

Approaches to studying the transmission of *Mycobacterium leprae*

MILEP2 STUDY GROUP*

Summary A collaborative study has been undertaken to establish the relationship between infection by *Mycobacterium leprae* and the development of immunity in a community in which multidrug therapy (MDT) has been used for more than 10 years, to elucidate the pathogenesis of infection in leprosy, and to develop and test an intervention strategy based on chemotherapy for interruption of transmission of the organism in the community. The first phase of the study included the establishment of laboratory facilities and pilot work in India. In the course of the second phase, the entire populations of three villages in India and one in Ethiopia have been surveyed, nasal swabs were obtained for detection of *M. leprae* DNA by means of the polymerase chain reaction (PCR), specimens of saliva were obtained for measurement of levels of anti-*M. leprae* IgA antibodies, and follow-up surveys have been carried out. A double-blind trial of chemotherapy among subjects whose PCR was positive is proposed, to determine if the course of the infection can be influenced by treatment. The performance of large numbers of PCR tests in endemic countries has required the development of rigorous internal and external quality control procedures. These have shown that many batches (as many as 50%) fail to meet quality control criteria, and must be retested. Despite this, development of these methods, and their application to field studies should provide tools for studying the transmission of *M. leprae*, and direct methods of testing innovative interventions.

Introduction

Understanding of the mechanisms and route of transmission of *Mycobacterium leprae*, including both routes of exit and portals of entry, is fundamental to the development of measures to prevent the spread of infection.¹ However, the information currently available with respect to the transmission of *M. leprae* is very limited, and is based mostly on measures of disease rather than on measures of infection. Research in this area has been seriously limited by the inability to cultivate *M. leprae* and to identify those individuals who are inapparently infected, and by the difficulty in measuring early immune responses.

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Measurement of the incidence of leprosy is difficult,² and measurement of the incidence of infection by *M. leprae* is not possible at present. However, incidence of disease is not a good proxy measure of incidence of infection, because only a proportion of the infected develops disease. Earlier work indicated that the nose is an important route of exit of *M. leprae* from the body,³ and it has been postulated that the primary lesions may be in the nasal mucosa,⁴ without necessarily excluding direct skin-to-skin contact as a route of transmission. Despite much investigation, the possibility of animal and environmental reservoirs of *M. leprae* has not been excluded. The major limiting factor is the lack of appropriate tools with which to study transmission of the organism.

The MILEP2 Study was designed to assess new tools that could be used to study transmission of *M. leprae* in endemic communities. The aim of the study is to define the means by which *M. leprae* is transmitted, and protective immunity within a leprosy-endemic population. The objectives are: (i) to establish the relationship between infection by *M. leprae* and the development of immunity in a community in which multidrug therapy (MDT) has been used for more than 10 years; (ii) to elucidate the pathogenesis of infection in leprosy; and (iii) to develop and test a chemotherapy-based intervention strategy for the interruption of transmission. The first phase of the study, the establishment of laboratory facilities and pilot work in India, has been completed.^{5,6} The second phase is currently being undertaken by collaborating partners in London, Bergen, Amsterdam, India and Ethiopia, with co-ordination based in Aberdeen.

Materials and methods

The principal methods to be employed in the research programme to study the transmission of *M. leprae* are the polymerase chain reaction (PCR) to detect small quantities of the *M. leprae* DNA, and measurement of mucosal immunity by assay of salivary IgA. The PCR method has been developed for use with nasal swabs,^{7,8} and was used in the preliminary work for this study.^{5,6} The IgA method is based on an ELISA assay, developed in the course of a previous study⁶ for use in salivary samples. The initial development work on both assays is aimed at simplifying the techniques for application to large numbers of samples.⁹

The study is being carried out in parallel in leprosy-endemic communities in India and Ethiopia by centres at Miraj, India and Addis Ababa, Ethiopia. Three villages, in which leprosy is endemic, and in which control programmes using multidrug therapy (MDT) had been in place for at least 10 years, were selected in India and one such village in Ethiopia. The villages are comparable in size, socio-economic status and prevalence of leprosy. Preparatory work included meetings with village leaders and villagers to explain the purpose of the study, the procedures, and the nature of the participation required. Initial surveys of each village included enumeration of the populace, collection of demographic information by household, occupational history, medical history including BCG and immunization, physical examination, and the collection of samples of saliva and nasal swabs. Children under the age of 3 years were excluded from the survey.

The surveys were to be repeated on three or four occasions in the course of 3 years in the villages in India, and on one occasion in the village in Ethiopia. Those individuals who are found to be consistently PCR-positive are to be considered for admission to a randomized, controlled trial of anti-leprosy chemotherapy. In addition, if the proportions of PCR-positive

individuals in villages are found to be similar, a community-based chemoprophylaxis trial is to be considered.

Current status

The development of simplified PCR and ELISA protocols has been completed in both Miraj and Addis Ababa, with technical assistance from the University College London and the Royal Tropical Institute in Amsterdam. The PCR-method required extensive work, and the development of internal and external quality controls. Batches of samples were run that included dilutions of positive controls, negative controls and external, quality control samples. Batches failing quality control standards were re-run. The technique has now been developed;⁹ however, experience has shown the importance of quality control for large-scale PCR work in this field. To assess the PCR technique, more than 200 anonymous nasal swabs collected in a non-endemic country were sent to India for analysis, along with six swabs spiked with *M. leprae* DNA. The results demonstrated that the technique was sensitive and specific.

Surveys of the entire populations of three villages in India and one in Ethiopia, to obtain nasal swabs for PCR for *M. leprae* DNA and specimens of saliva for measuring IgA levels against *M. leprae*, have been completed, and three follow-up surveys in India and one in Ethiopia have been conducted. The eligible population of the Indian villages was 3279, and that of the Ethiopian village was 841. The participation rate was 93% in India and 79% in Ethiopia. The compliance rate in successive follow-up surveys decreased with each survey, but remained good. To date, more than 7500 nasal swabs and saliva specimens are undergoing laboratory assay, and samples are being re-run if batches fail quality-control standards.

Endoscopic examination of the nose has been undertaken by a specialist in otolaryngology among those found to be PCR-positive, and biopsies for histopathological examination have been performed in a number of cases.

The proposed double-blind trial of chemotherapy among PCR-positive subjects must await the identification of individuals who remain consistently PCR-positive.

Discussion and conclusions

The study is ongoing, and analysis of specimens is continuing at the AHRI Laboratory in Ethiopia and at the Stanley Browne Laboratories of the Richardson Leprosy Hospital in India. The large numbers of PCR and IgA assays have demanded the development of rigorous internal and external quality control procedures. As many as 50% of the batches have failed to meet quality control criteria, and have had to be re-run. The quality-control procedures, including nasal swabs obtained in non-endemic countries, have demonstrated that it is possible to achieve good sensitivity and specificity. Development of these methods and their application to field studies should provide tools for studying the transmission of *M. leprae*, and direct methods of testing innovative interventions.¹⁰

Initial analyses of the PCR and IgA results will examine age and sex distributions, geographical patterns, temporal trends, relationships to BCG immunization and infection by *M. tuberculosis*, association with contact status, and persistence of the individual findings

over time. The follow-up analyses are likely to be the most useful in studying the patterns of PCR-positivity and IgA immune responses among individuals and in the four communities. This approach, employing PCR and salivary IgA to study endemic populations sequentially over a number of years to identify *M. leprae* in nasal swabs and mucosal immune responses in saliva, should contribute to our understanding of the transmission of *M. leprae*.

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