THE WHO PROGRAM FOR RESEARCH ON IMMUNOLOGY OF LEPROSY (IMMLEP)

A. <u>Background and Management</u> by H. Sansarricq^a, G. Torrigiani^b and J. Walter^C B. <u>Some Areas of Scientific Progress</u> by R. J. W. Rees^d

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A. BACKGROUND AND MANAGEMENT

Present methods for leprosy control need to be improved, especially because we are unable to identify the individuals at high risk of developing the disease, antileprosy drugs in use are slow acting, and there is no specific vaccine against the disease. One of the ways by which essential improvements may be obtained is intensified research in the immunology of leprosy. Thus, WHO has embarked on a special program in this area, which is based on a multidisciplinary approach and on the identification of successive objectives and steps.

I. Preparatory stages of IMMLEP

The importance of immunology for the understanding of leprosy has been recognized for a long time, and consequently the relevant studies have been of particular interest to WHO since the creation of the WHO Leprosy Unit in the late fifties. While early interest was mainly concentrated on the lepromin reaction, at a later stage the rapid advances in cell-mediated immunity also attracted scientists

Two meetings were convened by WHO, on the joint initiative of the Immunology and Leprosy Units, in Geneva (1970) (3) and New Delhi (1972) (4, 5). These meetings examined the clinical and pathological spectrum of leprosy, the immunological status in the various forms of the disease, the immunological responses in experimental leprosy, macrophage activity in leprosy, and made recommendations on areas for further research such as

44, 1 & 2 Sansarricq et al: WHO IMMLEP Program Development

studies on T-cell reactivity, circulating antibodies in connection with the different forms of the disease and with reactions, macrophage regulations, etc., and proposed a protocol for evaluating the transfer factor in leprosy.

These meetings helped to clarify some of the questions still unsolved and gave the opportunity to indicate further investigations which could provide the relevant answers.

However, new investigations were hampered because sufficient amounts of M. leprae and its antigens were not available.

Then, in 1971, Kirchheimer and Storrs (6) reported on the first successful experimental generalized leprosy in the nine-banded armadillo infected with <u>M. leprae</u>. Early in 1972 the WHO Leprosy Unit established collaborative research agreements with the centers of Kirchheimer (Laboratory Research Branch, US Public Health Service Hospital, Carville, USA) and Storrs (Gulf South Research Institute, New Iberia, La., USA). In 1974, Convit and Pinardi (1) succeeded in their attempts to transmit <u>M. leprae</u> to the seven-banded armadillo.

Following the second meeting on immunological problems in leprosy in New Delhi, it was decided to appoint as a consultant to the WHO Immunology Unit, T. Godal, who had made important contributions particularly on cellmediated immunity in leprosy. A crucial decision was then taken. Instead of giving first priority to the development of immunotherapy, it was decided to put to the test recent advances made in immunology, as well as in animal models, for the benefit of epidemiology and prevention of leprosy.

A special program for Research in Immunology of Leprosy (IMMLEP) was defined. At the same time a strategy was adopted which had already proved to be efficient in the WHO Human Reproduction Research Program, namely, the Task Force Strategy. While a variety of research projects in different areas related to leprosy, including immunology, continue to be coordinated by WHO, it was decided that WHO, in the management of IMMLEP, should play a leading role in providing the investigators with the opportunity to compare their results, to decide the long-term objectives for the future, and how they could share the different research activities and responsibilities in order to reach intermediate predetermined objectives. In other words, a truly coordinated research program was developed, based on a macro- and micro-network of activities.

II. The Special Program for Research and Training in Tropical Diseases

In the decades following the Second World War the increase of knowledge enabled substantial progress to be made in the control of communicable and non-communicable disease prevailing in developed countries (such as poliomyelitis and cardiovascular diseases). These advances have as yet hardly begun to be applied to the problem of tropical diseases, where methods of control and treatment have changed little in the last thirty years, with some notable exceptions such as yaws and smallpox. Moreover, the impact of communicable diseases on rural communities in the tropics is incredibly high. For example, if you happen to be born in and grow up in the African bush, you are liable to harbor four or more disease-producing parasites simultaneously, and in some areas the risk of your getting leprosy can be as high as 5 per cent or even 10 per cent.

As a consequence of such a situation, recommendations were made by the World Health Assembly that WHO should intensify its efforts to provide new remedies for either preventive or curative use in tropical diseases.

On these lines, a Special Program for Research and Training in Tropical Diseases was launched by WHO in 1974, under the directorship of H. Goodman, assisted by a Program Team (8).

Six diseases have been selected to be included in this program: malaria, schistosomiasis, filariasis, trypanosomiasis, leishmaniasis and leprosy. These diseases have been singled out on the basis of the need and opportunity to obtaine new remedies, but others may be added later and, when success is achieved and effective remedies are found, some may be deleted. At the beginning, the program will focus on one continent that carries the major burden, Africa. However, the fundamental concept is global. South America will be involved from the beginning through work on trypanosomiasis and leishmaniasis.

Concerning the investigators and institutions to participate in the program, for a variety of reasons the involvement of investigators and institutions in tropical countries is crucial for the successful implementation of the programs. On the other hand, it is also realized that, for the time being, the necessary know-how is to a large extent to be found among the scientists of the industrialized world and their involvement in the program is therefore equally important.

Within the Special Program for Research and Training in Tropical Diseases, two programs related to leprosy are at present considered:

- a) The Program for Research on Immunology of Leprosy (IMMLEP), started in 1974;
- b) The Program for Research on Chemotherapy of Leprosy (THELEP) which is in its pre-planning stage.

III. Organization of IMMLEP

As IMMLEP has already gained momentum during the last two years, it is considered as a pilot operation within the Special Program for Research and Training in Tropical Diseases.

From the organizational point of view, IMMLEP is mainly based on a Task Force and its Steering Committee.

44, 1 & 2 Sansarricq et al: WHO IMMLEP Program Development

The Task Force is a group of scientists of highest international standing whose duties are to review periodically the current status of immunology of leprosy, to set scientific directions for research, to make recommendations on the order of priorities to be followed and to identify the persons and institutions best qualified to participate in the program. The investigators selected are consequently invited to join the Task Force and to undertake a research project included in the program. Task Force members are chosen for their qualifications for the work in hand and this participation can change according to the phases of the work.

From the members of the Task Force, a Steering Committee has been designated, comprising members from outside the secretariat (WHO) and members of the secretariat. The Chairman of the Steering Committee, T. Godal, in coordination with the secretariat, is responsible for technical preparation of meetings and the analysis of scientific advances relevant to the program, as well as for the preparation of reports, etc. The Steering Committee in particular reviews and selects projects on the basis of priorities recommended by the Task Force and of the funds allocated, reviews proposals, maintains communication of recent developments to members of the Task Force, and establishes communication between IMMLEP and other organizations involved in leprosy research.

Up to date, eleven centers or laboratories are collaborating in IMMLEP. More will be included in the future. Unfortunately, so far only one participating center is located in the African continent, the Armauer Hansen Re-Search Institute (AHRI) in Addis Ababa.

IV. IMMLEP Current Developments

Three main objectives -- skin test, vaccine development and immunotherapy-were chosen, and the first meeting of the IMMLEP Project Group (now called the Task Force) under the able chairmanship of B. R. Bloom, took place in Geneva from 4-8 November 1974 (7). Eight protocols were designed, incorporating:

- 1. The supply of M. leprae.
- 2. Purification of M. leprae.
- 3. Antigen fractionation.
- 4. Taxonomic studies.
- 5. Induction of cell-mediated immunity to M. leprae.
- 6. Resistance to experimental infection.
- 7. Immunopathology.
- 8. Sensitization of human volunteers.
- 9. Development and trial of a specific soluble antigen for skin testing.
- 10. Preliminary considerations for a vaccine field trial.
- 11. Organizational structure.

Finally, the first meeting of the IMMLEP Steering Committee was convened in Geneva from 2-4 June 1975. During this meeting discussions took place on progress studies with skin test antigen, an armadillo bank, administrative procedures, ethical aspects of IMMLEP field activities, and on rules for publication, and a final report was prepared (2). International Journal of Leprosy

In general, we hope of course that the final aims of IMMLEP will be reached, even if this will mean in some instances a reorientation of the program. Nevertheless, we can foresee some possible difficulties. For instance, it may happen that it will be possible to develop a specific skin test which permits the identification of individuals with subclinical leprosy infection but not the identification of the subjects prone to develop leprosy. Another crucial question is if a vaccine will be able to prevent individuals from developing lepromatous leprosy.

V. IMMLEP Financial Aspects

There are, of course, financial implications in the implementation of the IMMLEP program. So far, financial assistance has been provided to the laboratories and institutions participating in the program, but this only covers additional expenses. However, the IMMLEP grants are considerably larger than the usual "token" grants provided by WHO.

The revised IMMLEP budget for the year 1974-1975 amounts to \$165,000 and the estimated budget for 1976 amounts to \$290,000. Funds have been provided by a contribution made by the Norwegian Agency for International Development (NORAD) of a yearly amount of \$75,000 for four years. Additional support is expected from the overall provision for the Special Program for Research and Training in Tropical Diseases.

B. SOME AREAS OF SCIENTIFIC PROGRESS

I. Distribution of M. leprae Infected Armadillo Tissues

For the IMMLEP program infected tissues have been provided from the Gulf South Research Institute, New Iberia, and the USPHS Hospital, Carville, and to date distributed to workers within the task force in Ethiopia, Norway, UK and USA. The earlier tissues were from both killed and dead animals, but since June 1975 from killed animals only.

II. Extraction and Purification of M. leprae

High priority and effort has been given to this. The method in current use which has given the best results, is as follows:

Infected liver or spleen is first exposed to 2.5 Mrad and then mechanically homogenized in buffered sucrose and from the homogenate the bacterial rich fraction is separated by differential centrifugation.

The bacterial rich fraction is then treated with collagenase and Pronase and the bacilli finally separated in an aqueous 2-phase system containing dextran and polyethylene glycol.

280



Fig. 1. Purification of M. leprae from infected armadillo tissue.

By electron microscopy the bacilli so prepared are free from contamination, including collagen.

To date 323 g infected liver and spleen have yielded 492 mg dry weight <u>M. leprae</u>, giving a mean yield of 1.5 mg dry weight <u>M. leprae</u> per 1.0 g wet weight infected tissue. The yields from batches have varied by less than 7%.

To date some 300 mg M. leprae have been distributed for the IMMLEP program. (The method is referred to by Dr. P. Draper on page 104 and detailed in Fig. 1).

Although the above method provides high yields of purified <u>M. leprae</u> from liver and spleen, it is not applicable to skin, presumably because of higher lipid content. Further techniques are being explored.

III. Preparation and Testing of Skin-Test Antigen

From batches of <u>M</u>. leprae purified as above, the bacilli were broken open by sonic disruption, centrifuged and the soluble supernatant was standardized on the basis of protein content. This soluble product constituted the skin-test antigen. The yields of soluble protein were approximately 25% of the M. leprae

By comparing the responses of guinea pigs sensitized to uninfected armadillo tissue with those sensitized to skin-test antigen, it has been shown that the antigen is contaminated by less than 0.5% "armadillo".

Preliminary and orientating tests on patients across the leprosy spectrum, read at 72 hours, showed the antigen to be inactive (<5 mm induration) in lepromatous patients and reactions of >5 mm induration in the majority of patients with active tuberculoid leprosy. The optimum skin test dose appears to be in the range $0.2 - 2.0 \mu g$.

With these promising preliminary results more extensive studies in Libya and Burma have been undertaken. In Burma some 5,000 tests have been carried out including patients across the leprosy spectrum, the population at large and close contacts. In the Burma study other similarly prepared mycobacterial antigens were also tested. The data has already been put on to the computer and is awaiting final analysis.

It is planned to carry out skin tests in the near future in two nonleprosy areas on volunteer medical students in Norway and the UK. 44, 1 & 2

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