

Leprosy vaccines

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Leprosy is a disease that has been identified since antiquity, associated during centuries with extraordinary stigma for patients. Even today it is considered a stigma in some social groups. Nevertheless, during the last century great advances have been made in research and treatment of the disease.

Many advances have contributed to our knowledge about this disease, and to the possibility of developing effective preventive and therapeutic vaccines for use in leprosy. These include the discovery by Armauer Hansen of the etiological agent of leprosy, *Mycobacterium leprae*; advances in the development of vaccines since the end of the 19th Century; development of BCG as the first mycobacterial vaccine, applied since 1921; establishment of armadillos as susceptible experimental animals which could be maintained in captivity and which produce enormous numbers of *M. leprae* (KIRCHHEIMER, 1971; MEYERS, 1977); and important advances in the knowledge of immunological aspects of the disease as well as the molecular biology of *M. leprae*.

The discovery of effective anti-leprosy drugs, the first being bacteriostatic dapsona during the middle of the 20th Century and subsequently including others such as rifampycin with bactericidal effects, led to the development of multidrug regimens for the treatment of leprosy in the 1980s. These combinations drastically reduced the possibility the development of bacterial resistance during the long periods of treatment that were required. This strategy led to guarded optimism that the disease could be controlled and possibly eradicated by chemotherapy.

Present Situation

It was logical and reasonable to think that multi-drug therapy (MDT) would be the most effective if not the exclusive focus in efforts to control leprosy. The myth that leprosy is an incurable disease has been largely destroyed, since patients with multibacillary leprosy were healed after two years of treatment. In the past endemic treponematoses was presumably eradicated by the use of penicillin; this pathology has re-emerged with vigor in much of the world. These observations did not reduce the hope that leprosy, quite a different disease, could be eradicated by a chemotherapeutic approach. During the last twenty years, with the emphasis on early detection and the use of MDT as essentially the only control measures, a radical decrease in the prevalence of leprosy has been observed. This provided the basis for optimism that the disease could be eliminated as a public health problem in a reasonably short period. Thus, in May, 1991, the 44th Assembly of the World Health Organization adopted a resolution to urge efforts to eliminate leprosy as a public health problem by the year 2000. Elimination was defined as a reduction in the prevalence to less than one case per 10,000 inhabitants.

This clear goal increased the interest of public health and non-government organizations (NGOs) in countries where leprosy is endemic. With an enormous joint effort, together they achieved the great accomplishment of sustaining a program for early detection of leprosy and application of MDT for more than 20 years. The dedicated efforts of public health and other agencies in implementing early detection and treatment, public health campaigns and education, as well as the availability of drugs provided without cost by WHO contributed to this outstanding endeavor.

The results of early detection and application of MDT have been continuous. The advances have been of enormous importance and the world must be grateful for the efforts of WHO as well as national public health and NGO participation in this effort.

Perhaps after more than two decades, the time has

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come to reflect on what has been obtained with chemotherapy and whether additional control measures should be considered to consolidate the advances that have been made.

It has been shown repeatedly that the drastic reduction in the prevalence of leprosy throughout the world, obtained through programs of early detection and MDT, has not been accompanied in most countries by a corresponding fall in the number of new cases detected each year (WHO, 1999). In the three-year period from 1998 to 2000, more than 700,000 new cases of leprosy were reported each year. This is a significant increase compared to the period from 1994-1996, when about 560,000 new cases were diagnosed annually.

Two examples of countries where the magnitude of the leprosy situation is quite different are Brazil and Venezuela. In Venezuela the prevalence has remained relatively stable, at about 0.6 cases per 10,000 since 1997. The detection of new cases was about 500 per year until 1998. During the last three years this rate has increased because of the intensification of active detection measures. These have been particularly intensive in distant rural areas, where the reported detection rate had remained at about 0.2 per 10,000 since the late 1970s.

Brazil, on the other hand, reported a prevalence of 4.57 cases per 10,000 inhabitants in the year 2000. This is a remarkable decrease from a rate of about 18 per 10,000 inhabitants in the second half of the '80s. Nevertheless, detection of new cases has exceeded 30,000 per year since 1990. More than 41,000 new cases were detected in the year 2000, corresponding to a detection rate of 2.4 cases/10,000 inhabitants; nearly 36,000 cases were detected in 2001, showing a modest decrease (Personal communication).

Two possible natural modes of infection by *M. leprae* have been suggested. Historically, transmission has been considered to be limited to contact among human beings. Much more recently, the detection of natural infection of armadillos with a microorganism, which is indistinguishable from *M. leprae* in the United States of America, led to the development of this model for production of large numbers of bacilli for preliminary studies in vaccine development and other research objectives. These infections as well as significant numbers of cases where no known contact with other human cases could be detected suggest other possible forms of transmission.

Epidemiological observations in Venezuela suggest the possibility of at least two modes of transmission of leprosy. The household or close contact transmission of disease with a long latent period, slow evolution, represents one; relatively frequent transmission from parents to children and predominance of the lepromatous form of disease. When this form of transmission occurs in distant and relatively isolated

areas, the resulting lepromatous cases are often not diagnosed until several years after the initiation of clinical disease. We consider this the classical form of family transmission.

Recent studies suggest another mode of transmission, which we have chosen to call community transmission. Small or medium-sized population groups present a disproportionate number of cases with special characteristics. Most cases are diagnosed as indeterminate, tuberculoid or different forms of borderline leprosy, many with a significant bacillary population. There are a few cases of lepromatous leprosy of short evolution. In these remote areas, annual re-examination of the populations reveals a significant number of cases that were not detected the year before.

The result of an epidemiological study carried out in two of the four states of central Venezuela where leprosy has not been eliminated as a public health problem revealed communities with a hyperendemic situation. Extensive studies showed three communities with more than 300 inhabitants that were free of leprosy, but we detected 38 communities with 40 to 5600 inhabitants where prevalence rates varied from 18.7 to 1294.3 per 10,000. Of a total of 20,450 persons who were examined, 441 cases were diagnosed, corresponding to a prevalence of 215.6/10,000. Of these cases, 75.68% were paucibacillary forms of disease. The re-examination of four communities with a prevalence of >300/10,000 after two years revealed 20 new cases among 946 individuals, corresponding to a new case detection rate of 211.4/10,000 in two years; 80.95% were paucibacillary forms (Unpublished data). These observations demonstrate the importance of evaluating remote areas of difficult access, where these hyper-endemic foci can occur, with possible community transmission of the disease. Examination carried out in successive years have continued to reveal new cases in many of these communities, even though MDT was immediately begun in all newly diagnosed cases. MDT of newly diagnosed cases has not modified the pattern of transmission of the disease in these hyperendemic foci.

The bacilli found in these patients have also shown differences in staining characteristics. In areas of classical transmission, there is a predominance of granular, degenerated bacilli. In contrast, in the areas of community transmission there is predominance, in a smaller bacterial population, of solid-staining bacilli without significant granular degeneration.

These two modes of transmission occur in different environments. The first is largely restricted to a household, while the second occurs in a more extensive and complex environment. These differences may require appropriate modifications in control programs.

Since the morphological differences we have observed are so striking, there may be even more fundamental differences in the strains of *M. leprae* involved in each mode

of transmission. More specifically, the degenerative phenomena observed in more than 50% of the genes of armadillo-derived *M. leprae* (COLE, 2001) may be very similar to the phenomena in classically transmitted *M. leprae*, where the bacilli are clearly adapted to the intracellular environment. The bacilli involved in community transmission might conserve a greater genomic integrity, since they are perhaps less dependent on the intracellular environment and more flexible in surviving under diverse environmental conditions. Comparative studies of these two forms of bacilli may be of exceptional interest.

Undoubtedly many subclinical or undetected cases of leprosy may be sources of new infections, which may not become evident for many years. The possibility also exists that some cases treated with MDT could still be sources of new infections. In a single report, relapses have been reported in 20% of patients with multibacillary leprosy after MDT treatment (JAMET, 1995). Epidemiological studies of the frequency of new cases in the residences of treated patients, particularly among children born after the completion of treatment, might be of interest.

To return to the issue at hand, careful and uninterrupted use of MDT for 20 years has profoundly affected the prevalence of leprosy throughout the world, but the detection of new cases has not shown a similar impact. This suggests the need for additional control measures to consolidate the gains that have been achieved (CONVIT, 2000). Since substantial interruption of transmission does not seem to be an option at present, we are confronted with the very real need for the development of an effective protective vaccine. There is important evidence available at present regarding the preventive effect of several candidate vaccines. It would seem appropriate to dedicate part of the effort and financial resources available for leprosy, which are clearly limited, to the development of a vaccine to prevent infection, even when the sources of transmission may persist. This position is reinforced by the epidemiometric models of leprosy developed by several investigators (LECHAT, 1977), where the introduction of a preventive vaccine in the model shows an impressive improvement in control of the disease.

Immunologic Considerations for a Potential Candidate Vaccine

There is substantial evidence indicating that a Th1 immune response is one of the factors associated with resistance to leprosy. Positive delayed-type hypersensitivity is one the manifestations of Th1 activity associated with variable degrees of resistance during the natural course of the disease.

The adjuvant effect of BCG as a stimulus of Th1 responses is widely recognized. BCG stimulates granuloma formation and the synthesis of pro-inflammatory cytokines.

Immuno-stimulatory CpG sequences were first recognized in BCG and other mycobacteria (TOKUNAGA, 1999). At present, BCG is perhaps the most acceptable Th1 adjuvant available for use in humans. It shares common antigens with *M. leprae*. Its routine application by the intradermal route seems particularly suitable for the induction of Th1 responses, perhaps in part because of the accessibility of appropriate antigen-presenting cells.

Two decades ago, research on the immunological response to *M. leprae* across the human spectrum of disease was very active. At present these lines of research are relatively inactive except in countries such as Brazil and India, as well as relatively isolated laboratories in other parts of the world. The most recent reports of the WHO advisory committee on the elimination of leprosy have pointed out that many groups which were previously active in leprosy research have moved on to other areas of investigation. We still do not know with certainty which are the protective antigens of *M. leprae*, or whether the protection may occur under some circumstances and in some individuals, but not in others. There is evidence that Th1 and Th2 responses are not clearly associated with resistance and susceptibility, respectively. The current situation partially reflects the extraordinary emphasis placed on early detection and prompt application of MDT as control measures. If we accept the possibility of transmission in pre-clinical stages of infection during a period which may extend for years and recognize the limitations in our knowledge of possible modes of transmission which may not even involve human contact, the limitations of MDT in elimination of leprosy as a public health problem become apparent. Definitive eradication doesn't appear to be an option with this strategy. This uncertainty occurs precisely at a time when increasingly powerful tools are available to the medical sciences for the development of new and more effective vaccines.

We might consider the great contrast that exists at present between research in leprosy and tuberculosis. Obviously the massive expansion of tuberculosis and the high mortality associated with the disease are contributing factors in the orientation of research efforts. Nevertheless, the efforts devoted to the development of an effective vaccine are impressive (BRENNAN, 2001; DOHERTY, 2002; YOUNG, 2002). Clear objectives have been defined, including the evaluation of new attenuated strains and of strains that carry genes for the synthesis of immuno-stimulatory cytokines, as well as the development of subunit vaccines to be administered, if necessary, with powerful adjuvants and of DNA vaccines. If we consider the substantial evidence of protection against leprosy afforded by BCG (CONVIT, 1956; CONVIT, 1993; PONNIGHAUS, 1992), it is possible that one or more of these new tuberculosis vaccines may provide even greater cross protection against *M. leprae* than BCG has. Where feasible, evaluation of that possibility should be

included in tuberculosis trials.

The *M. leprae* genome is known and is clearly very defective in the material available for study (COLE, 2001). The next logical step would appear to be the application of micro-array and proteomic technology to identify components of particular interest. Comparative genomic and proteomic studies of bacilli from the classical and community or environmental modes of transmission described previously are important research objectives.

Vaccines with preventive capacity in previous studies.

When we consider leprosy vaccines, it is important to refer to the Editorial published in Leprosy Review in 1996, Vaccination against leprosy — the view from 1996 (FINE, 1996). The authors consider the existence of three ironies, which are still valid. First, it has been repeatedly stated that there is no vaccine against leprosy. Nothing could be further from the truth. Not only is there a vaccine that is effective against leprosy, admittedly with variations from one country to another, but also this vaccine, BCG, is being applied at present to more individuals than any other vaccine in the world.

The second irony is that BCG is used as a vaccine against tuberculosis, but its protection against leprosy has been more consistent than protection against the disease for which it is used.

Finally, the authors mention that the focus of leprosy vaccination trials in Venezuela and Malawi emphasized the evaluation of the combination of killed *M. leprae* and BCG. In both trials, it was demonstrated that a simple, highly protective alternative would be repeated vaccination with BCG.

We should emphasize the efforts supported by IMMLEP/WHO for the development of a vaccine against leprosy, as well of the contributions of ILEP, Lepira and other NGOs. These efforts stimulated the collaborative trials in Venezuela and Malawi. The *M. leprae*/ BCG combination showed promise as a candidate vaccine, based on a series of previous studies showing favorable immunological changes in patients receiving the combination in immunotherapy protocols (CONVIT, 1979; CONVIT, 1982). At that time, an adequate supply of *M. leprae* had become available through the experimental infection of armadillos. The use of BCG as a second component of the candidate vaccine reflected the fact that BCG shares many common antigens with *M. leprae* but additionally stimulates immunological activity in lepromatous patients who were unresponsive to the leprosy bacillus. Additionally, BCG induces positive Mitsuda reactions in healthy Mitsuda-negative contacts (CONVIT, 1956), induces an immune granuloma with many immunologically active cells and shows other characteristics of adjuvant activity.

The results obtained with the *M. leprae*-BCG candidate vaccine were not promising in Venezuela (CONVIT, 1992), where problems in the preparation of the vaccine were detected after the trial was completed (WHO, 1995,) vaccines should be incorporated into leprosy control programs as they become available, since the present strategy has not significantly altered the rate of detection of new cases in many parts of the world. In the last ten years, more than five million new cases have been detected. While this is in part attributed to "hidden prevalence" of previously undiagnosed cases, there is little evidence that this situation will vary in the near future unless other elements are added to the control strategy. We might ask if it is ethically valid to pursue the present course, when we already have one or more candidate vaccines, which might show an impact on the appearance of new cases.

Several first-generation candidate vaccines have shown demonstrable protection to infection by *M. leprae*. The most widely studied is, of course, BCG. The evaluation of BCG as an anti-leprosy vaccine began in the 1940s; later studies, including those in Venezuela and Malawi (CONVIT, 1992; KARONGA, 1996; PONNIGHAUS, 1992), were largely based on the evaluation of BCG used as a placebo in the trials. These studies showed that repeated vaccination with BCG, especially if applied early in life before infection with *M. leprae* is established, had a protective effect that exceeded 70% in some areas. A single BCG vaccination has been associated with lower levels of protection. Sensitization to BCG decreases with time, and the duration of the protective effect is not yet clear. At present, the case-control studies carried out in Venezuela and Malawi firmly support the efficacy of repeated doses of BCG in the prevention of leprosy. In Venezuela, prevention of 56% was achieved with three vaccinations. No cases of multibacillary leprosy were observed in the individuals who received two or more BCG vaccinations. In Malawi, where multibacillary cases are relatively infrequent, higher protection was observed against borderline manifestations of the disease.

Trials have been carried out in Venezuela, Malawi and India using the killed *M. leprae*-BCG combined vaccine (CONVIT, 1992; GUPTA, 1998; KARONGA, 1996). The three trials included the use of BCG alone in the trial design. In India, the ICRC bacillus and *Mycobacterium W*, candidate vaccines developed in the national program, were also evaluated. In Venezuela and Malawi, the combined *M. leprae*-BCG vaccine did not show significantly higher protection than BCG alone, which was unexpectedly effective. In India, the *M. leprae*-BCG combined vaccine and the ICRC bacillus both induced about 65% protection, considered to be sufficient to be useful in public health programs. BCG alone induced 34% protection in India.

While other cultivable mycobacteria, including *M. vaccae*, *M. habana* and *M. marinum* have been used in much

smaller immunoprophylactic and immunotherapeutic trials, these results will not be reviewed here.

The technical difficulties associated with obtaining *M. leprae* in sufficient amounts for vaccine use seem to clearly preclude its consideration in future trials. We strongly consider the use of repeated vaccination with BCG to represent a viable first-generation candidate vaccine in leprosy control programs designed to compliment the fundamental approach of early detection and prompt application of MDT. BCG has been used in millions of individuals with very limited side effects. It is not recommended for use in individuals who have clinical signs of infection with the human immunodeficiency virus, but that limitation is relevant to many of the vaccines in use at present.

We would strongly urge the members attending the 16th International Leprosy Congress to consider a resolution to support the evaluation of the use of repeated doses of BCG as a support to leprosy control by WHO Advisory Committee members as well as national program leaders. In addition, we would urge the evaluation of the impact of new vaccines against tuberculosis on the prevalence and detection of new cases of leprosy in areas where the two pathologies co-exist.

We recognize that the use of repeated doses of BCG in leprosy control, whether applied to the contacts of leprosy patients or to entire communities where cases may depend

on other modes of transmission, does not represent an ideal long-term solution to preventive vaccination. Nor do we believe that MDT alone can achieve the elimination of leprosy as a public health problem, and much less its eventual eradication. We must begin to search for new tools if we are to consolidate the achievements that have been made. Genetic engineering may provide us with subunit vaccines, potent protective antigens of *M. leprae* inserted by genetic engineering into other vehicles with inherent adjuvant activity (even BCG itself) or combined with potent adjuvants.

The international community of investigators, clinicians, public health officials and many other individuals who concern themselves with leprosy and with other public health and social problems of developing countries must review progress and long-range goals from time to time. We feel that it is imperative to evaluate the long-range goals for leprosy control. Responsible individuals and groups must ask if the present program of early detection and appropriate treatment with MDT and even more powerful therapeutic drugs will really reduce infection rates with *M. leprae* to levels where the disease can no longer be maintained in nature. We invite those who share our concern about the need for additional measures, beginning with the use of repeated vaccination with BCG and supporting every effort to develop newer vaccines, to consider these issues.

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