Immunology, Epidemiology and Social Aspects of Leprosy
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VACCINE STRATEGIES FOR THE ERADICATION OF LEPROSY

BARRY R. BLOOM

Chairman, Department of Microbiology and Immunology
Albert Einstein College of Medicine
Bronx, New York 10461

Chairman, IMMLEP Steering Committee,
Special Programme for Research and Training
in Tropical Diseases
World Health Organization, Geneva

From ancient times, in virtually every culture, leprosy has evoked singular images of horror and fascination. There is no other disease whose sufferers were historically cast out of society, buried alive, or burned at the stake. There is no other disease whose very name, in some cultures, is taboo and cannot be written or uttered. Leprosy is thus a disease of the mind as well as of the body, and one of the peculiar aims of the scientific approaches to this disease is to deal with the unique fear and stigma associated with it. To this day leprosy remains an enormous problem and challenge:

i) The etiologic agent, *Mycobacterium leprae*, remains one of the very few pathogens of man that cannot be grown in culture;

ii) There is a long latency, perhaps 5 years, between presumed infection and manifestation of disease, and as a consequence the mode of transmission remains unknown;

iii) While 13 million people are estimated to have leprosy around the world, the disease has a relatively low prevalence, seldom exceeding 1-5/1000 in endemic areas;

iv) The reasons why leprosy disappeared from Europe at the end of the last century, yet is currently increasing in some developing countries, remain unclear;

v) At least one contributory factor is the recent emergence of both primary and secondary drug-resistant organisms. This has necessitated the recommendation for combined chemotherapy, a regimen a great deal more expensive than the standard dapsone monotherapy used for 20 years. What makes the study of leprosy most appealing from an immunological point of view is the fact that there is a well established immunologic basis for the manifestations of the disease and that there is no evidence supporting the view that the disease is ordinarily transmitted from animals to man, suggesting that it may be possible to design a vaccine that would be capable of eradicating this historic scourge of man from the face of the earth.

To accomplish this task will require enormous effort and commitment. It has been possible, largely through the auspices of WHO, to interest and engage scientists from all over the world who possess special expertise relevant to the common goal, and to create a network, a "laboratory without walls" to permit the exchange of ideas, information and scarce reagents. As will become evident, many of the newest techniques of immunology and molecular biology, as well as sophisticated epidemiology, are being brought to bear on the problem. While funds for the research effort have been limited, much has been learned and much already accomplished. A greater level of resources will be required to carry out field studies on large numbers of people to adequately test the effectiveness of any candidate vaccines. At another level, for any vaccine to be effective, there will have to be a major change in attitudes of the people in leprosy endemic countries about the disease, so that rather than shunning it and waiting until it is advanced before seeking treatment, they will want to be protected before they have the disease, or treated and cured at the earliest signs of disease. This will require the understanding, participation, and support of governments, health workers, community leaders and religious groups, without which the scientific efforts may prove fruitless.

The premise upon which the IMMLEP program was founded is that it would be possible to develop a vaccine which provided protection against clinical leprosy. That premise was based on an additional assumption that much of immunology, microbial biochemistry, mechanisms of bacterial killing and resistance to intracellular parasites, identification, purification and production of appropriate antigens of *M. leprae* or related microorganisms would

emerge from an intensive global research effort by many investigators. Optimism was derived as well from the availability of *M. leprae* grown in the armadillo. An enormous amount that has been learned in recent years about the complexities and interactions of cells of immune systems, networks, suppression, and inflammatory and cytocidal mechanisms, which, rather than definitively answering the basic questions, have opened new avenues and raised new questions. What has emerged from the acquisition of knowledge in recent years is clearly that there is not a single rationale for a single vaccine, but several rationales for several different types of vaccines which must be considered and explored in model systems and small scale trials, in order to make the wisest decision about what is likely to be most useful or effective in the field.

I. Premises

A. *Induction of Cell Mediated Immunity Will Confer Protection Against Infection.* The basic assumption of any vaccine is that induction of a state of immunologic reactivity to *M. leprae* antigens will lead to protection against it. Perhaps the key observation which established a relationship between immunity and protection derived from the study of the different courses of disease of patients across the spectrum of leprosy. Leprosy is a spectral disease: at one pole of the spectrum, the tuberculoid form of the disease, patients develop high levels of cell-mediated immunity and kill the bacilli in the tissues, albeit often with concomitant damage to the nerves around which the bacilli grow. At the lepromatous pole, patients are less able or unable to restrict the growth of the organism and lack cell-mediated immunity. In contrast, there appears to be a negative correlation between the level of circulating antibodies in patients and ability to restrict the growth of *M. leprae*, higher titers generally being found in lepromatous than in tuberculoid patients. The basic premise, then, is supported by a strong correlation between cell-mediated immunity and ability of patients to kill or restrict the growth of *M. leprae*. That is simply a correlation, however, not a proof or a guarantee that a person exhibiting cell-mediated immunity to leprosy bacilli cannot develop clinical leprosy. Obviously, patients with tuberculoid leprosy have some organisms which were able to grow, and do have a clinical disease. Of particular interest are the old findings of Chatterjee and Dharmendra that lepromin-positive subjects who contract leprosy develop only tuberculoid and not lepromatous leprosy.

The question remains how strong this correlation is. In order to provide protection against an intracellular bacterium, it is necessary to develop an immune response which produces three consequences: killing of the microorganism, degradation of the bacillus and clearance of antigen and ultimately of immune complexes. That is what is required to generate a disease-free state. At the present time the molecular mechanisms for intracellular killing of mycobacteria, particularly in macrophages, remain unclear, although there is recent evidence which indicates that oxidative cytocidal mechanisms involving the superoxide anion (O_2^-), hydrogen peroxide, (H_2O_2), hydroxyl radical ($OH^\cdot$) and hypohalide may be involved. Non-specifically or immunologically activated macrophages, under the influence of signals from T lymphocytes, have a greatly augmented ability to produce these oxygen radicals and metabolites which may be responsible for the killing event. What is important to emphasize, however, is that from many experimental studies performed in simpler organisms, the killing effects are quantitative rather than qualitative. The ability of a single activated macrophage to produce H_2O_2, for example, is limited. When that cell is infected with one or two microorganisms, the level of H_2O_2 produced may be sufficient to kill both organisms. When that cell is infected with five organisms, the level may still be sufficient to kill two, which means that three microorganisms will not be killed and may grow. It is probably for this reason that it has never been possible to show an absolute quantitative correlation between the diameter of skin test reactivity and the degree of resistance of animals to infection with micro-organisms.

A great deal more is known about the degradative enzymes within macrophages, which include a large list of proteases, nucleases, glycosidases, and lipases capable of destroying most normal biological materials. Yet mycobacteria have unique cell wall and lipid structure which render them much more resistant to degradation than almost any other organism, and it is for this reason that antigens persist for such long periods of time. Nevertheless, the correlation between cell-mediated immunity and resistance to growth of the organism in tuberculoid patients, the correlation between cell-mediated immunity macrophage activation and increased cytocidal oxygen metabolites and in degradative enzymes suggests that induction of immunity should lead to increased resistance, although that resistance cannot be conceived of as being absolute.

B. *Specific Cell-Mediated Immunity Can Be Induced by Immunization with Killed* M. leprae *or Other Mycobacteria.* The second specific experimental premise is that *Mycobacterium leprae* or other cultivable mycobacteria can produce cell-mediated immunity to antigens of the leprosy bacillus. Probably the first line of evidence to support that view is a modern reinterpretation of the Mitsuda test. The Mitsuda test would appear to be unique among all tests for cell-mediated immunity in that it is read not at 24-48 hours, but at 28 days. Since in almost all other systems it is possible to detect preexisting immunity by skin tests that are read at 48 hours, another simple interpretation of the Matsuda test is that it is not only a skin test which measures preexisting cell-mediated immunity, but is, in fact, a weak vaccine. As such, it has been designed to discriminate between individuals who are unresponsive to antigens of leprosy bacillus, either because they have lepromatous disease or because they have been unexposed to the bacillus or cross-reactive antigens, and those who have already been infected, clinically or subclinically, and for whom the Mitsuda test is a booster shot which augments weak prior existing sensitization, or in fact simply is able in 28 days to sensitize them. The fact that a significant percentage of normal individuals in leprosy nonendemic countries or areas become Matsuda positive suggests either that it is a weak vaccine, or that some individuals have been primed against cross reactive antigens.

A second line of evidence indicating that *M. leprae* is immunogenic derives from the studies on purified *M. leprae* carried out in mouse, guinea pigs and armadillos which indicate that in the absence even of oil adjuvants purified and killed *M. leprae* are capable of engendering delayed-type hypersensitivity. In the mouse there is convincing evidence that in addition to cell mediated immunity, high levels of protection against infection by viable *M. leprae* can be engendered. On the other hand the specificity remains unclear. At the moment there are very few unique antigens which distinguish *M. leprae* from all other mycobacteria. It is clear that vaccination of mice with BCG will protect against growth and dissemination of live *M. leprae*, and there is clear evidence that sensitization with *M. leprae* will lead to cross-reactions to a variety of other mycobacterial antigens. It is thus very difficult to establish what the unique and specific antigens of the lepra bacillus are, whether some must be included in the vaccine to induce protection, and whether other mycobacteria share these key antigens. The dilemma in interpreting cross reactive immunization is

compounded by the results of two large scale BCG vaccination trials in which the degree of protection against leprosy varied from 80% in Uganda to 20% in Burma. The reasons for the difference in these results remain unknown, although they suggest that BCG may provide some but not full protection against leprosy.

II. Vaccine strategies

There are at present two rationales for vaccination against leprosy. One is immunoprophylaxis, which is designed to protect a population at risk against developing clinical leprosy. The second is immunotherapy, which is designed to convert anergic lepromatous patients to a state of cell-mediated immunity, in the hope that they will then cure their infection, and ultimately their disease.

A. *A killed* M. leprae *vaccine.* Such a vaccine would be designed exclusively for immunoprophylaxis, since a vast amount of evidence indicates that lepromatous patients are immunologically unresponsive to the leprosy bacilli that they are harboring and to *M. leprae* antigens introduced in skin tests. The premise would be that a naive population would be primed to positive immune reactivity to specific antigens of the leprosy bacillus. When they became infected at some later time, the infecting organisms would serve to boost their already existing levels of cell-mediated immunity, and the patients would develop either subclinical leprosy and eliminate the organisms, or, at worst, develop a tuberculoid type of self-healing disease.

Such a vaccine has the potential for providing information on one of the key problems in leprosy, namely the identification of patients at high risk for lepromatous leprosy. If killed *M. leprae* were found to have a high conversion rate in the leprosy non-endemic population, and there were individuals in the leprosy endemic area who failed to convert to the vaccine, even upon revaccination, it might be argued that their unresponsiveness was due to the fact that they were incubating lepromatous leprosy and already anergic prior to the onset of detectable clinical symptoms of lepromatous leprosy. Thus nonresponders to the vaccine could, in principle, be considered at high risk, identified and then treated with chemotherapy, although this is expensive and logistically difficult.

B. *Killed or Live mycobacterial vaccines to provide crossreactive immunity against* M. leprae. As mentioned above, the first experimental tests of

this strategy were those using BCG vaccination to protect against leprosy, where the results in different parts of the world yielded vastly different rates of protection. There are studies of small numbers of patients with borderline and polar lepromatous leprosy who were vaccinated with BCG, in which clinical improvement was reported, although many of the patients developed reversal reactional symptoms. As a prophylactic vaccine against leprosy, BCG has provided some protection in Uganda, Burma and India, but except in Uganda, never above 25%, which is inadequate protection from a public health point of view.

Two cultivable mycobacterial strains have been reported in India to be effective, after being killed, at inducing cell-mediated immunity in lepromatous patients, and one must await with interest further scientific characterization of the strains and further data on their effectiveness. There are two difficulties with the use of such vaccines. The first is that in the absence of identifiable *M. leprae*-specific antigens, it is very difficult to know which mycobacteria have appropriate specific antigens cross-reactive with antigens required for protection against *M. leprae*. One hopes that as the biochemical purification of mycobacterial protein, glycoprotein and lipid antigens progresses, and monoclonal antibodies are developed, such specific antigens may be identified and cultivatable organisms screened for expression of those antigens. The second concern is that even if unique specific or cross-reactive antigens are found, how can one be sure that they will not engender immunological unresponsiveness or suppression, rather than priming for immunity to the key antigens? Another concern is the possible use of living microorganisms in populations some of whose recipients may have some immunodeficiency, or immunological unresponsiveness against mycobacterial antigens. The immunotherapy of cancer patients with live BCG vaccines and the unexpectedly high incidence of disseminated BCGosis serve to emphasize that concern. One of the appealing aspects of this strategy, however, is the ability to produce very large amounts of such a live cross-reactive vaccine very inexpensively, and they are likely to be effective longer than killed vaccines.

C. *A vaccine of killed* M. leprae *plus living BCG*. The basis for this vaccine derived from Convit's observations that when killed *M. leprae* were injected into the skin of lepromatous patients together with BCG, there was degradation and clearance of the *M. leprae* which was not seen when leprosy bacilli were inoculated alone. Based on these

observations Convit has demonstrated that such a vaccine of killed *M. leprae* plus BCG has strong immunotherapeutic effectiveness in patients with indeterminate, borderline, and, most recently, even in polar lepromatous patients, leading to skin test conversion, degradation of organisms in the skin and marked clinical improvement. The immunological rationale for this mixed vaccine is not fully clear, although it has fundamental implications for understanding the basic mechanisms of immune regulation in man. It may well be that BCG causes activation of T cells specific for its antigens, which then produce lymphokines which: i) have the ability to convert ordinary macrophages into antigen presenting cells, which then augment the ability of lepra antigens to be appropriately presented; and ii) expand small numbers of clones of T cells capable of recognizing specific *M. leprae* antigens. In any case, the data that you will hear provide evidence that this vaccine has therapeutic activity in patients who are otherwise anergic, and should have immunoprophylactic potential in the normal population. One major advantage of this vaccine would be that if there were contacts at high risk for lepromatous leprosy and harboring leprosy bacilli, this vaccine should force them to immunoconversion and serve therapeutically to cure their infection while it is still subclinical.

III. Problems Inherent in Vaccines against Leprosy

A. *Epidemiologic.* The only way that any of these vaccine strategies can be tested meaningfully is first by induction of resistance in appropriate animal models and then by field trials in man. Relatively small scale field trials can be set up to ask the question whether these antigen preparations are capable of inducing cell mediated immunity to antigens of the leprosy bacillus. It becomes a much greater problem to ascertain whether induction of cell-mediated immunity confers with it resistance to infection by *M. leprae*. For therapeutic trials, which in this case become the most feasible, one has simply to test relatively small numbers of patients with well defined stages of disease and look for therapeutic benefit as well as immunoconversion. With respect to protection of normal population, field trials become very complex. Leprosy bacilli are very slow growing organisms, the prevalence rate may be as low as 0.5 per 1000 population, and assuming that four out of five cases of leprosy are likely to be of the tuberculoid variety, this

means that one may have to vaccinate 1000 people to see a diminution in one detectable case of lepromatous disease over a decade. The third population for vaccination which is appealing is that of household contacts of patients with lepromatous leprosy, who are known to have a higher incidence of leprosy, yet the logistics of identifying those individuals and monitoring them with the vaccine are probably more cumbersome than larger scale mass vaccination in field trial areas in many countries.

Finally there is the question of the effectiveness of a vaccine in protecting individuals with subclinical infection. There is basically no precedent for using a vaccine against a disease of such long duration and low prevalence, and one must assume the study would have to be continued for 10-15 years before results could be evaluated.

B. "*The vaccine causes leprosy*". One prediction in vaccinating a large population in a leprosy endemic area is a likelihood that patients who are harboring indeterminate or borderline disease without having manifested clinical symptoms, after vaccination and the induction of relatively high levels of cell-mediated immunity, will begin to show the signs of tuberculoid leprosy. There are two consequences. The first that one can almost certainly expect is the cry from the public health and administrative authorities that the vaccine is causing harm and causing disease, and it will take a long process of education and preparation as well as careful monitoring and availability of appropriate treatment to minimize this problem. The more serious consequence is that some patients who are harboring the leprosy bacillus around the nerves, as they develop rapid cell-mediated immunity may be expected to develop nerve damage, and this must be anticipated and appropriate and rapid treatment provided. In this regard, the studies of Convit on the BCG plus *M. leprae* vaccine in patients with leprosy have been very encouraging in that only a low incidence of neurological symptoms have appeared at no greater prevalence than with chemotherapy alone, and these have been minor.

C. *The duration of sensitization.* Because of the low incidence of disease, and the long latent period before diseases are manifest, in order to provide protection over a long period of time it is necessary that such a vaccine have enduring sensitization. At the moment it is unclear how long a single vaccination confers cell-mediated immunity. In the guinea pig, a single high vaccine dose of killed *M. leprae* is capable of sensitizing animals to positive reactivity to first skin test one year later.

The duration of sensitization in man remains to be established, and if it is not able to confer high levels of sensitization over a ten-year period, then revaccination or booster vaccination of the population may have to be considered in any vaccine protocol.

D. *Unknown variables.* While a great deal of information is available, there remain a large number of scientific variables which will not be known at the time of vaccination trials. The mode of transmission of the disease is unknown. What factors determine the form of disease and whether one develops a positive or suppressed immune response to the specific antigens of the leprosy bacillus, what the role of the genetic constitution of the populations is, and what role environmental mycobacteria have in enhancing or suppressing responsiveness to the vaccine in different populations all remain unknown; yet each could play a significant role in determining the outcome in an individual and in a population.

IV. Future Directions

A. *Seroepidemiology.* The increasing availability of specific monoclonal antibodies should make it possible to identify new *M. leprae* specific antigens which should permit worldwide accurate and inexpensive epidemiological testing: (i) for infection by *M. leprae*; (ii) for studying the mode of transmission; (iii) for characterizing the latent period; and (iv) for identifying individuals at high risk for developing leprosy, hopefully predicting the form of disease to which they are prone. In addition, with such reagents it should be possible to engineer clones of *E. coli* or other microbial hosts expressing *M. leprae* specific epitopes, which could then be used as antigens for standardized epidemiologic testing. If using either *M. leprae*-specific antigens or monoclonal antibodies in immunoassays permits the identification of people in a population who have recently been infected with *M. leprae*, it would permit targeting any of the vaccines to the most susceptible group, thereby reducing the number of people required to be immunized and hopefully decreasing the cost of an eradication program.

B. *Recombinant DNA produced antigens for vaccines.* It is possible that some of these specific antigenic determinants may be important for developing protective immunity. Because of the limitations on vaccine

production in the armadillo, one hopes that it may be possible to produce effective vaccines by recombinant DNA technology in future. The difficulty, however, is that polypeptide antigens alone are unlikely to be as effective as mycobacteria in inducing cell-mediated immunity, as they would lack the extraordinary adjuvant activity possessed by the mycobacteria. Further development of effective adjuvants for inducing cell-mediated immunity in man is urgently required.

C. *Genetically engineered mycobacterial vaccines.* It is not totally unrealistic to conceive of the transfer of genetic information for leprosy-specific protective antigens into cultivable, non-pathogenic mycobacteria, such as BCG vaccine strains, that could provide an ideal vaccine, i.e., one that contains specific protective antigens, lacks tolerogenic determinants and possesses a potent adjuvant for cell-mediated immunity. Were such a vaccine strain to be developed and engineered with genes for protective antigens against other infectious agents, it could have enormous usefulness for immunizing against many diseases for which cell-mediated immunity is critical to resistance.

V. Conclusion

The present methods of control of leprosy are inadequate. The concept of treating patients who already have the disease, and may have been infectious and transmitting *M. leprae* for years before a diagnosis was made, is inadequate. Detection of patients by the traditional "case-finding" methods, more often than not passive rather than active, is clearly inefficient. Problems of the cost of combined chemotherapy, of persisting organisms even after chemotherapy, and the emergence of drug resistant organisms all argue that another strategy, based on preventing rather than treating leprosy, is needed.

Recent scientific developments indicate that at least one vaccine, and possibly others, are capable of providing specific immunity to the leprosy bacillus in man. New tools are being developed for the early diagnosis of infection and disease, and from the scientific point of view one awaits the results of field studies to confirm the feasibility of protecting the people exposed to infection against leprosy by vaccination.

Even if these scientific expectations and hopes are fulfilled, how are we to deal with the stigma and social problems associated with

leprosy? It is too late, in my judgment, to call the disease Hansen's Disease and fool the patients, their families or the community almost anywhere in the world. It is too late for social science questionnaires on people's attitudes toward leprosy. Everyone knows what an appropriate or acceptable answer is supposed to be, and it is unlikely that such surveys will uncover the real fear and prejudice associated with the disease. Clearly, education in a public health context is important, yet help from the sociologists will be crucial in specific contexts, e.g., in cultures where the use of the word "leprosy" is taboo. In my judgment, the most important thing that can be done to change attitudes is to have something tangible and substantial to provide the patients and their communities. This means new scientific tests to identify people with subclinical disease, and appropriate drugs or vaccines to prevent any clinical disease. Ideally, if there were an effective prophylactic vaccine, it should be possible to convince people of leprosy endemic countries that leprosy is a disease which, after centuries of producing destruction of bodies and minds, can be prevented and cured.

With the best available science and with understanding and support from national, community and religious leaders, in my judgment an effective vaccine has the potential to eradicate leprosy from the face of the earth in one generation or less. That makes the vaccine strategy for the eradication of leprosy a compelling one.