

In the "Final Diagnosis" portion, state the relevant diagnosis made prior to discharge, referral, or death and mark the box indicating the type of feeding being provided at discharge.

## Section 6: The Puerperium

This section provides spaces for entering the results of three postpartum control examinations. After "Time Elapsed Since Delivery or Abortion" enter that time. After "Temperature" enter the infant's temperature taken according to normal practice in three digits (e.g., 36.8°C), adding the letter "R" if the temperature was rectal and "A" if it was axillary. After "Pulse" enter the infant's heart rate as measured over a period of 60 seconds. After "Blood Pressure" enter the infant's systolic and diastolic blood pressures in millimeters of mercury. After "Uterine Retraction" state whether the retraction was good, poor, or absent (measure uterine height by using the pubis as a reference point, leading to results such as "eight cm above the pubis"). And after "Lochia" state the characteristics of the lochial flow.

In the "Maternal Discharge" portion, indicate whether the mother was healthy, ill, or referred elsewhere at discharge, or whether she died before discharge (during pregnancy, during delivery, or during the puerperium).

And finally, in the "Contraceptive Advice" portion, indicate whether or not the mother was given advice about family planning and indicate the method or methods about which advice was provided.

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## ELAYED-TYPE HYPERSENSITIVITY IN HUMAN VOLUNTEERS IMMUNIZED WITH A CANDIDATE LEPROSY VACCINE

### Introduction

One of the major goals of the IMMLEP (immunology of leprosy) program of the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases is the development of a vaccine against leprosy. A major step towards this goal was made in 1971 when Kirchheimer and Storrs (1) discovered that the nine-banded armadillo was extremely susceptible to infection with *Mycobacterium leprae*. This led to the availability of unprecedented amounts of bacilli and the possibility of developing a vaccine consisting of killed *M. leprae*.

Such a vaccine would have to meet two important requirements. First, it would have to be purified to remove contaminating host tissue by a process that would leave its immunogenicity

intact. And second, it would have to possess the ability to induce a protective cell-mediated immune (CMI) response in its recipients.

*M. leprae* produced in the armadillo is purified by a two-phase system developed by Draper (2), which has been further modified to ensure that the immunogenicity of the bacilli has been left intact (3). Shepard et al. have shown that the immunogenicity of *M. leprae* remains unaffected by the purification procedure and the heat treatment that is carried out in the preparation of this vaccine (4, 5).

As far as the second requirement is concerned, it was originally envisaged that killed *M. leprae* would have to be incorporated in an adjuvant in order to be able to elicit a CMI response. However, studies carried out in mice (6, 7) and in guinea pigs (8) showed that an irradiated, heat-killed preparation of *M. leprae* was extremely effective in inducing the CMI response, as assessed by delayed-type hypersensitivity (DTH) reactions. Moreover, Shepard et al. (6, 9) have shown that such a vaccine is capable of preventing the multiplication of live bacilli in the mouse footpad.

Having met the criteria of immunopotency in animal systems and the additional requirement of biological safety, the killed *M. leprae* vaccine was essentially ready for trials in man. However, owing to the low incidence and long incubation period of leprosy, field trials for the assessment of protective immunity would require that large populations be monitored over a long period of time. Before the start of such large-scale trials, it was considered necessary to assess the efficacy of the vaccine in small populations using appropriate indicators.

One such indicator is the ability of the vaccine to elicit a DTH response. DTH reactions are associated with resistance to intracellular bacteria such as *M. leprae* (10). This is most clearly observed in leprosy patients themselves, where an overall association between DTH and resistance, as measured by clinical, histologic, and immunologic criteria, is found (11, 12). We report here the results of a study carried out under the IMMLEP program that examines the DTH response among human volunteers in Norway who were immunized with this vaccine.<sup>1</sup>

## Materials and methods

A soluble antigen from ultrasonicated *M. leprae* (MLSA, ref. batch CD19) and purified protein derivative (PPD, RT 23 from Statens Serum Institute, Copenhagen) was used throughout the study. Both skin test antigens were provided by the IMMLEP *M. leprae* bank as

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<sup>1</sup> The main features of the study design were prepared by the IMMLEP Steering Committee in 1981 and were reported in Testing of Purified Armadillo-Derived *M. leprae* in Man, unpublished WHO document TDR/IMMLEP/SC/TEST/81.1, World Health Organization, Geneva, 1981.

coded samples. In addition, uncoded PPD was used for the initial skin testing of the volunteers. Skin tests were performed on the volar side of the forearm. The tests were read at 48 and 72 hours by recording the horizontal and vertical diameters of the induration reaction. The skin test reaction was expressed as a mean of these two diameters. The 72-hour reading was used in the results presented here because it gave a better measure of the response with much less interference from flare reactions.

Ethical clearance was obtained before the start of the trial from the Norwegian Radium Hospital, Oslo, the National Norwegian Drug Agency, and the WHO Secretariat Committee on Research Involving Human Subjects. Thirty-one vaccinees (12 males and 19 females) between 23 and 28 years old were initially skin-tested with PPD and subsequently assigned to four groups, so that an even distribution of the spectrum of PPD responses among these four groups was achieved. This was necessary in order to permit assessment of PPD-related side-effects in each of the groups.

A month after this initial PPD skin testing, the first group of volunteers was skin-tested again with coded antigens (PPD and *M. leprae*-derived skin test antigen—MLSA). Immediately after the 72-hour skin test reaction had been read, this group was given the lowest dose of *M. leprae* vaccine, i.e.,  $1.5 \times 10^7$  *M. leprae*. This was injected intradermally into three sites, using a standard grid (equilateral triangle of 3 cm), 0.1 ml being delivered into each site on the left deltoid region of the arm. The study was designed so that the group to receive the next vaccine dose attended the one-month examination of the vaccination site of the previous group. This permitted an assessment of the reactions to the vaccine and a decision as to whether a higher dose was acceptable. A control group of eight subjects, which was only skin-tested, was also included in the study.

At the completion of the study, the data were sent to WHO headquarters in Geneva where the code was broken and the results analyzed.

## Results

A marked increase in reactivity to the *M. leprae*-derived skin test antigen (MLSA) was observed in the vaccinated groups receiving the three highest doses of vaccine. In contrast, very little change was observed in their PPD reactivity. (As expected, their PPD reactivity was strong because they had been vaccinated with BCG during adolescence according to standard practice in Norway.)

The possibility that the PPD responses had influenced the responses to MLSA was considered. Cross-reactivity was assessed by comparing prevaccination responses to MLSA and PPD. A proportion of the volunteers responded in the prevaccination skin test to MLSA in a pattern related to their PPD prevaccination responses, suggesting cross-reactivity between the two antigens. However, the vaccine-related response to MLSA was not confined to prevaccination MLSA-positive responders. Indeed, the postvaccination MLSA activity was as strong in prevaccination negative as in prevaccination positive subjects.

The only clearly vaccination-related side-effects were observed locally. The local reactions, as measured by induration, reached a peak three weeks after vaccination and were clearly dose-related. Scar formation was observed in all subjects receiving the two highest doses of vaccine. The largest scar observed had a diameter of 9.5 mm. A few temporary petechiae were observed on the trunks of three subjects (two in group one and one in group three) three months after the vaccination. In the absence of a dose relationship, it seems doubtful that these petechiae were a result of the vaccination.

## Conclusion

The present study suggests that strong DTH reactivity can be induced by *M. leprae* in man with doses that do not produce unacceptable side-effects. The next step is to carry out similar studies in leprosy-endemic areas. Such studies would record the DTH reactions immediately after vaccination as well as the duration of the sensitization afforded by the vaccine. These studies should also compare the efficacy of *M. leprae* alone versus the efficacy of a combined *M. leprae* + BCG vaccine, since Convit et al. (13) have found that the combined vaccine is able to restore delayed-type hypersensitivity in unresponsive, indeterminate, and lepromatous patients. This suggests that leprosy could be one of the few infectious diseases for which a vaccine might be both prophylactic and immunotherapeutic.

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# **T**OXOCARA CANIS INFECTION OF CHILDREN IN A CARIBBEAN COMMUNITY

## **Introduction**

Toxocariasis is a public health problem in industrial countries, but few studies have examined the threat presented by this infection to the health of tropical communities. In a pilot study (1) we reported the use of enzyme-linked immunosorbent assay (ELISA) with embryonated *Toxocara canis* ova antigen (Toxocara-ELISA) (2, 3) as a method of detecting *T. canis* antibody in a sample population (n = 25) of Caribbean children. More than half of the children surveyed had significant antibody titers (3), indicating that the prevalence of toxocariasis among the population was unusually high.

Another study was therefore carried out to quantify the significant epidemiologic factors influencing the transmission of *T. canis* to children in a Caribbean community and to identify factors that might contribute to the high seroprevalence of infection.

This study attempted to examine all children between six months and six years of age living in the coastal village of Anse-la-Raye, Saint Lucia. The children accompanied their parents or guardians to the community health center, where a brief clinical history and physical examination were completed. Each child provided a specimen of