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EDITORIALS

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General Ideas Concerning a Vaccine Against Leprosy: A Basis for Discussion During the Eleventh International Leprosy Congress

One of the important programs of the World Health Organization, the Tropical Disease Research (TDR) program, is concerned with research and training in six of the so-called neglected parasitic diseases: leprosy, leishmaniasis, onchocerciasis, bilharziasis, trypanosomiasis and malaria. This program is primarily oriented toward the discovery of new procedures in the diagnosis, prevention and treatment of these diseases, as well as the formation of scientific personnel in the developing countries who would be able to carry out the mentioned objectives.

In leprosy, the development of a preventive vaccine is an objective of high priority of the group designated IMMLEP, which is the group within the TDR program concerned with the immunological aspects of leprosy. A preventive vaccine of high efficiency, low cost and free of secondary effects would constitute a decisive element in programs of control and eradication in those countries where leprosy is endemic.

A general discussion concerning the basic ideas in the development of such a vaccine, as well as some information about research in this area, might provide a useful contribution toward the orientation of future studies.

The three possible approaches toward the development of a vaccine against leprosy which have been considered are as follows:

1. Use of a cultivable mycobacterium which has an antigenic composition similar to *Mycobacterium leprae*.

2. Use of killed *M. leprae* isolated from the tissues of experimentally infected armadillos.

3. Use of a mixture of killed *M. leprae* together with another mycobacterium, such as BCG, with adjuvant activity, or with some other suitable adjuvant.

The use of a cultivable mycobacterium closely related to *M. leprae* in its antigenic composition, but with superior adjuvant properties, appears to have the soundest basis from a theoretical point of view but practical limitations are evident. The mycobacteria which share cross-reacting antigens with *M. leprae* either haven't been demonstrated to be useful in prevention of experimental disease, or application to human beings has been disappointing. For example, BCG, which has been used *a priori* in vaccination programs, confers immunity to infection in the mouse foot pad model,[1] pro-

duces cross-reactions of delayed hypersensitivity with *M. leprae* in guinea pigs,[2] and shares antigens with *M. leprae* demonstrable in precipitation reactions.[3] In practice, however, after eleven years of observation in trials carried out by WHO in Burma, its preventive value in leprosy has been shown to be very low. A cultivable mycobacterium, to be an efficient vaccinating agent, should possess not only the specific antigen(s) of *M. leprae* responsible for immunity induction but also a potent adjuvant effect; these synergistic qualities have not yet been demonstrated in BCG or other suggested mycobacteria, such as *M. vaccae*.[4] An antigenic analysis of numerous mycobacteria would be required to determine if there are cultivable mycobacteria which combine the characteristics mentioned above. In addition, this orientation in vaccine studies is severely limited by the inadequacy of present animal models, particularly in representing the lepromatous aspect of the leprosy spectrum, and in the lack of any precise knowledge concerning the nature of the antigens of *M. leprae* responsible for the induction of immunity.

The second orientation, which refers to the use of killed *M. leprae* from experimental lesions in the armadillo, could offer an adequate solution. Investigations concerning the induction of specific hypersensitivity to *M. leprae* in guinea pigs offer an encouraging basis, leading some investigators in this field to believe that this is the most attractive possibility currently available. This alternative, however, encounters limitations when extrapolated to human beings. It is well known that a relatively important percentage of the population either does not react or reacts slowly to *M. leprae;* this situation has not been demonstrated in guinea pigs. It is precisely this group of non- or slow reactors which must be protected, since nonlepromatous as well as lepromatous cases come from this group; in practice the use of *M. leprae* by itself would not be useful if protection were not afforded to this group.

It has been thought that the use of very large doses of killed *M. leprae* might transform the group with a defective response to *M. leprae* into good reactors. This idea would have to be tested first in the healthy population of poor reactors from endemic foci of leprosy, and perhaps in Mitsuda-negative patients with indeterminate leprosy in order to evaluate an effect on cell-mediated immune responses; the granulomatous response to very large doses of *M. leprae*, even in poor reactors, may constitute a limiting factor for this alternative. It must be borne in mind before initiating trials in the application of a vaccine against leprosy, that the trial period of observation should be at least ten years. Before undertaking an extensive trial, sound evidence that reasonably guarantees success would be most desirable.

It would appear to us that the third possibility, that of using a mixture of killed *M. leprae* from the armadillo together with BCG, offers the greatest possibility of success based on available studies. The bases for this opinion are presented in the following paragraphs.

Using the *M. lepraemurium* model, it has been demonstrated that the most efficient vaccination procedure in that experimental model was the use of a mixture of *M. lepraemurium* together with BCG.[5]

A series of studies carried out in our laboratories in lepromatous and Mitsuda-negative indeterminate leprosy patients and in Mitsuda-negative contacts have given the following results. The administration of relatively large numbers of heat-killed *M. leprae* (6.4×10^7 acid-fast bacilli) elicited formation of a nodule which was studied histologically at 30 days. In lepromatous patients this granuloma was composed of essentially pure macrophages; in Mitsuda-negative indeterminate patients and contacts there was a moderate number of lymphoid cells. A

[1] Shepard, C. C. Vaccination against experimental infection with *Mycobacterium leprae*. Am. J. Epidemiol. **81** (1965) 150-163.

[2] Goihman-Yahr, M., Raffel, S. and Ferraresi, R. W. Cross-reactivities of lepromin. Int. Arch. Allergy. **36** (1969) 450-468.

[3] Harboe, M., Closs, O., Bjorvatn, B., Kronvall, G. and Axelsen, N. H. The antibody response in rabbits to immunization with *Mycobacterium leprae*. Infect. Immunity. (In press, 1977)

[4] Stanford, J. L., Rook, G. A. W., Convit, J., Godal, T., Kronvall, G., Rees, R. J. W. and Walsh, G. P. Preliminary taxinomic studies on the leprosy bacillus. Br. J. Exp. Pathol. **56** (1975) 579-585.

[5] Hanks, J. H. and Fernandez, J. M. M. Enhancement of resistance to murine leprosy by BCG plus specific antigen. Int. J. Lepr. **24** (1956) 65-73.

characteristic common to all of these individuals was the persistence of large numbers of the inoculated *M. leprae* within the macrophages of the granulomata. In marked contrast, elimination of *M. leprae* and tuberculoid features of the granuloma were evident in Mitsuda-positive individuals. This test for competency in the clearance of bacilli (CCB test) clearly demonstrates the absence of such competency in the macrophages of persistently Mitsuda-negative individuals.[6]

Subsequently, we reported the following observation: when *M. leprae* in the concentration mentioned above was mixed with living BCG and injected intradermally into Mitsuda-negative individuals, histological study of the nodule demonstrated the absence of acid-fast bacilli in the macrophages of the granuloma, which was of the tuberculoid type. This induction of macrophage competency was limited by the experimental design to the observation of a local phenomenon.[7]

This phenomenon led to a new step in our studies, which was the study of possible systemic changes in cell-mediated immune response toward *M. leprae* in Mitsuda-negative individuals injected with a mixture of killed *M. leprae* and living BCG. After five years of observation, the following results have been reported: radical immunologic changes were observed after one or two applications of the mixture in persistently Mitsuda-negative contacts and indeterminate leprosy patients. All of the *in vivo* tests of cell-mediated immunity (48-hour test with lepromin supernate, Fernandez and Mitsuda reactions) became positive, as did the *in vitro* transformation test. In some of the indeterminate patients, lesions with a tuberculoid structure appeared, which regressed completely after treatment. The phenomena observed in the two groups constituted a clear demonstration of the preventive and curative action of the vaccine used.

In lepromatous patients, bacteriologically negative after sulfone treatment for more than ten years, less striking but nevertheless favorable immunologic changes were observed. Both the early and late reactions to lepromin became positive; however the 48-hour reaction to soluble protein antigen from lepromin and lymphocyte transformation remained negative. Histological study of the Mitsuda reaction showed a tuberculoid granuloma, but the macrophages were not competent in eliminating the bacilli.[8]

In summary, intradermal injection of the mixture of *M. leprae* and BCG produced favorable immunologic changes in the patients and contacts studied, suggesting the strong possibility of genuine efficacy of the mentioned vaccine.

We recognize that living BCG may not be the ideal mycobacterial adjuvant for use with killed *M. leprae*. This "ideal" adjuvant should have at least two characteristics: it should be widely distributed in nature, so that a large proportion of the population is sensitized, and it should be highly potent in adjuvant activity. An argument can also be made against the use of a living mycobacterium as an adjuvant.

Virulence factors in mycobacteria have not been fully analyzed, but the possibility exists that many species are not pathogenic precisely because they stimulate extremely strong immune reactions and are therefore unable to establish themselves in the host. Further studies are under way in our laboratories to evaluate the possible efficacy of using a killed mycobacterium of this type together with *M. leprae* in vaccination procedures.

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[7] Convit, J., Pinardi, M. E., Rodriguez-Ochoa, G., Ulrich, M., Avila, J. L. and Goihman-Yahr, M. Elimination of *Mycobacterium leprae* subsequent to local "in vivo" activation of macrophages in lepromatous leprosy by other mycobacteria. Clin. Exp. Immunol. **17** (1974) 261-265.

[8] Convit, J., Aranzazu, N., Pinardi, M. and Ulrich, M. Immunological changes observed in indeterminate and lepromatous patients and Mitsuda-negative contacts after the inoculation of a mixture of *Mycobacterium leprae* and BCG. (Submitted for publication)