period 1959-1968. Before 1958, rubella occurred in irregular three to 10 year epidemic cycles. After widespread use of the vaccine in the 1970s, rubella incidence declined markedly, but the endemic level of rubella activity remained unchanged.

Data on the relative occurrence of CRS before and after the introduction of rubella vaccine are very limited in most countries, including Canada. CRS was added to Canada's federal list of notifiable diseases only in 1979. A total of 67 CRS cases was reported by five provinces from 1979 to 1983. Analysis of CRS incidence data during this period indicates a declining trend of CRS per 100,000 live births. However, the numbers of CRS cases involved are too small to draw any definite conclusions regarding the impact of immunization programs on the occurrence of CRS.

Source: The foregoing is a modified version of the paper by J. Furesz, P. Varughese, S. E. Acres, and J. W. Davies entitled "Rubella Immunization Strategies in Canada" that was presented at the International Symposium on the Prevention of Congenital Rubella Infection held at Washington, D.C., on 13-15 March 1984.

IMMUNIZATION AGAINST LEPROSY: PROGRESS AND PROSPECTS[1]

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Recent years have seen tremendous progress in the field of leprosy immunology, and the availability of large quantities of Mycobacterium leprae, *grown in the nine-banded armadillo, has given impetus to the search for a vaccine specific for leprosy. Methods for production and purification of* M. leprae *have been developed, and the resulting preparation has been shown to produce good delayed-type hypersensitivity in mice and guinea pigs. Also, several small-scale studies in human subjects have shown that various preparations of* M. leprae *and BCG can induce cell-mediated immunity in Mitsuda-negative patients and contacts. It is now appropriate to consider field trials of vaccine preparations in selected groups before moving on to large-scale trials in different populations.*

The limitations of the current approach to leprosy control through mass treatment of patients are well-recognized. The long incubation period of the disease, the insidious onset, the chronic course, and the need for prolonged treatment have made control a formidable task, urgently requiring improved tools. A primary preventive approach through immunization would appear to be the answer, and efforts are now being made to develop a vaccine with high efficacy, acceptability, and cost-effectiveness.

Trials with BCG

In 1939, Fernández *(1)* injected BCG into healthy children who were lepromin-negative and found lepromin conversion in over 90%. He concluded that BCG might be efficacious in the prevention of leprosy. During the next 20 years a number of small-scale studies in selected populations also suggested BCG could be of value in preventing leprosy. In the 1960s, major

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field trials were undertaken to test the value of BCG against leprosy in Burma, India, Papua New Guinea, and Uganda. These trials yielded varying results; but in general, except in the Uganda trial, the protective effect of BCG seemed modest *(2-5)*. Factors responsible for the observed variations remain unclear.

Development of a Leprosy Vaccine

In the 1970s, it was discovered that *Mycobacterium leprae* could be grown in the nine-banded armadillo. The subsequent availability of large quantities of *M. leprae* gave impetus to the search for a vaccine specific for leprosy.

An underlying premise of this approach to leprosy control is that induction of immunologic reactivity to *M. leprae* antigens will lead to protection. This assumption is based on the observation that borderline tuberculoid (BT) and polar tuberculoid (TT) patients express strong levels of cell-mediated immunity and are able to restrict the growth of *M. leprae* (although in the process clinical problems may arise through damage to nerve and other tissues); but polar lepromatous (LL) and borderline lepromatous (BL) patients are less able or unable to restrict the growth of the organisms and lack cell-mediated immunity. In other words, there exists a correlation between cell-mediated immunity and the ability of patients to deal with *M. leprae*.

A second premise, based on experimental evidence, is that *M. leprae* and other cultivable mycobacteria can produce a cell-mediated immunity to antigens of *M. leprae*. The Mitsuda lepromin test itself, which is read at 28 days, does not detect preexisting immunity (as do other tests read at 48-72 hours) but actually sensitizes the individual, thus acting as a microvaccine. The test is thus able to discriminate between individuals who are capable of responding to *M. leprae* antigens and those who cannot, such as patients with lepromatous leprosy.

The supply of *M. leprae* is based on production in armadillos. While the possibility of using recombinant DNA technology to produce *M. leprae* antigens in *Escherichia coli* or other bacterial hosts, or of making *M. leprae* competent to grow, are clearly important options for the future, the supply for the next few years will be dependent on armadillos. Currently, IMMLEP[4] maintains over 300 armadillos in six centers for production of *M. leprae*, and the infected tissues are stored in a bank in London, England, to which scientists have access. The armadillos have to be caught in the wild in the southern United States, because to date attempts to breed them in captivity have been unsuccessful.

To ensure maximum systematic infection of about 5×10^{12} organisms in the shortest time possible, the animals are inoculated intravenously with at least 1×10^{8} organisms derived from either human cases or first-passage armadillo material. Since the supply is dependent entirely on production *in vivo*, it has not been possible to establish a "seeded" strain of *M. leprae*. For practical reasons, the human-derived *M. leprae* have been obtained from patients from different parts of the world; thus, any geographically-determined strain differences with important antigenic or immunogenic implications are likely to be represented in the bank.

Vaccine Testing

A method of purification has been developed which appears to give maximum yields of tissue-free bacteria with minimum damage to the organisms. Extensive *in vitro* and *in vivo* testing of *M. leprae* prepared using this method has shown no loss of identifiable mycobacterial antigens. Preparation of a vaccine involved both irradiation of the infected tissue and autoclaving of the final product. The preparation, even in the absence of adjuvants, produced good delayed-type hypersensitivity in mice and guinea pigs, optimum protective immunity in mice, and some immunity in armadillos against challenge with live *M. leprae*.

[4]IMMLEP is the Scientific Working Group on the Immunology of Leprosy set up in 1975 as part of the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases.

Studies in Human Subjects

An early study by Convit demonstrated the effects of repeated Mitsuda testing with standard lepromin; after four such skin tests, over 90% of the recipients in a nonendemic area converted to Mitsuda-positive reactivity.

In recent years, attempts to sensitize human subjects have been made by three different groups of workers with three different products.

Venezuela. In 1973, Convit and co-workers *(6)* immunized a group of 12 leprosy patients (with both lepromatous and indeterminate disease) and 16 adult contacts, all of whom were Mitsuda-negative, with a mixture of 6×10^8 *M. leprae* and BCG. These subjects were followed for seven years; no side-effects attributable to the vaccine were observed. The vaccine produced positive Mitsuda reactivity that persisted until the last observation in 1980.

In late 1979 a second group of 552 leprosy patients (with lepromatous, indeterminate, and borderline cases) and 25 contacts, all of whom were Mitsuda-negative, were inoculated several times with the same mixture of 6×10^8 *M. leprae* and BCG. Important immunologic changes related to induction of cell-mediated immunity were observed in this group, as indicated by one or more of the following:

a) 48-hour skin tests with soluble antigen from *M. leprae,* Mitsuda tests, and lymphocyte transformation tests: skin-test conversion to soluble antigen was demonstrated in 100% of the contacts, 93% of the indeterminate patients, 59% of the inactive BL/LL patients, and 32% of the active BL/LL patients.

b) Clinical improvement in terms of regression of lesions or development of reversal reactions.

c) Histopathologic evidence of reversal reactions resulting in a shift in the spectrum from LL to BL-BB and BT in over 80% of the patients.

Local reactions produced by the vaccine were found similar to those produced by BCG alone. As regards side-effects, 23 instances of neuritis were detected, most of them slight to moderate, among 351 immunized BL-LL patients. These were no more than what would be expected with routine chemotherapy, and were easily controlled with thalidomide or steroids.

India. Two Indian trials have employed organisms that are slightly different from *M. leprae* and that cross-react with it.

In one trial, a cultivable organism designated as the ICRC bacillus was used to prepare a vaccine *(7)*. The clinical trial was carried out, using organisms killed by cobalt irradiation, with 71 LL and 11 BB-BL subjects, all of whom were Mitsuda-negative. Each subject received a single intradermal injection of the vaccine containing 27-67 μg of protein. The vaccine produced no untoward side-reactions. Mild erythema nodosum leprosy appeared in 30% of the subjects with lepromatous cases, especially in those with a high bacillary index, but was easily controlled. It was reported that lepromin conversion occurred in 58% of the LL cases and 91% of the BB-BL cases. The histology of subjects' skin lesions showed significant morphologic changes.

The vaccine potential of another cultivable organism, designated *Mycobacterium W,* has also been explored in India *(8)*. Bacilli obtained from cultures were autoclaved and resuspended in saline at a concentration of 5×10^7 bacilli per ml.

A single dose of 0.1 ml of vaccine was then given intradermally to each of 32 lepromin-negative patients with lepromatous leprosy who had been treated for several years and who were bacteriologically negative. Lepromin sensitivity was retested four to eight weeks after vaccination, and 20 of the 32 subjects were found to have been converted to lepromin positivity.

IMMLEP Plans for Vaccine Testing

Preliminary Studies

Before undertaking large-scale trials of any vaccine, it is crucial to answer a number of questions by conducting small-scale studies. Studies on human sensitization have been designed by IMMLEP to establish (a) the optimum dose of *M. leprae* for sensitization, (b) the duration of sensitivity, and (c) the extent of possi-

ble side-effects in healthy BCG-immunized and unimmunized individuals in countries where leprosy is not endemic. The first of these studies has already begun in Norway. It has also been proposed that similar studies be conducted of healthy individuals in leprosy-endemic areas, particularly in potential vaccine trial areas, to determine whether there are major differences in the response to *M. leprae* antigens.

In addition, it has been proposed that tests be conducted, in collaboration with the Scientific Working Group on Chemotherapy of Leprosy (THELEP), to see whether *M. leprae* (alone or in combination with BCG) will effectively induce cell-mediated immunity in immunologically unresponsive, treated (with chemotherapy), and inactive lepromatous leprosy patients.

Finally, it will be important to ascertain whether immunization with killed *M. leprae* (with or without BCG) and later with other vaccines will provide protection against disease in high-risk groups. Protocols are being finalized for studies to examine the effects of *M. leprae* with BCG, as compared with BCG alone, in inducing specific cell-mediated immunity and in reducing the incidence of clinical leprosy among subjects who are contacts of leprosy patients and who give a negative response to the lepromin skin test.

It is hoped that performance of carefully controlled field studies will make it possible to decrease the number of vaccine preparations used in large-scale trials, and to focus on a small number of epidemiologically well-characterized trial areas. The human sensitization field studies found to employ the most effective vaccine preparation and regimen can then be expanded in some of these areas to a degree sufficient for them to constitute large-scale vaccine field trials.

Large-scale Vaccine Trials

The reasons for the different levels of protection against leprosy obtained in the four major trials of BCG are still not understood. In view of this variation in results, it is considered that trials of a leprosy vaccine may need to be carried out in more than one part of the world, taking into consideration the distribution of leprosy and geographic variations in the clinical spectrum.

Final selection of vaccine trial populations will require careful consideration of many factors, including:

a) The incidence of leprosy.

b) The clinical spectrum of leprosy. (Insofar as there is particular interest in the possibility of protection against multibacillary disease, the incidence of lepromatous forms may be the crucial factor in determining trial size.)

c) Population cooperation and stability, which are prerequisites for trials lasting several years.

d) Population representativeness. (Results of trials in small ethnic groups cannot be extrapolated with confidence to other populations.)

e) Population accessibility. (There should be easy access to good laboratory facilities.)

f) Risks of severe and widespread disease, malnutrition, and natural disasters should be low.

g) BCG status. (There should preferably have been low BCG coverage of the population.)

It is recognized that no population will be ideal with respect to all these features, and that compromises will be necessary.

At the same time, it is unclear whether a leprosy vaccine would be more effective in protecting individuals already infected with *M. leprae* or those not yet infected. Also, administration of a vaccine might cause a temporary increase in the incidence of reportable leprosy among infected individuals, by producing a sudden increase in their specific cell-mediated immunity. Moreover, the duration of protection of any such vaccine is unclear. These uncertainties have important implications for the planning of trials, especially because of the great investment of time and expense implicit in a general population trial.

In this vein, it may be advisable to begin with trials directed at high-risk groups (e.g., contacts), in order to obtain some results within a shorter time and at lower cost. Ideally, infected individuals in these groups should be identified at the time of recruitment into the trial, on the

basis of a serologic or skin test. In that event, these trials could provide an indication of the relevance of prior infection to the effects of a vaccine. In addition, the efficacy of the vaccine itself could be monitored through intermediate immunologic indicators. Large-scale vaccine trials in unselected populations will require preliminary information on the epidemiologic and immunologic characteristics of the population. Hundreds of thousands of subjects may need to be followed for a decade or more.

Immunotherapy Trials

Immunotherapy has so far received little attention, because until recently there appeared to be little hope of inducing cell-mediated immunity in specifically unresponsive lepromatous leprosy patients in the absence of an understanding of the nature of their immunoregulatory disorder. This view has been challenged by Convit's studies *(6)* claiming production of cell-mediated immunity and more rapid clearance of bacilli in patients treated with heat-killed *M. leprae* together with BCG. Moreover, the problems of long-term chemotherapy have made the development of immunotherapeutic methods, which could reduce the treatment period, a high-priority matter. However, it is not possible to recommend any trials of vaccine therapy without concurrent effective chemotherapy. The aims of these trials should be to ascertain the efficacy of *M. leprae* with BCG in inducing immunologic conversion, in accelerating clearance of antigen from the tissues, in reducing relapse rates, and in producing minimal side-effects.

Conclusions

Recent years have seen tremendous progress in the field of leprosy immunology and in the development of tools, such as leprosy vaccine, for control of the disease. However, there is a long way to go before these efforts can be translated into better methods of leprosy control. It is expected that over 10 years will pass, even under the most favorable conditions, before the protective efficacy of a vaccine could be clearly demonstrated. Until then, leprosy control will continue to depend on the optimum use of existing chemotherapeutic tools.

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