host immunity to *M. leprae* plays an important role in the clinical manifestations of the disease. More than 99% of the population is believed not to acquire the disease after having been in contact with the bacillus, due to protective immunity (Godal *et al.* 1972; Godal and Negassi 1973). Individuals that develop clinical disease can be classified into a 5 group spectrum (figure 1) (Ridley and Jopling 1966). On one side of the leprosy spectrum are lepromatous leprosy patients, which have a high humoral immune response and a low cellular response. These patients show an *M. leprae* specific cellular non-responsiveness and fail to clear the bacteria. On the other side of the spectrum are tuberculoid leprosy patients, which have a low humoral immune response but display both acquired cellular immunity and delayed type hypersensitivity against *M. leprae*. The bacterial load in these patients is low. The majority of the patients is positioned in between these two poles and is categorized as borderline lepromatous, borderline and borderline tuberculoid leprosy.

leprosy reactions

Superimposed on the leprosy spectrum, leprosy reactions can occur. Leprosy reactions can occur before treatment (Naafs and Wheate 1978), but are mainly observed

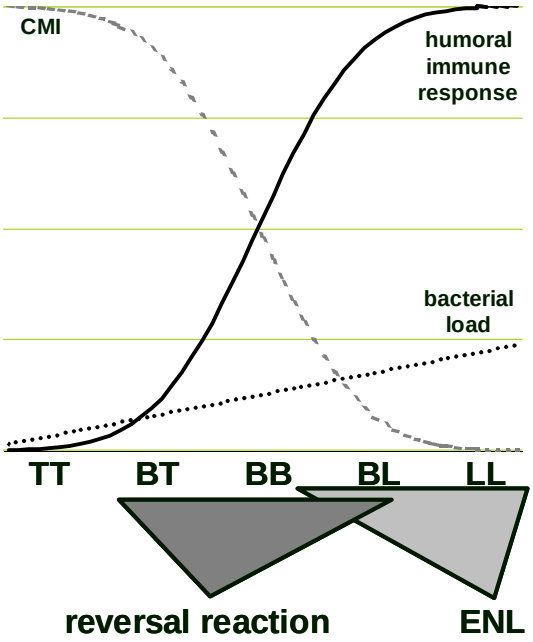

figure 1: the leprosy spectrum

during or after treatment and typically represent acute inflammatory episodes. Two commonly recognized types of leprosy reactions can be discriminated; 1) erythema nodosum leprosum (ENL) or type II leprosy reactions, which can occur in patients on the lepromatous side of the spectrum, and 2) reversal reactions (RR), also designated as type 1 leprosy reactions. The latter type occurs almost exclusively in patients of the borderline area of the spectrum, particularly during treatment (Lockwood *et al.* 1993). Reversal reactions are thought to represent episodes in which cell-mediated responses towards *M. leprae* are strongly increased, resulting in an inflammatory response in the areas of the skin and nerves affected by the disease (Modlin *et al.* 1983). Estimates of the overall prevalence of reversal reactions range from 8-30% in leprosy (Lienhardt and Fine 1994).

From the point of view of clearing bacteria, upgrading responses might be considered to be beneficial. However, the inflammation in nerve tissue often results in permanent damage and disability within a matter of days, if not treated adequately. Clinical neural involvement occurs in approximately 10% of pauci bacillary and 40% of multi bacillary leprosy patients, particularly in patients with reversal reactions (Richardus *et al.* 1996). It has, however, been suggested that sub-clinical damage takes place in virtually all leprosy patients and that 30% of the nerve fibers has to be destroyed before sensory impairment becomes detectable (Pearson and Ross 1975). Several pathogenic mechanisms may be responsible for the nerve damage in leprosy, like biochemical interference of *M. leprae* with host cell metabolism, mechanical damage due to the large influx of cells and fluid, or immunological damage. Since reversal reactions are accompanied by an increased cell mediated immunity (CMI), a role for the immune system in causing nerve damage during RR has long been suspected (Modlin *et al.* 1983; Naafs 1989). $CD4^+$ T cells are more abundant in skin lesions of patients with RR compared to lesions of leprosy patients without reactions. The T cells in the granulomas are predominantly $CD4^+$, whereas $CD8^+$ T cells are mostly present in the mantle area surrounding the granulomata (Modlin *et al.* 1983; Narayanan *et al.* 1984; Cooper *et al.* 1989). A strong increase of type-1 cytokines has been noted during reversal reactions (Cooper *et al.* 1989; Yamamura *et al.* 1991; Yamamura *et al.* 1992). T cell clones isolated from skin biopsies of patients with reversal

reactions are predominantly of Thelper (Th)1-type (Verhagen *et al.* 1997; Verhagen *et al.* 1998). The microanatomical location of serine esterase positive cells within tuberculoid granulomas and reversal reactions overlaps with the $CD4^+$ $CD45RO^+$ subpopulation (Cooper *et al.* 1989), indicating that these T cells contain cytotoxic granules. Analysis of *M. leprae* reactive $CD4^+$ cytotoxic T cell clones has confirmed that these cells are indeed highly cytotoxic (Mutis *et al.* 1993a). Thus, Th1 like cytotoxic T cells are believed to play a major role in the immunopathology of leprosy neuritis.

Demyelination and nerve damage could also be caused as a bystander effect of inflammation. Possible mediators are tumor necrosis factor (TNF)-α, proteases, and urokinase (Said and Hontebeyrie-Joskowicz 1992). Thus, besides the well documented protective effect of TNF-α during mycobacterial infections (Kindler *et al.* 1989; Appelberg 1994; Flynn *et al.* 1995; Kaneko *et al.* 1999), TNF-α may also be responsible for the observed pathology (Rook *et al.* 1989; Khanolkar-Young *et al.* 1995). In relation to leprosy, TNF-α mRNA and protein is more abundant in lesions of patients with reversal reactions (Yamamura *et al.* 1992; Khanolkar-Young *et al.* 1995). It is predominantly produced by *M. leprae* responsive type-1 T cells derived from patients undergoing reversal reactions (Verhagen *et al.* 1997), but also infected and activated macrophages can be responsible for the production.

early detection of leprosy reactions

Good markers for early detection of leprosy reactions are still lacking. Measurement of the acute-phase response and the ratio of serum amyloid A/C-Reactive Protein in particular have been suggested to be helpful in the clinical diagnosis of ENL (Hussain *et al.* 1995). Neopterin has recently been reported to be increased in patients with both ENL and RR (Hamerlinck *et al.* 1999). A marker more specific for RR is the presence of anti-PGL-1 IgM antibodies present in serum (Roche *et al.* 1991). Another risk factor correlated with the development of RR is post-pregnancy, since women are particularly susceptible 1 to 3 months after delivery (Rose and Waters 1991). Also the involvement of three or more body areas is related to an increased risk to develop reversal reactions (van Brakel *et al.* 1994). To prevent permanent nerve damage, prediction, early detection, and intervention are required. The treatment of choice for patients with RR is corticosteroids, suppressing detrimental T cell reactivity (Naafs 1996). ENL can also be treated with thalidomide, which strongly reduces TNF-α production in ENL (Barnes *et al.* 1992; Sampaio *et al.* 1993).

immunity against mycobacterial pathogens

T cells

T cells can be distinguished into two subpopulations based on their cytokine production profile. Polarized Th1 and Th2 cells were originally described among mouse $CD4^+$ cells (Mosmann *et al.* 1986). Th1 cells are defined by the production of interleukin (IL)-2, interferon (IFN)-γ and lymphotoxin, whereas Th2 cells are characterized by the production of IL-4 and IL-5. A third subset, designated Th0, secreted both Th1 and Th2 cytokines and is believed to differentiate into either Th1 or Th2 (Street *et al.* 1990). The same subsets have been identified in humans (Wierenga *et al.* 1990; Haanen *et al.* 1991; Romagnani 1991). The balance between Th1 and Th2 plays a crucial role during mycobacterial infections. In patients with tuberculoid leprosy, Th1 cells are dominant and correlate with bacterial clearance, while little or no Th2 cells can be found (Salgame *et al.* 1991; Haanen *et al.* 1991; Mutis *et al.* 1993b; Verhagen *et al.* 1997). Th2 cells have been documented in skin biopsies of patients on the lepromatous pole (Salgame *et al.* 1991; Verhagen *et al.* 1997), whereas they could not be found in peripheral blood (Mutis *et al.* 1993b). During RR, *M. leprae* reactive T cells derived from skin lesions almost exclusively produce IFN-γ and little

or no IL-4 (Verhagen *et al.* 1997).

Recently, a new $CD4^+$ T cell population was described, designated T-regulatory (Tr) (Groux *et al.* 1997). These Tr cells produce IL-10, but not IL-4 and exhibit immunosuppressive features. The presence of Tr cells in leprosy patients has not been analyzed in detail yet. Earlier studies, however, detected T cells in peripheral blood of leprosy patients which had suppressive effects on T cell proliferation and might thus explain non-responsiveness in lepromatous leprosy (Ottenhoff *et al.* 1986a; Mutis *et al.* 1994). These T cells do not suppress by IL-4 and IL-10 and thus may represent another, possibly unique subset of regulatory T cells.

Protective immunity against mycobacteria critically depends on the cell-mediated immune response. Animal studies using knockout models showed that both $CD4^+$ and $CD8^+$ T cells are required to eliminate mycobacteria from the host. $CD4^+$ T cells are essential for protection against tuberculosis, as illustrated by the increased incidence of tuberculosis in HIV patients (Barnes and Modlin 1996), and the increased susceptibility to experimental mycobacterial infections in CD4 -/- (Xing *et al.* 1998) and Major Histocompatibility Complex (MHC) class II -/- (Ladel *et al.* 1995) mouse models. Mycobacterium specific $CD4^+$ T cells expressing a Th1 profile and cytotoxic activity could be isolated from both patients and healthy contacts (Haanen *et al.* 1991; Mutis *et al.* 1993a). This population might therefore not only facilitate the elimination of bacilli by producing cytokines, but may also actively attack infected cells and kill them.

First evidence for the involvement of $CD8^+$ lymphocytes in the protection against mycobacterial infections came from the β2m -/- model, in which MHC class I expression at the cell surface is impaired and $CD8^+$ T cells are almost absent (Flynn *et al.* 1992; Ladel *et al.* 1995). Mortality was higher in β2m disrupted mice after infection with virulent *M. tuberculosis*, compared to wild type mice. Furthermore, cytotoxic $CD8^+$ cells could be demonstrated in tuberculosis patients at low frequencies (Lalvani *et al.* 1998) and in bulk cultures from leprosy patients (Kaleab *et al.* 1990a). The fact that both the MHC class I and class II deficient mice are devoid of protective immunity against mycobacteria illustrate the close cooperation of $CD4^+$ and $CD8^+$ T cells in eliminating mycobacteria.

natural killer cells

Natural killer (NK) cells preferentially kill target cells that do not express MHC class I molecules (Ljunggren and Karre 1985; Piontek *et al.* 1985). This phenomenon can be explained by the presence of NK receptors that inhibit NK cell activation upon recognition of MHC class I (reviewed in (Lanier 1998)). Activated killer cells presumably originate from NK cells and are efficiently induced by *M. leprae* (Kaleab *et al.* 1990a) and *M. bovis* Bacille Calmette-Guerin (BCG) (Wolfe *et al.* 1977; Kaleab *et al.* 1990a; Mizutani and Yoshida 1994). BCG induced killer cells reduce mycobacterial growth in *in vitro* infected macrophages (Denis 1991). Furthermore, NK cells could be isolated from a number of TB patients, healthy *M. tuberculosis* responders as well as non-responders (Restrepo *et al.* 1990). Freshly isolated PBMC from all three groups were tested on the NK sensitive target cell K562. Patients and non-responders showed low target lysis, while healthy responders efficiently killed the target. Cytotoxic activity of PBMC from patients and responders could further be enhanced by pre-incubation with *M. tuberculosis*, whereas this effect was marginal in the non-responder group. It is still unclear which NK/target cell interactions are essential for effective target cell lysis. Expression of Neural Cell Adhesion Molecule (N-CAM), also called CD56, on both target and effector has been hypothesized to be of importance (Lanier *et al.* 1989).

N-CAM expression has also been observed on some $CD4^+$ T cells in relation to multiple sclerosis (Vergelli *et al.* 1996; Antel *et al.* 1998). The inducing agent in this case was myelin basic protein. These T cells were able to kill N-CAM positive oligodendrocytes,

the central nervous system equivalent of Schwann cells, in an antigen independent manner. These studies also revealed that co-adhesion via other molecules, such as CD54 and CD11a, was essential and that homotypic N-CAM interaction alone was not sufficient to establish target lysis. This mechanism may also be involved in leprosy neuritis, since T cells derived from inflamed neural tissue show increased N-CAM expression when compared to peripheral T cells (Kaleab 1992).

mechanisms of killing of mycobacterium infected targets

Effective immunity to mycobacterial pathogens most likely involves lysis of infected cells as well as killing of the invading pathogen. Different mechanisms may be used to reach this goal. After recognition of infected targets via MHC/peptide/T cell receptor (TCR) interactions by $CD4^+$ and $CD8^+$ T cells, or via yet unknown interactions between targets and NK cells, these cells can secrete lytic granules, containing granulysin and perforins. These two components were reported to act in concert in attacking the infected target cells (Stenger *et al.* 1998). Perforin permeabilizes the eukaryotic cell membrane, allowing the granulysin to enter the cells. Granulysin subsequently attacks the intracellular bacteria by altering their membrane integrity, resulting in bacterial death. Knocking out perforin, however, does not alter the capability of mice to control *M. tuberculosis* or BCG infections, indicating that alternative killing pathways are likely to exist (Laochumroonvorapong *et al.* 1997).

Aside to granule-mediated lysis, killing of cells via the FAS or FAS related 'death-receptors' appears to be an important pathway. Interaction between FAS and FAS-L on target and effector respectively, initiates an intracellular cascade that finally results in apoptosis of the target cell (Nagata and Golstein 1995). There has been some debate on the issue whether this pathway also results in elimination of intracellular bacteria. Reduced bacterial viability has been reported by some (Oddo *et al.* 1998), while others see no effect (Stenger *et al.* 1997; Mazzaccaro *et al.* 1998). These latter results are supported by the finding that the course of *M. bovis* BCG infection in FAS-receptor-defective mice was not altered (Laochumroonvorapong *et al.* 1997).

A third mechanism of apoptosis induction is via extracellular Adenosine Triphosphate (ATP) (Zanovello *et al.* 1990). ATP, which may also be produced by immune effector cells, can interact with P2Y, or P2Z receptors on target cells and induce apoptosis (Pizzo *et al.* 1992). Extracellular ATP has not only been shown to kill targets via P2 receptors (Molloy *et al.* 1994), but also to affect the viability of intracellular bacteria (Lammas *et al.* 1997). Interestingly, individuals can be classified into three groups, based upon their response to ATP (Kumararatne *et al.* 1996): some individuals show responses to ATP in the absence of IFN-γ, while others only respond intermediately. The response of the intermediate group can be enhanced by IFN-γ. A third group does respond neither to ATP alone, nor to ATP in combination with IFN-γ. These responses are correlated with the level of P2 receptors on their APC and may thus explain differences in susceptibility to ATP mediated apoptosis between individuals.

TNF-α plays an important role in protective immunity against virulent mycobacteria (Kindler *et al.* 1989; Appelberg 1994; Flynn *et al.* 1995; Kaneko *et al.* 1999). As discussed above, it can also be involved in pathogenicity (Rook *et al.* 1989; Khanolkar-Young *et al.* 1995). TNF-α hardly has a toxic effect on Schwann cells on its own, but in combination with Transforming Growth Factor (TGF)-β it has been reported to cause significant Schwann cell detachment and lysis (Skoff *et al.* 1998). Little is known about the effect of TNF-α mediated target killing on mycobacterial survival. TNF-α mediated lysis has been reported to have a similar effect on mycobacterial viability as FAS/FAS-L mediated lysis (Oddo *et al.* 1998), but for both FAS and TNF-α this topic is highly disputed.

Cytokines determine the outcome of inflammatory immune responses in various ways. Chemokines, chemoattractive cytokines, such as RANTES (Regulated upon Activation, Normally T cell Expressed and presumably Secreted), Monocyte Chemotactic Proteins (MCP)-1, IL-6, and IL-8, may attract specific cell populations. Some of these chemokine genes have been disrupted in mice. IL-6 was reported to play a crucial role in cellular defense against mycobacteria. It is produced in an early stage of mycobacterial infection and inhibits bacterial growth in macrophages (Flesch and Kaufmann 1990; Orme *et al.* 1993). Disruption of the IL-6 gene greatly reduced the capability to clear *M. avium* and *M. tuberculosis* and these mice subsequently died (Appelberg 1994; Ladel *et al.* 1997). Similar results were obtained using other parasites (Kopf *et al.* 1994; Dalrymple *et al.* 1995). As shown in IL-6 -/- mice, IL-6 also attracts macrophages and T cells to the nervous system (Eugster *et al.* 1998). Monocytes and $CD4^+$ $CD45RO^+$ T cells are central components of reversal reaction granulomata (Cooper *et al.* 1989). MCP-1, RANTES, and IL-8 play an important role in recruiting these cells and initiating local immune responses (Taub *et al.* 1995) (Schall *et al.* 1990; Wilkinson and Newman 1992). Thus, the increase in chemokine production after infection of tissue cells, including Schwann cells, with *M. leprae*, may be an important event in the initiation of inflammatory responses in neural tissue.

The final result of inflammation is the net balance of Th1 promoting cytokines such as IFN-γ and IL-12, versus deactivating cytokines, including IL-4, IL-10, and TGF-β. Mice with genes disrupted for the genes encoding for IFN-γ -/- (Cooper *et al.* 1993) or IFN-γR (Kamijo *et al.* 1993) were unable to generate protective immune responses against mycobacteria. Also disruption of the IL-12 (Magram *et al.* 1996) or IL-12R gene (Wu *et al.* 1997) lead to strongly impaired Th1 responses. IL-18 disruption had similar effects (Takeda *et al.* 1998; Sugawara *et al.* 1999). Individuals deficient in receptors for either IL-12 or IFN-γ were highly susceptible for infection with low-pathogenic mycobacterial species, such as *M. avium* or *M. bovis* BCG (Newport *et al.* 1996; Jouanguy *et al.* 1996; de Jong *et al.* 1998; Altare *et al.* 1998). So far, microsatellite segregation studies did not yield indications that certain microsatellite markers are indeed associated with leprosy or tuberculosis (Siddiqui *et al.* 1999). Since deficiencies in these patients were often the result of single nucleotide mutations, more precise and informative markers will be needed to resolve these issues.

IL-12 production can be inhibited by Th2 like cytokines TGF-β, IL-4, and prostaglandin E2 (PGE_2) (van der Pouw Kraan TC *et al.* 1995; Skeen *et al.* 1996) and thus suppress Th1 responses (Salgame *et al.* 1991; Sieling *et al.* 1993). TGF-β also attenuates the IL-12 responsiveness of Th1 cells via down-regulation of IL-12Rβ1 and IL-12Rβ2 expression (Gorham *et al.* 1998; Bright and Sriram 1998; Zhang *et al.* 1999). Furthermore, TGF-β reduces MHC expression (Geiser *et al.* 1993) and suppresses iNOS and NO production (Vodovotz *et al.* 1996). Thus, in general TGF-β drives immune responses away from Th1, toward Th2-like responses in which DTH is suppressed. IL-10 suppresses macrophage function at various levels, including MHC class II expression (de Waal Malefyt *et al.* 1991; Koppelman *et al.* 1997) and proinflammatory cytokine production (Gazzinelli *et al.* 1996; Neyer *et al.* 1997) and has been suggested to be responsible for the latent phase during mycobacterial infection (reviewed in (Murray 1999)).

Schwann cells may well be actively involved in modulating cell-mediated immune responses via the production of cytokines. A number of studies reported the production of TGF-β (Flanders *et al.* 1991; Unsicker *et al.* 1991), which even increases 3-fold after damage to neural tissue (Kiefer *et al.* 1995). TGF-β may be partly responsible for immune suppression in neural tissue, thus creating an immune privileged site, like has been suggested for the eye (D'Orazio and Niederkorn 1998). This effect may be amplified by the production of PGE_2 (Constable *et al.* 1994), which also has a Th1 suppressing effect (van der Pouw Kraan TC *et al.* 1995). Furthermore, two studies have reported the production of

IL-6 by Schwann cells (Bolin *et al.* 1995; Murwani *et al.* 1996).

peripheral nerve system

anatomy of peripheral nerves

The peripheral nervous system (PNS) connects the central nervous system (CNS) with the periphery. It includes the cranial nerves, the spinal nerves with their roots and rami, the peripheral nerves and the peripheral components of the autonomic nervous system. A nerve fiber consists of an axon that is almost completely enveloped in a sheath of Schwann cells. Axons can be divided in myelinated and unmyelinated ones. Myelinated peripheral axons have a myelin sheath interposed between the Schwann cells and the axon (figure 2a). This myelin sheath is derived from the Schwann cells. Unmyelinated axons lack a myelin sheath and lie in deep grooves in the surface of the Schwann cells, with multiple axons enveloped by the same cell (figure 2b).

Externally, Schwann cells are covered by a basal lamina, which, in turn, is surrounded by endoneurial tissue (figure 2c). Several Schwann cell/axon units, which are embedded in endoneurial tissue, are surrounded by the relatively impermeable perineurium, consisting of randomly orientated and highly concentrated collagen fibers. Tight junctions between endothelial cells of the capillaries and the basement membrane separate the endoneurium from the circulation. This isolation, also referred to as blood/nerve barrier, is believed to be important for maintaining the appropriate physicochemical environment for the axons and for protecting them from harmful agents. However, the junction can provide a route through which bacteria or leukocytes can ultimately enter the PNS.

function of myelin

Myelin formation results from the dense winding of cell membranes around axons. These membranes partly fuse and thus form lipoprotein complexes. This substance is a perfect insulator and prevents almost all ion flow, increasing the resistance to ion flow through the membrane approximately 5000-fold. However, at the juncture between each two successive Schwann cells along an axon, a small, non-isolated area remains, the node of Ranvier, where ions can flow easily between the extracellular fluid and the axon. Action potentials can therefore only occur at the nodes, which increases the velocity of signals five-fold, as well as reduces energy used by axons a hundred-fold, since depolarization has to be established at the nodes only.

Schwann cells and myelin formation

Schwann cells are the myelin forming cells of the peripheral nerve system and are generated from the neural crest cells in embryonic life (Le Douarin *et al.* 1991; Anderson 1993). This process involves the generation of an intermediate stage, the Schwann cell precursor, that differs from both crest cells and immature Schwann cells in several ways, including survival requirements, antigenic phenotype, and morphology (Jessen *et al.* 1994; Gavrilovic *et al.* 1995). During late embryogenesis, these cells develop into S100-positive, bipolar immature Schwann cells, which in turn differentiate into myelin- or non-myelin-forming Schwann cells, depending upon the diameter of the axon they ensheath (figure 2a-b) (Jessen and Mirsky 1991). Myelin-forming Schwann cells wrap concentrically around a single large diameter axon (>1 μm), while non-myelin-forming Schwann cells ensheath multiple small diameter axons.

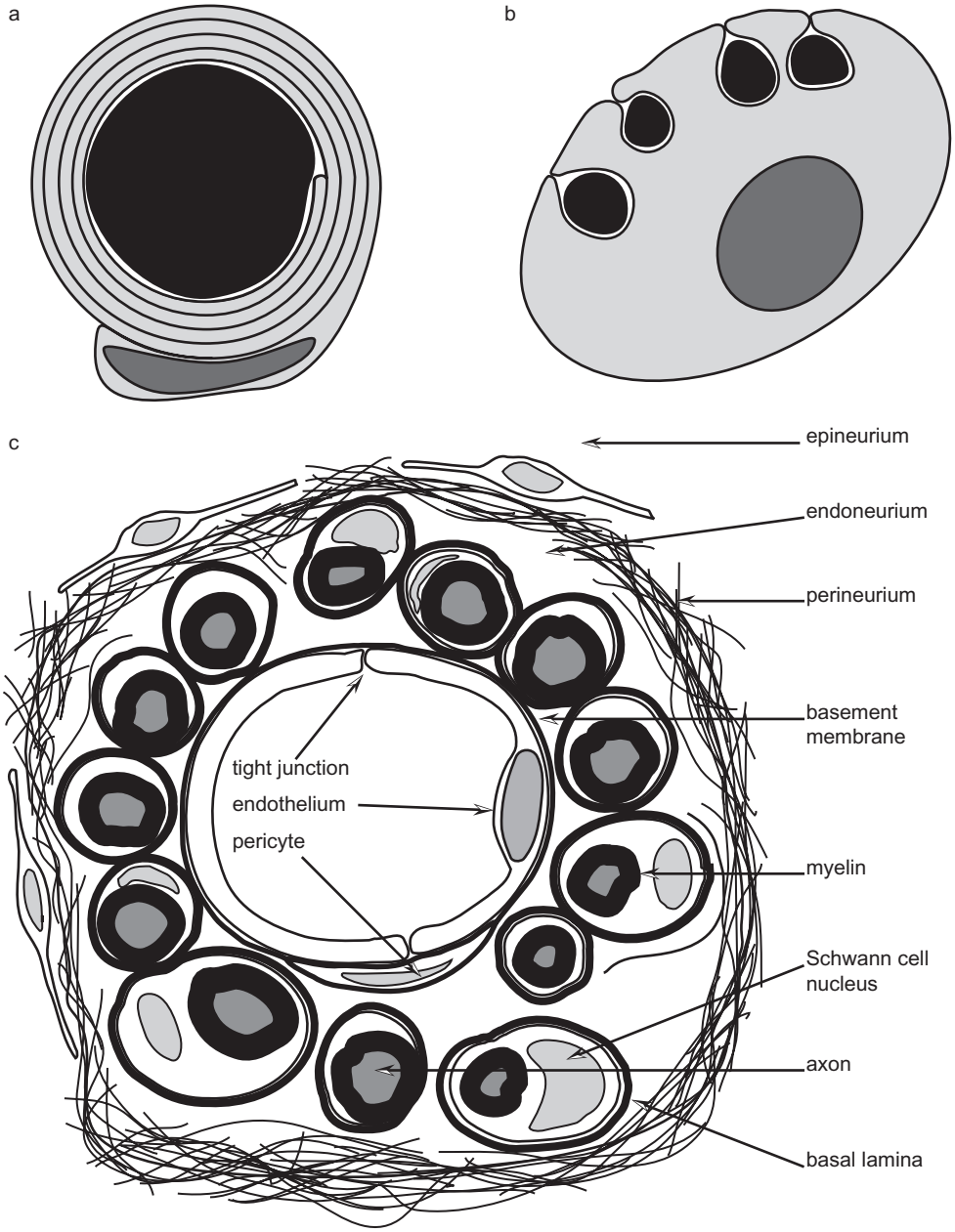

figure 2: Myelinating (a) and non-myelinating (b) Schwann cells and the structure of peripheral nerve tissue (c).

demyelinating neuropathies

Demyelinating neuropathies are characterized by disruption of the myelin sheath and segmental demyelination. A wide range of traumatic, hereditary, toxic, infectious and immune mediated processes may be associated with, or present as peripheral neuropathies. Examples of neuropathies in which the immune system may actively be involved in causing demyelination are chronic inflammatory demyelinating polyradiculoneuropathy and Guillain-Barré syndrome. Target tissues were shown to display increased expression of MHC class II (Pollard *et al.* 1986; Mancardi *et al.* 1988; Mitchell *et al.* 1991). A similar phenomenon was observed in leprosy, where Schwann cells were found to express MHC class II (Narayanan *et al.* 1990). Schwann cells may therefore well be actively involved in the immunopathology of leprosy neuritis by presenting *M. leprae* antigens to cytotoxic T cells. This hypothesis is supported by the finding that $CD8^+$ T cells were able to lyse murine Schwann cells in an MHC class I restricted, mycobacterial antigen dependent manner (Steinhoff and Kaufmann 1988). As a result of antigen recognition, Schwann cells were killed. Schwann cells were also able to stimulate $CD4^+$ T cells via MHC class II (Wekerle *et al.* 1986; Ford *et al.* 1993). So far, it is unknown to what extent these rodent studies can be extrapolated to leprosy neuritis, largely as a result of the inability to culture human Schwann cells, thus precluding such analyses in humans.

peripheral nerve system - *M. leprae* interactions

cellular receptors

Mycobacterium leprae, the causative agent of leprosy, resides intracellularly in macrophages and in the nerve surrounding Schwann cells (Boddingius 1974). Various receptor-mediated mechanisms, similar to those exploited for macrophage invasion, may play a role in invasion of human Schwann cells by mycobacteria. Candidates are Fc receptors (Vedeler *et al.* 1989), complement receptor 1 (Vedeler *et al.* 1989; Schorey *et al.* 1997), the fibronectin binding protein (Schorey *et al.* 1995), and mannose receptors (Schlesinger 1993). These mechanisms are, however, 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 laminin-α2, present in the basal lamina of Schwann cells (Rambukkana *et al.* 1997).

laminins

Laminins are extracellular matrix proteins that consist of alpha, beta and gamma chains (figure 3a) (Timpl and Brown 1994). In merosin, or laminin-2, the alpha chain is of the α2 isoform. The tissue distribution of laminin-2 is restricted to the basal lamina of Schwann cells, striated muscles, and trophoblasts of the placenta (Leivo and Engvall 1988; Engvall *et al.* 1990; Engvall 1993). The heavy α-chain can be subdivided into a number of domains (Engvall and Wewer 1996). The N terminal part or short arm of the α2 macromolecule spans the domains IV-VI. This short arm can interact with cellular receptors via various domains. Domain VI contains binding sites for integrin α1/β1 and α2/β1 (Colognato *et al.* 1997), and an integrin binding RGD site is present in domain IV (Aumailley *et al.* 1990; Aumailley *et al.* 1991; Schulze *et al.* 1996). The C terminus is characterized by the presence of five homologous G domains. These domains have been reported to be involved in binding to receptors as α/β dystroglycan (DG) (Yamada *et al.* 1996) and a number of integrins (Nomizu *et al.* 1996).

M. leprae exploits laminin-α2 and dystroglycan to bind to Schwann cells (figure 3b) (Rambukkana *et al.* 1998). It is likely that other receptors are also involved in *M. leprae*/

Schwann cell interaction, since blocking of the DG complex could not inhibit bacterial adhesion completely (Rambukkana *et al.* 1998). Candidates are integrins, which are also able to bind to laminin-α2. Furthermore *M. leprae* has been reported to interact with a yet uncharacterized 28-30 kDa phosphoprotein which is expressed by Schwann cells (Suneetha *et al.* 1997), thus providing other receptor/ligand interactions for *M. leprae* to bind to Schwann cells than via laminin-α2/dystroglycan alone.

mycobacterial receptors

Mycobacteria are well known to possess mechanisms to interact specifically with host cells. Recently, a laminin binding receptor of 21 kDa on *M. leprae* has been identified,

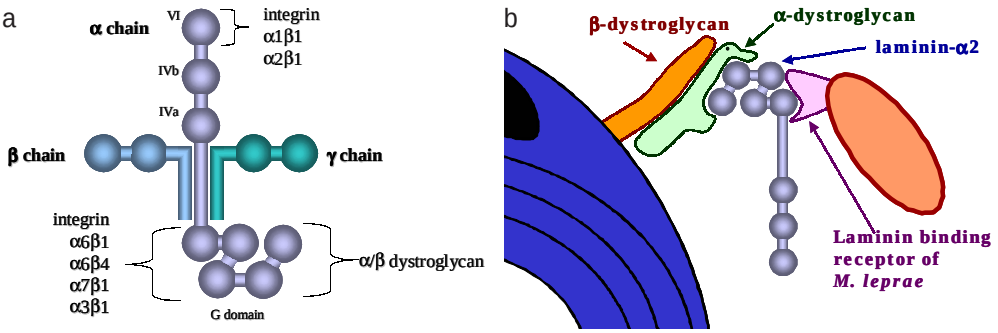

figure 3: a) Schematical structure of laminins. b) Molecules involved in *M.leprae*/Schwann cell interaction.

being a histone like protein (HLP) (Shimoji *et al.* 1999; Pessolani *et al.* 1999). This mycobacterial receptor may function as a critical surface adhesin that facilitates the entry of *M. leprae* into Schwann cells. Other mycobacterial receptors described to be involved in eukaryotic cell invasion include the mammalian cell entry (*mce*) proteins. Sequencing of the complete *M. tuberculosis* genome yielded four similar mce operons consisting of 8 open reading frames (Cole *et al.* 1998). Non invasive bacteria, as *Escherichia coli*, become invasive after introduction of *mce* genes (Arruda *et al.* 1993) and disruption of the mce gene of BCG reduced the ability to invade the non-phagocytic cells as compared to wild-type BCG (Flesselles *et al.* 1999).

aims and outlines of this thesis

The success of MDT in the control of leprosy is currently overshadowed by the increased frequency of leprosy reactions during treatment and the lack of decline of leprosy incidence. Particularly during the first 6 months of treatment, reversal reactions may occur in as much as 80% of the patients (Roche *et al.* 1991). Nerve damage is a prominent feature of leprosy reactions. Besides macrophages, Schwann cells are predilection sites for *M. leprae*. Nerve damage is at least partly immunologically mediated, since reversal reactions are clearly associated with an increased cell-mediated immune response. Many fundamental aspects of nerve damage in leprosy regarding the role of Schwann cells, T cells, and antibodies in the immunopathology of leprous neuritis have remained unresolved, partly as a result of the inability to culture human Schwann cells and *M. leprae*. This thesis aims to help resolving these issues by addressing the following questions:

- Is it possible to establish a human Schwann cell system for the study of immunological Schwann cell/T cell interactions?
- Are human Schwann cells able to present mycobacterial antigens to T cells?
- What are the consequences of T cell mediated antigen recognition for Schwann cell function, in particular Schwann cell killing as a cause of nerve damage?
- How do *Mycobacteria* interact with Schwann cells?
- To what extent can human Schwann cells modulate potentially harmful Th1 responses after exposure to *M. leprae*?
- In what respect do T cells from leprosy nerve lesions possibly differ from peripheral T cells regarding function and phenotype?
- Can T cell mediated, antigen independent cytolytic mechanisms be identified that are related to Schwann cell damage in leprosy?
- Can antibodies towards nerve related antigens predict nerve damage or leprosy reactions?

Chapter 2 deals with the establishment of a human *in vitro* cultured Schwann cell system. The antigen presenting capacity of these cells in relation to *M. leprae* (2a) and Schwann cell expressed alloantigens (2b) is described. The inability to establish long term Schwann cell cultures and to culture *M. leprae in vitro* have been important obstacles in studying these issues so far. Schwann cell cultures were phenotypically characterized and used to test the hypothesis that they function as non-professional antigen presenting cells for *M. leprae* specific $CD4^+$ Th1 cells, which are abundantly present in inflamed neural tissue during leprosy reactions. It is shown that Schwann cells are damaged by the cytolytic activity of these T cells during the process of antigen recognition. This may provide an important novel mechanism of immune mediated Schwann cell destruction.

In chapter 2b experiments are performed to test the capacity of human Schwann cells to prime antigen specific responses in an allogeneic model, using peripheral blood mononuclear cells of HLA-DR matched and mismatched individuals as responder cells. Artificial nerve grafts in combination with Schwann cells have already successfully been added to the lumen of such grafts in syngenic rat models to facilitate nerve regeneration. Rejection of allografted rat Schwann cells, however, has previously been reported. Since *in vitro* cultured human Schwann cells express MHC class II and can function as antigen presenting cells to activate specific T cells (chapter 2a), they may also induce immunological recognition and rejection of donor material by host cells. This study provides *in vitro* data confirming this hypothesis.

Chapter 3 describes the cytokine profile of *in vitro* cultured human Schwann cells and the effect of these cytokines on T cell activation. The outcome of local immune responses not only depends on a T cell/APC interaction, but also on the local balance between inflammatory and anti-inflammatory cytokines. In addition to functioning as non-professional antigen presenting cells, Schwann cells might also modulate local immune responses by releasing cytokines such as TGF-β, PGE_2, IL-4 and IL-10.

Lesional T cells are likely to be the most relevant T cell population to study in relation to nerve damage. Chapter 4 describes the characterization of *M. leprae* reactive T cells isolated from inflamed neural tissue of a leprosy patient. The study was focused on a special subset of T cells, expressing N-CAM. N-CAM is a neural cell adhesion molecule, which is expressed on neurons, Schwann cells, NK cells and some T cell populations. N-CAM expressing T cells have recently been reported to be involved in autoimmune mediated cell damage in multiple sclerosis. Homotypic N-CAM/N-CAM interactions may play a role in T cell mediated antigen independent Schwann cell killing. To address this issue, expression of N-CAM on T cells from nerve lesions and peripheral blood was compared.

Furthermore, N-CAM expression on peripheral T cells after exposure to *M. leprae* was analyzed in patients with and without type 1 or type 2 leprosy reactions. The relation between N-CAM expression and lysis of N-CAM positive targets was investigated.

In chapter 5 the presence of an *M. leprae* operon with high similarity to the mammalian cell entry protein of *M. tuberculosis* is described. Genes encoded by these operons have been reported to be directly involved in cell entrance. *M. leprae* may exploit these proteins for host cell invasion. The presence of HLP/Laminin-α2/Dystroglycan independent cell entrance mechanisms was also suggested by the finding that other mycobacteria, like *M. smegmatis*, are also able to enter human Schwann cells (chapter 2a).

Chapter 6 analyses the presence of antibodies in leprosy patients towards nerve related antigens. Neural antibodies are possible candidates for early detection of nerve damage. One such antigen is sulfatide, which is expressed as a surface determinant of myelin in the central and peripheral nervous system. Antibodies towards sulfatides have already been detected in several neuropathies, including insulin dependent diabetes mellitus, Guillain Barré syndrome, Miller Fisher syndrome and multiple sclerosis.

Finally, chapter 7 provides a synthesis of the described findings as well as their implications for the immunopathogenesis of leprosy Schwann cell and nerve damage.