Multidrug therapy against leprosy

Development and implementation over the past 25 years

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Improved knowledge and new hopes

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While the impossibility of cultivating *M. leprae* in artificial media has doubtless been the main obstacle to progress in experimental leprosy, researchers trying to elucidate the relationship between the leprosy bacillus and its human host were for many decades hampered by the extremely complex clinical and histological aspects of the disease. These challenges were taken up in the late 1950s – and the striking progress that was to be made during the 1960s and early 1970s is the subject of this overview.

The mouse footpad model

In 1960, Shepard (*1*) described the measurable – though limited – multiplication of *M. leprae* in the hind footpads of normal mice, which revolutionized experimental leprosy by making possible a wide range of new investigations. A few years later, Rees proposed another useful model, the thymectomized/irradiated (T/900r) mouse (*2*). Both mouse models proved to be invaluable in several critical areas, described below.

Generation time of M. leprae

In the logarithmic phase of growth in the mouse footpad, the generation time of *M. leprae* was calculated to be 12–13 days (3) – much longer than for any other bacterium. Such a prolonged generation time is consistent with the long incubation period and chronicity of leprosy.

Identification of purported isolates of M. leprae and monitoring of their viability

Most mycobacteria do not grow in the mouse footpad; those that do, show growth curves and histological features that are appreciably different from those of *M. leprae* (*3*). Thus, the mouse footpad method could be used for identifying *M. leprae* isolates from patients' nasal discharges and for monitoring of the viability of the organism.

Correlation between morphological aspect and infectivity

It was possible to demonstrate that only uniformly staining bacilli, the percentage of which determines the "solid ratio" (Shepard) or the "morphological index" (Ridley), are viable, as measured by infectivity for mice (*4*).

Use of the mouse footpad model in experimental chemotherapy

It is certainly in experimental chemotherapy that the mouse footpad model – in most instances Shepard's normal mouse – has been most widely used and has provided the most significant results (*3*, *5*, *6*). Applications of the model include the screening of new drugs, with determination of minimal inhibitory concentration and type of activity (i.e. bactericidal or bacteriostatic) against *M. leprae*; monitoring of drug trials; and demonstration of drug-resistant *M. leprae*. Developments of the mouse footpad model relevant to the preparation and confirmation of effectiveness of the 1981 combined drug regimens are discussed in Chapter 2 under the heading "Scientific factors (1972–1981)".

Chemotherapy

Progress made during the 1960s and 1970s in the field of chemotherapy of leprosy are discussed in detail elsewhere in this report. Here we recall only the most important milestones reached during those years:

- In 1964, the first cases of dapsone-resistant leprosy were demonstrated by the mouse footpad method.
- During the 1960s, the efficacy of clofazimine as an antileprosy drug and its anti-inflammatory activity were reported.
- In 1970, the rapid bactericidal activity of rifampicin against *M. leprae* was demonstrated.
- Although it had been known since the earliest days of the chemotherapy of leprosy that multibacillary patients can relapse if they stop treatment, it was only in 1974 that the existence of persisting viable *M. leprae* was detected for the first time in lepromatous patients treated for 10–12 years with dapsone. Thus, the concept of "persisters" was established.

The Ridley–Jopling spectrum

The concept of the leprosy spectrum, with a five-group classification system, was proposed by Ridley & Jopling in the 1960s (*7*, *8*). It is based on correlated clinical and histological features, the latter being interpreted as indicative of cell-mediated responsiveness. At the two ends of the spectrum are two stable forms of the disease – the polar tuberculoid (TT) highly resistant form and the polar lepromatous (LL) low resistant form. Between these two lie the intermediate borderline (BT, BB, BL) forms, which can undergo some evolution towards either end of the spectrum.

The Ridley–Jopling spectrum and classification represented a landmark, and the classification became the mandatory reference system for any scientific investigation involving leprosy patients. It was thus of crucial importance in two essential areas of studies on such patients – drug trials (for correct selection of patients) and immunological investigations. With regard to the latter, it is noteworthy that the spectrum concept was developed before the importance of immunological determinants was revealed by experimental studies in mice and more definitive studies in leprosy patients (*9*).

Immunology

The histopathological and clinical features of the Ripley–Jopling classification provided unequivocal evidence that the relationship between *M. leprae* and its host was dependent on the degree of the cell-mediated immune response of the host to the organism (*10*). The initial step appears to be the antigenic stimulation of the T (thymus-dependent) lymphocytes, either directly by the pathogen or after processing of the pathogen by macrophages. This leads to lymphocytic proliferation and release of lymphokines, some of which are able to enhance the antimicrobial capacity of the macrophages. It is an important feature of the lepromatous form of leprosy that the macrophages are unable to digest the organisms that they have phagocytosed.

During the late 1960s and early 1970s, intensive investigations were carried out, using all available techniques, with the main objective of establishing immunological determinants in leprosy, in relation to the Ridley–Jopling spectrum. The progress made was reviewed at a WHO meeting held in New Delhi in 1972 (*11*, *12*). Here, we provide an overview of the investigations of the relationship between *M. leprae* and its human host before the establishment of IMMLEP – the Immunology of Leprosy programme.

In vivo studies

- The correlation between the level of response to the Mitsuda reaction in the various forms of the disease is one of the characteristics of the Ridley–Jopling classification. "Lepromin positivity has become accepted as a measure of host resistance in patients with leprosy ... However, a positive lepromin reaction is not specific for leprosy" (*13*).
- Histological examination of lymph nodes in patients distributed over the whole disease spectrum showed that paracortical (thymus-dependent) areas were well developed in tuberculoid patients and extensively replaced by macrophages loaded with leprosy bacilli in lepromatous cases (*14*, *15*).
- No consistent relationship was found between the late lepromin reaction and reactions to purified protein derivative (PPD) and other antigens derived from cultivable mycobacteria in the various forms of leprosy (*13*, *16*).
- A high proportion of lepromatous patients failed to respond to two sensitizing agents (1-chloro-2,4-dinitrobenzene and 2-chloro-1,3,5-trinitrobenzene); healthy persons and tuberculoid patients did respond (*16*, *17*).
- Skin allograft rejection was delayed in patients with the lepromatous and, to a lesser degree, the tuberculoid form of the disease (*18*).

In vitro studies

- The lymphocyte transformation test (LTT) was established, using as antigen non-autoclaved *M. leprae* extracted from infected human tissues: TT patients responded quite strongly, whereas negative results were regularly obtained in LL patients (*19*, *20*). Patients in the BT group showed variable responsiveness and results in patients with untreated BL disease were usually negative (*21*). Although leukocytes from lepromatous patients did not transform in the presence of *M. leprae*, they responded to a varying degree to other mycobacterial antigens such as whole BCG and PPD (*21*). The level of reactivity appeared to be related to the status of treatment, i.e. reactivity was lower in untreated lepromatous patients than in patients who had received prolonged chemotherapy (*22*).
- Results of the leukocyte migration inhibition test (LMIT) also showed great variation, from strong responses to *M. leprae* in TT patients to a virtual absence of response in the LL group (*21*).

Humoral responses in leprosy

- The production of antibodies to antigens unrelated to *M. leprae*, such as typhoid/paratyphoid vaccines, appeared to be normal in patients with lepromatous and tuberculoid leprosy (*23*, *24*).
- Levels of circulating antibodies against a polysaccharide antigen common to *M .leprae* and other mycobacteria were high in a very large proportion of lepromatous patients and in a minority of tuberculoid patients (*12*).

Immunological complications in leprosy

- Some experimental evidence supporting a role for immune complexes in the pathogenesis of erythema nodosum leprosum (*25*, *26*).
- Evidence indicated that reversal reactions are due to a rapid increase of cell-mediated immune response to *M. leprae*, with a shift in histological classification – in both skin and lymph nodes – towards the tuberculoid end of the spectrum (*15*, *27*). These changes were associated with strong responses to *M. leprae* in vitro as measured by LTT and LMIT (*28*). In thymectomized/irradiated mice with lepromatous lesions, injections of syngeneic lymphoid cells resulted in changes in the lesions which resembled the reversal reaction in humans (*29*).

Vaccination

One of the main topics of discussion in the early 1970s was the possibility of using BCG as a tool for leprosy control, particularly in view of the shortcomings of dapsone-based treatment. Three BCG trials had been undertaken (*12*): in child contacts and relatives of known leprosy cases in Uganda; in persons of all ages in New Guinea; and in a population of children, mainly not exposed at home, in Burma (now Myanmar). Although conclusions were premature, the preliminary results collated in 1972 were strikingly different in the three trials: 80% protection was attributable to BCG in the Uganda trial, 46% in New Guinea, and 44.2% (restricted to the group aged 0–4 years at intake) in Burma.

The fact that numerous studies had demonstrated the effectiveness of BCG against experimental infection by *M. leprae* in mice featured prominently in the discussions (*30*).

Epidemiology

Existing knowledge of the epidemiology of leprosy had been reviewed by Newell (*31*) in 1966 at the request of WHO. Some important issues were investigated in subsequent years.

M. leprae portal of exit

It was shown that, in the early stage of the disease, lepromatous (BL, LL) cases excrete 10^6–10^9 leprosy bacilli daily in nasal mucus (*32*); that these organisms were indeed *M. leprae* was demonstrated by the mouse footpad method.

Survival of M. leprae outside the human body

The survival time of leprosy bacilli in nasal discharges kept under defined conditions for varying periods of time was also measured by the mouse footpad method (*33*).

Subclinical infection

It had long been observed that few of those exposed to heavy sources of infection in fact contract leprosy. Subclinical infection should therefore be common. Godal & Negassi (*34*) applied the LTT for the first time in investigating contacts and non-contacts of leprosy patients. They concluded that leprosy is more highly infectious than prevalence of the disease indicates, and that a subclinical infection commonly follows exposure to *M. leprae*. The relatively low response found in contacts of lepromatous patients suggests that, in these contacts, a "super exposure" to *M. leprae* can bring about a lowering in host resistance.

Establishment of global programmes for leprosy research

In the late 1960s and early 1970s, the means to prevent and cure tropical diseases (including leprosy) were unequal to the problem, yet less than 0.5% of the world's total medical research resources was devoted to tropical diseases. Moreover, a large proportion of these resources was spent in developed countries (*35*). As a consequence, the World Health Assembly of May 1974 adopted a resolution requesting WHO to initiate a coordinated effort for research in tropical diseases.

A few years earlier two meetings – in Geneva in 1970 (*36*) and in New Delhi in1972 (*11*, *12*) – had been convened by WHO on the joint initiative of the Immunology and Leprosy units. In November 1972, immediately following the second of these meetings, Howard Goodman, Chief, Immunology, and one of the authors, H. Sansarricq, then Chief, Leprosy, initiated joint activities aimed at coordinating and supporting investigations on the immunology of leprosy, on a global basis. In August 1973, Tore Godal, who had made important contributions particularly on cell-mediated immunity in leprosy, was appointed as a consultant by the Immunology unit, with the task of drafting a global plan for research on immunology of leprosy (*37*); financial support for this was requested from the Norwegian Agency for International Development (NORAD). The next logical step was the establishment of the Immunology of Leprosy programme (IMMLEP), which held its first meeting in November 1974 (*38*).

At the same time, Goodman had started to put in writing the ideas that served as a basis for discussion in a WHO Intra-Secretariat Planning Group set up in June 1974 for developing proposals for a Special Programme for Research and Training in Tropical Diseases (TDR).

The draft plan for IMMLEP was completed in mid-1974. At an informal meeting in August of the same year, at the suggestion of Professor Bergstrom from Norway, it was decided that IMMLEP should start immediately (with financial support pledge by NORAD) as a pilot activity for the research programme in tropical diseases then in preparation *(38)*.

Immunological investigations of leprosy had long been hampered by the unavailability of sufficient amounts of *M. leprae* and its antigens. In 1971, however, Kirchheimer & Storrs reported on the first successful experimental generalized leprosy in the nine-banded armadillo infected with *M. leprae* (*39*) – which would in principle provide a large supply of *M. leprae*. This success, plus the advent of new immunological methods, made it feasible to identify the development of a leprosy vaccine as a first objective for IMMLEP. Other objectives of the programme were the development of skin tests and further studies in immunopathology aimed at the development of immunotherapeutic measures.

At the request of the programme sponsors, detailed proposals for TDR were prepared during 1975 and 1976 and, in December 1976, the Special Programme was formally set in motion.

In 1976 the establishment of the programme for research on chemotherapy of leprosy (THELEP) – as a part of the normal growth of TDR – was to be an essential step towards the development of the 1981 Study Group regimens (see Chapters 2 and 6).

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