Recent advances and present trends in leprosy research

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Leprosy is a chronic communicable disease, naturally confined to man. Clinical symptoms affect mainly the skin and the peripheral neural system with frequent, extensive sequelae such as deformities of the face and extremities. Clinically manifest leprosy, untreated, may run a life-long course. The causative agent is an acid-fast bacillus, *Mycobacterium leprae*, which has not up to now been cultivated in vitro. The incubation period is between 3 to 10 years or longer. One of the most striking features of leprosy is that it presents a great variety of forms, depending on the cellmediated and not the humoral, immune status of each individual. These forms fit into a continuous spectrum, described by RIDLEY and JOPLING[1,2]. At one end, cell-mediated immune (CMI) response is very strong and leprosy bacilli very rare; this is referred to as polar tuberculoid leprosy. At the other end of the spectrum, polar lepromatous cases show a complete lack of CMI responsiveness and considerable multiplication of leprosy bacilli. Between both polar types, there is a continuous variation in CMI responsiveness and in the corresponding bacteriological status. The various CMI and bacteriological status correspond to a graduation in histological and clinical features[3].

The prevalence of leprosy frequently exceeds 10 per thousand on a countrywide scale in Africa and some parts of Asia, while it is around two per thousand for most South American countries. About 11 to 12 million cases of leprosy are estimated to exist in the world with little fluctuation over the past 10 to 15 years. Principal disease distribution is around the tropical and subtropical belts.

In 1960 the era of experimental leprosy was opened with the first successful inoculation of *M. leprae* into an animal, the mouse. Since then substantial advances have been made in the field of leprosy research. These advances appear to open up new avenues for further investigations which will result in a better understanding of the disease processes and can be expected to lead to the development of improved methods for diagnosis and detection of subclinical infection, for the treatment of the various leprosy forms and complications and, it is hoped, ultimately, the development of a vaccine.

This review, although not intended to be exhaustive, attempts to summarize the main recent advances in the field of leprosy research and the principal lines for further investigations with their expected possible results.

1. *Recent advances* 1.1 *Animal models*

1.1.1 *Mouse.* Observations on the method of growth of *M. leprae* in the foot pad of normal mice were published for the first time by SHEPARD in 1960[4,5]. REES[6-8] has used the mouse, immune-depressed by thymectomy and irradiation, for the same purpose.

In both normal and thymectomized-irradiated mice inoculated with a few (probably 10) uniformly stained (i.e. presumed viable) bacilli (see para. (c) below), the sequence of events comprises 3 phases, namely: a) a lag phase lasting about 90 days; b) a logarithmic phase of 60 to 110 days during which the bacilli multiply, and c) finally, a plateau phase.

In the immune-depressed mice, the events are identical to those in normal mice until the end of the logarithmic phase. In normal mice, the logarithmic phase ends abruptly with the appearance of a histological picture corresponding to the establishment of immune defence mechanisms and then there is no further increase in the number of bacilli. In immune-depressed mice, following the logartihmic phase, bacterial multiplication can continue although at a reduced rate, while lepromatous-like lesions develop locally and in other hairless sites.

Over the past 15 years, the mouse model has proved its value for the clarification of several important points[9,10].

a) Generation time of *M. leprae*. This has been calculated to be of 13 days in the logarithmic phase. Comparison can be made with the generation time of other bacteria (observed in culture media), e.g. approximately 20 h for *M. tuberculosis* and about 20 min for *E. coli*.

b) Identification of purported isolates of *M. leprae* and monitoring of their viability. The growth and histological patterns in the mouse foot pad are very important oriteria for identifying a strain of mycobacteria as being *M. leprae*. Most mycobacteria do not grow in the mouse foot pad and those which do show growth curve and histological features different from *M. leprae*. The mouse foot pad method has been used for the identification of *M. leprae* isolates, especially from nasal discharges and from arthropods fed on leprosy patients, in attempts to cultivate *M. leprae*, and for the monitoring of the viability of *M. leprae* in those materials.

c) Correlation between morphological aspect and infectivity. A correlation has been found between the proportion of uniformly staining bacilli (currently designated as 'morphological index' (MI, RIDLEY) or 'solid ratio' (SHEPARD)) in leprosy material and the degree of their infectivity when inoculated into the mouse.

d) Screening of new drugs. Two methods are available, namely, the continuous and the kinetic methods, the latter determining whether a drug is bactericidal, bacteriostatic or bacteriopausal. The administration of drugs in graduated dosages permits determination

of their minimal effective dosage and minimal inhibitory concentration.

e) Monitoring of drug trials. A loss of 99% of the pre-treatment rate of viable bacilli can be detected in the sufficiently sensitive mouse model, whereas only a 90% loss of pretreatment infectivity can be detected by measuring the decrease in the rate of solid staining organisms. Consequently the monitoring of drug trials by mouse foot pad inoculation provides more rigorous results than their assessment by measurement of the uniformly staining (bacilli) ratio.

With standard dapsone doses, the infectivity measured by the mouse foot pad method falls to undetectable levels in 3 to 4 months.

f) Demonstration of drug resistance. No other method is available to demonstrate that a patient's bacilli are resistant to drugs.

g) Pathogenesis of leprosy lesions. The mouse models, throughout their life-span show important features which can help in the understanding of pathogenesis of leprosy lesions in man. The main findings are the haematogenous spread from the site of inoculation, the multiplication of *M. leprae* in sites of predilection, namely, the dermis of the foot pad, ears and tail, the nose, the testes and dermal and peripheral nerves.

1.1.2 *Armadillo.* In 1971, KIRCHHEIMER and STORRS[11] reported a disseminated experimental *M. leprae* infection in the nine-banded armadillo (*Dasypus novemcinctus*, Linn.). This animal is an edentata which is common in South and Central America and in the southern part of the United States, but does not live naturally elsewhere in the world. Some of its biological features are particularly interesting for leprosy investigations, namely, a) low body temperature (32°–35 °C), b) long life-span (12–15 years), and c) regular production of litters of monozygous quadruplets. Unfortunately armadillos do not breed in captivity and all animals have to be caught in the wild.

Some 40% of armadillos inoculated with *M. leprae* develop, after about 1 year, a systemic and heavy infection with histological and bacteriological features of human lepromatous leprosy. The amounts of *M. leprae* which can be obtained from these infected animals are very high. A yield has been reported of 1.5 mg of dry weight of *M. leprae* per g wet weight of armadillo tissues.

In 1974, CONVIT and PINARDI[12] succeeded in their attempts to transmit *M. leprae* to the eight-banded armadillo (*D. sabanicola*). Recently, STORRS et al.[13] reported the successful inoculation of the leprosy bacillus in the seven-banded armadillo (*D. hybridus*).

At present armadillo colonies of 100 animals or more are maintained in the Gulf South Research Institute, New Iberia, La., USA, the US Public Health Service Hospital, Carville, La., USA, the National Institute of Dermatology, Caracas, Venezuela, and smaller colonies at the Center for Disease Control, Atlanta, Georgia, USA, and the Medical Research Council, London. Colonies are also being established in other centres (Paraguay, French Guyana).

1.2 *Chemotherapy of leprosy*

Dapsone (4,4'-diaminodiphenylsulfone) has been for over 30 years, and still is, the main antileprosy drug. Its tolerance is generally good, there are few side effects and it is cheap, costing only a few dollars per year per case. One of the disadvantages of dapsone, its slow action, has been well known since the beginning of its use.

As said before, it has been estimated by the mouse foot pad method that during the first 3 to 4 months of treatment of lepromatous cases with dapsone in full dosage, 99% of a patient's viable *M. leprae* are killed. There is no method available to monitor a subsequent decrease in the number of viable bacilli.

Even in lepromatous patients who have responded to dapsone, relapses occur. In the last 10 to 15 years it has been demonstrated that some relapses are caused by secondary resistance to dapsone. These have been observed at a frequency ranging from about 2% in Malaysia[14] to 3% per year in Ethiopia. Resistance to dapsone seems to develop mostly as a consequence of low-dose dapsone therapy and irregular treatment. The time from start of treatment to the appearance of secondary dapsone resistance ranges from 5 to 20 years, whereas in tuberculosis drug secondary resistance may appear in less than 1 year. These relapses require the use of alternative chemotherapy.

Very recently it has been shown that relapses in lepromatous cases, treated for more than 10 years with supervized dapsone therapy and having responded to dapsone, can be caused by the survival of small numbers of *M. leprae* fully susceptible to dapsone (persisters) probably in some privileged sites of the human body.

Other antileprosy drugs have recently been introduced. The most effective, rifampicin, is rapidly bactericidal against *M. leprae* (as against *M. tuber-*

[1] D. S. RIDLEY and W. H. JOPLING, Int. J. Leprosy *34*, 255 (1966).
[2] D. S. RIDLEY, Bull. Wld Hlth Org. *51*, 451 (1974).
[3] The Ridley-Jopling scale, in its simpler form, is as follows: TT, polar tuberculoid; BT, borderline tuberculoid; BB, borderline; BL, borderline lepromatous; LL, polar lepromatous.
[4] C. C. SHEPARD, Am. J. Hyg. *71*, 147 (1960).
[5] C. C. SHEPARD, J. exp. Med. *112*, 445 (1960).
[6] R. J. W. REES, Nature, Lond. *211*, 657 (1966).
[7] R. J. W. REES, M. F. R. WATERS, A. G. M. WEDDELL and E. PALMER, Nature, Lond. *215*, 599 (1967).
[8] R. J. W. REES and A. G. M. WEDDELL, Ann. N. Y. Acad. Sci. *154*, 214 (1968).
[9] C. C. SHEPARD, Bull. Wld Hlth Org. *44*, 821 (1971).
[10] R. J. W. REES, C. W. BINFORD, J. CONVIT, W. F. KIRCHHEIMER, Y. MATSUO, S. R. PATTYN, C. M. RIVAS, C. C. SHEPARD, E. E. STORRS and A. G. M. WEDDELL, Int. J. Leprosy *41*, 446 (1973).
[11] W. F. KIRCHHEIMER and E. E. STORRS, Int. J. Leprosy *39*, 693 (1971).
[12] J. CONVIT and M. E. PINARDI, Science *184*, 1191 (1974).
[13] E. E. STORRS, G. P. WALSH and H. P. BURCHFIELD, J. trop. Med. Hyg. *78*, 216 (1975).
[14] M. F. R. WATERS, J. M. H. PEARSON and R. J. W. REES, Leprología *19*, 243 (1974).

culosis). Viable *M. leprae* cannot be detected in skin biopsy specimens obtained 3 to 4 days after a single dose of 1200 or 1500 mg or after treatment for 3 to 4 days with daily doses of 600 mg. In view of its rapid killing effect on *M. leprae*, rifampicin would have much to recommend it. However, it is a very expensive drug and attempts to use intermittent dosages so as to reduce the cost have met with toxic effects which are sometimes very severe, resulting from the production of antibodies to the compound.

Acedapsone (diacetyldapsone, DADDS) an insoluble derivative of dapsone, and clofazimine, a red phenazine dye, like dapsone and rifampicin have been found to be active against *M. leprae* both in the mouse and in controlled clinical trials, but they also have important disadvantages. A few other drugs, ethionamide, thiacetazone and streptomycine offer some promise.

1.3 *Immunology of leprosy*

1.3.1 *Lepromin reaction*[15]. The lepromin proposed initially by MITSUDA (1923) and HAYASHI (1933) is a suspension of the whole autoclaved homogenized leproma, including tissue elements. Injected intradermally, lepromin elicits an early (FERNANDEZ) reaction after 48 h and a late (MITSUDA) reaction after 4 weeks. There is a good correlation between the level of posititivity of the late lepromin reaction and the clinical-histological status of leprosy patients throughout the RIDLEY-JOPLING spectrum. In healthy persons living in endemic areas, the late lepromin reaction is negative in the first months after birth. Then the proportion of positive reactions and the degree of positivity increase steadily with age, and at 15 years of age and over most individuals have a very strong or strong positive reaction. On the other hand, populations in areas where leprosy is not endemic show a high proportion (about 80%) of positive MITSUDA reactions. Therefore the MITSUDA reaction, although indicative of resistance to *M. leprae*, cannot be considered as being only related to specific immunological changes provoked by the organism.

1.3.2 *Recent studies on the immune response in leprosy*. Since 1971 various in vitro tests for the monitoring of cell-mediated immune response have been applied to leprosy[16–18]. The lymphocyte transformation test (LTT) with non-autoclaved *M. leprae* as antigen has been the most widely used.

In leprosy patients, a continuous decrease in cell-mediated immunity levels has been found throughout the RIDLEY-JOPLING scale from the tuberculoid towards the lepromatous end.

Keeping in mind the reservations which should be made on the specificity of available in vitro CMI tests[19], these results, however, give strong support to the opinion that clinical and histopathological lesions in leprosy are determined by the capacity of each individual to show a specific CMI response to *M. leprae*.

The levels of serum precipitins in leprosy patients have been investigated by using double-gel diffusion techniques with *M. duvalii* as antigen, since *M. leprae* was not available in sufficient quantities[18]. The levels of these precipitins increase from the tuberculoid towards the lepromatous end of the RIDLEY-JOPLING spectrum in complete contrast to the levels of CMI response. This raises the question of whether the impairment of CMI in lepromatous leprosy is related to the high levels of circulating antibodies.

The above findings and other studies also suggest preliminary explanations, which have still to be confirmed, for two types of well-defined reactions occurring in leprosy, namely, erythema nodosum leprosum (ENL) and reversal reactions[18].

ENL occurs only in highly bacilliferous patients (LL and BL), especially when they are put under treatment. The available information suggests that in ENL immune complexes resulting from the presence of vast numbers of bacilli and high levels of circulating antibodies are at the origin of histological lesions very similar to Arthus reactions, and of clinical symptoms many of which are similar to those of serum sickness.

Reversal reactions may occur in leprosy patients, except those in polar groups, and they are characterized clinically by a movement towards the tuberculoid end of the spectrum. Here a rapid increase in CMI response to *M. leprae* with the corresponding histological reaction seems to be the origin of nerve and other tissue damage.

1.3.3 *CMI in vitro tests in contacts of leprosy cases*. In 1972, GODAL et al. in Addis Abeba[19–21] applied for the first time CMI in vitro tests to healthy individuals who had various degrees of previous contact or no contact at all with leprosy cases. In subsequent studies, they found that about 50% of individuals having occupational contact with leprosy cases for more than 1 year, as well as household contacts, gave a positive response.

Positive results are interpreted as resulting from a subclinical infection by *M. leprae*. These investigations give support to the opinion that in leprosy, as in many other communicable disease, a large number of individuals become infected and only a small proportion of them will later develop clinical disease, whereas the rest succeed in combating the causal agent.

Confirmatory studies of this type are in progress. The interpretation of such investigations requires some clarification, since there is a certain level, still to be determined, of interference by the tuberculosis infection in the CMI reactions observed[19].

1.3.4 *BCG vaccination against leprosy*. Because of some similarities between *M. leprae* and *M. tuberculosis*, the idea has been developed for a long time that the BCG vaccine might have a preventive effect against leprosy[22].

Results of recent trials carried out with BCG to assess its protective effect on leprosy are contradic-

tory[22]. Claims of having reached an 80% protection in one trial (Uganda) could not be confirmed by the WHO Leprosy BCG trial in Burma (using the same brand of vaccine) in which a temporary low protective, effect, reaching its peak with 39% in its 5th year and decreasing to about 10% in the subsequent years, was reported. A third trial (Karimui, New Guinea) using a different BCG preparation, repeated yearly, had protection rates of about 50%. Perhaps more important than these different results is the recent (1974, 1975 and 1976) appearance of lepromatous and borderline cases in the vaccinated and control groups in the Leprosy BCG trial in Burma.

1.4 *Transmission of M. leprae*

1.4.1 *Portal of exit.* The early leprologists were convinced that *M. leprae* was spread by infectious cases from the naso-pharyngeal lesions through nasal discharge and respiratory droplets. Then, for a long time, little attention was paid to this opinion and the hypothesis of transmission through 'skin to skin' contact prevailed among many authors.

Recently, PEDLEY[23,24] showed that acid-fast bacilli are not found on intact skin of lepromatous cases. At the 10th International Leprosy Congress (Bergen, 1973), DAVEY and REES[25], PEDLEY, BARTON et al.[26], reported that lepromatous cases excrete leprosy bacilli through their nasal mucus at an early stage of the disease, in amounts of 10^6 to 10^9 bacilli per day. These organisms were indeed *M. leprae* as demonstrated by inoculation in the mouse foot pad.

1.4.2 *Survival of M. leprae outside the human body.* Recently, DAVEY and REES[25] made investigations on the survival time of *M. leprae* in nasal material. This material was kept in defined physical conditions for different periods of time, after which samples were inoculated into the mouse. From the reported results, it can be concluded that in nasal discharges left in the dark at about 20 °C the great majority of leprosy bacilli can survive for more than 1 day and less than 2 days.

2. *Present trends in leprosy research*

2.1 *Unsolved problems*

2.1.1 *In vitro cultivation of M. leprae.* Mass in vitro cultivation of *M. leprae* would undoubtedly result in rapid progress towards obtaining a better armament against leprosy by enabling simpler methods to be developed for essential tools such as drug screening and evaluation, preparations for in vivo and in vitro immunological assessment, and possibly a vaccine.

PATTYN[27], at the request of WHO, recently reviewed the problem of in vitro cultivation of *M. leprae*. No substantiated claim has been made concerning the growth of *M. leprae* in cell-free media and the application of the tissue culture technique has been equally disappointing. More recently, SKINSNES[28] reported having succeeded in the cultivation of *M. leprae* in cell-free media containing hyaluronic acid. This claim, however, has not yet been confirmed.

According to PATTYN[27], only an improved understanding of the metabolism of *M. leprae* could indicate the way for its successful cultivation. This approach is the reverse of the usual sequence of studies in which empirical in vitro cultivation has permitted biochemical requirements of bacteria to be established.

Investigations on the metabolism of *M. leprae* are in progress and interesting findings have been reported in recent years, especially on various enzymatic activities[27]. However, it appears that much time will be required before sufficient information is accumulated to enable cultivation attemps based on specific metabolic requirements to be made.

2.1.2 *Improved case-detection methods.* The diagnosis of early leprosy is still based on its clinical features and therefore requires individual training and experience. Concerning the lepromatous cases, which represent the core of the leprosy problem, it appears that when these are detectable by routine diagnostic methods they have already been contagious for a substantial period of time, and it is to be assumed that a proportion of contacts are already infected. Therefore, it would be extremely important to develop a method permitting the very early detection of these lepromatous cases.

In addition, it would also be of great interest to have a test for detection of subclinical infection, especially if this test could permit the identification of individuals at high risk of contracting the disease. Such a test could also be an important epidemiological tool if its variations in populations were closely related to the level of leprosy prevalence.

2.1.3 *Improvement of present treatment methods.* It is very frustrating to see that, compared to the progress made in the treatment of other bacterial diseases over the last 30 years, few antileprosy drugs have been proposed as alternatives to the well-established dapsone.

Although primary resistance to dapsone has not yet been observed, it is bound to occur and this would result in a difficult epidemiological situation. When considered individually in comparison with dapsone, the new drugs of proven efficacy on *M. leprae* (ace-

[15] *Immunological Problems in Leprosy Research:* 1, Bull. Wld Hlth Org. *48*, 345 (1973), Memorandum.
[16] W. E. BULLOCK, JR. and J. FASAL, J. Immunol. *106*, 888 (1971).
[17] T. GODAL, B. MYKLESTAD, D. R. SAMUEL and B. MYRVANG, Clin. exp. Immunol. *9*, 821 (1971).
[18] T. GODAL, B. MYRVANG, J. L. STANFORD and D. R. SAMUEL, Bull. Inst. Pasteur *72*, 273 (1974).
[19] T. GODAL, Leprosy Rev. *45*, 22 (1974).
[20] T. GODAL, M. LOFGREN and K. NEGASSI, Int. J. Leprosy *40*, 243 (1972).
[21] T. GODAL and K. NEGASSI, Br. med. J. *3*, 557 (1973).
[22] *Immunological Problems in Leprosy Research:* 2, Bull. Wld Hlth Org. *48*, 483 (1973), Memorandum.
[23] J. C. PEDLEY, Leprosy Rev. *41*, 31 (1970).
[24] J. C. PEDLEY, Leprosy Rev. *41*, 167 (1970).
[25] T. F. DAVEY and R. J. W. REES, Leprosy Rev. *45*, 121 (1974).
[26] J. C. PEDLEY, Int. J. Leprosy *41*, 511 (1973).

dapsone, rifampicin, clofazimine) offer some advantages (greater ease in use or greater initial efficacy) and have some disadvantages (higher cost or higher toxicity). Above all, when used as single drugs, they would face the same problems of drug resistance and microbial persistence as dapsone. On the other hand, it appears reasonable to envisage that different combinations of these drugs with dapsone could more effectively deal with the problems of resistance and persistence, and consequently make the treatment of lepromatous cases more effective and shorter.

Other problems have to be considered in relation to treatment of leprosy. In lepromatous cases, the action of drugs is not supplemented by CMI as is the case in tuberculosis. Even the great load of dead bacilli remain in their host cells for years, and the hypothesis has been raised that these dead bacilli would play a role in pathological mechanisms. In reversal reactions, it would be necessary to reduce the adverse effects of CMI reaction which causes nerve and other tissue damage.

2.1.4 *Improvement of preventive methods.* The contradictory results of BCG vaccine in the prevention of leprosy have been mentioned above (see under 1.3.4).

Chemoprophylaxis with dapsone has been tried. In two different trials, a protective effect of about 50% was noted in children exposed to sources of leprosy infection, but the partial protection ceased when the drug was stopped. Apart from such limitation, logistic and even ethical reasons rule out this form of prevention for practical reasons, with perhaps the exception of some limited population isolates.

2.1.5 *Epidemiological problems.* a) Role of genetic factors. Leprosy appears to be concentrated preferentially in certain families and there is strong evidence that the degree of contact with infectious cases is correlated with the incidence of new cases. However, the question is still open whether genetic factors play a role in the transmission of the disease, and especially in determining the development of the immune deficient lepromatous form.

b) Portal of entry of *M. leprae*. There is fairly good evidence that the main portal of exit of *M. leprae* is the nasal mucosa, but the question of entry of the organism is still undecided. Since from the few observations of fortuitous inoculations in man it has been noted that a local lesion develops at the site of inoculation, some would think that *M. leprae* enters into a new host through a small skin wound. Recently the hypothesis has been formulated of the possibility of the respiratory tract being the portal of entry for *M. leprae*. REES and MCDOUGALL submitted immune-depressed mice to aerosols containing *M. leprae*. They observed first that immediately after exposure the leprosy bacilli were found in the lungs and not in the nasal passages. Then during the 2 years following exposure 30% of the mice developed experimental disease with bacilli present in lung and some peripheral sites (nose, ears, foot pad). This does not, of course, prove that the respiratory tract is the actual way of infection in human leprosy, but it is consistent with that hypothesis.

2.2 Areas for further investigation

To comply with the above needs, and in view of recent advances, suggestions can be made for further investigations along the lines which we shall review below.

WHO is actively collaborating in the efforts of the scientific community aimed at the solution of existing problems. In particular, WHO is stimulating a special endeavour in the areas of immunology and chemotherapy of leprosy by means of what is called 'task force strategy', within a Special Programme for Research and Training in Tropical Diseases[29].

2.2.1 *Research on immunology of leprosy.* a) *Objectives.* In view of the present needs three objectives can be fixed for further investigations in this field, namely: a) the development of simple tests for leprosy subclinical infection and early detection of leprosy cases, b) the development of a vaccine, and c) methods for immunotherapy.

b) *Approaches.* 1. Tests for subclinical infection and early diagnosis. The LTT and other CMI in vitro tests already constitute valuable tools for the detection of subclinical infection. It appears, however, that their specificity should be improved and the methods simplified. Micromethods are already in use.

Even when these tests are simplified, their use will still require skilled laboratory personnel and some equipment. Therefore, an attempt should be made at the same time to develop simple skin-tests to be used under field conditions. With the available supply of great quantities of *M. leprae* from the armadillo, it now appears feasible to produce well-standardized preparations which may be tested for antigenic properties.

2. Development of a vaccine. In view of the non-cultivability of *M. leprae*, there seem to be three possible approaches to the search for a vaccine against leprosy[18], namely: a) Killed *M. leprae* in an appropriate adjuvant to stimulate cellmediated immunity; b) Killed cross reactive mycobacterium, closely related to *M. leprae* in adjuvant; c) A live non-pathogenic cross-reactive mycobacterium.

It is considered that killed *M. leprae*, if it can be supplied in sufficient quantities, is likely to be superior to any killed cross-reactive organism. The armadillo can provide the required amounts of *M. leprae*. The search for a live vaccine (i.e. cross-reactive and non-pathogenic) could be pursued while the field trial with killed *M. leprae* is being undertaken.

The methodology to screen the three above possibilities in animal models by CMI testing or resistance to experimental infection is available. The same applies to CMI testing in human volunteers. Thus, it should

be possible to obtain a considerable quantity of information before the time-consuming and relatively expensive field trials ultimately have to be carried out.

3. Immunotherapy. So far, four different methods have been tried to boost host defence mechanisms in lepromatous leprosy, namely: diphtheria toxoid, BCG, allogeneic leukocytes, transfer factor. The overall conclusion that may be drawn from the results obtained so far is that a few injections do not give significant results[18]. However, carefully controlled studies with repeated injections of BCG have not yet been undertaken. With allogeneic cells, the results are conflicting, but because this form of therapy poses additional risk factors, it should not be encouraged at present. Other possible forms of immunotherapy are levamisole and thymosin. However, it is suggested that further studies in immunotherapy should not be considered before results of the transfer factor studies currently in progress are available.

c) *Present achievements*[30]. Efforts are being made to increase further the production of infected armadillo tissues. To date, a method for purification of *M. leprae* has been established. Three antigenic preparations have been developed and tested in laboratory and field trials. Immunochemical studies and investigations on the relationship between *M. leprae* and other mycobacteria are in progress.

2.2.2 *Research on chemotherapy of leprosy*. The objectives in this area are two-fold: a) the better use of existing drugs, and b) the development of new drugs.

The suggested approaches with regard to the first objective include the collection of more precise information on the risk of relapse with dapsone-resistant *M. leprae* strains, studies on pharmakinetics of drugs, establishment of animal models for resistance and persistence phenomena, clinical trials with two or more drugs.

To achieve the second objective, attempts should be made to obtain the synthesis of new compounds in different classes (analogues of clofazimine, derivatives of rifampicin and inhibitors of mycobacterial dihydrofolate synthesis) to be tested in the mouse.

2.2.3 *Epidemiology of leprosy*. Two main lines of investigation are suggested. It seems that the use of available immunological techniques (such as HLA antigen studies) in large population sections could provide clearer answers on the question of the role possibly played by genetic factors in the epidemiology of leprosy.

On the other hand, attempts should be made to identify unknown risk factors and high risk groups by means of prospective incidence studies in large population groups.

The recent developments mentioned above on the nose as a source of large numbers of *M. leprae* in early untreated lepromatous leprosy are important in deciding what variables to measure in incidence studies. Anthropological methods for measuring inter-personal contacts are also now available, and can contribute substantially to such studies. Also, prospective incidence studies can be continued with comparisons of different treatment regimens.

2.2.4 *Biology of M. leprae*. The need for improved knowledge on the biology of the pathogen has been indicated above. It seems that modern techniques for cell biology and biochemistry, as well as the use of *M. lepraemurium* as a model, might help in the clarification of some unsolved problems.

Conclusions

Over the last 5 years, important progress has been made in the field of leprosy research. While the mouse foot pad model has confirmed its value, especially for the assessment of antileprosy drugs, in vitro methods for the monitoring of CMI have provided strong support for the concept of the leprosy spectrum developed by RIDLEY and JOPLING. The same methods have also provided evidence that in many instances exposure to *M. leprae* is followed by subclinical infection which, in most cases, will be overcome, only a few of the infected cases developing overt disease. The role of nasal mucosa lesions as the main source of living leprosy bacilli from contagious cases has been proved.

On the other hand, as regards therapy of leprosy, the shortcomings of the well-established dapsone monotherapy have become increasingly apparent, with growing evidence of secondary resistance to the drug and with the recent discovery of the bacterial persistence phenomenon. At the same time, a few new antileprosy drugs have been proposed.

Presently available leprosy control methods require improvement. But it may be possible through clinical trials to develop better therapeutic regimens, especially by combining two or more existing antileprosy drugs. In addition, several groups of new compounds show promise of being active against *M. leprae*.

The generalized experimental infection obtained in the armadillo has opened a source of unlimited amounts of *M. leprae* antigens which, unfortunately, cannot be provided at present by in vitro cultivation. This, together with the recent advances in the field of immunology of leprosy, will lead to new investigations aimed at the development of skin tests for subclinical infection, immunotherapeutic methods and, hopefully, a vaccine.

It is, of course, realized that the availability of fully adequate therapeutic and preventive methods for leprosy control will not automatically lead to the reduction of the leprosy problem. The successful application of these methods will depend on the solution of operational problems. But research has to come first.

[27] S. R. PATTYN, Bull. Wld Hlth Org. *49*, 403 (1973).
[28] O. K. SKINSNES, E. MATSUO, P. H. C. CHANG and B. ANDERSSON, Int. J. Leprosy *43*, 193 (1975).
[29] H. SANSARRICQ, G. TORRIGIANI and J. WALTER, Int. J. Leprosy *44*, 276 (1976).
[30] R. J. W. REES, Int. J. Leprosy *44*, 280 (1976).