Combination chemoprophylaxis and immunoprophylaxis in reducing the incidence of leprosy

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Abstract: Leprosy is a complex infectious disease caused by *Mycobacterium leprae* that is a leading cause of nontraumatic peripheral neuropathy. Current control strategies, with a goal of early diagnosis and treatment in the form of multidrug therapy, have maintained new case reports at ~225,000 per year. Diagnostic capabilities are limited and even with revisions to multidrug therapy regimen, treatment can still require up to a year of daily drug intake. Although alternate chemotherapies or adjunct immune therapies that could provide shorter or simpler treatment regimen appear possible, only a limited number of trials have been conducted. More proactive strategies appear necessary in the drive to elimination. As a prevention strategy, most chemoprophylaxis campaigns to date have provided about a 2-year protective window. Vaccination, in the form of a single bacillus Calmette–Guérin (BCG) immunization, generally provides ~50% reduction in leprosy cases. Adapting control strategies to provide both chemoprophylaxis and immunoprophylaxis has distinct appeal, with chemoprophylaxis theoretically buttressed by vaccination to generate immediate protection that can be sustained in the long term. We also discuss simple assays measuring biomarkers as surrogates for disease development or replacements for invasive, but not particularly sensitive, direct measures of *M. leprae* infection. Such assays could facilitate the clinical trials required to develop these new chemoprophylaxis, immunoprophylaxis strategies, and transition into wider use.

Keywords: mycobacteria, treatment, antibiotics, T-cell, vaccine, prevention Hansen's disease

Epidemiology and etiology of leprosy

Leprosy, also known as Hansen's disease, is historically associated with disfigurement, social ostracization, and removal of basic human rights. These situations have now thankfully been improved and legally rectified in most countries. Although it is now known that leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, various myths such as the disease being a divine curse, karma for past misdeeds, or a genetic predisposition, among others, persist. Leprosy can, however, be controlled and is curable.

Leprosy is an extremely complex disease as it manifests across a wide array of symptoms, and various forms can be distinguished and characterized. *M. leprae* has a predilection for the skin and, uniquely, also the nerves. The World Health Organization (WHO) has established diagnostic criteria as the presence of one or more of the following key signs: appearance of hypopigmented or reddish lesion with hypoesthesia, presence of acid-fast bacilli in lymph node smears, and compatible skin lesion histopathology. Although nonfatal and typically characterized by the appearance of skin lesions, leprosy is one of the most common worldwide causes of nontraumatic

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peripheral neuropathy. Neuropathy arises not only from the infection and damage of peripheral nerves by *M. leprae* itself but also from the inflammatory and immunologic responses to the infection. In addition, as many as half of all patients may be affected by one of the two major clinical types of leprosy reactions, which are acute inflammatory complications that can develop during the course of leprosy irrespective of treatment status. The inflammation associated with reactions can be a medical emergency that often requires hospitalization. If nerve damage is allowed to progress, it can become disabling or, through sensory loss, lead to traumatic injury.¹⁻³

Once diagnosed, leprosy is treatable and patients are operationally defined into one of two categories, paucibacillary (PB) and multibacillary (MB), for treatment purposes. The Ridley–Jopling scale characterizes five forms of leprosy through the use of clinical, histopathological, and immunological methods: lepromatous leprosy (LL), borderline lepromatous, mid-borderline, borderline tuberculoid (BT), and tuberculoid leprosy (TT).^{4,5} A pure neural leprosy presentation, which is PB, also exists. PB leprosy patients, encompassing TT and a number of BT forms, are characterized as having one or few skin lesions and granulomatous dermatopathology with low or absent bacterial indices (BI). At the extreme PB pole, TT patients demonstrate a specific cell-mediated immunity against *M. leprae* and have an absent, or low, BI. Control of bacterial growth by PB patients indicates that these individuals mount a strong, but not necessarily curative, immune response against *M. leprae*. MB leprosy encompasses LL, borderline lepromatous, mid-borderline, and a number of BT forms. At the extreme MB pole, LL patients demonstrate high titers of anti-*M. leprae* antibodies but an absence of specific cell-mediated immunity.⁴ In the absence of a strong cellular immune response, LL patients do not control bacterial replication and have high BI. Because most of the clinicians and health care workers who are seeing patients typically have limited facilities that cannot readily determine Ridley–Jopling classification, the simplified WHO diagnostic criteria have been widely adopted as the preferred diagnostic strategy. Reliance on only one key criterion for the operational MB/PB diagnosis presents limitations; however, because not all lesions are obviously hypopigmented or erythematous, and they are not always anaesthetic. The clinical diagnosis may be supported by the histopathological analysis of a skin lesion, especially when bacilli and/or a neural infiltration are found, but these methods do not have good sensitivity. This is especially true for patients with indeterminate or TT presentations.

Treatment for leprosy has evolved over time. Since 1995, based upon the annual reporting of new cases, WHO has disseminated a cocktail of antibiotics for free of charge in the form of multidrug therapy (MDT). The widespread provision of MDT and revised, shortened treatment regimens have been major contributors to the massive reduction of registered leprosy cases. Although 16 countries reported >1,000 new cases during 2009, all but a handful have yet to achieve a prevalence rate of less than one case per 10,000 persons, the threshold considered indicative of eliminating leprosy as a public health problem.⁶ In all WHO regions and at a global level, the number of new cases reported during the reporting year has stabilized in recent years, and continued or renewed vigilance appears necessary.⁷ Leprosy is not evenly spread and localized regions with higher incidence rates can generally be distinguished within most reporting countries.⁸⁻¹¹ Several Indian states are reporting the maintenance of, or reemergence of prevalence rates to, levels above the national target, and an abundance of new cases are now being detected in the Amazon region of Brazil.¹²⁻¹⁴ Many cases are likely not being diagnosed and the reported numbers probably significantly underrepresent the real leprosy situation.¹⁵

Interestingly, conditions with which to culture *M. leprae* in vitro have not been determined, and how *M. leprae* enters the body to establish infection is not definitively known. Many patients anecdotally connect the site of their first obvious lesion with some previous injury or skin break, and entry through the skin is implied. Similarly, it is unclear how transmission occurs, although person-to-person transmission and subsequent expulsion of *M. leprae* directly in the skin and nasal epithelia are strongly suggested.¹⁶ MB cases typically have large numbers of acid-fast bacilli deep in the dermis, and although there are reports of *M. leprae* in the desquamating epithelium, there are no reports of acid-fast bacilli being found in the epidermis. It is, therefore, unclear if *M. leprae* reach the skin surface in large enough numbers to represent a meaningful mechanism of transmission.¹⁷ LL patients have relatively large amounts of *M. leprae* in the superficial keratin layer of the skin, suggesting that the bacteria may exit along with the sebaceous secretions.¹⁸ The quantity of *M. leprae* within nasal mucosal lesions of LL patients can be as high as 1×10^7 bacilli, and most lepromatous patients have bacilli that are expelled into their nasal secretions by simply blowing the nose.¹⁹⁻²¹ Research, therefore, increasingly favors the respiratory route as the major mode of expulsion and potential transmission to others.^{22,23} Alongside genetic factors, one of the most significant risk factors for developing leprosy

is long-term contact, typically through sharing the same residence, with an untreated MB leprosy patient.

Treatment strategies

In order to develop preventative strategies to reduce the incidence of leprosy, it is instructive to understand how the disease is treated and to consider the current limitations and potential complications that may arise.

Chemotherapy

Treatments for leprosy have undergone considerable evolution in the past century: from chaulmoogra oil in 1915 to promin, a sulfone drug that successfully treated leprosy but required many painful injections in the 1940s, to dapsone monotherapy in 1946, and then eventually to MDT in 1982 (Figure 1). By the 1960s, resistance to dapsone had started to develop, and dapsone-resistant *M. leprae* strains are now prevalent. In 1981, a WHO study group recommended MDT, a combination of dapsone, rifampicin (RIF), and clofazimine. This cocktail is safe and effective and provided in convenient monthly calendar blister packs. Since 1995, WHO has been providing free MDT for all patients in the world, initially through the drug fund provided by the Nippon Foundation and since 2000, through Novartis and the Novartis Foundation. As part of Novartis's commitment to the 2012 London Declaration on Neglected Tropical Diseases, the company renewed its pledge to work to end leprosy by extending its donation of MDT through 2020. This includes MDT and support costs, to aid WHO with the donation and logistics, worth >US\$40 million with an overall expectation of reaching an estimated 1.3 million patients.

MDT, consisting of RIF, clofazimine, and dapsone for MB leprosy patients and RIF and dapsone for PB leprosy patients, alters the course of disease in leprosy patients and is the most common way to limit the dissemination of *M. leprae* to others. Interestingly, RIF is the only component that is strongly bactericidal for *M. leprae*.^{24,25} Dapsone is a sulfone antibiotic for which anti-inflammatory and immunomodulatory effects have been recognized, but the precise mechanism of action is not known. Clofazimine has a weakly bactericidal action against *M. leprae*. Because clofazimine is an orange-colored iminophenazine dye, it often causes discoloring of the skin. This skin pigmentation gradually resolves, but it may require up to 2 years once the drug is discontinued to return to pretreatment levels, and patients often feel marked and stigmatized by this and withdraw clofazimine from their treatments as a consequence.

Figure 1 Timeline of the major treatment and prevention strategies for leprosy.

Notes: Drug treatments and preventative vaccine strategies are shown and indicated in the timeframe of the first trial. Current strategies are boxed and shown in bold. Potential new strategies and timelines are indicated at the bottom.

Abbreviations: BCG, bacillus Calmette–Guérin; MDT, multidrug therapy; ROM, rifampicin, ofloxacin, and minocycline; U-MDT, uniform multidrug therapy; *M. w.*, *Mycobacterium w.*; ICRC, Indian Cancer Research Center.

Although MDT is effective in the majority of current cases, as mentioned, dapsone resistance is relatively widespread. When combined with clofazimine noncompliance, this means that many patients may unwittingly be taking RIF monotherapy. This has the potential to be highly conducive for the emergence of resistance and several investigators have indeed observed multidrug-resistant strains of *M. leprae*.^{26–31} The WHO Global Leprosy Programme initiated a Sentinel Surveillance Network to monitor drug resistance in leprosy to proactively monitor the situation.³² The widespread

emergence of drug-resistant *M. leprae* would undermine the efforts of the WHO-MDT campaign.^{29,33–36}

Relapse rates after taking MDT are generally low (~1%), but wide variations are reported in different regions and can be unacceptably high in some areas.^{37,38} Relapse rates are dependent on several operational factors. A 10-year prospective study in the Philippines noted a significant difference in the relapse rates of MB patients followed at a referral center versus those observed in field clinics (9% and 3%, respectively).³⁹ In southern India, a much higher relapse rate, equivalent to 20/1,000 person-years, was observed among MB patients given MDT for 2 years. This rate was reduced to 10/1,000 person-years in patients who were treated until they became smear negative.⁴⁰ In general, higher relapse rates are observed in patients with a high BI at the time of diagnosis, indicating that these patients likely require longer treatment.^{37,41} When relapse does occur, it is often related to poor MDT compliance.

Over time, the duration of leprosy treatments has gradually been shortened: dapsone was given over many years; when first introduced, MDT was administered to MB and PB patients for 2 and 6 months, respectively; since 1998, MDT has been given to MB patients for 1 year.⁴² Even with shortening treatment times, patients can become weary with the length of treatment and may also experience intolerance, side effects, and toxicity from each of the components of the regimen.^{38,43} Despite concerns that a uniform MDT regimen provided to all patients for 6 months may undertreat MB patients, especially those with a high initial BI, and simultaneously overtreat PB leprosy patients, such a regimen has been trialed on the basis that it is operationally more convenient and could therefore be more effective in the context of the integration into general health care services.

While there appears to be a need for alternative bactericidal agents and more combinations that can be used to treat leprosy, these are currently limited. Ofloxacin, as well as other quinolones, has been reported to have a rapid and highly bactericidal activity against *M. leprae* in mouse experiments and human trials. Although moxifloxacin/pefloxacin/ofloxacin, minocycline, and clarithromycin have all demonstrated greater activity than both dapsone and clofazimine in clinical trials, clinical application of these has been largely confined to the use of single-dose RIF, ofloxacin, and minocycline for single-lesion PB leprosy patients in trials.^{44–51} To evaluate the efficacy of a 4-week ofloxacin-containing regimen for PB leprosy, we enrolled PB patients in a randomized, double-blind trial.⁵² One group received the standard 6-month WHO-MDT regimen, whereas the other received

28 daily supervised doses of RIF 600 mg + ofloxacin 400 mg, plus 5 months of placebo. Both regimens appeared generally efficacious and resulted in few relapses. While the addition of ofloxacin and minocycline as secondary treatments could attenuate the spread of drug resistance among *M. leprae*, it is noteworthy that ofloxacin resistance has been found in at least two relapses.^{32,34,44,49,53–56} Moxifloxacin, gatifloxacin, and linezolid are all licensed for human use and are used to treat several bacterial infections. PA 824, now called pretomanid, is in an advanced stage of development. Although the efficacy of moxifloxacin, gatifloxacin, linezolid, and PA 824 as antimycobacterials has been demonstrated in tuberculosis (TB) models, these drugs have had only limited amounts of testing in the mouse footpad model of *M. leprae* infection.^{57,58} Evaluations of these agents against replicating *M. leprae* have been undertaken in the mouse footpad model.⁵⁹ A dose–response curve was observed for linezolid activity against *M. leprae*: 25 mg/kg five times weekly was bacteriostatic, 50 mg/kg five times weekly was partially bactericidal, and 100 mg/kg was fully bactericidal. The strong bactericidal activity of moxifloxacin against stationary *M. leprae* was extended by demonstrating activity against “rapidly” multiplying *M. leprae*. PA 824 was found to lack any activity against rapidly multiplying *M. leprae*. Thus, experimental evaluations of emerging antimycobacterials that are being driven by TB research provide an important transition to inform their potential, or lack thereof, for treating leprosy.

Immunotherapy

Another strategy with which to reduce the duration of treatment is to adjunct chemotherapy with immune therapy, and the concept of using a vaccine in conjunction with drugs for treatment of leprosy has already been studied. Katoch et al⁶⁰ evaluated untreated high BI cases that were allocated to one of three treatment groups. All patients received a modified MDT regimen; but in addition, one control group received distilled water, another group received bacillus Calmette–Guérin (BCG), and yet another group received killed *Mycobacterium w* (*M. w*) every 6 months, until *M. leprae* was no longer observed in skin slit smears. Despite inducing cell-mediated immunity, the incidence of reactions was not increased by provision of the vaccines. Viable bacteria were detected by outgrowth in mouse footpads in samples from patients on MDT alone up to 24 months of therapy, whereas there was no indication of living *M. leprae* in either of the two immunotherapy groups after 12 months. Patients in both the immunochemotherapy groups showed histological upgrading and accelerated granuloma clearance.

In a similar study, untreated MB patients with moderate BI were provided MDT for 12 months and one of three treatments (saline, intradermal BCG, or *M. w*, each administered at 3-month intervals for four total doses).⁶¹ By 12 and 24 months, the patients in BCG group demonstrated a significantly greater improvement in clinical score compared to those in the *M. w* group, with both the BCG and *M. w* groups showing reduced clinical scores compared to the MDT only control group. BI declined by 2.40 units per year in patients receiving BCG, 2.05 units per year in the *M. w* group, and 0.85 units per year in the control group. The incidence of type 2 reactions, neuritis, and development of new deformities was decreased compared to the controls.

These studies indicate that cellular responses can be induced even in leprosy patients with high BI without exacerbating disease and that the addition of immunotherapy to MDT can reduce the effective treatment period required for bacterial clearance.

Preventative strategies

Although the free, widespread provision of MDT has massively impacted and reduced the global prevalence of leprosy, there are many indications that further effort is required to maintain control and continue onward toward eradication. Active case-finding programs generally record case numbers at rates many fold greater than those detected and reported by the current, passive detection strategies. *M. leprae* infection does not always cause disease, and estimates are that up to 75% of infections may be spontaneously cleared without causing significant symptoms.^{62,63} Together, these indicate that reliance on the appearance of clinical symptoms to prompt treatment leaves a large population of *M. leprae* infected individuals with the potential to transmit infection to others who could propagate disease. Focusing on immediate contacts of patients as recruits within trials aiming to reduce the incidence of leprosy is a logical and practical strategy, although large enrollments are still necessary to provide the statistical power required to interpret efficacy.

Chemoprophylaxis

Various chemoprophylaxis trials have demonstrated a benefit to treating individuals lacking the symptoms of leprosy, especially in those at the highest risk of infection.^{64–72} RIF treatment had a significant impact in Indonesian communities where everybody was given the drug but had no effect in similar communities where only household contacts and direct neighbors were treated.^{64,73} Single-dose RIF treatment showed a protective effect of 57% in household and other contacts

when used as a prophylactic strategy in Bangladesh.⁷⁴ These results are in line with older dapsone prophylaxis programs, which were also found to be more effective when provided to everyone residing in affected communities.⁷⁰ Although generally successful, the relative failure of this approach in a subset of seropositive contacts of MB patients could be that their bacillary load may have been too high to be eliminated by a single dose (or as in Indonesia, two doses) of RIF by the time the chemoprophylaxis is given.

Although each chemoprophylaxis trial has reported a benefit, different magnitudes and the relatively short duration of protection indicate functional limitations. This cannot be entirely unexpected because drugs can only protect those already harboring *M. leprae*, and thus, while chemoprophylactic strategies can reduce the incidence of leprosy in some situations, the nature of drug activity restricts efficacy to a subset of individuals: anyone infected after the chemoprophylaxis is given is not protected. There are also indications that a more extensive regimen may be needed for those nondiseased individuals harboring higher levels of *M. leprae*.

Vaccines

By promoting a lasting adaptive immune response, a vaccine, unlike drug treatment, has the potential to provide active and sustained protection. Consistent with exposure or low-level infection, many contacts of leprosy patients exhibit *M. leprae* antigen-specific inflammatory responses and the majority do not develop disease. Thus, the ideal vaccine against leprosy would induce strong, long-lasting T-cell responses directed against *M. leprae* antigens that would limit infection, prevent disease, and, furthermore, reduce bacterial transmission to others.

Although attempts have been made to develop a vaccine based on whole mycobacteria, at present, the BCG vaccine more typically associated with TB is the only vaccine administered for the prevention of leprosy. The presence of a BCG scar has been recognized as a protective factor for leprosy, but, as clearly indicated by the persistence of leprosy in countries where BCG use is widespread, BCG vaccination does not provide perfect protection against the disease.¹⁰ The degree of protection afforded by BCG against leprosy has varied dramatically between studies. Systematic meta-analyses indicate that BCG has a wide-ranging protective efficacy with an average ~50% and protection appears to be better against the MB than the PB form.^{75,76} The use of different BCG strains may be a factor in the varied protection reported across various studies, although this remains unclear.⁷⁷ As with TB,

the protection afforded by BCG against leprosy is greatest in children and wanes with aging.^{78–80} Computer modeling, based on the 2003 leprosy situation in hyperendemic districts of Bangladesh, indicated that the incidence of leprosy would be substantially reduced by, among various other factors, good BCG vaccine coverage of infants.⁸¹

Some studies indicate that multiple BCG vaccinations enhance protection and it has been relatively common to recommend the immunization (or reimmunization) of leprosy patients and their contacts.^{82,83} Because no substantial benefit of BCG revaccination is observed against TB; however, WHO guidelines for TB do not support BCG revaccination.^{84–86} Models including a second BCG vaccination for the prevention of leprosy have not been generated and the efficacy of this approach is debated.^{75,87–89}

A major research and development area in the TB field is the refinement of BCG to make it more immunogenic and to provide protection over a longer period. Investigators have genetically refined the bacteria and several recombinant BCG (rBCG) vaccines are being evaluated. The protection that most of these rBCG vaccines can afford against leprosy has not been evaluated, and it is therefore unclear what impact they could have on the incidence of leprosy. Only some rBCG vaccines have been produced with consideration of leprosy.^{90–94}

M. leprae itself has been assessed in various trials, often to see if it can add to the protective effect of BCG. Large-scale human trials were conducted in Venezuela, Malawi, and India to measure the efficacy of BCG with and without killed *M. leprae*.^{82,83} In Venezuela and Malawi, 5–9 years after vaccination, the incidence rate of all new leprosy cases was reduced across all ages, but the BCG/*M. leprae* vaccine did not enhance the protection afforded by a primary BCG vaccination alone. Although the observed leprosy incidence rates in a similar trial in South India were not high enough to ascertain the protective efficacy of the vaccines in surveys conducted within the 8 years following immunization, it was determined that BCG/*M. leprae* improved protection to 64% whereas BCG alone provided 34.1% protection.⁹⁵ The reason for this discrepancy is unclear, but it is noteworthy that even if *M. leprae* contributed to protection over BCG, further development of a killed *M. leprae*-containing vaccine would be enormously constrained by the difficulties associated with mass production. Reproducibly generating a consistent product in immune-compromised mice or armadillos would appear to be extremely difficult, if not impossible, although the data do suggest vaccine improvements over BCG are possible.

Several alternate, cultivatable mycobacteria have also been evaluated as leprosy vaccines. In the aforementioned South India trial, one additional group was immunized with the alternative mycobacteria *M. w*, while another group was immunized with Indian Cancer Research Center (ICRC) bacilli.⁹⁵ Both *M. w* and ICRC bacilli have been demonstrated to protect mice against experimental inoculation of *M. leprae*.^{96,97} Of all the immunization groups in the South India trial, ICRC bacilli provided the best protection at 65.5%. Despite evaluations indicating that *M. w* provided the lowest protective efficacy (25.7%) of all the vaccines evaluated, a large-scale, double-blind trial of a *M. w* vaccine in index cases and their household contacts was conducted in Uttar Pradesh, India.⁹⁸ When index cases, and not the contacts, received the *M. w* vaccine, surveys at 3, 6, and 9 years after the initial vaccination indicated protective efficacies of 43%, 31%, and 3%, respectively. When only contacts received the vaccine, protective efficacies of 69%, 59%, and 39% were observed. When both patients and contacts received the *M. w* vaccine, the protective efficacy was 68%, 60%, and 28% at each follow-up time. Thus, the protective effect of the *M. w* vaccine in that follow-up trial was sustained for a period of ~7–8 years. However, *M. w* has either become widely used in India nor has been evaluated in other leprosy-affected regions.

Combined strategies

As indicated by combined treatment strategies, the simultaneous provision of chemoprophylaxis and immunoprophylaxis is suggested as an active control strategy with the greatest potential of reducing the incidence of leprosy. Given the live mycobacterial basis of the BCG vaccine, it cannot be administered at the same time as any chemoprophylaxis. A combined strategy involving the staggered provision of RIF and BCG is, however, currently under evaluation.⁹⁹ Unlike BCG, immunization with nonliving vaccines, such as a killed whole mycobacterium or a subunit vaccine, could be provided at the same time as drug treatment. A defined (subunit) vaccine produced by standard methods could negate the quality control concerns associated with whole bacterial vaccines, but such a vaccine is still lacking for leprosy. Experimental immunizations with crude antigens have demonstrated that proteins within the *M. leprae* cell wall, cell membrane, and cytosol all provide protection when administered with an adjuvant before infection.^{100,101} The 35 kDa Ag85B and hsp65 antigens have all been shown to confer protection when expressed in a DNA vaccine.^{102–104} Purified and/or recombinant 10, 25, and 65 kDa proteins have also provided protection in the experimental mouse footpad model.¹⁰⁵

Vaccination of mice with the Ag85 proteins purified from BCG culture filtrate, in conjunction with Freund's incomplete adjuvant, protected by inhibiting *M. leprae* growth.¹⁰⁶ In a conflicting report, however, recombinant Ag85A/B did not protect when administered with either Freund's incomplete adjuvant or monophosphoryl lipid A.¹⁰⁷ As with the use of killed *M. leprae*, the use of crude *M. leprae* antigens in a vaccine is severely constrained by the need to cultivate large quantities of *M. leprae*. Selection and production of recombinant antigens have, however, been simplified by the completion and publication of the *M. leprae* genome in 2001, and a defined subunit vaccine appears within reach.^{108–110}

Interpreting protection

While the primary goal of any intervention is to reduce overall leprosy incidence, slow development of the disease and the relatively low incidence rates (even in leprosy hyperendemic regions) pose logistical problems for statistically powered evaluations. Leprosy incidence rates are typically reported earlier historically reported levels in trials, probably because of both increased awareness of leprosy within the study population and the requirement for closer observance leading to active case finding.^{11,73,111} This, and variance in year-to-year leprosy incidence rates, suggests that experimental trials are better suited to distinguish protective strategies than observational studies.⁷⁵ Observational studies do, however, yield results more quickly. Past studies have either relied on long-term follow-up and comparison of new case detection between untreated and treated groups or on skin slit smears and biopsy to determine how bacterial burden and histological responses of patients have been affected. Surrogate endpoints predictive of response could significantly shorten trials and expedite the adoption of new strategies. The identification of surrogate endpoints indicated by simple biomarkers that could replace, reduce, or negate the need for invasive skin slit or biopsy procedures would also make trials more tractable over larger populations.

The majority of untreated MB leprosy patients can be identified by robust antibody responses at the time of diagnosis and, similar to anti-phenolic glycolipid (PGL)-I IgM responses, IgG responses against protein antigens appear to correlate well with bacterial burden.^{112–117} This suggests that as *M. leprae* are killed and removed from the body, these responses should diminish. Accordingly, the IgM responses against PGL-I, as well as the IgG responses to the 35 kDa Ag85A and Ag85B proteins, are all documented to decline during treatment.^{118–125} Decreases during and after MDT in the antibody responses to recombinant protein antigens,

including leprosy IDRI diagnostic-1 (LID-1), which is now being used in rapid diagnostic test formats, have also been reported.^{126–130} Significant declines in IgG levels are observed among MB patients after completion of MDT, but declines appear to be less pronounced for anti-PGL-I IgM.^{126,131,132} The rate of decay of anti-PGL-I levels after the initiation of treatment has been reported to range from a linear decline and quick conversion to seronegative through to the retention of positive responses for many years.^{121,124} While it is unclear how antigen-specific antibodies emerge, develop, and retreat in nondiseased individuals, long-term follow-up of serum antibody responses in a large population, such as is desired for vaccine trials, appears entirely feasible.

Interferon- γ (IFN- γ) release assays are now commonly used for TB and have tended to demonstrate high antigen-specific IFN- γ levels at the time of diagnosis that subsequently decline with treatment. We have identified multiple antigens that are recognized in whole blood assays for leprosy patients.^{115,133} It is well documented through the use of whole *M. leprae* or crude antigen fractions that PB patients have strong antigen-specific cellular responses, and this has been corroborated by the observation of IFN- γ secretion in whole blood assay (WBA) involving the incubation of untreated PB patient blood with either peptides or recombinant proteins.^{115,117,134–138} Although MB patients are usually considered anergic because they have low or absent cell-mediated immunity to crude fractions of *M. leprae*, “upgrading” of responses upon vaccination or treatment indicates otherwise. The decreased IFN- γ production seen ~2 years after MDT completion in a recent study of PB patients could possibly be explained by the elimination of bacilli and clearance of antigen from the body.¹³¹ Indeed, an IFN- γ recall response to LID-1 has been observed among MB patients shortly after the conclusion of MDT.¹³¹ Among the recombinant proteins assessed, this property was unique to LID-1, indicating that the cellular responses of MB patients are antigenically restricted. Why the cellular response against LID-1 emerges in MB leprosy patients after MDT is unclear, but an improved understanding of how successful, and even unsuccessful, treatment affects antigen-specific responses of leprosy patients holds the potential to identify markers that could be used to expedite the introduction of treatments and interventions.

Conclusion

Leprosy can be treated, and the disfiguring disabilities associated with advanced nerve damage can be prevented. The most effective treatment requires early diagnosis; however, and this requires continued vigilance. In addition, although

current MDT regimens are highly effective, compliance issues and the potential emergence of drug resistance will continue to be of concern. Although alternate drug or adjunct immune therapies with the potential for use in new, shorter, or simpler treatment regimen appear possible, trials to support their widespread use are limited. As a strategy to reduce the incidence of leprosy, even though estimates show that chemoprophylaxis alone provides a 2-year protective window, chemoprophylaxis appears as the best currently available strategy. While effective vaccination programs have the potential to provide a more sustained protective window, adapting control strategies to provide both chemoprophylaxis and immunization has distinct appeal and likely provides the greatest opportunity for sustained reductions in the incidence of leprosy. Further research on alternative therapies and new leprosy control strategies need to overcome economic, political, or operational barriers and require advocacy and sponsorship from pertinent stakeholders. Chemoprophylaxis could provide an immediate, short-term protection, with immunization generating a longer-term protection. Simple assays measuring biomarkers as surrogates for disease development or invasive, but not particularly sensitive, direct measures of *M. leprae* infection could facilitate the trials required to transition these new control strategies into wider use.

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Disclosure

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