Paper for second meeting on Strategy of Leprosy Control, Mogadishu, 3 October - 5 November 1982

# RESEARCH NEEDS AND RECENT RESEARCH ADVANCES IN LEPROSY

S. K. Noordeen
Leprosy Unit, WHO, Geneva

Leprosy continues to remain a serious public health problem in the developing countries, particularly if one considers that the populations exposed to the risk of contracting the disease are very large (about 1.4 billion), and that more than one third of leprosy patients face the threat of permanent and progressive physical and social disability. The WHO global estimate of leprosy patients continues to remain at 10.6 million patients, an estimate considered by many as rather conservative. It should be emphasized here that the problem of leprosy is far more serious than what is represented in the numbers alone, particularly in terms of the intense human suffering involved resulting from the social problems related to leprosy.

A major problem in leprosy in recent years has been resistance of M. leprae to dapsone, the most widely used drug. Over the past 15 years secondary dapsone resistance has been reported with increasing frequency among the patients at risk, that is, multibacillary patients subjected to dapsone monotherapy. To cite just one example, Malaysia, where the prevalence of secondary dapsone resistance was estimated to be only 1 to 3 per 1000 in 1966, by 1981 the prevalence estimates had gone up to as high as 100 per 1000. Still the indications are that there are countries with even higher prevalences. It should be mentioned that whenever dapsone resistance has been sought among treated and relapsed patients of lepromatous leprosy it has been found. The number of countries identified with dapsone resistance is now probably over 25 spread over all parts of the world, and the prevalence is steadily increasing in many countries. The situation with regard to the

occurrence of primary dapsone resistance is even more disturbing and the rate of its incidence appears to be increasing at a faster pace than that of secondary resistance.

In spite of the progress with chemotherapy the need to develop a method of primary prevention continues to be of great relevance to leprosy control. In the past, in the absence of an M. leprae-derived vaccine against leprosy, BCG has been tested for its preventive effect against leprosy in large-scale prospective trials in Uganda, Burma, Papua New Guinea, and India. Long-term follow-up of the study subjects in these trials indicates that BCG is capable of protecting against leprosy to a variable degree. Whereas the study in Uganda indicated that the overall protection was as high as 80%, the three other studies showed that the overall protection was only moderate ranging from 28% to 46%. Thus BCG by itself cannot be a very effective tool against leprosy, and the need for developing a highly effective vaccine remains a major goal in the ultimate control of leprosy.

In relation to the above research needs, the most important development in recent years is the organized goal-oriented research efforts being made through the UNDP/World Bank/WHO Special Programme in Research and Training in Tropical Diseases. The Programme includes two important components on leprosy, one on immunology (IMMLEP) and the other on chemotherapy (THELEP).

Immunology of Leprosy

The objectives of the IMMLEP Scientific Working Group which was set up in 1975 are - (a) development of an anti-leprosy vaccine, (b) development of immunological methods for detection of specific immune responses to M. leprae, and (c) development of an increased understanding of immunopathological mechanisms.

The following are the major contributions made by IMMLEP in recent years:

1. Expansion of the supply of armadillo-derived M. leprae with the establishment of a bank of infected tissues and purified bacilli, and making available M. leprae to a large number of scientists.

2. Development of methods of purification of M. leprae from infected tissues with minimal tissue contamination, with several modifications and refinements.

3. Induction of strong cell-medicated immunity by purified bacilli in mice, guinea pigs and armadillos.

4. Protection of mice against infection with viable M. leprae by sensitization with killed bacilli without added adjuvants.

5. Production of purified killed M. leprae under licensed premises of acceptable potency, standardization and toxicity for use in man.

6. Initiation of molecular biological studies on nucleic acid base composition of M. leprae DNA and application of recombinant DNA techniques using plasmid and cosmid vectors for expressing M. leprae gene products and proteins in E. coli.

7. Application of DNA hybridization techniques for elucidating taxonomic relationships of M. leprae to other mycobacteria and corynebacteria species.

8. Development of monoclonal antibodies specific for M. leprae antigens and application of sophisticated immunochemical and biochemical methods for their characterization.

9. Identification of a glycolipid antigen uniquely associated with M. leprae, and identification of glycoprotein antigens that may contain unique antigenic determinants while sharing cross reacting determinants with other mycobacteria.

10. Development of immunofluorescence, RIA and ELISA techniques for measuring serological reactions to M. leprae antigens in patients, contacts and endemic populations.

11. Detection of circulating immune complexes and complement breakdown products in serum indicating antigen-antibody reactions in tissues.

12. Development of an animal model for immunological unresponsiveness to M. leprae antigens.

13. Establishment of several mechanisms of immunological suppression in in vitro studies on lymphocytes of leprosy patients.

14. Demonstration that repeated Mitsuda testing can convert skin test negative normal individuals to positive skin test reactivity.

15. Application of skin test and serological test to obtain basic information relating to the questions of the extent and distribution of M. leprae infection in endemic areas and of the factors that influence that distribution and host responses to infection, including genetic studies on association with HLA antigens.

16. Analysis of the results of four large scale BCG vaccination trials for their efficacy against leprosy and of the factors which might have influenced the differences observed.

Outside of IMMLEP a major development in recent years is the identification by Convit of the possibility of immunotherapy in leprosy based on the vaccination of Mitsuda-negative indeterminate and LL leprosy patients and persistently Mitsuda-negative contacts, using a mixture of live BCG and killed purified M. leprae. The vaccination according to him led to destruction of M. leprae at the vaccination site. All the contacts had become Mitsuda positive and also positive by skin test to a cell-free extract of M. leprae (SPA) after one, or at the most, two vaccinations. The patients required more vaccinations but more

than half of them eventually became positive. There was histological and clinical evidence of an increase in ability to destroy bacteria and of increased immunological competence against M. leprae, sometimes resulting in a 'reversal reaction' and a move along the leprosy spectrum towards BT. Mild neurological reactions had occurred but had been readily controlled.

Another important development outside IMMLEP is the identification of a natural leprosy-like infection in the sooty mangaby monkey, a West African species. Immunological and bacteriological tests has indicated that the organism was extremely similar to M. leprae at the least. The infection had responded to conventional antileprosy chemotherapy, and the animals had shown characteristic signs of nerve damage. Transfer of infection to other mangabey monkeys had been achieved.

Chemotherapy of Leprosy

In the field of chemotherapy substantial contributions have been made by the Scientific Working Group on Chemotherapy of Leprosy (THELEP), particularly in the area of definition of the problem of dapsone resistance and clinical trials.

Dapsone resistance

At the inception of THELEP in 1977, the situation on Dapsone resistance was confused, with an alarming incidence (3 per 100 per year) of relapse with secondary dapsone resistance reported from Ethiopia, and prevalence estimates of 2.5-8 per 100 reported from Costa Rica, Israel and Malaysia, but hardly any information from the rest of the world. Since then the problem of dapsone resistance has been thoroughly investigated. Secondary resistance to dapsone appears indeed to be a serious problem, with estimated prevalence rates of 10 per cent of those at risk in areas as diverse as Gudiyatham Taluk, South India, and Shanghai Municipality, China. Even more alarming are the prevalence estimates for primary dapsone resistance resulting from infection with dapsone-

resistant M. leprae; these are 60 per cent in Addis Ababa (Ethiopia), and over 30 percent in both Bamako (Mali) and Chingleput (India). The accumulation of all this information highlighted the urgency of the situation resulting in development of multi-drug regimens for leprosy control programmes through a meeting of the WHO Study Group in October 1981.

Chemotherapy trials

Although the need for combined chemotherapy was understood, the significance of persisting M. leprae a problem demonstrated earlier by Waters et al, was not at all clear, nor were the means to deal with them known. Therefore, THELEP undertook to study, through formal clinical trials, a number of combined-drug regimens, which had been selected not so much for their eventual utility as in the hope that they would provide information on the effects of regimens of varying degree of intensity on the subpopulation of microbial persisters. Two trials were initiated, one in Chingleput and another in Bamako in 1978. Because the study of each patient requires three years from the time of his recruitment, only fragmentary data on persisting M. leprae are available; the few data already available are sufficient to suggest that the "maximal" regimens may be no more successful in eliminating persisters than the less intensive regimens with which they are being compared. A completely unexpected result of the two trials had been the finding that almost one third of the patients in each centre appear to have been infected with dapsone-resistant M. leprae. THELEP is also carrying out field chemotherapy trials to evaluate the effect of fixed duration multi-drug regimens on occurrence of relapse.

Laboratory studies

The detection of persisting M. leprae requires the use of immunosuppressed rodents capable of surviving for most of the life-span of an immunologically normal animal. Whereas at one time only the thymectomized irradiated mouse

model was available for study of persisters, progress has since been made through THELEP with other models such as the neonatally-thymectomized rat (NTR), the congenitally athymic rat, and the nude mouse.

The compliance of leprosy patients with chemotherapy had long been known to be poor. Because good compliance is essential to the success of chemotherapy as a means of leprosy control, so long as chemotherapy is to be largely self-administered, new methods of ascertaining compliance were needed for use in the field. THELEP supported work on an ELISA technique for measurement of dapsone and its metabolites in urine, has resulted in simple "kits" for field use.

Drug Development

There is a considerable need for additional drugs for leprosy as at present there are only four bactericidal drugs available - dapsone, rifampicin, clofazimine and ethionamide/prothionamide. The major problem in this area is the lack of simple procedures for screening of potentially new drugs. One recent and promising development in this area is the use of surrogate mycobacteria to screen compounds.

Other Areas of Research

Apart from immunology and chemotherapy of leprosy there are other areas in which research progress is badly needed. These include *in vitro* cultivation of *M. leprae*, mechanism of transmission of leprosy, epidemiological and socioeconomic factors favouring spread of leprosy, and operational problems in relation to disease control. With the momentum developed in leprosy research in the last few years the day is not far off when we will have sufficient knowledge to deal with leprosy in a much more effective manner than we are capable of at present.