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

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CHAPTER 7

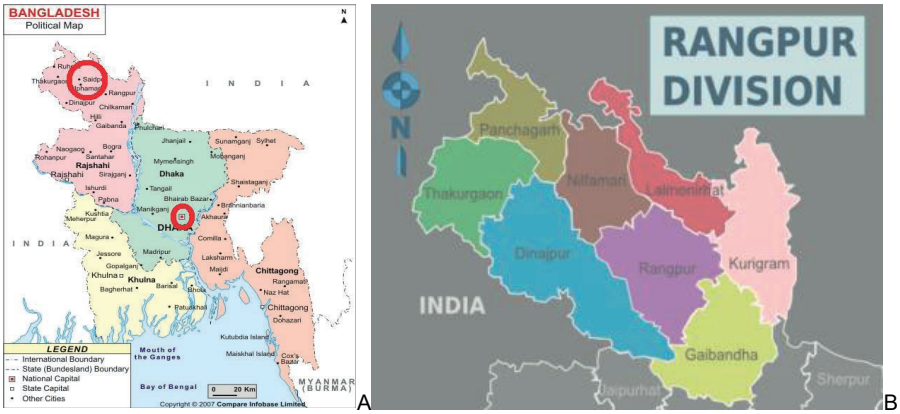
7

General Discussion

CHAPTER 7: GENERAL DISCUSSION

This thesis is centered around the MALTALEP trial, a cluster randomized controlled trial conducted from 2012 to 2019 in northwest Bangladesh among 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.

Figure 1A: Map of Bangladesh, with the research area indicated with the larger circle (the smaller circle indicates the capital city Dhaka).
Figure 1B: the MALTALEP study was conducted in the districts of Nilphamari, Rangpur, Thakurgaon and Panchagarh in northwest Bangladesh.



A B

Figure 2: Population and number of new cases in the four districts Nilphamari, Rangpur, Thakurgaon and Panchagarh in the northwest of Bangladesh.

	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Population	7214063	7320833	7429183	7539136	7650715	7763948	7878854	7973399	8069079	8190035
New cases	1043	867	572	651	641	765	801	666	862	967

This thesis is centered around the following three 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?

Research Question 1: What are the potential causative mechanisms underlying development of leprosy following BCG vaccination?

In 1989, Bagshawe[74] already said that BCG vaccination may precipitate clinical signs and symptoms of tuberculoid leprosy in people carrying *M. leprae* and cause upgrading of existing lesions. Duppre *et al*[60] also hypothesized that the high number of cases with tuberculoid leprosy found 2-10 months after BCG vaccination in Brazil, is caused by BCG stimulating the already present anti-mycobacterial immunity in individuals infected with *M. leprae* before or immediately after BCG vaccination. In line with these studies, we also found an unexpectedly high proportion of healthy contacts of leprosy patients presenting with PB leprosy within 12 weeks after receiving BCG vaccination (33,6% of all cases diagnosed during the 2-year observation period)[75 83].

It is well known that in tuberculoid leprosy, macrophages have a classical activation phenotype (M1), while macrophages in lepromatous disease show alternative activation (M2)[76-79]. BCG has shown to direct macrophages preferentially towards pro-inflammatory M1 activation[76]. In the case of subsequent *M. leprae* infection, higher levels of inflammatory cytokines will be released; the immune system can then clear the bacilli more effectively or tuberculoid leprosy may develop[76]. Thus, BCG vaccination causing increased pro-inflammatory immune responses, may also render already infected contacts more prone to developing PB leprosy by shifting the immune response to destructive Th1 responses. Rhodes *et al*[80] showed that BCG revaccination provides a higher and longer lasting IFN- γ+ CD4+ T-cell response than primary vaccination in humans, with a peak around 30 days.

When addressing the research question what immunological processes play a role in leprosy activation following BCG vaccination, it is instructive to observe immunological responses during BCG-related complications: large local reactogenicity after BCG vaccination possibly correlates with an adequate immune response, and thus less chance of developing the more severe lepromatous forms of leprosy, which are an expression of a suppressed immune response.

In our trial, adverse events were observed in 0.34% of the contacts of leprosy patients that received BCG vaccination[75]. These complications consisted primarily (80%) of skin ulcerations. We investigated what type of immune profile is associated with BCG-related complications[75]. Similar to the increased pro-inflammatory Th1 immunity and high levels of pro-inflammatory cytokine observed for tuberculoid leprosy patients, contacts with adverse events at the site of BCG vaccination showed elevated IFN-γ levels in response to *M. leprae* specific proteins in whole-blood assays (WBA). In addition to the elevated IFN-γ levels, we found that lower levels of $sCD40L_{NIL}$ and GRO_{WCS} were significantly associated with BCG complications. sCD40L and GRO (CXCL1) both play a role in T-cell regulation; a decrease in their levels may potentially cause uncontrolled T-cell immunity damaging

the skin. Finally, regulatory T-cells secrete CC chemokine ligand 4 (CCL4), which in turn suppresses Th1 cells[12]. In our study[75], a reduction in CCL4 (although not significant) was found in those contacts that developed complications after BCG. This may indicate decreased T-cell regulation and a shift towards excessive Th1-type immunity with inflammation at the BCG vaccination site as a result. Skin complications after BCG vaccination therefore may be surrogate markers for protective immunity against leprosy, although there may be a higher risk of developing tuberculoid leprosy.

The Mitsuda reaction shows if an appropriate immune response to an intradermal injection of lepromin (which is a heat-killed leprosy bacilli) is formed. In case of a negative skin reaction, it has a good prognostic value for susceptibility to the lepromatous form of leprosy; a positive skin reaction indicates resistance to lepromatous leprosy[77]. In the same way, individuals with high local reactogenicity after intradermal BCG administration have less risk for lepromatous leprosy onset[78]. In 12 tuberculin skin test and Quantiferon negative, BCG-naive adults in the Netherlands, BCG vaccination induced significant Th1-type immunity in those with large local inflammation responses. However, in low inflammation responses, significantly increased regulatory $CD8^+$ T-cells were found[79].

The sudden increase in leprosy patients after BCG vaccination is caused by a boosted cell-mediated immunity by homologues of *M. leprae* antigens in BCG. The mechanism may be similar to the immune reconstitution inflammatory syndrome (IRIS) seen in human immune deficiency virus (HIV) patients on highly active antiretroviral treatment (HAART), who develop leprosy. Deps *et al.*[81] defined IRIS in leprosy as leprosy and/or Type 1 reaction and erythema nodosum leprosum (ENL or Type 2 reaction) within 6 months after start of HAART. 89.5% of the leprosy/IRIS cases presented with TT or BT leprosy. After starting HAART, IRIS was initiated after a mean of 8.7 weeks. Immune restoration in leprosy as IRIS after starting HAART is based on an increase in circulating $CD4^+$ T cells[81].

We suggest that a comparable process takes place, namely stimulation of the Th1 cascade, leads to presentation of clinically apparent tuberculoid leprosy and augmentation of type 1 reactions after BCG vaccination in contacts of leprosy patients. Our trial is unique with respect to several aspects, one of which is that it has its first follow-up moment relatively soon after BCG vaccination (within 8-12 weeks). By finding the new cases at an early stage, early treatment was also possible, possibly preventing complications. In fact, BCG vaccination given to household contacts of leprosy patients could actually identify this important group. However, it should not be used as a legitimate diagnostic test for pre-clinical leprosy, since it is unclear whether BCG vaccination only alters the incubation period or changed the course of the infection from self-limiting, subclinical infection to manifest disease.

Research Question 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?

The MALTALEP trial[82] was designed to evaluate whether combining SDR with BCG (re)vaccination provides additional value in preventing leprosy. Strengths of our trial[83] is that it is a randomized-controlled trial that includes an extensive number of leprosy contacts (14,988). Also, because it is based in a leprosy-endemic area, implementation lies close to clinical field practice. However, on the basis of the trial results, we cannot justify the introduction of a combination of BCG and SDR in the field to prevent the development of leprosy amongst household contacts of new leprosy patients. This is due to several reasons:

Firstly, in the first year after BCG (re)vaccination, the reduction in incidence of leprosy in the SDR+ arm was 42% compared to the SDR- arm, which shows a clear effect of this chemoprophylactic intervention[83]. However, the low number of cases prompt us to designate this effect as a trend, as it was not statistically significant ($p>0.05$), due to lack of power. No additional effect of SDR was seen in the second year, which is within the line of expectation because SDR is not a vaccine and no long-lasting immunological response is induced[84]. The COLEP trial describes an overall effect of SDR of 57% in the first two years[70]. However, chemoprophylaxis with SDR was most effective in contact groups with relatively low perceived *a priori* risks, such as contact groups of PB index patients, in contacts not living in the same household, or without close blood relationship to the index patient. It is assumed that infected contacts in these groups have had less exposure to *M. leprae* prior to SDR provision and therefore lower bacterial loads than those who are closer to an index patient. Thus, one single dose of rifampicin should be enough to clear the bacterial load in these low-risk groups, whereas for more heavily infected individuals (due to either genetic susceptibility and/or long-term exposure to an untreated MB patient), treatment with SDR is less effective. In the COLEP trial in the group of blood-related household contacts the effect of SDR was around 25% only, while it was around 50% in non-blood-related and neighbouring contacts, and up to 75% in social contacts[70 85]. For the high-risk group that is possibly incubating MB leprosy, a diagnostic test indicating the extent of infection would justify an extended treatment regimen, possibly a full course of MDT. Contact screening including a field-friendly diagnostic test could represent an efficient strategy to reduce transmission of *M. leprae* in the community. The goal of early contact tracing and subsequent provision of SDR is three-fold: firstly, with contact tracing early cases of leprosy can be detected; secondly, provision of SDR prevents leprosy in infected contacts without clinical signs of leprosy; and thirdly it contributes to the interruption of transmission of *M. leprae* to others[84]. The finding that SDR

is efficient for contacts who are not living in the same household is in line with the few other studies available on this subject. These show that blanket chemoprophylaxis of a whole community, mostly including individuals with less exposure and as such with no or low bacterial load, is more effective than chemoprophylaxis to household contacts only in reducing new case detection rates in the community[69 86]. Our study[83] had a larger proportion of household contacts, probably explaining why SDR was less effective in our study (42%), when compared to COLEP (57%), where a wider group of contacts were included[70].

Secondly, the effect of BCG in protecting against leprosy among leprosy contacts in the MALTALEP trial appeared to be smaller than anticipated[83], compared to previous studies on BCG immunoprophylaxis, for example from Brazil[60]. The incidence rate in our study in Bangladesh is 33.72 per 10,000 PYAR in the BCG only arm at 2 years of the MALTALEP trial[83]. The incidence rate of leprosy at 2 years among the household contacts and next-door neighbours in the non-intervention arm in the COLEP study was 39.35 per 10,000 PYAR[70]. This implies a 14.3% reduction of leprosy incidence by BCG vaccination compared to no intervention. In the Brazilian study amongst leprosy contacts[60], the protection conferred by a booster BCG vaccination was 56% and was not substantially 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. This might also explain the lack of effect of BCG in the MALTALEP trial when compared to no intervention: in Bangladesh a larger proportion of leprosy patients develop the PB form (66% of total leprosy patients) when compared to other parts of the world (50% of total leprosy patients)[2]. Furthermore, the BCG strain used may also have effect on the efficiency of BCG vaccination[87]. In Bangladesh, the Moscow strain 361, Tokyo strain 172 and the Aventis-Pasteur strain are used. Elsewhere the use of other more virulent BCG strains for vaccination could lead to different results. The Tokyo strain, for example, is known to be a non-virulent strain; restoration of its lost T-cell epitopes in the future may lead to new, more powerful BCG vaccine strains[88]. Furthermore, genetic or nutritional differences between populations, environmental influences such as sunlight exposure (vitamin D), poor cold-chain maintenance, or exposure to environmental mycobacterial infections may lead to variation in the efficacy of BCG[89].

Thirdly, we found no statistically significant difference between the development of leprosy in contacts who were revaccinated as part of the trial (with visible BCG scar) *versus* contacts who were vaccinated for the first time in their life as part of the trial (BCG scar naïve)[83]. This is in line with the conclusions of Merle *et al.*[59] that revaccination might give extra protection to adults for whom the effectiveness of the first vaccination decreased over time, but there may be no use of revaccination when it is performed in school children. Our study[83] has a relatively high proportion of school

children between 5 and 14 years of age (15%), which may explain why revaccination had little benefit.

Finally, we had expected to prevent the excess cases in the first year after BCG as described previously[60 74], by giving chemoprophylaxis in the form of SDR 8-12 weeks after BCG vaccination. We had not anticipated however, to find such a large proportion of new leprosy cases (33.6%) in the three-month observation period before providing SDR (Table 2[83]). This renders the bactericidal capacity of SDR redundant and therefore less prominent.

Table 2: New leprosy cases among contacts of newly diagnosed leprosy cases identified according to the time points of diagnosis

	8-12 weeks	1 year	2 years	Total
BCG				
PB	23	24	24	71
MB	0	3*	0	3
BCG and SDR				
PB	26	14	23	63
MB	1	5	6	12
Total	50	46	53	149

*Only 1 new MB leprosy case had a BI of 2+ (BL), the rest of the MB cases were smear negative (MB BT).

The Brazilian trial[60] only described an augmentation of new leprosy cases 2-10 months after BCG vaccination. It is possible that this trial did not describe earlier cases, because follow-up did not occur in the first two months, although this is not mentioned specifically in the trial description. Future trials could consider providing SDR before BCG. Giving BCG and SDR at the same time within the MALTALEP trial was not possible, due to the bactericidal effect of SDR on BCG, which is a live vaccine. When designing the trial, we chose not to administer SDR before BCG, because of the logistic implications as an extra follow-up time point would have been necessary, which is difficult in a country like Bangladesh with a limited infrastructure and political instability.

Although we do not recommend a combined strategy with BCG and SDR based on the results of our trial, there is sufficient evidence to continue advising administration of SDR to household contacts of new leprosy cases. However, the direct immunological effect of SDR on infection has not yet been investigated, nor its effect on *M. leprae* infection in the community. Future studies will assess this in more detail in Bangladesh in the next three years. The advantage of targeting household contacts in general is that they are a clearly defined group, who are easily reachable. Because of the social stigma associated with leprosy, new leprosy patients may be less willing to give disclosure when

asking people outside the direct contacts to participate in prophylactic campaigns. Furthermore, since new leprosy cases are becoming rarer in most endemic countries, it is not cost-effective to apply interventions such as chemoprophylaxis and immunoprophylaxis to total populations within a blanket approach due to the enormous numbers needed to treat to prevent a case of leprosy. Targeted interventions towards well-defined high-risk groups is preferable.

Research Question 3: Can immune markers be identified in contacts of leprosy patients that predict the development of clinical leprosy?

It has been established that antibodies directed aganst the *M. leprae*-specific phenolic glycolipid I (PGL-I) cannot be applied in predicting the development of clinical leprosy in the Bangladeshi context. Combined biomarker tests, however, are increasingly proving to be useful.

Our findings in contacts in Bangladesh[90] are in line with previous literature[91-96], where it has been shown that the development of leprosy amongst leprosy contacts was not associated with the level of anti-PGL-I seropositivity among these contacts at intake. Although positivity to anti-PGL-I and development of leprosy in healthy contacts was associated, choosing contacts for prophylaxis based on anti-PGL-I response would miss more than half of future leprosy cases, particularly PB[91-94]. To understand the value of anti-PGL-I Ab as a predictor of leprosy in those at risk of developing leprosy, we analyzed the anti PGL-I Ab levels in the blood of 224 contacts of leprosy patients in a highly endemic area in the northwest part of Bangladesh and followed them over a period of 6 years[90]. Six of these 25 (24%) contacts who developed leprosy had a positive anti-PGL-I Ab level of >0.150 at intake. Thirty five out of 199 (17.6%) contacts who did not develop leprosy had a positive anti-PGL-I Ab level of >0.15 at intake. No significant association was found for the anti-PGL-I Ab levels at baseline (OR: 1.01 (0.78, 1.31), 95% CI p=0.94) between the two groups. Furthermore, changes in anti-PLG-I Ab levels did not predict disease progression in contacts of new leprosy patients in Bangladesh. These results clearly indicate that also in Bangladesh anti-PGL-I Ab tests alone are not able to diagnose leprosy amongst leprosy contacts at an early time point.

Most of the leprosy patients' contacts in our study, however, developed PB leprosy (21 out of 25), which offers an explanation for the lack of increase of anti-PGL-I titers at leprosy diagnosis. In this respect it is important to realize that in Bangladesh the percentage of PB cases amongst new leprosy cases is generally higher (67%) than in other countries in Asia or the rest of the world (on average around 50%)[2]. In southeast Asian countries, such as Indonesia, predominantly MB patients are found, which is probably due to a combination of genetic factors as well as lack of early case detection, since more PB cases are found when active case finding strategy is applied[84]. Therefore, in other leprosy endemic countries, where more MB leprosy occurs, the longitudinal pattern of anti-PGL-I Ab levels could have more prognostic value. Furthermore, anti-PGL-I antibodies could be a useful tool for monitoring how effective the treatment of leprosy (reactions) is, since effective treatment leads to a decrease in antibody levels[97].

Importantly, combining humoral and cellular biomarkers (instead of serology alone) gives more possibilities in distinguishing *M. leprae* infected from non-infected individuals, patients from

contacts, or lepromatous from tuberculoid patients[50 98]. Field-friendly tests based on a recently developed lateral flow test format (UCP-LFA) using biomarker signatures instead of single markers, were useful in identifying which contacts are at risk of developing leprosy, as well as individuals infected with M. leprae without clinical symptoms[50 98]. Other studies by our group have focused on this immunodiagnostic research line as part of the IDEAL project[50 99] and projects situated in leprosy endemic areas outside Bangladesh[54].