Preventing Leprosy: Epidemiological and immunological aspects of chemo- and immunoprophylaxis in leprosy patients' contacts

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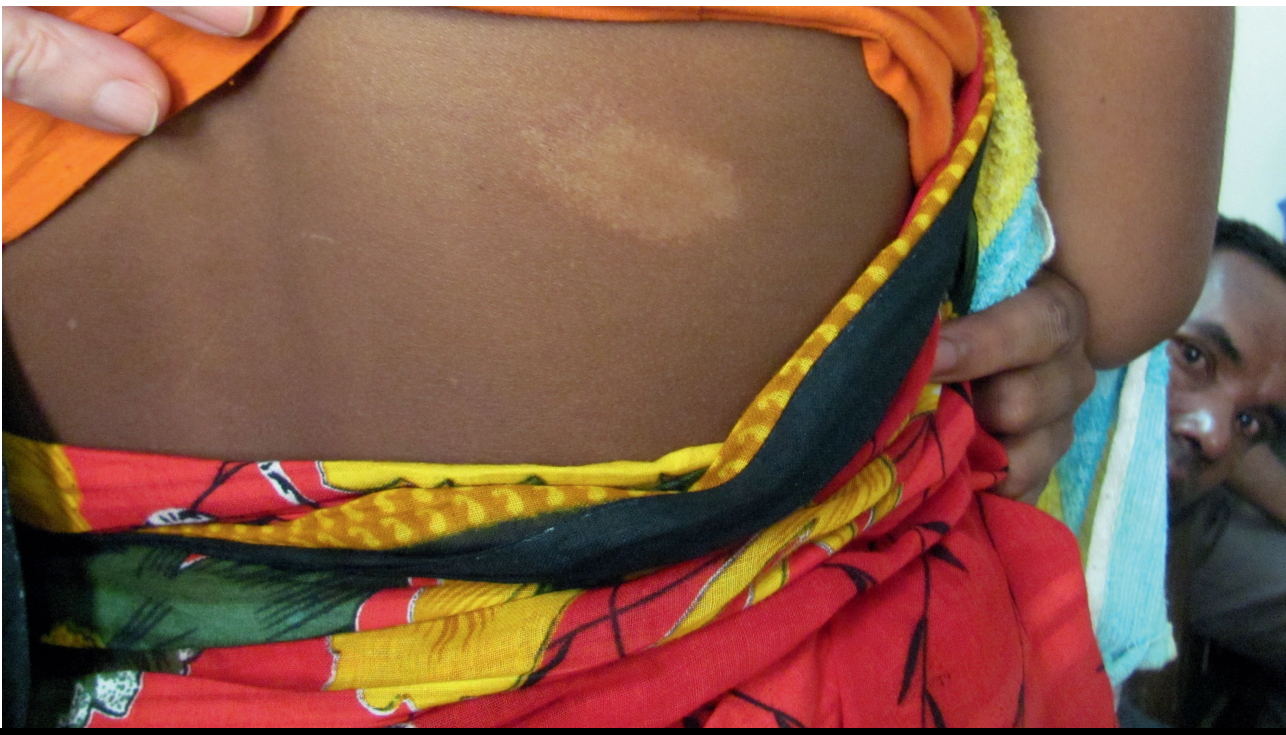
CHAPTER 1

General Introduction



CHAPTER 1: GENERAL INTRODUCTION

Leprosy

Leprosy is caused by *Mycobacterium leprae*, and primarily affects the skin and nerves. Leprosy is feared because of the deformities it can cause, consequently inducing social stigma and discrimination[1]. Grade 2 disability is defined as visible deformities in hands/feet and/or visual impairment as a result of leprosy. In order to prevent nerve damage, early diagnosis and subsequent treatment with multi-drug treatment (MDT), is crucial. Leprosy still presents a significant public health problem. Around 200,000 new cases of leprosy are detected each year, with highest numbers in India, Brazil and Indonesia[2]. *Mycobacterium leprae* closely resembles *Mycobacterium tuberculosis*, the bacillus causing tuberculosis (TB). *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) vaccination is known as a vaccine against tuberculosis[3], but is also known to cross-protect against leprosy[4]. New TB vaccines often contain antigens with homologs in *M. leprae*, implying there is room to integrate new TB and leprosy vaccine research[5].

Classification of leprosy

Leprosy is diagnosed when at least one of the following cardinal signs is present: one or more pale or reddish skin lesions with definite sensory loss; thickened peripheral nerves; and a positive skin smear result for acid-fast bacilli. Patients with negative smear results at all sites and who have no more than five skin lesions are defined as having paucibacillary (PB) leprosy, and those showing positive smear results at any site or who have more than five skin lesions as multibacillary (MB) leprosy[6]. The proportion of PB versus MB leprosy cases varies per geographic region. In Bangladesh approximately two thirds of the leprosy patients present with PB leprosy, whereas one third develops MB leprosy[2]. Worldwide, however, these proportions are more evenly divided, with a little over 50% of the patients categorized as MB leprosy[2].

Besides the pragmatic division into PB and MB leprosy as provided by the World Health Organisation (WHO), the Ridley-Jopling classification[7] classifies leprosy based on histopathological features, bacillary load and immunological response into tuberculoid leprosy (TT), borderline tuberculoid leprosy (BT), borderline borderline leprosy (BB), borderline lepromatous leprosy (BL) and lepromatous leprosy (LL). In smears stained by the Ziehl-Neelsen method, living leprosy bacilli appear as solid staining, bright pink rods. These slit-skin smears are usually taken from 6 places, including both earlobes and active sites of infection, through a small incision in the skin, from which dermal tissue is taken. The Bacterial Index (BI) indicates the number of leprosy bacilli in smears. According to

Ridley's logarithmic scale, it ranges from zero to 6+ and is based on the number of bacilli seen in an average microscopic field of the smear. In practice, however, often only clinical criteria are used for classifying individual patients, since skin-smear services are not always available and dependable, but this may depend on the possibilities in a country or region. Since PB leprosy is characterized by a low bacterial load and thus bacilli-negative smears, diagnosis of leprosy in Bangladesh, as a country with a majority of PB cases, is extra complicated. Other skin diseases, such as fungal infections, nutritional deficits, vitiligo, pityriasis alba and versicolor, psoriasis, post-kala-azar dermal leishmaniasis, etcetera, may also complicate diagnosis.

Immunopathology of leprosy

Most individuals who have been in contact with the leprosy bacterium, clear the bacteria and never develop an infection. In the remaining small percentage, one or a few ill-defined hypo-pigmented or faintly erythematous patch(es) of indeterminate leprosy (I) may develop. Leprosy often starts with the indeterminate form and is therefore often not recognized. Indeterminate leprosy may heal without treatment, persist as indeterminate leprosy or become one of the definite (determinate) types of the disease. It is also possible for individuals to develop definite types of leprosy directly; and also for PB leprosy to spontaneously heal again[8].

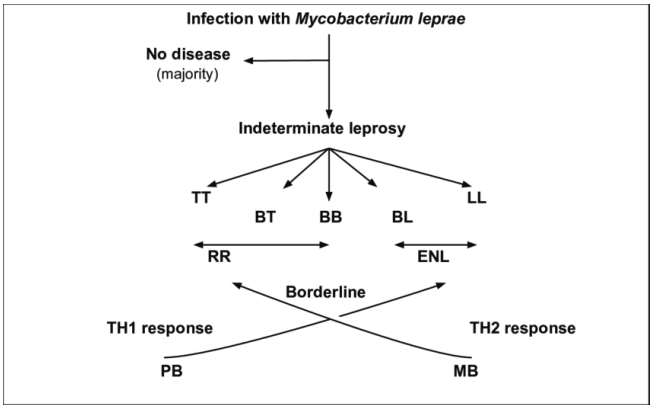


Figure 1. The Ridley-Jopling classification of leprosy.

Neder L, Rondon D, Cury S, da Silva C. Musculoskeletal manifestations and autoantibodies in children and adolescents with leprosy. Jornal de pediatria 90(5)·April 2014. DOI: 10.1016/j.jped.2014.01.007..

Leprosy depends on the infected individual's resistance to the disease. Macrophages have a classical activation phenotype (M1) in tuberculoid leprosy, while in lepromatous disease there is a pathway of alternative activation (M2). The M1 pathway stimulates $CD4^+$ T helper 1 (Th1) cells to produce pro-inflammatory cytokines such as interferon- γ (IFN-γ). These cytokines activate macrophages to

eliminate bacilli[9], leading to bacterial control, but also to secondary tissue damage due to the inflammation. Together, these macrophages form well characterized granuloma with few bacilli, leading to few and well-defined skin lesions[7].

The pronounced and local immune reaction in PB leprosy causes damage to melanocytes, sensory nerves, sebaceous glands and sweat glands. The skin lesions of TT leprosy consist of a single or few hypo-pigmented, oval or round, well-defined patches. Less melanocytes leads to hypopigmentation. Sensation is impaired, and the lesions are either hairless or with sparse hairs. Sweating is impaired in the affected area, causing the leprosy patch to be drier than the skin surrounding it. Sometimes an enlarged cutaneous nerve enters the lesion visibly. The related peripheral nerve trunk is usually enlarged. Damage to nerves leads to loss of sensation, pain, tingling and muscle weakness or paralysis.

In primary neuritic leprosy one or more peripheral nerve trunks are involved, without evidence of skin lesions. First sensory loss occurs, then motor loss; paralysis may lead to disabilities like claw hand or toes, wrist- or foot-drop, lagophthalmos, etc.

T cells of lepromatous leprosy patients are anergic to *M. leprae* and their tissues are ideal for the multiplication of leprosy bacilli. However, patients with lepromatous leprosy are not immune deficient in general. Macrophages in lepromatous disease prefer alternative activation (M2), which is not favorable for induction of Th1 responses[10 11]. Additionally, suppressor type CD8 + T cells also play a role in T cell anergy towards M. leprae in LL, which downregulate macrophage (M1) activity[12-15]. Furthermore, the production of anti-inflammatory cytokines such as interleukin-10 (IL-10) results in disseminating, progressive infection[16 17]. Patients with lepromatous leprosy also have higher levels of regulatory CD25+ CD4 + T-cells (Tregs), which play a role in *M. leprae*-Th1 unresponsiveness in lepromatous leprosy[18-20]. In lepromatous leprosy, there is also a high production of antibodies, which leads to accumulation of immune complexes, activating the complement system[21].

In lepromatous leprosy, granulomas are disorganized and filled with foamy macrophages and numerous bacilli. Skin lesions are more numerous, shiny, symmetrical and nodulous, furthermore they are less well defined and less anaesthetic. Loss of eyebrows and –lashes may occur. Patients with lepromatous leprosy have more elaborate and serious effects of nerve damage.

Leprosy Reactions

During the usually chronic course of leprosy, acute episodes (reactions) may occur[22 23]. It is mainly the borderline forms of leprosy that are immunologically unstable and therefore most likely to develop leprosy reactions[23]. Reactions may occur spontaneously, but are also associated with co-infections with helminths or HIV [23-27] and genetics[28]. Reversal reactions usually occur in the first 6 months of starting treatment. This is probably due to the bactericidal effect of rifampicin, which kills high amounts of bacilli. *M. leprae* antigens are released, which in turn trigger inflammatory reactions. The precipitating factors may not be obvious in some cases. There are two kinds of hypersensitivity: Type 1 (reversal reaction), which occurs mostly in patients with borderline tuberculoid leprosy, as a result of inflammation in skin and nerves caused by Th1 helper cells[29]; and Type 2 reaction (erythema nodosum leprosum), which occurs mostly in patients with borderline lepromatous leprosy, in which antigen and antibody complexes of the humoral immunity cause damage in tissues with systemic complications[30]. During reactions, inflamed skin lesions and nerves can be very painful and tender; irreversible nerve damage may occur if treatment with prednisone is not started soon enough. Reversal reactions must be differentiated from relapses, so that proper treatment can be given. Individuals who have received inadequate chemotherapy or those that have drug-insensitive organisms are more at risk of relapse.

Leprosy epidemiology

The global number of new leprosy cases has remained relatively stable over the past years (figure 1)[31], suggesting that transmission is ongoing; treatment of new cases alone seems insufficient. In order to be able to interrupt the transmission of leprosy, it is necessary to know the transmission routes of *M. leprae* and the risk factors of developing leprosy. A combination of factors (see below) play a role; predicting which *M. leprae* exposed individuals will progress to disease is therefore complicated.

The highest numbers of new leprosy cases are detected in India (135,485 in 2016), Brazil (25,218 in 2016) and Indonesia (16,826 in 2016)[31]. In Bangladesh, the number of new cases was 3,000 in 2016, compared to 3,141 new cases in 2013[31]. The incubation period of leprosy is generally between 3 and 5 years, although a great variability is known (between a few weeks to 45 years have been described)[32]. The exact mechanism of transmission of *M. leprae* is not known. Possibly, the bacterium is transmitted by skin-to-skin contact between cases of leprosy and healthy persons or by the respiratory route[33]. The respiratory route is considered the most important.

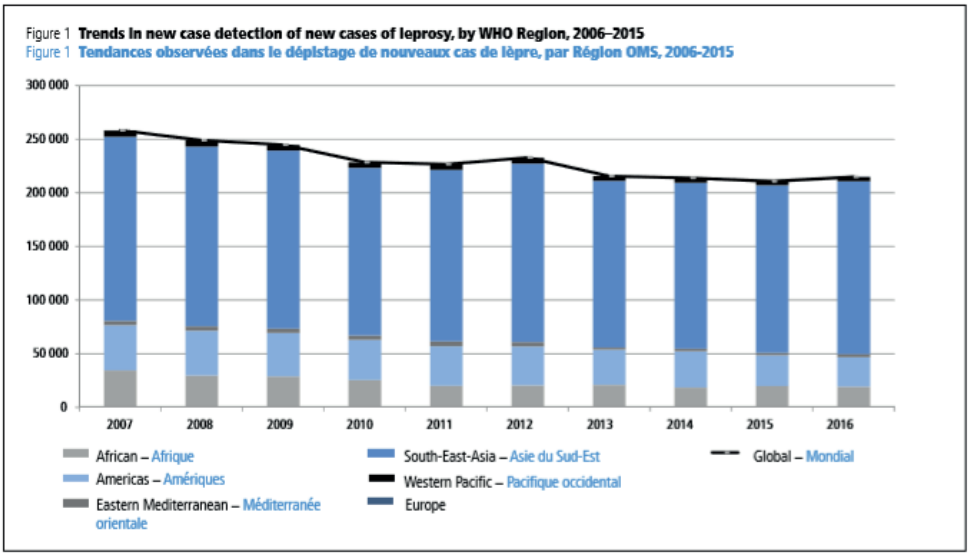


Figure 2. Trends of new case detection of leprosy between 2006 and 2015 by WHO region[31].

Nowadays the main reservoir for *M. leprae* is most likely human. Undetected (MB) cases in the community probably cause continued transmission[34]. Household contacts of MB patients have a higher risk of developing leprosy than PB cases, probably due to the high bacterial load[35]. Armadillo's are another possible reservoir of leprosy in the Southern States of the USA[36] and Brazil[37], although a recent article showed that *M. leprae* infected armadillo's may not always represent a source of infection in a specific area in the Brazilian Amazon region[38]. Possible, viable *M. leprae* resides in the soil and water mainly in areas with high prevalence of leprosy[39 40]. In Bangladesh, *M. leprae* DNA was found in 16.0% of soil from houses of leprosy patients[41]; possibly environmental sources can be (temporary) reservoirs for *M. leprae*, although further research is needed.

A low socioeconomic status and specifically nutritional deficits are known risk factors for leprosy in general[42 43]. A recent period of food shortage probably reduces the cell mediated immunity of individuals which harbour *M. leprae*, leading to clinical leprosy disease[43]. In tuberculosis, malnutrition is associated with low levels of leptin, a hormone secreted by adipose tissue[44]. Low leptin concentrations in turn, suppress macrophage functions due to elevated glucocorticoid levels, leading to decrease of bacterial killing and increased risk of disease[44]. Possibly, similar processes play a role in leprosy. Genetics[45] and co-infections[46] are also risk factors for developing leprosy. Helminth infections, specifically, have immunomodulatory properties, and can skew the host immune response

towards Th2[47 48]. The Th1 response is crucial in combatting mycobacterial infection, therefore helminth co-infection may stimulate *M. leprae* growth by upregulating Th2 cytokines or CD4 + CD25+ regulatory T cells (Tregs).

The main risk of exposure to *M. leprae* is in close contacts of new, untreated cases. Higher age of the contact, a higher bacterial load of the index patient, and close physical and genetic distance are independently associated with the risk of a contact acquiring leprosy[35]. The chance of finding a household contact with previously undiagnosed leprosy is ten-fold higher compared to the general population, for different categories of neighbours and social contacts this is between three and five-fold[49]. Contact management therefore seems an important pillar in stopping the spread of leprosy. *M. leprae* infected individuals may carrying large numbers of bacteria, without showing clinical symptoms, leading to a continued transmission. The WHO declared leprosy eliminated in 2000, after this there was a dramatic decline in leprosy control activities. This has led to a decrease in contact management and lower new case detection rates. Possibly worldwide large numbers of unreported cases remain undetected[34], also causing continued transmission.

Tools for early detection of leprosy

Enzyme-Linked Immunosorbent Assay (ELISA) techniques are used in Interferon-Gamma Release Assays (IGRAs) and are used more frequently than before. However, laboratory facilities are needed that are not found at all health centers in leprosy-endemic areas. Lateral flow assays (LFAs) are easily usable immunochromatographic assays, which find target analytes in samples without needing expensive equipment. Since diagnosing leprosy as early as possible is critical, LFAs provide new possibilities for rapid detection of leprosy patients in early stages of the disease or of *M. leprae* infected individuals without clinical symptoms[50-52 53 54]. Identification of predictive biomarkers is complicated, due to the long incubation time and low incidence of leprosy. By comparing immune profiles of leprosy contacts and following them over longer periods of time, it is becoming possible to identify which biomarkers correlate with progression to disease[50]. However, this requires large numbers of new cases indicating long-term follow-up studies in multiple endemic areas of which this study is one of the very few examples in leprosy research.

The role of phenolic glycolipid I

M. leprae phenolic glycolipid I (PGL-I) is an antigen found on the outer surface of the mycobacterium[55]. The finding of high levels of IgM antibodies (Ab) to PGL-I in leprosy patients, lead to the the development of several tests that were investigated extensively for diagnostic purposes[51]

[55 56]. In contrast to MB leprosy patients, anti-PGL-I Ab titers are not useful in detecting PB leprosy patients, since they develop cellular (not humoral) immunity and for this reason often lack Abs to PGL-I[51 55 56]. Schuring *et al.*[57] confirmed that anti-PGL-I seropositivity was associated with BI, which explains why the vast majority of PB patients have negative anti-PGL-I Ab titers.

The role of lateral flow assays using leprosy-specific biomarker profiles

Because of the broad disease spectrum of leprosy, biomarkers for both cellular and humoral mediated immunity are necessary in diagnostic tests in order to detect of *M. leprae* infection . This was demonstrated in a study[50] that used lateral flow assays (LFAs) for four immune markers (anti-PGL-I antibodies, IL-10, CCL4 and IP-10) in a field-trial in Bangladesh. Different biomarker profiles, not single markers, distinguished groups that were infected with *M. leprae* from those that were not, patients from household contacts and endemic controls, or MB from PB patients. This study is an example of how field-friendly LFAs are helpful tools in efficiently monitoring the different stages of infection and disease in leprosy contacts, facilitating early treatment of infected contacts and preventing the development of actual disease.

The role of BCG in leprosy protection

There are many studies into the use of immunoprophylaxis (vaccination) and chemoprophylaxis to prevent leprosy in contacts of leprosy patients. *Mycobacterium bovis* BCG vaccination is known as a vaccine against tuberculosis[3] and is part of the neonatal immunization scheme in a lot of areas in the world. In Bangladesh, the coverage of BCG vaccination at birth is estimated to be 98% (http://www.who.int/immunization/monitoring_surveillance/data/bgd.pdf). BCG is also recognized as protecting against leprosy[58-60]. The reason BCG can be used as a vaccine against leprosy is because of the many homologous antigens present in *M. bovis* (found in BCG vaccines) and the *M. leprae* and the *M. tuberculosis* genomes[4]. This gives a cross-reactive, protective immune response to *M. leprae* after BCG vaccination. Furthermore, live-attenuated vaccines such as BCG can give non-specific effects (NSE), besides protection against the specific micro-organisms for which it was meant[61]. The first possible immunological mechanism to explain NSE is heterologous immunity, in which T-cell memory responses to a specific antigen also cross-protect against other pathogens[62]. The second hypothesis is ‘trained immunity’, in which immunological memory is developed by the innate immune system[63].

One meta-analysis[58] showed that BCG vaccination offers an average protection of 26% against leprosy in experimental studies and 61% in observational studies; the observational studies thus

overestimating the protective effect of BCG vaccine in leprosy. The protection was better for MB leprosy compared with PB leprosy, since BCG could lead to an increase in the milder tuberculoid and indeterminate forms of leprosy, since host immunity may have improved after BCG vaccination[58]. Another meta-analysis[59] found a protective effect of BCG of 41% for trials and 60% for observational studies. There was a greater variability of the BCG vaccine effect against PB forms; for MB leprosy the estimates were more homogeneous, but a statistically significant different effect was not found. The protective effect of the BCG vaccination was significant higher if studies were conducted among household contacts instead of the general population. This is shown in a cohort study of 3536 contacts of 1161 leprosy patients in Brazil[60], whereby the protection conferred by a booster BCG vaccination was 56% and not clearly affected by previous BCG vaccination. This effect was 83-85% for the indeterminate and MB forms, but a non-significant effect of 26% was found for the PB forms. The risk of tuberculoid leprosy in the first months was high among BCG vaccinated contacts: among the 58 new cases detected during 18 years of contact follow-up, leprosy was diagnosed in 21 of these contacts (36%) within 2-10 months after vaccination; 18 out of these 21 contacts had PB leprosy.

Merle *et al*[59] performed a meta-analysis which showed no statistical difference in BCG protection in studies where patients are vaccinated once versus twice or more. The two large trials compared in this meta-analysis had very different results. The first was a cluster randomized trial[64] in Brazil among 99,770 school children aged 7–14 years, which were followed for 6 years. In the vaccinated group, an incidence rate ratio of leprosy of 0.99 was found compared to the control group, showing that revaccination did not give extra protection. By contrast, a randomized controlled trial[65] in northern Malawi showed that a second BCG vaccination gives a 49% protection against leprosy. The main difference between these two studies are the characteristics of the revaccinated population: in Brazil only school children were studied, whereas in Malawi infants to adults took part. Revaccination might give extra protection to adults for whom the first vaccination has become less effective over time, but revaccination might be less useful in school children[59].

Concluding, BCG vaccination provides a variable protective effect against leprosy in different studies[58-60] and seems better for protection against MB than PB leprosy, since improved host immunity after BCG vaccination could lead to an increased occurrence of milder forms of PB. The benefit of BCG seems dependent on the population receiving vaccination, with more benefit in adults than in children[59].

The role of SDR and previous BCG vaccination in leprosy prevention

Chemoprophylaxis entails the use of a drug to prevent the development of a disease. Dapsone and rifampicin (together with clofazimine) are drugs that are used as part of the MDT cocktail to treat newly diagnosed leprosy patients. However, these antibiotics have also been studied singularly as chemoprophylactic drugs in contacts of new leprosy patients since the 1960s. A meta-analysis of three studies using dapsone[66-68] as chemoprophylaxis, showed a reduction of 40% of leprosy incidence amongst contacts using dapsone versus placebo. A chemoprophylaxis trial in five Indonesian islands[69] was started in 2000, in which a blanket group (rifampicin prophylaxis given to all eligible persons), was compared to a contact group (rifampicin prophylaxis given to all eligible contacts of former/treated and newly diagnosed leprosy patients) and a control group (no chemoprophylaxis given). After three years, the cumulative incidence of leprosy was significantly lower in the blanket group, but no difference was found between the contact and control groups. Thus, rifampin prophylaxis seems most effective in communities where everybody was given the prophylaxis in contrast to only household contacts and direct neighbors[69]. A possible explanation is that the bacillary load in close contacts is already too high to be eliminated by a single dose of rifampicin.

In the COLEP study[70], a cluster randomized controlled trial conducted in a leprosy endemic area in the Northwest of Bangladesh between 2002 and 2009, the effect of single dose rifampicin versus placebo in preventing leprosy in close contacts of newly diagnosed leprosy patients was studied. The COLEP study showed that a single dose of rifampicin (SDR) in contacts of newl leprosy patients reduced the incidence of leprosy in the first two years with 57%[70]. In the subgroup analysis it was discovered that those contacts with a low *a priori* chance of developing leprosy, benefited most of the chemoprophylaxis (i.e. if the contact was not blood-related to the index patient, if the index patient had PB leprosy, and if the contact was a social contact rather than a household contact or neighbor). Furthermore, the COLEP study showed that the effect of SDR depended on the BCG status of the contact. If the contact had received BCG vaccination as neonate (presence of a BCG-scar), the protective effect of SDR was 80%[71]. Childhood BCG vaccination and SDR both had a protective effect for leprosy in contacts of about 60%, but if a contact who had previously received BCG vaccination also received SDR, the protective effect appeared to be additive. Based on these experiences, a trial was started in Bangladesh to assess the effectiveness of a combined strategy (the MALTALEP study). In this cluster randomized controlled trial, contacts of newly diagnosed leprosy patients received either BCG alone, or BCG plus SDR. In particular, the main aim was to determine whether the excess cases in the first year after immunoprophylaxis[60] can be prevented by chemoprophylaxis with SDR.

Recently, the World Health Organisation (WHO) has included SDR as guideline in their leprosy elimination strategy[72]. Implementation studies on SDR as leprosy post-exposure prophylaxis (LPEP)[73] are currently ongoing, which are designed to study the effectiveness, impact and feasibility of contact tracing and PEP for leprosy. However, the direct immunological effect of SDR on infection has not yet been investigated, nor its effect on *M. leprae* infection in the community.

Aims and outline of the Thesis

Chapter 1 gives an introduction on leprosy. Furthermore, tools are described for early detection of leprosy. The role of BCG and SDR in leprosy prevention is discussed. Finally, the rationale behind a combined strategy is introduced.

Chapter 2 describes the design and purpose of the MALTALEP trial, a cluster randomized controlled trial in the Northwest of Bangladesh among around 15,000 close contacts of new leprosy patients, to evaluate the effect of BCG only versus BCG and SDR as prophylactic measure to prevent the development of leprosy.

Chapter 3 provides an analysis of the clinical and demographic parameters of the unexpectedly high proportion of healthy contacts of leprosy patients presenting with paucibacillary leprosy within 12 weeks after receiving BCG vaccination in the first 1,5 years of the MALTALEP trial (0,40% of vaccinated contacts). It also describes the various immunological mechanisms that could underlie this phenomenon.

Chapter 4 describes the immune- and genetic profiles associated with adverse events after BCG vaccination in a leprosy endemic area in Bangladesh. Cytokine profiles induced by BCG vaccination in whole blood assays of contacts with and without vaccine-associated complications are compared.

In Chapter 5, the anti-PGL-I antibody levels of leprosy contacts are followed to determine whether these can be utilized as a prognostic biomarker for leprosy by predicting which individuals will progress to disease.

In Chapter 6, the primary and secondary outcomes of the MALTALEP trial are described. The difference between the number of new leprosy patients among leprosy contacts that emerge in either of the two intervention arms (BCG only versus BCG and SDR) within two years of intake is compared. Secondary data analysis is carried out in order to define special groups at risk for developing leprosy.

Finally, in Chapter 7, the following three research questions are discussed.

Research Questions:

1. What are the potential causative mechanisms underlying the development of leprosy following BCG vaccination?
2. Do the results of our trial justify the introduction of a combination of BCG and SDR in leprosy health care programs in Bangladesh to prevent the development of leprosy amongst household contacts of new leprosy patients?
3. Can immune markers be identified in contacts of leprosy patients that predict the development of clinical leprosy?