Determinants for the development and course of leprosy: findings from a prospective cohort study
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Chapter 1

General introduction
1 Mycobacterium leprae

1.1 Mycobacterium family

*Environmental.* Most species from the *Mycobacterium* family are free living or environmental, so typically present in soil and water. They can be ingested by phagocytic cells, which for environmental mycobacteria would be typically amoebae. Amoebae feed on microorganisms by engulfing them (phagocytosis) and digest them in stomach-like compartments (vacuoles). Some mycobacteria have the pathogenic ability to proliferate inside phagocytic cells. After phagocytosis, by either amoebae or other phagocytic cells, these pathogenic mycobacteria can interrupt the digestive processes of their host, and even start to use the intracellular nutrients, thereby feeding on the predator.

*Human.* Mycobacteria may enter the human body, for instance through aerosols. The human immune system includes phagocytes (white blood cells) which apply similar mechanisms as amoebae to kill invading microorganisms. [1-3] The ability to infect human phagocytes and cause disease has been shown for several mycobacterial species, including *Mycobacterium ulcerans*, cause of Buruli ulcer, *M. avium*, a significant cause of death in immunocompromised people, and the most famous ones, *M. leprae* and *M. tuberculosis*, causing leprosy and tuberculosis (TB), respectively. However, clinical disease is a rare outcome, especially if one takes into account the numerous interactions that occur daily between humans and (in particularly the environmental) mycobacteria. [4-6] Even when infected with TB one has a lifelong risk of only 10% to develop clinical disease. [7]

Unlike leprosy, TB is very infectious, potentially deadly, and highly prevalent throughout the world, effectively meaning it generates
more resources for drug development, disease control and research. Besides the higher incidence, TB can be grown *in vitro*, making it easier to study. Analogies between *M. leprae* and *M. tuberculosis* led to comparative studies, extrapolation of study results, like transmission routes and risk groups, [8-9] and even shared control strategies, like contact surveys and BCG vaccination. [10-11]

In the following paragraphs details on *M. leprae*, its effect on humans, disease characteristics, and progress in disease control are given, concluded with a summary of the current research needs and the outline of this thesis.

**1.2 *Mycobacterium leprae* cell characteristics**

*General characteristics.* *M. leprae* is an acid-fast, rod-shaped bacillus with the very impermeable cell wall that is typical for the mycobacterium family. Many favourable aspects are attributed to this cell wall, with respect that it allows a relative long survival in harsh conditions, like exposure to acids, alkalis, detergents, oxidative bursts, lysis by complement and (certain) antibiotics. [12]

*Intracellular localization.* *M. leprae* is an intracellular bacterium which evidently lost its capability to proliferate outside particular environments. [13] This has frustrated scientific progress, since *in vitro* culture has for a long time been a prerequisite to reveal metabolic pathways and virulence factors. The bacterium targets human Schwann cells and phagocytes, like macrophages and dendritic cells. [14] While phagocytes live to ingest pathogens like *M. leprae*, the invasion of Schwann cells is an unusual trait. A possible mechanism for this may be the binding of specific *M. leprae* cell wall components like phenolic glycolipid-I (PGL-I) and 21kDa protein [15,16] to parts of the basal lamina layer of Schwann
Hereafter, uptake may be facilitated by alpha-dystroglycan.

**Slow growth.** Within the mycobacterium family there is a distinction between rapid and slow growers. *M. leprae* has an exceptionally slow doubling time of 11-12 days (determined using a mouse model [19]). Since most of the pathogenic mycobacteria, like *M. tuberculosis*, *M. avium*, *M. bovis* and *M. leprae*, are slow growers, some have hypothesized that the (slow) growth rate itself or a growth rate determining virulent trait, may enhance pathogenicity. [12,20-22]

**Temperature.** While normal human body temperature does not affect the *M. leprae* membrane, the optimum metabolic activity is definitely not 37°C. Lahiri et al. (2005 [23]) concluded that the optimum growth temperature for *M. leprae* is 33°C or lower and a relatively short-term incubation at 37°C has clear deleterious consequences. [24] It is therefore not surprising that *M. leprae* is most prevalent in the cooler parts of the human body (such as fingers, toes, nose and earlobes), and that the nine-banded armadillo, which has a core body temperature of 33°C, supports massive systemic growth of *M. leprae*. [23]

### 1.3 Genome

Like most intracellular organisms, *M. leprae* has a relative small genome. The complete genome sequence revealed an extreme case of reductive evolution: 3.27 megabase for the *M. leprae* genome compared to 4.41 megabase *M. tuberculosis*. [13] Reductive evolution should be considered as a niche-specific refinement and not as decay to a certain limit. [25] The gene comparison with *M. tuberculosis* revealed that *M. leprae* has remarkably few protein-coding genes, suggesting that *M. leprae* encodes just enough to permit intracellular growth. [13] Although the *M. leprae* genome consists for about half of both...
pseudogenes and non-coding regions, [13] this does not mean that this half is non-functional. Akama et al. (2009 [26]) pointed out that the relatively high amount of pseudogenes, and their altering expression level following macrophage infection, [27] does indicate some biological function. Moreover, the same authors stated that the high expression of unidentified non-coding regions may indicate a yet unrecognized function. [26]

All \textit{M. leprae} strains known to date can be attributed to a single clone whose global spread from Eastern Africa or the near East happened after its reductive evolution. [28] The genome is now very stable and differences seem to have no known effect on disease outcome. [28,29] However, recently a new mycobacterium species, \textit{M. lepromatosis sp novin}, was identified from two lepromatous leprosy patients, which led to the statement that some of the clinical and geographic variability of leprosy may be explained by different causative agents. [30]

The completion of the genome sequencing of a number of mycobacteria kick-started research into metabolic, biochemical and pathogenic parameters and pathways. [14] New leprosy biomarkers may be derived from essential metabolic pathways and likewise maybe reveal new targets for vaccines, drugs and diagnostics.

\section*{2 Reservoirs and transmission routes}

\textit{Humans.} \textit{M. leprae} is present in patients, although this can be hard to prove: in about 70\% of the patients the bacteria cannot be detected by microscopy on acid-fast stained smears or biopsies. [31] Polymerase chain reaction (PCR) and serological techniques may show the presence of \textit{M. leprae} DNA or specific antibodies, but, again, not all
patients give PCR- or seropositive results. [32,33] This is illustrative for the low profile that the bacterium often maintains in the human host. If bacterial presence can be established by microscopy, it is typically seen in the facial/nasal area, the peripheral nerves or skin lesions. Likewise, patients are generally thought to shed the bacteria via the nasal cavity and, potentially, the skin. [33]

It can be concluded from both leprosy and TB studies that the bacterial load of a patient is an important risk factor for transmission to contacts. However, it is certainly not the only risk factor, [8,34] suggesting that all patients should be considered as possible sources of infection, but the degree of infectiousness varies. As a consequence, the emphasis in leprosy control is very much on early detection and treatment of patients, since this stops their infection potential, which is thought to lead to a reduction in incidence. Screening of contacts of leprosy patients for early signs and symptoms is not systematically done in most control programs. Also, the absence of an early diagnostic test hampers the early identification of leprosy.

*M.leprae* is thought to be present in healthy individuals in endemic areas. Surveys with PCR and/or serology tests suggest that subclinical infection is far more common than overt disease. Studies showing presence of *M.leprae* DNA in the nasal cavity support this hypothesis. [35-40] Serological tests performed on endemic individuals showed that the antibody prevalence varies greatly from 1.7-30%. [41] It is currently still under debate whether transmission occurs through these sub-clinically infected individuals. [8]

*Other (potential) hosts and sources: armadillos, soil and water.* Besides humans, the only other naturally occurring reservoir host is the nine-banded armadillo, Dasypus novemcinctus, [42-44] which may host large numbers of *M.leprae*. [45] There is increased proof that humans can get infected by armadillos but at the moment it remains to be seen
if this transmission route needs to be—or even can be—controlled. [45-48] Others animals, like mice (mouse footpad) and mangabey monkeys, can be experimentally infected with *M. leprae* as well, but the disease prevalence in these animals under normal conditions is unknown.

Infection with *M. leprae* occurs from person to person, but indirect routes may be possible as well, since the bacterium is viable for some time outside the body. [49] Soil and water are considered as potential (intermediate) reservoirs, [50] in analogy to other environmental mycobacteria that occasionally cause disease in humans. [51] Lavania et al. (2008 [50]) showed, using DNA and RNA detection techniques, that viable *M. leprae* were present in 50% of soil samples collected in the direct vicinity of a leprosy patient. Furthermore, fewer, but still 15%, of samples taken from areas not directly associated with a leprosy patient, also showed presence of viable *M. leprae*. Although still preliminary, this may indicate a potential non-human intermediate environment. Whether *M. leprae* is capable of proliferation in an environmental phagocyte, like other mycobacteria, has yet to be determined. So far, the phagocyte *Acanthamoeba castellanii* was shown to ingest and support viability of *M. leprae*, [50] but whether proliferation of *M. leprae* in an environmental phagocyte is possible still remains to be determined. Moreover, considering its niche-specific reductive evolution one can wonder whether *M. leprae* remains sufficiently competitive with other organisms/mycobacteria to survive on a large scale outside known hosts.

No control policy is currently active to target non-human infection sources or transmission pathways.
3 Host – pathogen interaction

3.1 Entry of the pathogen into the host

More than 95% of the infected people do not develop overt disease [53,54] as the immune system will kill any invading *M. leprae* before disease symptoms occur. So, only in less than 5% of the infections *M. leprae* will have some degree of success in evading the human defence system. Although not completely understood, there are some insights in how *M. leprae* succeeds in this.

For instance, the surface molecules PGL-I and ManLAM are both involved in cleavage of C3, a protein from the complement system that promotes phagocytosis via complement receptors CR1 and CR3. However, phagocytosis via these receptors does not result in an effective oxygen burst, lacking reactive nitrogen which is needed to kill the bacteria. Mouse knockout models showed that *M. leprae* could not be killed by reactive oxygen alone, but that reactive nitrogen was required; [55,56] *M. leprae* PGL-I and superoxide dismutases SodC and SodA are responsible for neutralizing reactive oxygen. [14,57] Natural antibodies may even aid the bacteria in this favorable mechanism, since they were shown to facilitate cleavage of C3 to the *M. leprae* surface. [58]

When phagocytosis has taken place by effective receptors, like the mannose-, SIGN- or lagarin receptors, the initial phagosome-lysosome fusion may be hampered by live bacteria. [59] Additional stimulation by IFN-gamma, produced by other cells, is then required for secondary fusion. However, these cells have to be recruited first from the lymphoid tissue. And since the effect of IFN-gamma stimulation decreases in a time dependent manner with the intracellular presence of *M. leprae*, [59-61] it is clear that a quick response is essential for bacterial clearance. Thus, fast DC migration after encountering a
pathogen is thought to be essential for optimal immune response. Intracellular *M.leprae* may suppress antigen presentation needed for recruiting other immune cells by down-regulating the antigen presentation complex, MHC I/II in DCs. If ultimately the DC succeeds in expressing antigens, the expression of PGL-I on its cell surface has further immunosuppressing capabilities. This was demonstrated by Hashimoto et al., [62] who showed that after masking the expressed PGL-I, T-cell proliferation as well as IFN-gamma production were up-regulated. Furthermore, innovative research by Van Helden [63,64] illustrate another possibility for a delayed immune response; bacteria lacking lipopolysaccharide (LPS) do not trigger DCs to lose their podosomes, which are surface molecules that prevent DCs to migrate quickly.

Initial interaction of *M.leprae* with dendritic cells (DC) can result directly in a favorable maturation process: Krutzik and coworkers (2003 and 2005 [65,66]) showed that interaction and maturation—mediated by the TLR1/2 pathway—led to the maturation of the DCs into the DC-SIGN and DC-CD1 subtypes. DC-SIGN will attempt to kill the bacteria through phagocytosis, but without help from the stimulus factors produced by DC-CD1 the bacteria may circumvent being killed. Successful killing requires enough DC-CD1 subtype cells that will recruit other immune cells and activate secondary phagosome-lysosome fusion. The DC-SIGN subtype has also been shown to cause IL-10 derived immunosuppressing by binding ManLAM of *M.leprae* or *M.tuberculosis*. [67-70]

*Schwann cells*. Besides for macrophages, *M.leprae* has a host tropism for the Schwann cells of peripheral nerves. Possibly, bacterial invasion occurs through direct binding to Schwann cells in the dermis or through accumulation in epineural lymph and blood vessels followed by entering of the endoneural area through the blood supply. [71] Intracellular proliferation proceeds slowly and at some point the
Schwann cell may express antigen on its surface with MHC II molecules. [72] Once recognized by cytotoxic T-cells, the Schwann cell is destructed and subsequent release of inflammation factors within the endoneural area can lead to further nerve damage. [73]

### 3.2 Host immunogenetics

Leprosy offers an opportunity to investigate the association between gene functioning and human immunology, since leprosy presents itself as an immunological spectrum from tuberculoid to lepromatous leprosy, which poles correlate with the two types of adaptive immune responses to *Mycobacterium leprae*. [74]

Genetic diversity of the host has great potential to modulate susceptibility to *M. leprae* infection, especially for those genes directly involved in the above described mechanisms, e.g. lysosome maturation, oxidative burst, and antigen presentation. Numerous genes have been reported to influence susceptibility or disease outcome, many in immunomodulating pathways of TLR/LIR-7, VDR, TNF-alpha and TGF-beta1. [75]

In the COLEP study the human PARK2/PACRG and Toll-like receptor (*TLR*) genes were studied, and as this thesis describes the results of the COLEP study, we will concentrate on these genes.

Sub-optimal protein degradation after oxidative stress and antigen presentation may be particularly important for *M. leprae* susceptibility. [76,77] *PARK2* and *PACRG* are genes linked to the ubiquitin tagging system, which tags unneeded proteins to be degraded. Whether mutations cause susceptibility for leprosy infection and/or modify clinical outcome is still under debate. [75] Unpublished results of the COLEP study indicate that the single nucleotide polymorphism (SNP)
PARK_e01(-2599) is related to susceptibility to leprosy per se (T allele OR 1.25; 95% CI 1.04-1.52) and the genotype TT is related to MB leprosy (OR 1.76; 95% CI 1.17-2.66). Interestingly, all eleven patients who had developed ENL reaction, had the TT genotype (unpublished results).

TLRs interact directly with the pathogen and initiate inflammatory responses. [78] Each TLR is able to recognize a specific class of pathogen ligands and subsequently two TLR-mechanisms can be activated. Firstly, stimulation of DC maturation enables adaptive immune responses, [79] and, secondly, recognition by TLR may lead to macrophage maturation and ensuing activation of antimicrobial activity and phagocytosis. [80,81] The TLR1/2 pathway was shown to lead to maturation of DC into the DC-SIGN and DC-CD1 subtypes, as described above. [65,66]

It is quite difficult to single out a genetic prognostic marker, because of the diversity of mechanisms and the possibility that they are interlinked, as well as possible other factors that may influence the course and outcome of infection and disease. [75] The availability of the whole human genome sequence, allowing gene comparison and genome wide-scans, may further increase our understanding of host immunology, potentially leading towards a multiple marker test.

4. Leprosy

4.1 Clinical leprosy

For most people the infection with and clearance of the bacilli and its antigens goes unnoticed (subclinical infection). However, depending on the immunological response of the host, a whole range of clinical and histopathological features may appear. Ridley and Jopling have
categorized clinical leprosy into five types: tuberculoid (TT) and lepromatous (LL) leprosy with three borderline groups, BT, BB and BL, and an indeterminate group (I) based on these patterns. [74] Tuberculoid leprosy patients have a fairly successful *M. leprae* specific cell-mediated immune response. Their lesions are characterized by epithelioid cell granulomas, participation of lymphocytes (mainly of Th1 type), and few if any detectable bacilli. In contrast, in the lepromatous form, the specific cell immunity against *M. leprae* is virtually absent [82], with diffuse dermal infiltrates characterized by poorly differentiated young macrophages with a heavy load of bacilli and a small number of T cells predominantly of the Th2 type. [83] In the spectrum of borderline leprosy there are varying degrees of cell-mediated immune response declining from the tuberculoid to the lepromatous pole. [74]

The balance between the immune response and *M. leprae* is not necessarily stable: spontaneous fluctuations known as leprosy reactions may occur. Reactions are acute events of inflammatory response, and since nerves may be involved they are considered as medical emergencies. There are two types of reactions: reversal reaction (RR) and erythema nodosum leprosum (ENL). Reversal reactions are a spontaneous increase of T-cell reactivity to *M. leprae* antigens that occur in 30% of the borderline patients. [73] Common clinical characteristics are neuritis and inflammation of skin lesions (swelling, redness, local heat, loss of sensation and tingling). [84] ENL is a systemic inflammatory response characterized by high circulating concentrations of TNF-alpha and systemic toxicity, which mainly occurs in BL and LL patients. [85,86] A common clinical characteristic are inflamed, painful and red nodules. Due to its systemic nature, ENL reactions affect the whole body and patients feel general malaise and fever. [87]

As described above the tropism of *M. leprae* for Schwann cells causes peripheral nerve damage. The risk for nerve function impairment (NFI) is highly increased during reactions. Preventing permanent
disabilities due to nerve function impairment remains a major concern in leprosy control, [88] in particular because NFI can take place before, during and/or after leprosy treatment. Early detection (within 6 months) and corticosteroid treatment may prevent further decline of or even revert nerve function. [84,89,90]

4.2 Diagnosis

Early diagnosis—of subclinical infection, disease, and reactions—is a major topic in leprosy control, since a patient may become a source of infection and develop nerve function impairment at an early stage. Bakker et al. reviewed several studies for the risk of PB and MB contacts and estimated that contacts of patients had an increased risk—PB contacts had a two times increased risk, MB contacts 5 to 8 times increased risk—compared to non-contacts. [34]

*Diagnosis of subclinical infection.* Diagnostic tools for the phase before clinical symptoms become apparent would be invaluable to prevent transmission. There are initiatives ongoing to develop such early diagnostic tools (e.g. the IDEAL collaboration [91]), but currently no test is routinely implemented, even though a number of targets are under evaluation. For instance, Geluk et al. [92] report the development of a leprosy-specific T-cell assay with novel antigens selected from the genome sequence. Although some of these antigens showed to be highly sensitive for leprosy, many healthy controls also had a positive test result, decreasing the specificity. Duthie et al. [93,94] reported a good association with an *M. leprae* antigen-construct LID-1 (LID-1= fusion construct of ML0405 and ML2331) and future development of leprosy, but for PB patients the positive predictive value was rather low (6 out of 30).
The low incidence of clinical leprosy effectively means that a biomarker should be both very specific and sensitive, and have a good predictive value for the development of clinical disease. Moreover, there is always the possibility of spontaneous healing/bacterial clearance, decreasing the positive predictive value of any test. Also, immune responses may be different between subclinical infection and clinical disease, in the same respect as differences between PB and MB disease.

In general, antibodies are not effective against intracellular pathogens, which are shielded off by a host cell, but they may facilitate the non-lethal phagocytosis by complement receptors. [95] The most widely used serological test today is based on PGL-I. [41] Seropositivity indicates a non-protective, humoral immune response against \textit{M.leprae}. Besides a sign of infection, antibodies may indicate successful proliferation of \textit{M.leprae}. Potentially, initial seropositivity is an indicator for a continuing and later mainly humoral immune response of an individual with an increased susceptibility to develop clinical/MB disease. [35,96,97]

\textit{Diagnosis of clinical disease.} Leprosy is diagnosed when finding any one of three cardinal signs, 1) one or more hypopigmented, anaesthetic skin lesions; 2) one or more thickened peripheral nerves; or 3) presence of acid-fast bacteria. [98]

Clinical features, in particular the skin lesions, are the most profound indicators for leprosy, but may take a long time to develop. [99] Neuropathy assessment is normally done with a monofilament test and voluntary muscle test. [101] With the monofilament test, nylon monofilaments are used to monitor touch sensation on the hands and feet. [102] Instead of filaments, a regular ball-point pen may be used as well. [103]
The number of acid-fast bacteria is expressed in a bacterial index, a logarithmic scale ranking from zero to six. After acid-fast staining of skin smears or biopsies, the bacteria are counted under a microscopy. [100] As described above (section 4.1) histopathology can also be used for diagnosis and classification.

Anti-PGL-I IgM serology has a strong correlation with microscopy results and overall systemic bacterial load. [32] About 15-40% of the PB patients and 75-100% of the MB patients are seropositive. [41] The test is not suitable for diagnosis, but may contribute to correct classification [104-106] and the identification of high risk groups for NFI [107] and future development of leprosy. [108] And to detect a leprosy relapse in an early stage. [109]

Analogous to other micro-organisms, the detection of the pathogen by PCR could play an important role in diagnosis. A number of very sensitive PCR-techniques have been developed over the years, [110-112] but so far these are hardly used in routine leprosy control programs.

*Diagnosis of reactions and nerve function impairment (NFI).* Management of nerve function impairment is an important aspect in leprosy control as timely treatment may prevent permanent damage. Neuropathy assessment can be performed as described above. A recent publication of the INFIR study group (2008 113), an initiative to compare diagnostic tests for neuropathy assessment, concluded that detection was best when using an electrophysiological test (sensory nerve conduction) or measuring thermal threshold (warm temperature perception test). Symptoms could be detected 12 weeks earlier than with the common monofilament test.

Testing all patients on a regular basis puts a high burden on health care workers. Therefore patients should be taught to inspect their body on a daily basis and attend to any injuries promptly. [114,134]
Self reporting and self care needs to be promoted continually to prevent permanent nerve damage.

NFI risk factors and an NFI prediction rule have been determined based on data from the Bangladesh Acute Nerve Damage Study (BANDS 115). The prediction rule categorizes patients into NFI risk groups based on their World Health Organization (WHO) classification (ie, PB or MB leprosy) and the presence of NFI at diagnosis.

### 4.3 Classification of disease

The classification according to Ridley and Jopling as described above is still in use, and is especially helpful for research. Nowadays, the most widely used classification system for treatment purposes is the one designed by the WHO, which is based on clinical features of skin lesions only. Microscopy results can be taken into account as well, but this is optional, since it requires laboratory facilities, which are often absent in the field. After a number of modifications, the WHO classification nowadays divides leprosy patients into multibacillary (MB) patients with 6 or more skin lesions and/or a positive bacterial index and paucibacillary (PB) patients who have up to 5 skin lesions and a negative bacterial index. [116]

### 4.4 Risk factors

Even though transmission patterns in leprosy are difficult to study, there are a number of well-recognized individual risk factors for leprosy such as sex, age, bacterial load of the index patient and genetic and physical distance to a patient. (reviewed by Bakker et al. and Moet et al. [8,34]) Males are at higher risk than females, especially for MB leprosy and children and elderly are frequently reported to have a higher leprosy incidence. [8,34] The exact causative mechanisms for these
increased risks are not always well-established, but are generally accepted to be related to immunological and/or exposure differences.

As described above, patients in which bacteria can be demonstrated are considered to be most infectious, thereby increasing the risk for their contacts. [8, 34] *M. leprae* specific antibodies were shown to be associated with leprosy in a few prospective studies. [32,35,93,94,96,117] Potentially, seropositivity is an indicator for a continuing humoral immune response in an individual, which is associated with (lepromatous or MB) leprosy.

Leprosy is often associated with poverty, but evidence is not easily obtained. [118] Ponnighaus et al. (1994 [119]) found an association with two poverty-related traits and leprosy development: lower house quality and less education independently increased the risk. Kerr-Pontes (2004 [120]) showed that inequality, population growth, and presence of a railroad were associated with higher leprosy prevalence. It was speculated that population growth and social-economic inequality may cause over-crowding, thus facilitating transmission of *M. leprae*. In addition, these inequalities may hamper social needs and so impair health. [121]

Lietman et al. (1997 [122]) reported that infection with *M. tuberculosis* protects against leprosy. Fine et al. (2001 [123]), showed evidence for cross-protection from natural exposure to certain environmental mycobacteria, which may explain the geographic distribution of mycobacterial diseases like leprosy and tuberculosis. Sterne et al. (1995 [124]) concluded that there is a marked geographic variation in the incidence of leprosy, not explained by socioeconomic or cultural factors. Besides, national/local policies and quality of leprosy control have an obvious impact on leprosy risk as early detection and treatment have an effect on ongoing transmission. So, policies on diagnosis, interventions (both prophylactic and curative), and contact
surveys influence infection risk and leprosy incidence. Finally, (the characteristics of) geographical location may be considered as a risk factor as well.

4.5 Global prevalence and incidence.

The current global leprosy situation is monitored by the WHO and shows a decrease in overall new case detection since 2001 (table 1). [125]

Before 2001, WHO have been reporting a decrease in the global disease burden from 5.2 million in 1985 via 805,000 in 1995 and 753,000 at the end of 1999. [126] Furthermore the WHO states that the global prevalence rate of the disease has dropped by the year 2000 to a level of less than 1 per 10,000 inhabitants. Subsequently, the WHO declared that leprosy has been eliminated as a public health problem at a global level (prevalence <1/10,000 cases). At a country level this was achieved in 113 out of 122 countries where leprosy was considered as a public health problem in 1985. [126]

However, the above statements undervalue that, despite the prevalence drop, the majority of leprosy endemic countries are still detecting new cases at a steady level, even though the numbers may be relatively low. India is the remarkable exception with incidence dropping from 473,658 in 2002 to 137,685 in 2007. [125] Since about two thirds of all new cases come from India, the statistics from this country have a large impact on global figures. The steep decline in South-East Asia—primarily India—is questioned since it is unlikely that the MDT policy and elimination activities changed transmission that dramatically. [127-129] Besides, overall numbers for regions can be misleading since control activities and political commitment may vary per country and over time.
Moreover, the number of reporting countries differs over time, making interpretation difficult. [130]

**Table 1:** Trends in the detection of new cases of leprosy, by WHO region, 2001-2007 (excluding European Region). [125]

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<th>2001</th>
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<td>45 179</td>
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<td>174 118</td>
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<td>6 216</td>
<td>7 137</td>
<td>6 190</td>
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<td><strong>620 638</strong></td>
<td><strong>514 718</strong></td>
<td><strong>407 791</strong></td>
<td><strong>299 036</strong></td>
<td><strong>265 661</strong></td>
<td><strong>254 525</strong></td>
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</table>

**5. Leprosy control**

For centuries leprosy was a mysterious, chronic and untreatable disease and exclusion from society was common practice. Modern disease control was unthinkable, until two turning points in leprosy history shed some light on the mystery and changed the perspective of patients: the discovery of the causative agent, *M. leprae* by Armauer Hansen (1873) established leprosy as an infectious disease, and the discovery of dapsone in the 1940’s turned leprosy into a treatable disease.

Even without the benefits of this knowledge, leprosy was already largely gone from Europe by the end of the 17th century, [131] even though the reasons for this are poorly understood. Several hypotheses have been raised, yet proving them may be impossible. Were people less susceptible because of increased social-economic standards? [119,120] Or was the competition of other pathogen(s) interruptive enough to hamper *M. leprae* transmission? [122, 132] Was it the
isolation of patients [133] and/or improved hygienic behaviour? Although answers are lacking, the phenomenon does illustrate that elimination is possible.

5.1 Treatment

A major step forward was the introduction of multi drug treatment (MDT) in 1982, consisting of a regimen of rifampicin, dapsone and clofazimin for MB leprosy and rifampicin and dapsone for PB leprosy (see figure 1). [134]

Treatment with rifampicin leads to a large reduction of viable bacteria after the initial dose, [135,136] effectively stopping infection potential. The shortened treatment period compared to dapsone in combination with cleaning of registers resulted in a steep decline of registered patients in the 1980’s. It was hoped that MDT would permit control of the disease and ultimately interruption of transmission. This led to the development of the concept of “leprosy elimination”. [129]

“Leprosy elimination by the year 2000” was first proposed in 1986 and accepted at the 44th World Health Assembly in 1991, modified by the postscript “as a public health problem”. Leprosy elimination was thus defined as a disease prevalence of less than one case per 10,000. The subsequent leprosy elimination activities had some notable successes, but also revealed the epidemiological, medical, and political problems of a time-bound concept. [137,138]

Several articles have been written stressing the weaknesses of the elimination strategy. [14,127,132,137] The elimination strategy includes several non-sustainable elements for the long term. [127] In addition, mathematical modelling by Meima et al. [133] calculated that the incidence decline with the elimination strategy would be gradual
### The standard adult treatment regimen for MB leprosy is:
- Rifampicin: 600 mg once a month
- Clofazimine: 300 mg once a month, and 50 mg daily
- Dapsone: 100 mg daily
  
  *Duration: 12 months (12 blister packs)*

### The standard adult treatment regimen for PB leprosy is:
- Rifampicin: 600 mg once a month
- Dapsone: 100 mg daily
  
  *Duration: six months (six blister packs)*

### Standard child (ages 10 – 14) treatment regimen for MB leprosy is:
- Rifampicin: 450 mg once a month
- Clofazimine: 150 mg once a month, and 50 mg every other day
- Dapsone: 50 mg daily
  
  *Duration: 12 months (12 blister packs)*

### The standard child (ages 10 – 14) treatment regimen for PB leprosy is:
- Rifampicin: 450 mg once a month
- Dapsone: 50 mg daily
  
  *Duration: six months (six blister packs)*

**Figure 1:** Treatment regimens for leprosy. [125]

(2-12%), even under favourable circumstances. Many questions [130,133,139] were thus raised for the steep prevalence declines reported by some countries in recent years. [130] Currently, the emphasis on “elimination” is abandoned in the WHO strategy guide. [134]
5.2 Prophylactic interventions

As mentioned in section 5.1, MDT did have an impact on prevalence of the disease, but its impact on incidence is debatable. Hence, the research community investigated novel approaches to reduce the incidence. One of the approaches studied was chemo- and/or immunoprophylactic interventions.

Chemoprophylactic regimens in leprosy are protective against leprosy. A meta-analysis by Smith and Smith (2000 [140]) showed protection by dapsone, and two recent studies showed that rifampicin chemoprophylaxis is protective for a limited period of time. [141,142] In a randomized controlled trial by Moet et al. (2008 [141]) a single dose of rifampicin gave a 57% reduction in leprosy incidence during the first two years. Bakker et al. (2005 [142]) showed 75% reduction after 33.5 months with two doses of rifampicin supplied to the complete population of three small islands; no reduction, however, was seen in a neighbouring island population where only spatially defined contacts of leprosy patients received rifampicin. [142] Both studies found that the protective effect was strongest in the contact groups furthest away from the index patients, suggesting that close contacts require a more extensive regimen, possibly due to higher initial bacterial load.

The World Health Organization’s (WHO) Expanded Program of Immunization lead to the current widespread use of BCG vaccination and is thought to have had a major effect on leprosy incidence. [143] The protection of BCG vaccine is clearly demonstrated, Setia et al. (2006 [11]) and Zodpey (2007 [144]), who both published a meta-analysis. None of the analyzed studies reported a negative protective effect of BCG. The overall protective effect was lowest for experimental studies as reported by Setia et al. (26%; 95% CI 14-37%); the highest effect was seen for cohort studies by Zodpey (62%; 95% CI 53-69%). By
preventing disease, but also by altering PB/MB ratios, BCG is thought to give protection against the more infectious MB leprosy. [145]

6 Research themes for leprosy control

In the past years emphasis in leprosy control has been on the availability and accessibility of control activities, which include diagnosis, treatment with MDT, patient and family counseling, community education, prevention of disabilities/impairments, rehabilitation, and referral for complications. [134] While these control activities need to be continued, innovative approaches are necessary to further lower the incidence of leprosy.

A consultancy meeting [146] on “Innovative Approaches To Further Reduce Leprosy Burden In Countries”, held in September 2008, postulated technical, operational and strategic needs for improving integration of leprosy services into the primary health care system, quality of services and monitoring. Among the given priorities were prevention and management of nerve function impairment and reaction (theme 1), improved chemotherapy (theme 2), operational research to improve sustainability and integration of leprosy services (theme 3), and diagnostics to identify individuals at high risk of developing leprosy (theme 4). The recommendations are likely to be included in the upcoming WHO strategy for 2011-2015.

Research needs were also postulated in the report of the 9th Technical Advisory Group meeting on Leprosy Control, which was held in March 2008. [147] The research needs were identified based on an analysis of the necessary criteria for “leprosy eradication”. The research priorities are: a test for infection (theme 5), understanding transmission (theme 6), understanding the development of a protective immune response (theme 7), and development of effective, safe,
Chapter 1

acceptable and inexpensive interventions (theme 8). Besides these research needs, feasibility of leprosy eradication mainly depends on the technical feasibility, economic resources and political commitment. The Technical Advisory Group considered leprosy to be not eradicable at this moment.

7 This thesis

Data from this thesis are derived from the COLEP trial in northwest Bangladesh, covering the data and samples collected during intake and the first two follow-ups. [148]

The COLEP study was designed to determine the effect of chemoprophylaxis with single-dose rifampicin. It was designed as a large double-blind and placebo-controlled trial. The study population consisted of newly diagnosed leprosy patients (1037) and contact groups, of roughly 20 contacts per patient (total contacts included: 21,708). In the second month after the start of treatment of the patient, all contacts were visited for inclusion. During this survey, an examination for signs and symptoms of leprosy was done, blood samples were collected and either prophylaxis or placebo was distributed to the whole contact group.

A sample from the general population was also included in the study to compare their characteristics with the patient and contact groups. In a random cluster survey, twenty clusters of 1000 people were examined during house-to-house visits.

Two follow-up surveys were performed after two and four years to enable comparison of new case detection rates between contact groups and the general population.
The study was conducted in northwest Bangladesh, in the districts Nilphamari and Rangpur. At a nation-wide level, Bangladesh has reached the elimination goal of the WHO, but some districts remain above the 1/10,000 prevalence level. By the end of 2002, the prevalence in Nilphamari was 3.0 and Rangpur 1.3 per 10,000. [149] The active surveys done for the COLEP study revealed that actual prevalence in the general population was six times higher than the registered prevalence. [150]

The field work was performed by the Rural Health Program (formerly DBLM), which started its leprosy control activities in 1977 in Nilphamari and in 1986 in Rangpur. This centre has experience with performing high-quality international research: it was involved in BANDS and the TRIPOD studies, both studies focusing on nerve function impairment.

This thesis addresses the following research themes:
- Chapter 2 addresses the influence of host genetics on susceptibility to leprosy (research themes 1, 4, 7).
- Chapter 3 describes the serological, demographic and clinical patients characteristics (research themes 2, 4).
- Chapter 4 proposes an improved prediction rule for nerve function impairment (NFI) and discusses its implications (research themes 1, 8).
- Chapter 5 shows the potential of two preventive strategies for leprosy: vaccination with BCG and chemoprophylaxis with rifampicin (research theme 8).
- Chapter 6 describes the contribution that serology can make to identify individuals at high risk to develop leprosy (research themes 4, 5, 6).
General introduction

Compound of family houses with men made pool for bathing and fishing

Typical house, this one is used by two adults (one is a leprosy patient)
Chapter 1

Data entry by Kallyan Kundu

Ziehl-Nielsen staining by Kanu Ram Chowdhury
General introduction

Early start: pick up by driver from the guesthouse in Nilphamari

COLEP staff meeting during 3rd follow-up
COLEP staff meeting 3rd follow-up—staff making a group assignment

COLEP staff meeting 3rd follow-up—staff discussion on calculations
General introduction

Field visit near Nilphamari—asking permission to enter family premises

Field visit near Nilphamari—gathering of the index patient’s contacts
Chapter 1

Pictures showing blood and data collection of the patient's contacts
Arriving in a village near Rangpur for another follow-up visit
Blood and data collection

Drying of bloodcards
Afterwards we were invited for tea and a photo was requested.
Field visits in the hill-track area in Chittagong is done by boat (Chittagong is not a part of the COLEP study area)

Arriving at a little island with a few houses
Arriving at a patient house where the local housing situation was shown
The kitchen

The bedroom
Staff helping with digitalization of patient information cards in 2005.
Front page of patient information card, the so-called redcard
On the back page the clinical features of the patient are drawn. Here the lesion size is indicated: 13.6 by 4.5 cm


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