

# THE IMMUNOBIOLOGY AND EPIDEMIOLOGY OF LEPROSY; A COLLABORATIVE PROJECT BETWEEN UNIVERSITIES OF THE USA AND THAILAND

Sir,

Thank you for your interest in our programme project grant, 'The Immunobiology and Epidemiology of Leprosy', which is supported by an award to the University of Illinois by the National Institutes of Health. The institutional collaboration is between the University of Illinois College of Medicine, the University of Cincinnati, the University of New Mexico in the USA and Chiang Mai University and the McKean Rehabilitation Institute in northern Thailand.

The overall objectives of this programme are to further understanding of the immunopathology, epidemiology and genetic susceptibility to leprosy and its complications. The US-Thai collaborative research programme consists of four interrelated projects, namely: a study of the epidemiology and seroepidemiology of leprosy infection and disease (Project 1); an HLA genetic study of leprosy susceptibility (Project 2); a study of the immunoregulatory abnormalities and immunopathology of leprosy and its complications (Project 3); and a study of phagocytic cell abnormalities in leprosy (Project 4).

The goals for the current year were to develop detailed epidemiologic protocols and data collection forms for use in the field, to develop serologic assays for the specificity and sensitivity in the detection of *Mycobacterium leprae* infections and to characterize the study population by detailed review of McKean records.

A modification of the Fluorescent Leprosy Antibody-absorbed (FLA-abs) test described by Abe has been developed in Chiang Mai and in Chicago. Results from preliminary studies are being evaluated for sensitivity and specificity.

The records of 568 patients seen at McKean Hospital in the past 5 years were reviewed to begin to develop a data base for the epidemiologic studies and the studies of HLA haplotypes. At least one 'close' blood relative of 228 index cases (40.1%) of these leprosy patients also had leprosy. It appears from this review, that the population of readily available patients and families will be quite adequate for the proposed epidemiologic and genetic studies.

Instruments have been developed for the uniform collection of data on leprosy patients and patients undergoing leprosy reactions. Detailed clinical, biochemical, immunological and epidemiological data have been obtained from patients who will be followed prospectively and studied according to protocols developed in the project.

Instruments have been developed and pre-tested for the collection of epidemiologic and clinical data in the field surveys. These forms have been modified based upon their pre-testing in the field (Chiang Dao) and have been translated into Thai. Surveys of two New Life Leprosy Villages in Thailand (about 1,000 persons) have been completed.

We are studying patients with various forms of leprosy and their families to document an association of leprosy with genes in the major histocompatibility complex. We shall determine if genes in this region influence susceptibility to or the clinical outcome of *M. leprae* infection.

At the University of Illinois, we are performing HLA-A, B and DR determinations and mixed lymphocyte cultures on immigrant leprosy patients, family members of leprosy patients and leprosy project personnel. Two Thai co-investigators are trained, equipped and prepared to carry out these activities in Chiang Mai.

We plan additional studies of families in the US. Study of Chicago patients allows us to develop methods for export to Thailand, to anticipate difficulties which may arise in Thailand, as well as to collect data from several racial groups not available in Chiang Mai.

Determination of a strong major histocompatibility complex (MHC) association with the polar forms of leprosy would provide further evidence that susceptibility to leprosy is in

part determined genetically. Such an association is expected from twin studies and family studies of leprosy which have already been reported in the literature and from knowledge of the function of the MHC in man and mouse. Studies of the HLA-A and B locus of unrelated individuals with leprosy have not been revealing. Family studies of haplotype and the HLA-D, -DR locus are expected to be more productive. Such data might provide an explanation for the dichotomous immunologic, bacteriologic, morphologic and clinical findings in the two polar forms, tuberculoid and lepromatous leprosy. Demonstrations of an HLA-D association with lepromatous leprosy would lend support to the hypothesis that immune response genes linked to the HLA complex determine, in part, immunologic reactivity to *M. leprae* and colour the clinical picture of *M. leprae* infection. Association of depression of non-specific or *M. leprae* specific parameters of cellular immunologic reactivity or increased serologic activity with prevalent HLA-B and HLA-D alleles in lepromatous leprosy patients would strengthen the hypothesis that the course of leprosy is partially determined by immune response genes linked to HLA genes.

The goals for the current year included standardizing basic clinical immunology laboratory tests to be used in evaluating leprosy patients in Chiang Mai, to initiate work on immunoregulatory cell populations in patients with leprosy and in controls, and to train Dr Sanit Makonkawkeyoon, Head of the Department of Clinical Immunology at the Faculty of Medical Technology and the director of the laboratory aspects of the Leprosy Research Project in Chiang Mai, in these procedures and to initiate studies of immune complexes in blood and other tissues of leprosy patients.

Dr Ward Bullock initiated the research on immunoregulatory cell population in year one by making a site visit to Chiang Mai with Drs Nelson and Schauf where research protocols of this project could be developed and the needs of the project in the field could be assessed. Subsequently, Dr Bullock has recruited to his faculty, Dr Susan Watson, a cellular immunologist who is very active in the investigation of regulatory disturbances associated with chronic mycobacterial infections.

Specimens of frozen skin biopsies of patients with various types of leprosy and its complications and serum samples were sent from Thailand to the laboratory of Dr Kenneth Tung, University of New Mexico, for assays of circulating immune complexes and tissue-bound immune complexes.

The major goal of the coming year is to make certain that our assays have, in fact, been well established in Thailand and are capable of providing reliable data for the study of leprosy patients. Normal values will be established by studying the peripheral blood leucocytes of healthy Thai volunteers, and studies of leprosy patients will begin. Some of these patients who appear to be more reliable will be entered into a 4-year longitudinal study of their immunoregulatory cell functions.

In the second year of the grant, we plan to obtain cross-sectional data from leprosy patients for circulating immune complexes (CIC). We will also study patients with various types of leprosy and reactional states for the presence of tissue-bound immune complexes. The ultrastructural characteristics of the tissue injury associated with immune complexes in patients with leprosy and its complications will also be studied by electron microscopy.

In addition to the cross-sectional studies described above, a group of patients will be studied longitudinally for the sequential appearance and disappearance of circulating and tissue-bound immune complexes.

The goals for the current year included development of assays to examine the effects of *M. leprae* infection on the phagocytic and metabolic properties of macrophages and neutrophils. Utilization of these assays for study of cells from leprosy patients requires a standardized source of *M. leprae* or *M. leprae*-derived antigens.

Although progress in studies of phagocytic cells from leprosy patients has been hampered by lack of a readily available source of standardized *M. leprae*, development of

assays which can be applied to cells from leprosy patients has been undertaken in the laboratories of Dr Burton Andersen and Dr Paul Gudewicz. Dr Andersen's efforts will be concerned primarily with the role of neutrophils in leprosy. Dr Gudewicz's research interests centre on the function and metabolism of macrophages in a variety of pathophysiologic states. Dr Gudewicz has established the following assays of activity by macrophages: (1) phagocytic uptake of  $^{125}\text{I}$  gelatin-coated latex particles; (2) radiolabelled leucine incorporation into TCA precipitable material; (3) radiolabelled uridine incorporation into TCA precipitable material; and (4) radiolabelled glucose oxidation by macrophages. Mr Sichon Songsiri, from Chiang Mai, Thailand began a 6–8 month training period in the laboratories of Dr Andersen and Dr Gudewicz in July 1980. Arrangements have been made to develop some of the phagocytic cell assays in the mouse *M. lepraemurium* model in the US, as greater availability of the mycobacteria and cells from controlled sources will speed progress in the earlier phases of this study. Experiments for standardization of assays for phagocytic and metabolic functions with use of normal cells are currently being done. Mr Sichon will participate in all phases of animal and human cell experiments in the US and then apply techniques learned to study the larger patient population identified in Chiang Mai, upon his return there.

Goals for the remainder of 1980 and 1981 included: (a) transfer of the mouse *M. lepraemurium* model from Lexington to Chicago and (b) collection of cells from selected leprosy patients for study of phagocytic and metabolic properties of neutrophils and macrophages during the course of the disease process and treatment. Study of the mouse model in both early and late stages of the infectious process will allow evaluation of any progression of cellular defects in phagocytic and metabolic responses to leprosy.

The results from these studies should contribute significantly to the understanding of most defence mechanisms (and failures thereof) operative in leprosy. In this research programme, the efforts of multiple investigators in both the US and Thailand, who are focusing on special aspects of immunology, immunogenetics and epidemiology, are being co-ordinated by establishment of (a) core programmes to identify a well-characterized patient population for study and (b) a central data file, so that information obtained by any investigator is available to others. Results are expected to further define populations at risk, to augment studies of vaccine development and to lead to new strategies of treatment and control of the disease. Moreover, the results should lead to increased definition of cell–cell interactions and some other aspects of regulation of immune responses during natural infection by an intracellular micro-organism.

*Department of Preventive  
Medicine and Community Health  
University of Illinois at  
the Medical Center  
Chicago, Illinois, USA*

VICTORIA SCHAUF, K E NELSON and  
W E BULLOCK