Induction of delayed-type hypersensitivity in human volunteers immunized with a candidate leprosy vaccine consisting of killed Mycobacterium leprae

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A killed armadillo-derived Mycobacterium leprae vaccine was examined for its ability to induce a delayed-type hypersensitivity (DTH) response in purified protein derivative (PPD)-positive human volunteers living in a leprosy non-endemic country. Four groups of individuals aged between 23 and 28 years were given $1.5 \times 10^7$, $5 \times 10^7$, $1.5 \times 10^8$ and $5 \times 10^8$ M. leprae intradermally. 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 while there was very little change observed in their PPD reactivity. No unacceptable side-effects attributable to the vaccine were observed. The killed armadillo-derived M. leprae vaccine thus appears to be able to induce a DTH response in man at doses which do not cause unacceptable side-effects.

One of the major goals of the IMMLEP (immunology of leprosy) programme of the UNDP/World Bank/WHO Special Programme 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 & 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. Firstly, it would have to be purified to remove contaminating host tissue by a process that would leave its immunogenicity intact. And secondly, the vaccine 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.6

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 (3, 4). 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 (5, 6) and in guinea pigs (7) 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. (5, 8) have shown that such a vaccine was 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 large populations to 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 (9). This is most clearly observed in leprosy patients themselves where an overall association between DTH and

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resistance, as measured by clinical, histological and immunological criteria, is found (10, 11). We report here the results of a study carried out in 1983–84 under the IMMLEP programme which examines the DTH response in human volunteers in Norway immunized with this vaccine.

**MATERIALS AND METHODS**

**Skin test antigens**

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 this study. Both skin test antigens were provided as coded samples by the IMMLEP *M. leprae* bank at the National Institute for Medical Research, Mill Hill, London. In addition, uncoded PPD was used for the initial skin testing of the volunteers (see study design). 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 as it gave a better measure of the response with much less interference from flare reactions.

**Study design**

Ethical clearance was obtained prior to 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 (SCRHIS). Thirty-one vaccinees (12 males and 19 females), aged between 23 and 28 years, were initially skin-tested with PPD and subsequently assigned to four groups (7–9 subjects in each) so that an even distribution of the spectrum of PPD responses among these four groups was achieved. This was necessary to allow 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 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 that was to receive the next vaccine dose attended the one-month examination of the vaccination site of the previous group. This enabled an assessment of the reactions to the vaccine and a decision on whether a higher dose was acceptable. A control group (8 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. The results were then analysed by the investigators.

**RESULTS**

As shown in Fig. 1, 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

![Graph showing DTH responses to MLSA](image)

**Fig. 1.** Comparison of the pre- and post-vaccination DTH responses to MLSA. Four groups of individuals were injected intradermally with graded doses of killed *M. leprae*. These individuals were skin-tested for responses to MLSA 72 hours prior to vaccination and 3 months after vaccination. While the pre- and post-vaccination DTH responses were not significantly different in the control group and in group 1 which received a dose of $1.5 \times 10^7$ *M. leprae* ($P>0.5$, student's *t*-test), they were significantly different in the other groups ($P<0.001$).
change was observed in their PPD reactivity (Fig. 2). 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 pre-vaccination responses to MLSA and PPD. As shown in Fig. 3, a proportion of the volunteers responded in the pre-vaccination skin test to MLSA in a pattern related to their PPD pre-vaccination responses suggesting cross-reactivity between the two antigens. However, the vaccine-related response to MLSA was not confined to MLSA pre-vaccination MLSA-positive responders. As shown in Fig. 4, the post-vaccination MLSA activity was as strong in pre-vaccination negative as in pre-vaccination positive subjects.

The only clearly vaccination-related side-effects were observed locally. The local reactions, as measured by induration, reached a peak 3 weeks after vaccination and were clearly dose-related (Fig. 5). 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 trunk of 3 subjects (two in group 1 and one in group 3) at 3 months after the vaccination. In the absence of a dose relationship,
it seems doubtful that these petechiae were a result of the vaccination.

**DISCUSSION**

The development of a vaccine requires a systematic examination of all the criteria necessary for its success. Thus, the criteria of purity, efficacy and safety in animal studies having been met, the next logical step was to examine its performance in human subjects. In order to avoid possible interference from natural exposure to *M. leprae*, it was decided that the vaccine should first be studied in non-endemic countries. Here it was necessary to make a distinction between a BCG-vaccinated population and a non-vaccinated population for the same reason. The present study was carried out in Norway where the population is vaccinated with BCG.

This study shows that purified armadillo-derived *M. leprae*, killed by irradiation and autoclaving, is able to induce a strong delayed-type hypersensitivity reaction in man. The DTH reactions obtained were as strong as the responses to PPD in this BCG-vaccinated population. This is in agreement with studies carried out by Smelt et al. (12). They showed that 6 out of 7 normal individuals vaccinated with $2 \times 10^8$ killed armadillo-derived *M. leprae* became positive to a soluble *M. leprae* skin test with a mean increase in induration of 10.4 mm.

BCG and *M. leprae* have been shown, in serological assays (13), to share a number of antigens. Thus, the possibility of cross-reactivity in DTH reactions has been assessed. The comparison of pre-vaccination reactions to MLSA and PPD revealed a pattern of reactivity which suggests a varying, but considerable cross-reactivity. Consequently, the possibility that the killed *M. leprae* vaccine merely boosted an existing response to cross-reactive antigens has to be considered. While the possibility cannot be excluded entirely, in this study, it seems unlikely since the responses were as good after vaccination of MLSA pre-vaccination negative vaccinees as MLSA pre-vaccination positive vaccinees. Moreover, *M. leprae*-specific T-cell clones have now been developed from the vaccinees (18).

Recent studies by Mehra et al. (14) indicate that the suppressor T-lymphocytes associated with multibacillary leprosy respond to phenolic glycolipid, an epitope unique to *M. leprae*. Henceforth, the deletion of such "suppressor" epitopes may be desirable in the development of a vaccine (13). In this regard, it is noteworthy that the present vaccine does not contain detectable levels of phenolic glycolipid (D. B. Young & T. M. Buchanan, personal communication, 1984). Furthermore, none of the vaccinees developed antibodies to phenolic glycolipid as tested by a sensitive ELISA method (16). This finding may be very important as it suggests a means of studying the epidemiology of infection and seroconversions to live *M. leprae* infection in vaccinated populations.

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. (17)
have found that the combined vaccine is able to restore delayed-type hypersensitivity in unresponsive, indeterminate and lepromatous patients. Thus, leprosy could be one of the few infectious diseases where a vaccine may be both prophylactic and immunotherapeutic.

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RÉSUMÉ

INDUCTION D'UNE HYPERSENSIBILITÉ RETARDÉE CHEZ DES VOLONTAIRES IMMUNISÉS AU MOYEN D'UNE PRÉPARATION PROPOSÉE COMME VACCIN ANTILÉPREUX ET CONSTITUÉE DE MYCOBACTERIUM LEPRAE TUÉS

Parmi les principaux objectifs du programme MMLEP (Immunologie de la lèpre) du Programme spécial PNUD/Banque mondiale/OMS de recherche et de formation concernant les maladies tropicales, figure la mise au point d'un vaccin contre la lèpre. Des travaux récents effectués chez des animaux ont montré que Mycobacterium leprae tué possède des propriétés adjuvantes intrinsèques et est capable de susciter une forte réponse immunitaire à médiation cellulaire in vivo. Après la mise au point de méthodes adaptées pour l'isolement de M. leprae destinés à l'utilisation chez l'homme, l'essai d'un vaccin tué a été envisagé chez des volontaires.

La présente étude décrit les résultats de cet essai, entrepris sur des volontaires positifs pour le dérivé protéinique purifié (PPD), résidant dans un pays exempt d'endémie lèpreuse (Norvège). Quatre groupes de sujets âgés de 23 à 28 ans ont reçu 1,5 x 10^7, 5 x 10^7, 1,8 x 10^8 et 5 x 10^8 M. leprae par voie intradermique. Une augmentation sensible de la réactivité vis-à-vis de l'antigène de M. leprae lors d'une épreuve cutanée a été observée dans les groupes ayant reçu les trois plus fortes doses de vaccin, alors qu'on n'observait qu'une très faible modification de leur réactivité au PPD. Aucun effet secondaire indésirable pouvant être attribué au vaccin n'a été observé. Le vaccin constitué de M. leprae tués obtenus sur le tatou semble donc capable d'induire chez l'homme une réponse de type hypersensibilité retardée, sans effets secondaires inacceptables. Cette étude ouvre la voie à l'essai d'un tel vaccin dans des régions d'endémie lèpreuse.

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