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In Honour of Dr R. J. W. Rees, C.M.G.

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Special Issue in Honour of Dr R J W Rees, CMG

A Dedication

For the past thirty years Dr RJW Rees, known to his friends and colleagues as Dick Rees, has been closely associated in so many aspects of the international scientific battle against *Mycobacterium leprae*.

It is in recognition and appreciation of his inestimable contribution to this battle that LEPRA has dedicated this special issue of *Leprosy Review* to him. It will not only provide up to date information on all aspects of leprosy research in which Dick Rees is particularly interested, but also should inspire others with his determination, drive and energy until the battle is won and leprosy is no longer feared by people who live where the disease is still endemic.

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Dr R J W Rees CMG

A Retirement Tribute

The face of leprosy research has undergone a complete transformation from the grey days of the 1920's and 30's, when leprosy was of all diseases the most isolated from the main stream of medicine and of no appreciable interest to medical research workers as a whole. A small but highly dedicated company of leprosy specialists did, however, keep the flame of research alight, and British leprosy workers were prominent among them, fostered over many years by the British Leprosy Relief Association. Leonard Rogers, Ernest Muir, John Lowe, Gordon Ryrie and Robert Cochrane were all well-known pioneers in the specialty. Rogers' and Muir's *Leprosy* was the standard textbook for over 20 years from 1926. This group, in common with international colleagues from across the world were almost all of them first and foremost clinicians, as were their immediate successors. While able to bring highly sensitive powers of observation to clinical and pathological aspects of leprosy, they were in the very nature of things unable significantly to penetrate the fundamental mystery at the heart of the disease, namely the precise nature of the leprosy bacillus and its behaviour in the human body in relation to the body's defences.

By the 1950's things were changing. The science of microbiology was expanding rapidly. Cochrane's *Practical Textbook of Leprosy*, published in 1947, and still more, the first edition of his *Leprosy in Theory and Practice* published in 1959 presented leprosy in a manner entirely in line with current thinking in general medicine, so robbing the disease of its isolation and awakening the interest of specialists in other disciplines to the fascinating problems posed by almost every aspect of leprosy. It was at this opportune moment that the decision was taken to add a leprosy unit to the existing celebrated tuberculosis department at the National Institute for Medical Research at Mill Hill, London. Equal wisdom and foresight were displayed in the decision not to introduce a specialist leprologist from overseas, but instead to give responsibility to Dr Rees, a brilliant member of the scientific staff of the Institute since 1947; an expert in the microbiology and pathology of tuberculosis. Dr Rees was thus able to approach leprosy problems both with an entirely open mind and against a firm background of general medicine.

It quickly became apparent that a new star had arisen in the leprosy firmament, and one of first magnitude. Under Dr Rees' leadership the leprosy

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unit soon established a reputation of the highest order, and the reasons are not far to seek.

1 Based on the standards already attained in tuberculosis research, the leprosy unit was able to establish a level of technical excellence that immediately placed it in the top rank of leprosy research centres in the world. One cannot recall a single instance over the past 20 years in which a hasty judgement or technical inadequacy challenged the authority of the unit, which thus became an extremely important reference point for leprosy workers everywhere.

2 Dr Rees could easily have devoted his time to esoteric research of harmless academic interest. Instead he chose immediately to address himself to the intensely practical and difficult problems confronting field workers, which for decades had been evocative of diverse and sometimes contradictory judgements on important issues. He established a mouse colony and proceeded to confirm Shepard's astonishing findings on the limited but significant growth of *Mycobacterium leprae* in the mouse footpad. This was then carried further, using thymectomized and irradiated mice, in which he was able to observe a form of leprosy closely akin to human lepromatous leprosy, and several important points of pathological and immunological interest resulted.

With Dr D'Arcy Hart a method was devised for the accurate counting of *M. leprae*. Equally important, the morphology of living bacilli, as distinct from dead bacilli, was clearly established, settling an argument that had been going on for decades.

This firm bacteriological foundation opened up new levels of precision in chemotherapeutic research, and Dr Rees now turned his attention to this crucial subject. A Medical Research Council Field Research Unit was opened at Sungei Buloh in Malaysia in the charge of Dr Michael Waters; another at Addis Ababa under Dr John Pearson, and more recently, a third at Hyderabad in South India. A long series of highly important studies followed on the assessment of various types of chemotherapy in leprosy, studies which have had a great influence on current thinking on these subjects. Just at the time when Dr Rees began his leprosy studies, the first significant evidence began to arise of drug resistance to dapsone. His bacteriological work formed a reliable basis for the study of this ominous phenomenon and he applied himself to this with a vigour commensurate with its great importance, and with conspicuous success.

The early 1970's provided an opportunity for studies on the nasal discharge in leprosy, in which the writer was one of those able to work with Dr Rees from the uniquely placed independent field centre at Dichpalli, Central India. These shed important new light on the transmission of leprosy, all made possible by the unimpeachably high standard of the Mill Hill laboratory.

In 1969, Dr Rees had been officially appointed Head of the Laboratory for Leprosy and Mycobacterial Research at Mill Hill. By 1973 he was at the height of his authority as a research scientist in leprosy. The Centenary International Leprosy Congress was held at Bergen that year and Dr Rees was a central figure,

contributing to no less than 15 scientific papers, all of them important, a great tribute to his scientific acumen and energy.

Typical of his scientific alertness was his subsequent visit to the Gulf Southern Research Institute, USA to see for himself the work of Dr E Storrs on the nine-banded armadillo, a new experimental animal of potential importance, and to study its husbandry in captivity. The result was the establishment at Mill Hill of one of the first experimental armadillo colonies outside America.

3 Only passing reference is made here to Dr Rees' important contribution on the international level. For the past 20 years no congress or consultation on leprosy and related aspects of tropical medicine has been complete without his participation. In one international leprosy congress after another the microbiology section has owed a great deal to his sound judgement and up to the moment contribution. He has been an indispensable member of medical boards and the councils of organizations connected with leprosy, including the Expert Leprosy committees of the World Health Organization; member of Council of the International Leprosy Association; associate editor of the *International Journal of Leprosy*; chairman of the LEPRA Medical Advisory Board and vice-chairman of the editorial board of *Leprosy Review*.

4 Quite integral to this record of achievement is the personality of Dr Rees. It cannot have been an easy decision for him to accept a career in leprosy research. It was a career offering no open door to fame and fortune. Leprosy is too closely associated with the world's poor and rejected to offer lucrative prospects to anyone. The kind of person who enters this field and stays in it is one whose primary motivation is concern for the suffering of those who cannot help themselves, a concern which accepts some diminution in personal ambition and which thrives on colleagueship. It is characteristic of Dr Rees that in only a few of the numerous scientific papers that bear his name does he appear as the single author. He has consistently been the supporter and encourager of others, seeing most new discovery as essentially the result of team work, and though his personal contribution has been crucial he prefers to see his name in second or third place rather than first on the list of authors. It is part of his lifestyle not to seek the limelight, but to accept responsibility without hesitation.

This quality has been all important in maintaining the relevance of the Mill Hill unit to immediate issues. For leprosy workers across the world a visit to London was unthinkable without a visit to Dr Rees. The experience of the writer is surely typical, that a morning spent with Dick Rees was the most memorable and stimulating event of one's leave, always opening up some exciting possibility for future work on return to one's station overseas.

A succession of leprosy workers have been helped to gain precious experience in Dr Rees' department. One of the first was Dr B R Chatterji, observer of some growth of *M. leprae* in hybrid mice, and granted a Fellowship to Mill Hill where his work could be assessed against the impeccable laboratory conditions there.

So as Dr Rees comes to the formal point of retirement, a host of friends and

colleagues will want to unite both in expressing their admiration for all that he has been able to accomplish, and wishing him continuing happiness and fulfilment in the years ahead. It would be an irreparable loss if retirement was allowed to signal a withdrawal from the leprosy scene; rather let us anticipate that in a situation of greater relaxation, his penetrating mind and practical wisdom will continue to be addressed to the changing problems of leprosy, to advise and encourage leprologists everywhere. Certainly it can be said that as a result of his life's work, leprology, and indeed leprosy itself, will never be the same again. His friends across the world rejoiced when in 1980 he received the honour of Commander of the Order of St Michael and St George. Perhaps he himself took even greater pleasure in the award to him the same year of the Manson Medal of the Royal Society of Tropical Medicine and Hygiene, the Society's highest mark of distinction. In writing of Dr Rees' retirement, Professor Jagadisan referred to him as 'this outstanding medical scientist who is so eminently human and humane'. That just about says it all.

T F DAVEY

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Recent changes in leprosy control

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In recent years the limitations of the dapsone-based approach to leprosy control have become increasingly obvious. More recently, it has been found necessary to recommend multidrug regimens which, it is hoped, despite their relative complexity, should result in significant progress in the control of the disease.

This has coincided with an increasing interest in leprosy on the part of governments of many endemic countries. One result of this interest has been the development of concerted efforts in research on leprosy, particularly through the Scientific Working Groups on Immunology of Leprosy (IMMLEP) and Chemotherapy of Leprosy (THELEP),* aimed at the development of better tools for control. Also, many voluntary agencies have strengthened their collaboration with governments and WHO, and have substantially increased their contributions to leprosy activities.

On the whole, leprosy control is now at a turning point at which the secondary prevention strategy must undergo a change from the dapsone monotherapy approach to a multidrug therapy approach, and when research is opening up avenues for developing a primary prevention element to be added, hopefully, to the available armaments against the disease.

1 Magnitude of the problem

When expressed in terms of numbers, the leprosy problem does not at first sight seem very impressive. However, there are several factors which give the problem a far higher importance than that of mere statistics. The most important of these factors are the very large populations exposed to the risk of contracting leprosy, the chronicity of the disease, the progressive and permanent disabilities which

* The IMMLEP and THELEP Scientific Working Groups are components of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

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occur in a proportion of patients, and the social ostracism to which the patients and even their families are subjected.[1]

Three global surveys of the leprosy situation were made by WHO in 1966,[2] 1972[3] and 1976[4] respectively. Since the last survey, the Organization continues to make efforts to update figures. It is evident that these figures are in many cases approximate and give only a very rough idea of the real situation.

In the 1976 WHO global survey, as shown in Table 1, the total number of leprosy cases existing in the world was estimated to be 10,595,000. This figure does not differ significantly from the estimates derived from previous surveys, which were 10,876,000 and 10,407,200 in 1966 and 1972 respectively.

The estimated total of 10 to 11 million leprosy cases has only an indicative value. However, it is doubtful whether a higher estimate would better reflect the true situation, for reasons given in an earlier publication.[5]

Table 1. Estimated leprosy cases by WHO regions (1975)

WHO region	No. of cases
Africa	3,500,000
Americas	400,000
Eastern Mediterranean	160,000
Europe	25,000
South-East Asia	4,510,000
Western Pacific	2,000,000
Total	10,595,000

2 The classical strategy for leprosy control

2.1 ELEMENTS OF STRATEGY

In the 1950's, it was believed that a secondary prevention approach based on dapsone monotherapy of all infectious cases would, by reducing the reservoir of infection, result in the control of the disease. Leprosy control programmes were based on early case-finding, follow-up of contacts, and prolonged chemotherapy of patients by dapsone, both to limit transmission of the infection in the community and to prevent the disabilities that characteristically occur in various forms of leprosy. This strategy, which was recommended by WHO,[6–9] has been adopted during the last 30 years in virtually all endemic countries. Dapsone is safe and inexpensive. However, the treatment of paucibacillary cases requires 3–5 years and lepromatous patients must be treated for life, thus resulting in poor

patient compliance with self-administered treatment. Thus, in many countries, it proved operationally very difficult to mount and sustain field operations, including the two main elements of case-detection and prolonged chemotherapy, on a sufficiently wide scale to make a significant impact on the problem.

In view of the difficulties inherent in the secondary prevention strategy, attempts were made to add to the dapsone-based approach a primary prevention component, i.e. protection of the exposed individuals particularly by a vaccine. In the absence of a vaccine derived from *Mycobacterium leprae*, BCG was 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 other three studies showed that the overall protection was only moderate, ranging from 28% to 46%.[10] Therefore, BCG by itself cannot be a very effective tool against leprosy, and the need to develop a highly effective vaccine remains.

2.2 ACHIEVEMENTS

The present achievements summarized below are approximately those corresponding to the period during which the secondary prevention strategy for leprosy control based on dapsone monotherapy has been applied.

2.2.1 *Registered cases*

(a) *Present information*. When the latest figures of registered cases for each country available in the WHO leprosy unit (October 1982) are compiled they lead to the total by continent shown in Table 2. Thus, there are at present more than 5,300,000 leprosy patients registered in the world.

(b) *Increase in the number of registered cases*. Table 3 shows the increase in the number of registered cases worldwide compared to the first WHO global survey (1966). The total number of cases of leprosy known in the world has increased substantially. The increase is almost 2,500,000, i.e. 88%, between 1966 and 1982.

Table 4 shows the changes in the number of registered cases over the same period for each continent.

These figures reveal the following important points.

(a) In Africa the number of known cases is lower now than in 1966 by 406,860, i.e. a decrease of 23·7%. Such a decrease results partly from the release from control of a substantial number of patients.

(b) In Asia the increase in registration has been dramatic: 2,800,000 more cases on the registers in 1982 than in 1966 (306·8% increase). However, the increase took place mainly between the 1976 WHO survey and the 1982 compilation: 1,882,838 registered cases in 1976 and 3,724,400 in 1982. In fact, this

Table 2. Registered leprosy patients by continent (1982)

Continent	No. of countries surveyed	Estimated population (millions) around 1978*	No. of registered patients	Known case prevalence per 1,000
Africa	48	415	1,305,272	3·14
Americas	40	587	267,549	0·46
Asia (excl. USSR)	33	2,385	3,724,400	1·56
Europe	19	771	16,616	0·02
Oceania	15	22	13,509	0·61
Total	155	4,180	5,327,346	1·27

* *World Health Statistics, Annual 1981.* Geneva, 1981.

Table 3. Increase in registered leprosy cases in the world

	No. of countries or territories reporting	No. of registered cases	Increase when compared with 1966 WHO global survey
1966 WHO global survey	151	2,831,775	—
1972 WHO global survey	124	2,887,481	+55,706 (+1·96%)
1976 WHO global survey	148	3,598,167	+766,392 (+27·06%)
Latest available information in WHO in October 1982	155	5,327,346	+495,571 (+88·13%)

Table 4. Changes in number of registered leprosy cases by continent (from 1966 WHO global survey and 1982 WHO information)

Continent	Increase/decrease in number of registered cases and corresponding percentage	
Asia	+2,808,875	(+306·8%)
Africa	−406,860	(−23·7%)
Americas	+89,736	(+50·4%)
Europe	−8	(−0·05%)
Oceania	+3,828	(+39·5%)

increase results to a great extent from new registrations made in India during the same period: 1,320,000 registered cases in 1974 (1976 WHO global survey) and 2,800,000 in 1982,[11] and from the inclusion of 200,000 (an approximation) known cases[12] reported by The People's Republic of China, which had not been included in the 1976 WHO global survey.

2.2.2 *Treated cases*

The figures reported in the 1976 WHO global survey showed, for four WHO regions, average proportions of treated cases varying from 71% to 86%, and average proportions of regularly treated patients in three regions from 42% to 53%.

2.2.3 *Cases released from control*

The information on these is particularly scarce and inaccurate. Based on the 1976 WHO global survey, an attempt had been made to estimate the number of cases released from control annually. The estimate was of 120,000 to 150,000. If it is assumed that the release of patients from control has continued at the same level, it may tentatively be concluded that between 1 and $1\frac{1}{2}$ million leprosy patients have been released from control in the last decade.

Although the global impact of leprosy control has not been impressive, there are a few countries or areas where control activities were conducted in a well-organized and sustained manner over periods of 10 years or more and comparable evaluations have been possible. In such programmes reductions in prevalence of up to 80% or more were achieved, but a parallel decline in incidence was not observed.[5,13]

3 Recent changes

3.2 PROBLEMS RECENTLY ARISEN

3.2.1 *Resistance of* Mycobacterium leprae *to dapsone*

A major problem in leprosy in recent years has been resistance of *M. leprae* to dapsone. The first cases of *M. leprae* resistance to dapsone were proven by the mouse footpad method in Malaysia in 1964.[14] 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. The number of countries where dapsone resistance is prevalent is now probably more than 25 spread throughout the world, and the prevalence is steadily increasing in many countries.[15]

When lepromatous patients relapse with *M. leprae* secondarily resistant to dapsone, they can infect their contacts with these resistant bacilli, and those contacts who subsequently develop clinical leprosy will have primary resistant disease. Thus, primary resistant leprosy can occur in any form of the disease. Primary resistance to dapsone was proven for the first time in 1977, and subsequent studies show that its prevalence appears to be increasing at a faster pace than that of secondary resistance.[15]

3.2.2 *Persisting* M. leprae

In 1974 it was demonstrated that microbial persistence, a feature of tuberculosis and other infectious diseases, is also a feature of lepromatous leprosy.[16]

3.3 RECENT CHANGES IN CHEMOTHERAPY OF LEPROSY

In recent years the introduction of a few new drugs, and particularly rifampicin, has improved the prospects for better treatment. Nevertheless, the high cost and comparatively greater toxicity of newer drugs have limited their wide application in the field. The main drugs with bactericidal activity against *M. leprae* are rifampicin (high bactericidal activity), ethionamide and protionamide (intermediate bactericidal activity), dapsone and clofazimine (both with low bactericidal activity).

Secondary resistance of *M. leprae* has, up to now, been reported[17,18] in 7 patients treated for approximately 4 years with rifampicin monotherapy and in a small number of patients who had received 5 years of ethionamide monotherapy.[19] As for clofazimine, despite its widespread use, only 1 proved case of resistance has as yet been reported.[20]

Microbial persistence has been observed in patients treated with rifampicin for 5 years,[21] and with clofazimine for 10 years.[22]

As early as 1976, in view of the problem of secondary resistance of *M. leprae* to dapsone, the WHO Expert Committee on Leprosy had recommended that all active cases of multibacillary leprosy be treated with at least two effective antileprosy drugs, including rifampicin.[23] However, relatively few countries and individual centres have introduced multidrug therapy as a routine practice in their leprosy control programmes.

Because of the growing threat resulting from the increase of secondary and primary resistance to dapsone, as well as of the need to prevent resistance to other drugs, a WHO Study Group on Chemotherapy of Leprosy for Control Programmes was convened in October 1981,[15] in order to define effective chemotherapeutic regimens that were practicable under field conditions. The Study Group proposed standard regimens for all categories of patients in two groups: multibacillary and paucibacillary. The regimens are based on the supervised administration of monthly doses of rifampicin. In multibacillary

patients the treatment also includes a supervised monthly dose of clofazimine and self-administered daily clofazimine and dapsone. This regimen is to be given for at least 2 years. In paucibacillary patients monthly rifampicin and daily self-administered dapsone are to be given for 6 months.

The Study Group also recognized that so far no drug alone appears to be capable of eliminating persisting *M. leprae*. The possibility that combinations of drugs may be able to eliminate such persisting organisms is currently under investigation in THELEP-controlled field trials.

To prevent the further increase of dapsone resistance and the emergence of resistance to other drugs, the Study Group recommendations should be implemented as soon, as widely and as accurately as possible. If combinations of bactericidal drugs *appropriately designed* according to the Study Group recommendations are not put into practice: (a) the problem of secondary and primary resistance to dapsone will continue to increase, and (b) resistance to other drugs will emerge and spread.

As a result, until new drugs become available (which will require at least another decade) the leprosy problem will become unmanageable.

In view of the seriousness of the situation, WHO is giving top priority to the implementation of these Study Group recommendations.

It is obvious that no improvement in the efficacy of therapeutic regimens can increase the efficiency of leprosy control if operational aspects are not improved at the same time. The Study Group reviewed the relevant problems related to case-detection, drug delivery and case-holding. The most urgent needs are related to three areas: (a) adequate additional training of all categories of personnel involved in leprosy control, (b) reorganization of leprosy control activities, and (c) mobilization of additional financial resources.

4 Other needs and prospects

4.1 NEED FOR IMMUNOPROPHYLACTIC METHODS

Present and future treatment methods may be able to solve the problems of resistance to dapsone and other antileprosy drugs, and the problem of persisters, but the need for arduous case-finding and case-holding activities would remain. Adequately trained personnel, sufficient financial resources and good logistics would still have to be provided. Therefore, the development of a tool for primary prevention, i.e. a vaccine of good protective value, would be an invaluable asset.

Such a vaccine may even be a *sine qua non* for effective leprosy control. Even after potent drug regimens have been put into use, one fears that their epidemiological impact will not be as great as one would wish. In those programmes based on dapsone monotherapy and conducted under the best possible conditions, there was a substantial reduction in prevalence but little

decrease in incidence, suggesting that before infectious cases receive treatment they have already spread infection with *Mycobacterium leprae* among a large proportion of their contacts. It seems likely that the use of more potent chemotherapeutic methods, even if it reduces the infectious period of lepromatous cases by a few months as compared to dapsone monotherapy, would not greatly affect the spread of *M. leprae* in the community. However, such a hypothesis remains to be investigated.

If it were only to overcome the difficulties related to rigorous case-finding and case-holding, we would still need an effective vaccine against leprosy. This is the main objective of the IMMLEP programme, and work on such a vaccine is progressing satisfactorily. We believe that it may be possible to launch field trials for the IMMLEP vaccine in the next few years and have results after about 10 years.

4.2 NEED FOR IMPROVED DIAGNOSTIC TOOLS

In view of what has just been discussed, a test to identify individuals incubating lepromatous leprosy would be of great value. Some of the tests currently being developed by the IMMLEP SWG may meet this need. Such individuals could then be put under close surveillance or even given prophylactic treatment. They could also benefit from the immunological conversion resulting from the method recently proposed by Convit.

4.3 IMMUNOTHERAPY

Recently, Convit[24] reported that in leprosy patients consistently Mitsuda-negative, the repeated inoculation of a mixture of heat-killed *M. leprae* and living BCG results in clinical, histopathological and immunological changes towards the tuberculoid end of the spectrum. If these results are confirmed, this would be a significant contribution to the treatment of infectious cases and hence to the secondary prevention of the disease.

Conclusion

The implementation of the secondary prevention strategy for leprosy control based on dapsone monotherapy had to face many difficulties. The main global results obtained during the period of the dapsone monotherapy approach may be summarized as follows:

(a) *Worldwide*. More than 5 million leprosy cases out of an estimated total of about 10 million existing cases are now under treatment. It can be estimated that from 1 to $1\frac{1}{2}$ million leprosy patients have been released from control during the last decade.

(b) Under favourable circumstances reductions of prevalence of 80% were achieved in a few countries or areas.

The shortcomings of dapsone monotherapy have been increasingly realized over the last 15 years. A new approach to secondary prevention of leprosy through multidrug therapy of all cases has been recently recommended by WHO. The contribution of the THELEP Scientific Working Group to the development of the newly recommended regimens has been essential, clearly demonstrating transfer of the results of research to control efforts. For the first time the results of worldwide research efforts, stimulated and coordinated at the global level, have been translated into important changes in the strategy for leprosy control.

However, in the long term, primary prevention methods, the most important being an effective vaccine, are an essential need in an effective strategy for leprosy control. In addition, immunological tools which would allow the identification of individuals at high risk of developing lepromatous leprosy will be of great help.

In any case, it is unlikely that conclusions on the efficacy of a vaccine will be available within the next decade, or that new potent drugs can be developed.

Therefore, for the years to come, and despite the shortcomings and limitations of the secondary prevention approach, the implementation of effective chemotherapeutic regimens based on combinations of bactericidal drugs is a must if we do not want the leprosy problem to become unmanageable and the gains made so far to be lost. Consequently, in the WHO leprosy programme for the next quinquennium top priority has been given to the implementation of multidrug therapy.

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Bacteriology of *Mycobacterium leprae*

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Summary A review is presented on the morphologic features, chemical characteristics, growth capacity, drug sensitivity, metabolic activity and antigen structure of *Mycobacterium leprae*. Since the availability of large numbers of *M. leprae* from armadillo tissue, knowledge, particularly on the chemical characteristics has increased. It may be expected that knowledge on the metabolic activities will increase, leading perhaps, one day, to the long-awaited *in vitro* cultivation of the organism.

Morphology

Classically *Mycobacterium leprae* is stained by the Ziehl–Neelsen method, and shows up as an acid–alcohol-fast (AAF) rod 0·3–0·4 μm × 2–7 μm. Metachromatic granules may be observed. The molecular basis for the acid–alcohol fastness has not been elucidated although it is generally believed to be related to the cell wall composition, particularly the mycolic acids. Nocardia containing nocardomycolic instead of mycolic acids, differing mainly from mycolic acids by a shorter carbon chain length, are not AAF, although this difference is not absolute: some individual cells of nocardiae may appear acid-fast, while some mycobacterial species, particularly the rapidly growing ones, may contain a fraction of non-acid-fast organisms.

It has been claimed[1] that treatment of *M. leprae* containing preparations with periodic acid renders more organisms stainable by an acid-fast staining technique. It has been shown[2] that such organisms are present in human biopsy material but that the failure of staining by the standard acid-fast technique is a characteristic of dead *M. leprae* unable to infect the mouse footpad, whereas organisms recovered from mouse footpads during their logarithmic multiplication, stain equally well by the standard technique as after periodate treatment.

It was claimed[3,4] that treatment of *M. leprae* containing smears with pyridine rendered them non-acid-fast while all other mycobacteria tested are 'pyridine-fast'. There has been some controversy on this point[5,6] but it seems now that the

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reaction is valid if highly purified pyridine is used as shown by McCormick and Sanchez.[7]

Most *M. leprae* organisms are also granular. It has long been thought that these irregularly staining bacteria are degenerate, dead forms, particularly since during the later phases of treatment, in patients who are approaching bacteriological negativity, only AAF 'dust' can be found. Rees and co-workers[8, 9] showed in an elegant series of experiments that this is indeed the case and this has been amply confirmed by use of the mouse footpad technique: granular, 'non-solid' forms of *M. leprae* are degenerate, unviable.[10] This has led to the notion of the morphologic index.[11] However, determination of the morphologic index requires carefully standardized fixation and staining techniques, the use of a perfectly regulated microscope and very careful examination of 100–200 individual bacteria, a practice only attainable in reference laboratories and not realizable in field conditions.

In thin sections *M. leprae* has a cell wall 15–20 nm thick consisting of two electron-dense layers and an outer electron-transparent layer. Mesosomes continuous with the cytoplasmic membrane are sometimes numerous, but their function and meaning is unknown, they might even be artefacts.[12]

In electron microscopy the walls of *M. leprae* may show band-like structures parallel to the short axis of the cell[13] and 'paired fibrous structures'.[14] Electron microscopic examination of tissues infected with *M. leprae* has established the presence of an electron-transparent zone surrounding the bacilli[15–17] which may be considered as a capsule of *M. leprae*, composed of a specific mycoside.[18, 19]

Chemical structure of cell walls

The wall of *Mycobacterium leprae* consists of peptidoglycan[20] to which are attached arabinogalactan (polysaccharide) chains bearing mycolic acids. Associated lipids are phosphatidyl-inositol-mannosides (PIM), attenuation indicator lipid (AIL), phtioldimycocerosate (DIM) and mycoside.

The cross-linking tetrapeptides in the peptidoglycan are unique: only half of the usual amount of alanine is present, and this is present as the D-enantiomer, while equimolar amounts of glycine are present, suggesting that the L-alanine in the *M. leprae* peptidoglycan tetrapeptide is replaced by glycine. This structure is rare and is found in some plant pathogenic corynebacteria.[12] The mycolic acids of *M. leprae* are also different from those of other mycobacteria in that they contain only α- and keto-mycolates[21, 22] whereas other mycobacteria have 3 mycolates.

Phosphatidyl-inositol-mannosides (PIM) which occur in all corynebacteria, nocardiae and mycobacteria have also been found in *M. leprae*[23] as well as DIM, phtioldimycocerate and AIL, attenuation indicator lipid, a methylated-phenol-

phtiocerol-dimicocerosate, originally found in attenuated strains of *M. tuberculosis*.[23, 24]

Finally, the mycoside of *M. leprae* is closely related to mycoside A from *M. kasasii*, but contains a different and unique trisaccharide composed of 3,6-di-O-methylglucose, 3-O-methylrhamnose (both not previously found in nature) and 3,6-di-O-methylglucose.[19] Large quantities of the glycolipid are also present in infected armadillo liver residue freed of *M. leprae*, suggesting that it may correspond to the electron-transparent zone surrounding the organism in infected tissue.[19] All in all many particularities of the chemical structure of *M. leprae* have now been revealed. They should be most useful for the identification of claims concerning *in vitro* grown strains of *M. leprae*.

Recently, the first results on DNA studies of *M. leprae* were published.[25] The genome size was $1{\cdot}1–3{\cdot}2 \times 10^9$, the G + C content ranged from 55·6 to 71·5 mol%. Homology of *M. leprae* DNA was higher with a strain of corynebacteria (68·4%) than with mycobacteria of which *M. tuberculosis* and *M. scrofulaceum* showed the highest homology: 51·8% and 57·9% respectively. Clearly, these results await confirmation.

Growth

Mycobacterium leprae multiplies in the cooler parts of the body of mice, rats and hamsters: footpads, ears and testicles, with a generation time during the logarithmic phase of 12–14 days.[26] The minimal infective dose in the mouse footpad (MFP) is 1–3 organisms[10] and growth stops when $\pm 10^6$ bacilli are present. Rees[27] has shown in the very early years of the MFP era that this limitation was of an immunologic nature, and introduced the immunosuppressed mouse (thymectomized and irradiated). In these animals multiplication of *M. leprae* continues beyond the 10^6 ceiling in the MFP and gives rise to generalized infection with all the characteristcs of lepromatous leprosy in man.[27, 28] The same occurs more rapidly after intravenous injection. Later other immunosuppressed rodents were introduced: the newborn thymectomized Lewis rat,[29] the athymic mouse[30, 31] and the athymic rat (Colston, unpublished).

Immunosuppressed rodents have been mainly used to detect small numbers of viable *M. leprae* among large numbers of dead bacilli in patients u
so called persisters, because in these animals large numbers of even dead bacilli do not lead to an immune response, arresting the growth of a small inoculum of viable *M. leprae*. (Recently it has been shown that normal mice can also serve this purpose.)[32] Kirschheimer & Storrs[33] found that a high proportion of nine-banded armadillos (*Dasypus novemcinctus*) is sensitive for *Mycobacterium leprae* and develop a generalized infection, particularly when intravenously injected. Klingmüller & Sobich[34] found that this is also the case in the hedgehog (*Erinaceus europeus*) as confirmed by McDougall *et al.*[35]

The availability of large amounts of *Mycobacterium leprae* from armadillos has allowed all the chemical analyses referred to above and opened perspectives for the development of an antileprosy vaccine.

Drug sensitivity

Strains of *Mycobacterium leprae* from previously untreated patients, isolated during the 1960's, were all very sensitive to dapsone: 0·0001% in the diet producing serum concentrations of 0·01–0·03 g/l.[36] This situation is now much changed due to the widespread and increasing occurrence of secondary and primary dapsone resistance.

Minimal inhibitory concentrations of other drugs for wild strains of *M. leprae* have been determined, the pattern of which is characteristic for *M. leprae*.[37]

Metabolic activities

There has been much debate about the metabolic activity of *Mycobacterium leprae* recovered from human biopsies. Now that greater amounts of bacteria can be purified from infected armadillo livers, it may be expected that considerable progress will be made in this field in the near future. Prabhakaran *et al.*[38] detected a dopa (3,4-dihydroxyphenylalanine) oxidase in *M. leprae*, confirmed by autoradiographic studies.[39] Wheeler & Gregory[40] could not detect catalase in *M. leprae*, but a low activity peroxidase and a superoxide dismutase (more easily detected at pH 7·8 than at pH 10).

Antigenic structure

Immunodiffusion studies did not show any specific antigens in *Mycobacterium leprae*.[41] Antigens detected by crossed immunoelectrophoresis cross-react with other mycobacteria, with a particular situation for antigen 21 related to *M. tuberculosis*.[42] However, mycoside A from *M. leprae* has recently been shown to be a suitable antigen for taxonomic and perhaps serological studies.[18, 19, 43]

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Recent studies of antileprosy drugs

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Summary As a part of the programme of the Therapy of Leprosy (THELEP) Scientific Working Group, a number of compounds with potential activity against *Mycobacterium leprae* were prepared in other laboratories. We report here the results of studies of their activity against *M. leprae* with the use of the kinetic method in mice. A modified protocol is described that facilitates comparison of drugs in the same experiment. Two analogues of cycloserine, glycylhydroxamic acid and beta-analylhydroxamic acid were inactive in a dosage of 0·1% in the diet. Isoetam, (D-2,2′-(ethylendiimino)-di-l-butanol)di-isoniazid methane sulphonate was also inactive at this dosage. Three compounds related to dapsone, 4-nitro-N′-phenylsulphonamide, 4-amino-N′-phenylsulphonamide, and 4,4′-diaminobenzene sulphonic acid phenyl ester, had little or no activity at dosages of 0·01% in the diet in experiments with strains shown to have normal susceptibility to dapsone. Two thiosemicarbazones, pyridinal-4-thiosemicarbazone and pyridinal-2-thiosemicarbazone, were inactive in dosages of 0·01%; the latter was inactive at 0·01% in an experiment where thiacetazone was shown to have bactericidal-type activity at a dosage of 0·1% and marginal activity at 0·01%. Brodimoprim, a dihydrofolate reductase inhibitor, which is related to trimethoprim but has a longer half-life, was inactive in a dosage of 0·1%; it had no synergistic effect with 0·01% dapsone against a dapsone-susceptible strain. It was also inactive against a dapsone-resistant strain, alone or in combination with dapsone. The cyanimino analogs of ethionamide and prothionamide were inactive in a dosage of 0·1% against an ethionamide-susceptible strain. Experiments with a series of compounds related to chaulmoogric acid were unsuccessful because the compounds were too toxic. Experiments with a series of compounds related to clofazimine were unsuccessful because their pharmacokinetics were unfavourable for study at dosages where clofazimine itself was active. The limitations imposed by the mouse-foot-pad system are discussed and related to those in other experimental systems.

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Introduction

In collaboration with the Therapy of Leprosy (THELEP) Scientific Working Group, we have examined a number of new drugs for activity against *Mycobacterium leprae* in mice in the last several years. The drugs were designed and synthesized by the members of the Scientific Working Group named below. The meetings of the Scientific Working Group were a vital part of the development of these new drugs, in that they provided a focus for scientific attention and a forum for discussion that extended far beyond any one individual's competence, from drug design and synthesis to pharmacokinetics, microbiology and application to leprosy field situations. Some of the results of drug testing were published elsewhere in a collaborative study (Shepard *et al.*, in preparation). We present here the remainder of the results to date. Although little activity was observed with the new drugs, the results can be helpful to others interested in the design of new antileprosy drugs.

Materials and methods

We used a recently described modification of the kinetic method[1] and slightly modified it further (see below). In brief, CFW female mice, 6–12 weeks old, were infected in a rear foot pad with 5×10^3 *M. leprae* of a 'fast' strain[2] in mouse passage. The strains used were isolated from untreated patients before 1965, that is, before the recent increase in primary dapsone (DDS) resistance in several parts of the world, and most had already been shown to be susceptible to 0·0001% DDS in the diet. The growth curve of *M. leprae* in the various groups was monitored by harvests of the infected foot pad tissues and microscopic counts of acid-fast bacteria (AFB) in the tissue suspensions. Each count was carried out on a pool of four mice per group, and there were four (untreated) control groups. The harvests were carried out at approximately 28-day intervals during the logarithmic growth phase of the controls (roughly 98–182 days, when the average count rose from $10^{4·3}$ to $10^{6·0}$ AFB/mouse), and at 56-day intervals thereafter. Harvests were discontinued in treated groups when they reached plateau levels (usually $10^{6·0}$ to $10^{6·2}$ AFB/mouse).

The statistical significance of the difference between groups was estimated as described[1] or by a slight modification. In the described procedure, comparisons between a treated group and the control groups are taken care of automatically by the protocol, and in general growth delays as little as 25 days are detectable ($P < 0·05$). Based on a generation time of 12·5 days, a value that fits our results well, 25 days of growth delay corresponds to a loss in numbers of viable *M. leprae* of 75%. A disadvantage is that when comparisons of treated groups are important, adjustment of the protocol is usually needed to increase the number of counts from the particular treated groups. This adjustment has proved difficult to

accomplish sometimes while maintaining the 28-day counting interval and carrying out other laboratory activities. Consequently, when comparisons of treated groups are important, a modified protocol has been used in which counts are carried out on four individual mice from each group. The significance of the differences between groups at a particular time is estimated by the two-sample rank test (Mann-Whitney test) with the use of tables giving the exact probability value, for example Table J in Siegel.[3] The period selected for comparison is that when one, or both, of the groups being compared are in the logarithmic phase. As in previous studies[1] the *P* values from each successive period are multiplied to give the final *P* value for the differences between groups.

Results

GLYCYLHYDROXAMIC ACID AND BETA-ANALYLHYDROXAMIC ACID

These compounds, which are sterically related to cycloserine, were prepared by J B Hines, University of South Carolina. Their antimycobacterial properties have been described.[4,5] At a dosage of 0·1% in the diet, they were inactive against *M. leprae* (Table 1).

ISOETAM

D-2,2′-(ethylendiimino)-di-l-butanol)-di-isoniazid methane sulphonate, is a drug that has been used in the treatment of patients with tuberculosis. It was received from J Colome and J Castillo, Ferrer Internacional, Barcelona, Spain. At a dosage of 0·1% in the diet, it was not active against *M. leprae* (Table 1).

SULPHONE-RELATED COMPOUNDS

Three compounds (4-nitro-N′-phenylsulphonamide or SAL1, 4-amino-N′-phenylsulphonamide or SAL2, and 4,4′-diaminobenzene sulphonic acid phenyl ester or SAL3) were prepared by J K Seydel, Forschungsinstitutborstel, Borstel, West Germany.[6] He had found them active *in vitro* against certain cultivable mycobacteria at about the same levels as DDS. Accordingly, in experiment M1-23-79 we tested SAL1 and SAL2 against *M. leprae* at dosages near the minimum effective dosage (MED) of DDS. DDS, at 0·0001% in the diet, had the expected activity, but SAL1 and SAL2 were inactive even at 0·001% in the diet. In a second experiment, M6-30-80B, the drugs were tested at 0·01%, a dosage of DDS that produces levels of DDS in the plasma and tissues that are about the same as those produced in man by standard daily dosages. SAL2 had a minimal amount of activity with an estimated 29 days of growth delay, But SAL1 and SAL3 were inactive. DDS again had the expected amount of activity; it

Table 1. Results of studies of several compounds for their activity against *M. leprae* by the kinetic method[a].

Experiment and drug	Dosage (%)	Time given (days)	Growth delay (days)	Probability value[b]
M 10-30-78[c]				
GHA	0·1	70–126	0	
BHA	0·1	70–126	0	
Isoetam	0·1	70–126	0	
M 1-23-79[c]				
SAL1	0·001	70–126	0	
,,	0·0001	70–126	0	
,,	0·00001	70–126	0	
SAL2	0·001	70–126	8	NS
,,	0·0001	70–126	4	NS
,,	0·00001	70–126	0	
DDS	0·0001	70–126	74	< 0·02
,,	0·00001	70–126	14	NS
TSCL1	0·01	70–126	28	< 0·06
TB1	0·01	70–126	0	
M 6-30-80[c]				
DDS	0·01	70–126	> 114	< 0·0016
SAL1	0·01	70–126	26	NS
SAL2	0·01	70–126	29	< 0·008
SAL3	0·01	70–126	4	NS
TSCL2	0·01	70–126	0	
TSCL2	0·1	70–126	0	
TB1	0·01	70–126	17	< 0·04
,,	0·1	70–126	> 100	> 0·0016
M 11-18-81[d]				
DDS	0·01	70–98	87	< 0·001
BDP	0·1	70–98	18	< 0·017
DDS(0·01%) + BPP(0·1%)		70–98	54	< 0·002
M 2-10-82[d]				
ETH	0·1	70–126	> 56	< 0·024
DPS28	0·1	70–126	5	NS
DPS29	0·1	70–126	0	NS
M 5-21-82[d, e]				
DDS	0·01	70–98	14	NS
BDP	0·1	70–98	6	NS
DDS(0·01%) + BDP(0·1%)		70–98	15	NS

[a] *Abbreviations:* GHSA, glycylhydroxamic acid; BHA, beta-analylhydroxamic acid; SAL1, 4-nitro-N′-phenylsulphonamide; SAL2, 4-amino-N′-phenyl-sulphonamide; DDS, dapsone; TSCL1, pyridinal-4-thiosemicarbazone; TB1, thiacetazone; SAL3, 4,4′-diaminobenzenesulphonic acid phenyl ester; TSCL2, pyridinal-2-thiosemicarbazone; BDP, brodimoprim; ETH, ethionamide; DPS28, cyanimino analog of prothionamide; DPS29, cyanimino analog of ethionamide.

[b] For difference from untreated control.

[c] Protocol as described in 1.

[d] Modified protocol. See text.

[e] Tested against a dapsone-resistant strain of *M. leprae*.

manifested bactericidal-type activity with >114 days of growth delay, well in excess of the 56 days of drug administration. (Pure bacteriostasis contemporaneous with the period of drug administration would have produced 56 days of growth delay.)

THIOSEMICARBAZONES

Two compounds, pyridinal-4-semicarbazone (TSCL1) and pyridinal-2-semicarbazone (TSCL2), were received from Dr Seydel. In these compounds the acetylaminobenzene portion is replaced by a pyridine group. TSCL1 had questionable activity in one experiment at a dosage of 0·01%, and TSCL2 had no activity in another experiment at dosages of 0·01% and 0·1%. In the former experiment thiacetazone (TB1) was inactive at 0·01%; in the latter, 17 days of growth delay was observed with a dosage of 0·01% and >100 days with 0·1%. By the continuous method of drug administration, TB1 has previously been found to be partially active in a dosage of 0·01% against most strains of *M. leprae* and completely active in a dosage of 0·1%.[7,8]

BRODIMOPRIM

This drug, a 2,4-diaminopyrimidine, is a dihydrofolate reductase inhibitor. For some years now, one has hoped to find a dihydrofolate reductase inhibitor that will be highly active in combination with dapsone, as a result of sequential blockade in the folate biosynthetic pathway. It differs from trimethoprim in that the central methoxy group is replaced by a bromine, and it has a longer half life. Trimethoprim, in a dosage of 0·1%, has previously been found to be inactive against *M. leprae* when administered alone, and it had no additive effect when administered with dapsone.[7] In our studies, 0·1% brodimoprim was inactive alone, and it did not increase the effect of dapsone (Table 1). Against a DDS-resistant strain it had no effect alone or in combination with dapsone (experiment M 5-21-82).

CYANIMINO ANALOGS OF PROTHIONAMIDE AND ETHIONAMIDE

Study of these compounds was suggested by Michael Tute, Pfizer Central Research, Sandwich, Kent, United Kingdom, and the compounds were synthesized by M Hooper and D P Self, Sunderland Polytechnic, Sunderland, United Kingdom (under a WHO project grant 790490). The precedent was the replacement of the thione group of metiamide by a cyanoimino group to yield cimetidine, a potent H_2 antagonist with low incidence of side-effects. Thus, the $-CSNH_2$ group of prothionamide and ethionamide was replaced with a $-CNCNNH_2$ group. DPS 28 and DPS 29, however, were found inactive in a concentration of 100 μg/ml against a strain of *M. tuberculosis* that was susceptible

to 1·2 μg prothionamide/ml and 0·6 μg ethionamide/ml. Table 1 shows that the compounds were also inactive against *M. leprae*.

OTHER COMPOUNDS

In this same period, three inconclusive experiments were also carried out (results not shown). In one, a series of new clofazimine-related compounds were tested along with clofazimine controls. Although the clofazimine controls had the expected growth delay, the new compounds were inactive. Although demonstrating good activity *in vitro* the new compounds had basic substitutions that increased their solubilities and altered their distributions in the body, as evidenced by lack of pigmentation of the fatty tissues. Since the compounds were all administered in dosages of 0·001% and 0·0001%, that is, at dosages near the MED of clofazimine, the inactivity of the new compounds could have arisen from altered pharmacokinetics.

Two inconclusive experiments were carried out with chaulmoogric acid and several related compounds. The compounds were administered in propylene glycol by injection. In one experiment they were given intraperitoneally 5 days a week in a dosage of 0·6 mg, but this proved too toxic, and after 15 days, the dose was lowered to 0·2 mg. Deaths continued, however, and the dead mice had a knot of intestine that was tied together with peritoneal adhesions. Two few mice remained alive to allow reliable conclusions. In the other experiment the drugs were given subcutaneously in a dose of 2 mg three times a week. The mice developed subcutaneous lesions and many died. In both experiments only the mice receiving the vehicle remained well.

Discussion

Now, after 23 years of use, the mouse foot pad remains the most convenient and reliable laboratory system for the study of drugs for activity against *M. leprae*. Certain strains of rats have been proposed rather than mice on the basis that their metabolic disposition of dapsone is more similar to that of man,[3] but this feature would be helpful only for particular purposes. Nine-banded armadillos have been proposed on the basis that they develop a disease similar to that of human lepromatous disease and that their uniovular quadruplets provide a set of genetically uniform animals. The mouse is much the more convenient, however, especially in view of the fact that the statistical requirement for numbers of animals per group does not decrease when the experimental animal is large or inconvenient to work with. The mechanism of action of nearly all antimicrobials is against some particular metabolic feature that is characteristic of the microorganisms and not the host. Fortunately, the mouse foot pad provides an environment in which the *M. leprae* can be exposed to a drug during the equivalent of at least six generations (75 days) of logarithmic bacillary growth in

the controls. In this sense, at the present the mouse is providing the most convenient substitute for a favourable artificial medium. Of course, genetic homogeneity can be provided by inbred lines of mice in very large numbers.

Compared with potential *in vitro* systems, however, the disadvantages in the use of the mouse are distinct. It is true that analogs of a compound known to be active in low dosages can be compared with the known compound at its minimal effective dosage. Thus it is possible to test analogs of dapsone or rifampicin when only 10–20 mg is available if the pharmacokinetics of the analog is similar to that of the parent compound. Nevertheless, the first screening of a new compound usually requires 10–15 g of drug (usually the maximum tolerated dosage, often about 0·1% in the diet or 10 mg/day by injection, is administered for about 2 months). With many antimicrobials, these dosages provide plasma and tissue concentrations of 10–30 μg/ml. Thus *in vitro* procedures would be needed to screen entirely new drugs at this level if they are available in only milligram amounts. Unfortunately, reliable methods for the cultivation of *M. leprae in vitro* through even one generation of growth do not appear to be available yet.

Other disadvantages of an *in vivo* model were displayed in this work. The drug to be tested may be too toxic or the pharmacokinetics (absorption, distribution, biotransformation, excretion) may be unfavourable. It might be argued that these unsuitable properties of a compound would prevent its use in man anyhow. Toxicity and unsuitable pharmacokinetics, however, may be a function of a part of the molecule that is distinct from that responsible for its antibacterial activity, and it may be possible to design active congeners with more suitable properties if an adequate degree of antibacterial activity is present.

In the case of the compounds related to clofazimine that we tested, the altered pharmacokinetics were revealed rather simply by their failure to pigment the body fat. Changes had been expected because the compounds had a basic substitution and greater solubility in water. In the case of the new compounds listed in Table 1, they were analogs of known compounds, and it is unlikely that they had unsuitable pharmacokinetics. If necessary, firm evidence that the compound is reaching the site of the microorganism can be provided by the development and application of a procedure for the specific determination of the active compound in body fluids, in a manner similar to that used for the study of thioamides against *M. leprae* in mice (Shepard *et al*, in preparation).

When one is studying drugs that have already been shown to be active in mice against other infectious agents, toxicity and unsuitable pharmacokinetics are much less likely to interfere. For example, in a current experiment we are studying a series of beta-lactam antibiotics; they have already been shown to be active in animals and man against other bacteria.

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Immunology of human leprosy—current status

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It gives me great pleasure to contribute to a volume honouring Dr RJW Rees. Just over a decade ago, as a Nuffield Fellow, I first met him in an art gallery, at a party hosted by the British Society of Immunology. Over the years, like many others, I worked with him and learned a great deal about leprosy as well as human values. His ability to cross national borders and interact with leprosy workers of diverse interests and motivations has been invaluable in developing constructive group activity. His immediate response to a frantic call for reagents/chemicals has made our personal research work possible. My colleagues and I wish him many years of continued productive work in leprosy.

Introduction

In the last decade leprosy has proved to be an intriguing challenge to immunologists. Though absolute proof regarding the non-variability in the pathogenicity of *Mycobacterium leprae* is not available, it is generally conceded that the spectrum of clinical types seen in leprosy is due to variation in host immunity. Prolonged and sustained antigenic stimulation with leprosy bacillus leads to a complex array of immunological responses. The end result is reflected as a wide spectrum of immunopathological aberrations. Both humoral and cellular responses are elicited and immune-complexes have been seen in the circulation of leprosy patients. Since *M. leprae* is located intracellularly, cell-mediated responses may be more important for its elimination. Effective immunity and high resistance appear to be linked to T-cell functions. The mechanisms responsible for the non-responsiveness, particularly of the antigen-specific type, are being gradually unravelled. In recent years progress has been made in relation to HLA-linked genes, cells mediating suppression, antigenic components of *M. leprae* and possible tools for the diagnosis of subclinical infection.

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Ridley & Jopling[1] introduced a classification of clinical leprosy based on the concept that it was a five-point spectral disease. Tuberculoid individuals of polar (TT) and borderline (BT) type have paucibacillary, localized self-limiting disease whereas the patients with lepromatous leprosy (polar LL and borderline BL) have disseminated multibacillary disease which requires long-term chemotherapy. The borderline forms of this disease (BT, BB and BL) are unstable in their immunological responsiveness and prone to reactions. Most of the studies described in this review have used the above classification. The present review is limited to the progress made in the last 5 years in the understanding of the immunological aspects of leprosy. The reader is referred to earlier excellent reviews for investigations prior to this time,[2,3] and other articles in this volume pertaining to reactions and immunoprophylaxis.

Nature of the causative organism

SPECIES-SPECIFIC ANTIGENS OF *Mycobacterium leprae*

The identity of species-specific antigens of *M. leprae* with biological activity has received impetus since the availability of infected armadillo tissues, containing abundant bacilli. Comparison of armadillo and human-derived *M. leprae* showed similarity in delayed hypersensitivity tests[4] and in lymphocyte transformation tests in leprosy patients.[5] The immunogenicity and heat stability of the armadillo-derived bacilli was also established.[6] That some antigens were lost due to treatment with alkali and pronase during purification of bacilli from liver tissue led to the adaptation of improved procedures.[7,8] In most studies the biological relevance of these antigens has been established by demonstrating the presence of antibodies directed against these components in leprosy sera.

Earlier studies[9] using indirect immunofluorescence have been confirmed[10] and indicated that antibodies recognizing *M. leprae* specific antigens were present in patients across the leprosy spectrum. A search for protein antigens with sensitive techniques has revealed both cross-reacting and species-specific components in *M. leprae*. Lepromatous sera as well as hyperimmune sera from immunized rabbits showed 7 distinct bands which cross-reacted with other mycobacteria.[11,12] Subsequently it was shown that antigen 7, which was equivalent to antigen 60 of BCG, was recognized by the patients' sera. Moreover, acetone-killed *M. leprae* from the armadillo tissues was shown to have a protein antigen which was resistant to heat and pronase treatment.[11] The application of hybridoma technology is expected to produce monoclonal antibodies which may be helpful in dissecting the various antigenic components of *M. leprae*. Gillis & Buchanan[13] reported the production of 11 monoclonal antibodies which reacted against *M. leprae* and 18 other mycobacteria as assessed by ELISA and immunofluorescence techniques. Of these, two seemed to be specific for *M. leprae*. Other workers using SDS–PAGE fractionation of armadillo-derived *M. leprae* antigen and then

reacting them with antibodies in lepromatous sera, detected two specific antigenic bands of 33 KD and 12 KD.[14]

As early as 1970 it had been reported that lepromatous patients had antibodies which reacted with phospholipids (namely, mannophosphoinositides) of *M. tuberculosis*.[15] Recently, attention has been drawn to the presence of *M. leprae* specific lipid antigens with biological activity. Lipids of bacterial origin were shown to be present in the lesional skin of leprosy patients.[16, 17] A species-specific lipid antigen in infected armadillo tissues has been reported.[18] It has been chemically characterized to be related to phenolic mycoside A. The distinctiveness, uniqueness and species-specificity of this phenolic glycolipid lies in the trisaccharide appendage. The only other closely related component would seem to be the mycoside A from *M. kansasii*, though it shows some differences in the oligosaccharide composition and mobility on thin-layer chromatography. This lipid antigen is present in large amounts in the infected armadillo tissues (2·2 mg/g dry weight of liver with 9×10^{10} AFB/g). It can be obtained even after the bacilli have been removed, suggesting that it may be responsible for the electron-transparent 'foam' surrounding the organisms in the tissues. That it is immunogenic has been indicated by the presence of antibodies recognizing phenolic glycolipid in 80% of leprosy patients.[19–21] This antigen may serve as a unique marker to identify subclinical leprosy, though its biological relevance in the host immune response to the bacillus requires further investigation.

An implication that some antigenic components of *M. leprae* may selectively induce delayed-type hypersensitivity skin reactions has been suggested.[22] An anionic component of electrophoresed Dharmendra antigen, when injected intradermally in tuberculoid patients, produced delayed-type skin tests with a lympho-mononuclear infiltrate. The corresponding cationic component showed an early skin reaction with a predominant polymorphonuclear infiltration.

Immune responses in patients with leprosy

Both humoral and cell-mediated responses appear to be elicited by the pathogen in the human host. A decade of investigations has indicated that high resistance with concontaminant elimination of the organism as exemplified in tuberculoid leprosy is associated with good T-cell functions. The multibacillary low-resistant form of lepromatous leprosy is associated with enhanced humoral and reduced cellular responses. Moreover, it has been suggested that other environmental mycobacteria may modulate the immunological responses of the host to *Mycobacterium leprae* infection. Studies by Stanford *et al.* have drawn attention to the concept that protection induced by BCG as well as leprosy types may be related to the species of mycobacteria present in a given area to which the population is first exposed.[23, 24]

(a) ANTIBODY RESPONSES IN LEPROSY

Serum immunoglobulins, particularly of IgG class and other mycobacterial antibodies, had been consistently found to be raised in lepromatous leprosy individuals. Using sensitive, crossed immuno-electrophoresis (CIE), radioimmunoassays (RIA) and immunofluorescence, recent studies have indicated that *M. leprae* specific antibodies are seen throughout the leprosy spectrum, though the proportion of positive cases and the titres were highest at the lepromatous pole. Using a sensitive radioimmunoassay where *M. leprae* specific antibodies with titres of 10^5 could be measured in a lepromatous serum pool, Harboe *et al.* reported that 61 of 62 polar lepromatous, all of 24 borderline sera and 20 of 48 tuberculoid were positive. On the other hand, 38 sera from control tuberculin-positive individuals from a leprosy non-endemic region were negative.[25] In subsequent studies, the same group has identified antibodies to antigen 7 of *M. leprae* to be of significance and present in the sera of leprosy patients.[12] Using purified preparation of this antigen it was shown that the antibodies were directed against determinants other than arabinogalactan and arabinomannan in 12 out of 14 lepromatous leprosy sera.[25] Antibodies against antigen 7 appeared to be good markers of intrauterine infection in leprosy.[26] By means of solid phase RIA,[27–29] it was possible to identify the class specificity of *M. leprae* specific antibodies. High IgG anti-*M. leprae* antibody levels were detected in lepromatous leprosy. In tuberculoid leprosy they varied from negative to strongly positive in patients with active lesions. High levels of IgM anti-*M. leprae* antibodies were also found in lepromatous patients, which showed a marked fall in titres in the first year of treatment. It was further shown that as a result of intrauterine infection 30 and 50% of cord sera of children born to BL/LL mothers had IgA and IgM anti-*M. leprae* antibodies respectively. Babies born to tuberculoid mothers did not show specific antibodies. Interestingly, a few months after birth, children from both groups of mothers had similar levels of species-specific IgA and IgM, but reduced levels of IgG antibodies.[28]

The presence of anti-lipid antibodies has also been reported in untreated patients from Thailand. 9/9 LL, 2/2 BL, 5/10 BT and 7/8 TT patients were found to have significant levels of antibodies to the specific phenolic glycolipid antigen. The titre of antibodies was highest in the lepromatous group ($10{\cdot}8 \pm 4{\cdot}6$) as compared to the tuberculoid patients ($2{\cdot}2 \pm 1{\cdot}9$).[21]

Antibodies have been demonstrated not only in the blood of patients, but have also been shown to be locally synthesized in the lesional skin *in vitro*. IgG as well as *M. leprae* specific antibodies have been shown to be synthesized in the skin biopsies from lepromatous patients but not in tuberculoid individuals.[30, 31]

In addition to the development of mycobacterial antibodies, the disease appears to elicit autoantibodies. Anti-Ig, anti-nuclear and anti-thyroid antibodies have been reported in many lepromatous patients.[2] However, in one study no differences were noted in the incidence of autoantibodies in the control and

patient population of California.[32] Circulating antibodies to connective tissue microfibrils appear to be also present in lepromatous sera.[33]

(b) CELL-MEDIATED IMMUNE RESPONSES

It is now well established that T-cell mediated responses provide effective immunity in leprosy. These responses are found to be intact in tuberculoid leprosy and show a graded reduction towards the lepromatous pole. A general impairment of T-cell functions has been reported in some lepromatous patients and refuted in others.[3, 34] However, there is universal agreement that patients of polar lepromatous leprosy show long lasting anergy to specific antigens as judged by *in vivo* skin tests and *in vitro* lymphocyte functions. Details of earlier studies have been extensively reviewed elsewhere.[2, 3]

In recent years it has been suggested that the lymphocyte transformation test (LTT), considered to be an *in vitro* correlate of cell-mediated immunity, may be measuring hypersensitivity rather than the resistance status of the patients.[35, 36] LTT in babies has been reported to be depressed by plasma factors from leprosy mothers[37] and in individuals receiving DDS (dapsone 4′,4′-diaminodiphenyl sulphone).[38] An interesting study has indicated that a natural killer cell activity was depressed during erythema nodosum leprosum though not altered in the stable form of the disease.[39]

Diagnosis of subclinical infection

Apart from indicating the status of humoral immunity, *Mycobacterium leprae* specific antibodies could be used for the early detection of subclinical infection in the community, obviating the operational problems inherent in large-scale delayed hypersensitivity and T-cell function tests. Most of the antibody detecting systems employed in the above studies could be utilized for this purpose. Where possible, lepromin testing could be combined with antibody assays to evaluate high risk groups.

Abe *et al.*[40] and Bharadwaj *et al.*,[41] using indirect immunofluorescence, showed that healthy contacts of leprosy patients had detectable *M. leprae* specific antibodies. In addition, the latter compared antibody and lepromin reactions and found that 52% of individuals with positive serology showed negative lepromin reactions and may thus constitute a high risk group. A similar study using ELISA to estimate antibodies and skin tests with various antigens investigated the relationship of these parameters to protective immunity in an Iranian population.[42]

Studies using crossed immunoelectrophoresis,[11, 12] radioimmunoassay,[25] solid phase radioimmunoassay,[27–9] ELISA for mycobacterial and phenolic glycolipid antigens[21] have been developed with a view to detecting early infection. Results on

follow-up studies are awaited to evaluate the relative merits of the presently available systems.

Mechanisms for unresponsiveness in leprosy

The mechanisms underlying the depression of host immune responses in leprosy have yet to be elucidated. Recent studies in this field have investigated the role of (a) suppressor T cells, (b) adherent cells, and (c) HLA-linked genetic factors.

(a) ROLE OF SUPPRESSOR T CELLS

Since the demonstration of a subpopulation of murine T cells which exert immune regulation by suppressing immune responses,[43] an avalanche of publications is available on the role of these cells in the normal and diseased states. Investigators working independently and concurrently in Ethiopia, India and the USA have investigated the role of suppressor cells in human leprosy. Bjune,[44] working on a well-defined population in Ethiopia, showed that *Mycrobacterium leprae* antigens in general suppressed the *in vitro* PHA responses of all leprosy patients and healthy contacts.

Mehra *et al.*,[45] using patients from the USA, showed that the addition of Dharmendra antigen-suppressed Con A induced lymphocyte transformation selectively in lepromatous and not in tuberculoid patients or healthy individuals. They further showed that this suppression was due to the classical suppressor T cells bearing TH2+[46] and T8+ phenotypic markers.[47] Using the fluorescent cell sorter, they were able to show that these cells functionally suppressed the mitogenic responses of allogeneic lymphocytes from healthy individuals. These workers implied that suppressor T cells were responsible for the lack of cellular immune responses in lepromatous leprosy. However, these studies did not clearly define the treatment status, and the ethnic nature of the patients studied. These factors are important if immune regulation is being studied at a given point of time during chronic infection.

Nath *et al.* studied untreated patients from a hyperendemic and a low endemic area of India and used four different methodologies for suppressor cell activity. Care was taken to ensure HLA compatibility in cell mixture studies. Concanavalin A-induced suppressor cell activity was found to strongly and selectively suppress autologous mitogenic responses of tuberculoid patients but not that of lepromatous leprosy individuals.[48] *M. leprae* antigen suppressed Con A stimulated lymphocyte transformation in the majority of tuberculoid, but only in a few lepromatous patients. Many of the latter as well as healthy contacts showed enhanced responses. These differential effects were observed on day 4 of culture. If the cultures were prolonged to 6 days the results confirmed the findings of Bjune[44] in that suppression was uniformly noted in all individuals.[49] Moreover,

enumeration of T cells with Fc receptors for IgG—thought to represent a suppressor T-cell subset[50]—showed normal values in tuberculoid and reduction in lepromatous patients.[51] These studies, therefore, would indicate that suppressor T-cell activity was generated during infection with *M. leprae* in individuals with high resistance and capable of strong cellular immune reactions. They may serve to inhibit reactions and unwanted antibody formation. Suppressor cells have been reported to frequently develop during a strong immune response.[52] The lack of suppressor T-cell activity in lepromatous leprosy may explain the excessive production of antibodies and autoantibodies, a situation analogous to systemic lupus erythematosus where suppressor cells have been found to be reduced. The above results were definitively confirmed in further studies on siblings of leprosy families. Using HLA-D matched lymphocyte–lymphocyte co-cultures, it was shown that tuberculoid leprosy individuals had natural suppressor lymphocytes that inhibited antigen and mitogen responses of HLA identical siblings. Lepromatous individuals lacked such cells in the circulation.[53] Though the preferential migration of suppressor cells away from the blood to other sites cannot be ruled out, the paucity of absolute numbers of cells with phenotypic T8 markers in lepromatous lesions would suggest that the skin is not a major site.[54]

A lack of suppressor T cells in lepromatous leprosy has also been reported from Ethiopia.[55] There was a gradual development of *M. leprae*-induced suppressor activity in healthy individuals exposed to subclinical infection, implying an association of suppressor cell activity with resistance to leprosy infection.[56] Bullock *et al.* also studied patients resident in the USA and found that T8+ (suppressor/cytotoxic) cells from lepromatous donors when co-cultured with normal B cells failed to suppress the response to pokeweed mitogen. They also postulated a loss of regulatory function by T8+ suppressor T cells in lepromatous patients.[56b] Studies in Carville, USA, also indicate that suppressor cell activity is associated with tuberculoid and not lepromatous individuals.[57] Moreover, armadillos with disseminated disease lack suppressor activity whereas those resistant to repeated reinfection possessed suppressor T-cell function (EJ Shannon, personal communication). Functional studies have been more valuable in elucidating this phenomenon than studies on phenotypic markers for functional subsets of T cells. The numbers of T3+ (pan T cells), T4+ (helper/inducer) and T8+ (suppressor/cytotoxic) cells seem to be the same in all leprosy types[58] (and personal observations). As indicated elsewhere these markers were of value in the study of lesions.

Thus it would seem that most of the studies on defined populations of leprosy patients show that suppressor T-cell activity is generated during strong cell-mediated immune responses in high-resistant individuals as part of the natural course of *M. leprae* infection. The lack of this activity is commonly observed in disseminated leprosy, indicating that suppressor T cells may be helpful in inhibiting unwanted antibody production and allergic reactions.

(b) ROLE OF ADHERENT CELLS/MACROPHAGES

M. leprae reside within cells of the mononuclear phagocyte series and lead to granulomatous reaction in the host. The earlier claim,[59] that macrophages from lepromatous individuals have an inherent defective killing capacity has been refuted by others.[60, 61] The methodology used in these studies may not have been sensitive enough to assess *M. leprae* viability/multiplication.

Using allogeneic macrophage–lymphocyte cell combinations, Hirschberg drew attention to defective macrophage function in lepromatous patients which inhibited *M. leprae*-stimulated lymphocyte transformation.[62] Nath *et al.*, using HLA-D identical co-cultures, confirmed these findings. In addition, they showed that lepromatous lymphocytes when combined with tuberculoid macrophages responded to *M. leprae* antigens, indicating thereby the presence of antigen-reactive T cells in lepromatous leprosy.[53] Additional studies from our laboratory have confirmed the above and indicate that antigen processing by lepromatous macrophages is capable of inducing *M. leprae* lymphocyte transformation in HLA-compatible tuberculoid individuals. In addition, we have evidence to show that adherent cells in the peripheral blood of lepromatous individuals with macrophage characteristics exert suppressive effects on antigen-induced lymphocyte transformation of tuberculoid patients and healthy contacts.[63] This suppression appears to be mediated by soluble factors from macrophages.

Studies from another group in India indicate that lepromatous macrophages show receptor, biochemical and functional alterations after phagocytosis of viable but not killed *M. leprae*. Protein synthesis as assessed by 3H-leucine uptake has been shown to be selectively defective in the macrophages from lepromatous patients.[64] Such macrophages show a reduction in the density of Fc receptors on exposure to *M. leprae* and not to other mycobacteria.[65] Lysates from these macrophages reduced the protein synthesis of normal macrophages and inhibited lymphocyte transformation.[66]

The recent studies refuting a macrophage defect[63] are not necessarily in conflict with the above results since our experience has shown that the concentration of macrophages required to constitute *in vitro* responses may vary from individual to individual and dose-related assays are required in these studies.

The above data taken together suggests that macrophages may negatively modulate *in vitro* lymphocyte responses and may contribute to the non-responsiveness seen in lepromatous leprosy. It is not possible as yet to comment on whether this defect is inherent, HLA-linked, or secondary to the disease. However, a macrophage defect alone may not explain the specific antigen-related unresponsiveness, unless mediated through specific T cells.

(c) THE ROLE OF HLA

Following the observations that the resistance of mice to certain antigen/virus was controlled by genes linked to the histocompatibility genes, intensive efforts

are in progress to find a causal relationship between HLA and human disease. The diverse clinical types in leprosy related to the varied host responses to *M. leprae* would suggest an association of leprosy with genes linked to immune responsiveness. However, a number of population studies carried out in various ethnic groups seeking an association with HLA-A, -B and -C antigens have not provided a clear-cut pattern in leprosy.[67–73]

Using the argument that susceptibility to leprosy may be HLA linked but not necessarily identical to the HLA-A and -B alleles, De Vries *et al.* studied siblings and parents of multicase families in the Surinam population and thereby observed a haplotype association with leprosy. Though no association was found with a particular HLA-A-B-C antigen or haplotype, yet siblings affected with tuberculoid leprosy shared a parental haplotype with a higher frequency than expected.[74] These results were confirmed in another ethnic population of Wardha, India.[75] Similar association was also observed in tuberculoid siblings of 75 families of South India where neither parent was diseased.[76]

HLA-D is thought to be the human equivalent for the murine immune response genes (Ir). Studies on HLA-D-related (DR) antigens in the Wardha area showed that siblings affected with tuberculoid leprosy inherited DRW2 significantly more often than was expected. The frequency of DRW6 on the other hand was much less. These findings suggested that susceptibility and resistant genes for tuberculoid leprosy was linked to DRW2 and DRW6 respectively.[75, 77] However, no such association with DR2 could be found when non-familial sporadic tuberculoid leprosy patients were compared with a well-matched normal control population.[78] This apparent discrepancy was due to the fact that the control population of that area had a high frequency of DR2. It is possible that the high prevalence of tuberculoid leprosy seen in this area may be linked to the high frequency of DR2 in the general population. A similar though less marked increase in frequency of DR2 was reported in Mexican borderline tuberculoid patients.[79] On the other hand, in the Japanese studies, both tuberculoid and lepromatous patients showed a higher frequency of DR2.[80] It is interesting to note that in the Negroid–Caucasoid population of Surinam, HLA-DR3 was associated with tuberculoid leprosy and seemed to be protective against the low-resistant and unstable forms of leprosy.[81]

The Ethiopian studies examining the influence of HLA-D in *M. leprae*-induced lymphoproliferative assays do not support the concept of an HLA-linked, specific unresponsiveness in leprosy.[82] Nevertheless, it is possible that HLA-linked genes may play a role in determining the type of leprosy by regulating mechanisms of host responsiveness to *M. leprae* infection. The success of a leprosy vaccine may be dependent on further elucidation of this association.

In situ characterization of cellular infiltrates in dermal lesions

Membrane receptors present on lymphocytes and cells of the mononuclear

phagocytes, as well as lysosomal enzymes, are useful markers for the identification of functional subsets of cells in cryostat sections of leprosy lesions. Ridley *et al.* reported that epithelioid cells of tuberculoid leprosy had C3 receptors and macrophages in lepromatous lesions lacked C3 but possessed receptors for the Fc fragment of IgG, suggesting thereby that Fc receptors were lost on epithelioid cells.[83] More recently, using haemoadsorption with AET-treated sheep erythrocytes, Gupta *et al.* found that the predominant lymphocyte in the leprosy granulomas was the T cell. There was a graded reduction in the number of T cells from tuberculoid to the lepromatous lesions. These workers did not find any differences in the density of Fc and C3 receptors on mononuclear phagocyte series across the leprosy spectrum.[84] Working independently, the same group[54] and others[85, 86] confirmed the T-cell nature of the lymphocytes by means of monoclonal antibodies to phenotypic markers of T cells and their subsets using indirect immunofluorescence and immunoenzymatic techniques. Moreover, it would appear that the proportion of T4+ (helper/inducer) to T8+ (suppressor/cytotoxic) T cells was higher in tuberculoid lesions and varied from high to low in borderline and subpolar lepromatous leprosy.[54, 85] The few lymphocytes seen in polar lepromatous lesions were T8+. It is difficult to give a biological significance to the presence of T8+ cells in view of the paucity of absolute numbers of such lymphocytes in the lepromatous lesions. It was interesting to note that typical granulomas of tuberculoid leprosy had T8+ cells were arranged in a concentric manner in the mantle whereas T4+ cells were seen both in the mantle as well as scattered amongst the epithelioid cells.[54, 85] All three studies showed DR/Ia-like antigens on the lymphocytes and macrophages indicating the presence of activated T cells and macrophages. Some epithelioid cells appeared to lack these antigens.[54] Ridley, using formalin fixed tissues which may destroy these antigens, reported contradictory results showing a selective absence of Ia-like antigens on macrophages of lepromatous lesions.[87] It would thus appear that activated T cells are the predominant lymphocytes in leprosy granulomas and are reduced in number from tuberculoid to lepromatous pole. B cells are infrequent in these lesions,[88] (personal observations). The distinction between epithelioid cells and macrophages could not be determined fully by the surface markers used in the above studies.

Immunomodulation of host responses

With a view to improving the anergic state of lepromatous patients, several approaches at modulating immune responses have been undertaken.

Transfer factor therapy has been shown to produce temporary improvement in the form of positive skin tests and reversal reactions.[89–92] Injection of leucocytes[93] and thymus grafts[94] had similar effects. It would appear that the injections of mycobacteria and particularly of BCG alone or with *M. leprae* are

effective in converting to skin positivity, producing reversal reactions and causing clearance of bacilli. In recent years good results have been reported by repeated injections of BCG with *M. leprae*.[95, 96] Positive skin tests and leucocyte migration inhibition have been reported in lepromin-negative patients injected with a saprophytic mycobacterium.[97] A cultivable bacillus from leprosy skin grown in conditioned medium called ICRC bacillus has been shown to be immunogenic in mice and produce positive skin tests with bacterial clearance.[98] Evaluation of the effectiveness and duration of immunomodulation in these studies is awaited. However, it is clear that partial restoration of some T-cell functions is possible by injection of armadillo-derived *M. leprae* and other mycobacteria. Their role in immunoprophylaxis will be discussed elsewhere in this volume.

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Immunotherapy and immunoprophylaxis of leprosy

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The clinico-pathologic spectrum of leprosy in its diverse forms is the expression of the immunologic response of the human host to infection by *Mycobacterium leprae*.

In this spectrum, the lepromatous pole is characterized by a total lack of cell-mediated effector immunological responses; this lack is also seen *to a lesser degree* in borderline lepromatous (BL) as well as in other forms, which present a variable degree of deficiency of this type of immunity as they are located along the spectrum towards the tuberculoid pole of the disease.

There is substantial evidence that the immunological defect which reaches its maximum expression in lepromatous patients is also present in other population groups, including a small portion of the healthy population. This evidence includes the following observations:

(a) A significant proportion of patients with the indeterminate form of leprosy show immunological behaviour similar to that of the lepromatous patient, characterized by a lack of cell-mediated manifestations toward *M. leprae*. Without adequate treatment, this indeterminate form of leprosy shows a tendency to progress to lepromatous leprosy (LL). We have seen this phenomenon of progression towards the LL pole in indeterminate patients who, after many years of treatment, have stopped their medication due to various reasons and suddenly re-appear with LL or BL disease. On the other hand, indeterminate lesions in Mitsuda-positive patients show a tendency to regress spontaneously, or to evolve towards the highly resistant forms of the disease.[1]

(b) Studies of the Mitsuda reaction in the general population, both in endemic and non-endemic areas, show that a small proportion of the general population is persistently Mitsuda-negative. In 1978 we studied the persistence of this negativity towards lepromin in the population of a non-endemic area of Venezuela and found that 1·25% of the general population remained

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persistently Mitsuda-negative after 4 injections of lepromin during an 8-month period.[2]

In 1955, Dharmendra & Chatterjee[3] reported the results of a study carried out in endemic areas in India which gave extremely interesting results. They found that among a group of 650 healthy contacts of leprosy patients, 126 were Mitsuda-negative. Of these, they tested 109 with a series of 3 applications of lepromin during the course of 1 year; a group of 16 remained negative even after the 3 lepromin injections. After 15–20 years, a new examination showed 10 active cases of leprosy among this group of 16 persons; 8 of them were lepromatous cases. No less than 55% of the lepromatous cases found in this study had developed in that 2·3% of the population which remained persistently negative after repeated lepromin injections. The other 45% appeared among those persons who were Mitsuda-negative in the first test, of whom only 1 had responded to repeated injections of lepromin; the rest came from the 17 initially negative contacts who did not receive several injections of lepromin. Not a single lepromatous case appeared in the group of 524 Mitsuda-positive contacts. These observations suggest an immunological defect in healthy persons before the development of the clinical disease, and they give the bases for the selection of high-risk populations in immunoprophylaxis studies. They also open the possibility of important studies on the mechanism of the immunological defect in persons who do not present the possible side-effects of active lepromatous leprosy and/or prolonged treatment.

After a decade of active research, it seems evident that the important immunological defect in lepromatous leprosy is a specific defect towards *M. leprae*. These patients do not present the clinical complications characteristic of generalized immuno-deficiency; apparently there is no increase in susceptibility towards viral, mycotic or malignant processes.

One of the most significant contributions toward the production of skin-test reagents for use in the immunological and epidemiological evaluation of reactivity to *M. leprae* and/or the vaccine itself has been the development of a suitable procedure for the purification of *M. leprae* from the tissues of experimentally infected armadillos. This procedure, designed to eliminate tissue contaminants while conserving the immunogenic and antigenic properties of *M. leprae*, has been developed at the National Institute for Medical Research by Dr Philip Draper, in close collaboration with Dr Dick Rees.

When using SDS-polyacrylamide electrophoresis methods to analyse a soluble extract obtained from bacilli purified according to the 1/79 IMMLEP Protocol developed by Draper, we found more than 40 proteic and polipeptidic bands, without considering the lipid and polysaccharide fractions or the insoluble fraction.

This extract produces important cross-reactions in guinea-pigs sensitized with BCG, an observation which clearly demonstrates the presence of common

antigens between *M. bovis* and *M. leprae* in this preparation. In any case, lepromatous patients *do not* recognize these common antigens, even though they recognize specific antigens of other mycobacteria. More than 65% of a group of 231 lepromatous patients give strong reactions, with an average of 30·5 mm of diameter, to 2 units of PPD. In this same group, 96% give completely negative reactions to a soluble antigen from *M. leprae*. This same extract produces reactions over 10 mm in diameter in 70–75% of the contacts tested in endemic areas of Venezuela. As we shall see later, the usefulness of this type of antigen for detecting the non-reactive population is unquestionable, but positive reactivity cannot be attributed to a response towards specific *M. leprae* antigens.

In our laboratories we are specially interested in the possible importance of a macrophage defect in the cellular alteration observed in the progressive forms of leprosy. Lepromatous macrophages possess the necessary enzymatic machinery to digest *M. leprae* if they are activated by another immunological mechanism which undoubtedly requires the intervention of sensitized lymphocytes.[4] But the importance of the macrophage is not limited to its response towards lymphocytic stimuli; these cells play a fundamental role in the processing and presentation of antigens to the lymphoid system in the induction of the immunological response, in immunosuppression, etc.

The following could be postulated: a primary defect in the processing and/or in the presentation of *M. leprae* antigens by the non-reactive macrophages would result in an inadequate immunological response, characterized by the activation of sub-populations of suppressor cells or lack of an adequate stimulus to helper lymphocytes. This possibility seems attractive when interpreting the results of immunotherapy with *M. leprae* and BCG in lepromatous patients. One of the more obvious consequences of the use of this combination is the forced digestion of *M. leprae*, a process which could produce the generation of the respective immunogens.

The concept that the immunological defect in leprosy is irreversible has been expressed frequently. This concept is backed by the observation that lepromatous patients treated successfully with chemotherapy do not develop positive reactions towards *M. leprae* even when they are stimulated by procedures which produce an immunological conversion in normal persons.[5]

The results we will see with immunotherapy with a mixture of *M. leprae* and BCG indicate that the defect is reversible in a high percentage of cases, if an adequate immunotherapeutical procedure is used. This observation gives preliminary but substantial support to the idea that prophylactic and therapeutical vaccination could offer an important option in leprosy control.

Immunotherapy

A decade ago we showed that Mitsuda-negative persons, including healthy individuals and leprosy patients, do not eliminate a suspension of heat-killed

Mycobacterium leprae from their tissues in a period of 1 to several months, but that they do eliminate BCG. In the first case, a macrophagic granuloma is formed which retains the injected *M. leprae* in its cells.[6] In the second, there is development of an immune granuloma and the BCG is eliminated. When both mycobacteria are injected together in the same site a typical immune granuloma is formed and both mycobacteria are eliminated in a short period.[4] These results suggest that the use of this mixture *M. leprae* plus BCG could provoke the digestion of *M. leprae* and the liberation of some of its antigens which could stimulate the immune system in persons normally unable to carry out this digestion. BCG would act as a stimulant of the digestive process through the activation of macrophages. Both components of the vaccine, heat-killed *M. leprae* and BCG, have been used in intracutaneous injections in human beings during many years without producing adverse side-effects, so there would be sufficient reason to expect that a mixture of both would be well tolerated by non-reactors to *M. leprae*.

Based on these considerations, in 1973 we started using a mixture of heat-killed *M. leprae* and live BCG in 6 patients with inactive lepromatous leprosy, 6 with indeterminate leprosy, Mitsuda-negatives, and in 6 persistently Mitsuda-negative contacts. In the contacts we used only one dose of the vaccine; the patients received several injections during a period of 1 or 2 years. During a 6-year observation period, we have seen important immunological changes, including the regression of indeterminate lesions and a strong and persistent positivization of the Mitsuda reaction.[7] Due to the favourable changes seen and the lack of adverse side-effects, we have proceeded to evaluate this procedure in 577 persons within the immunotherapy programme of the National Institute of Dermatology.

This group includes 25 persistently Mitsuda-negative contacts, 46 patients with indeterminate leprosy, also Mitsuda-negative, 155 patients with inactive BL and LL and 351 patients with active BL or LL. Before vaccination we did a detailed clinical, dermatological and neurological examination, biopsy of active lesions and humoral and cellular immunity tests, *in vivo* and *in vitro* (skin reactivity to PPD, to soluble antigen from *M. leprae* and to Mitsuda antigen, *in vitro* lymphocyte transformation and determination of suppressor cells and antibodies against *M. leprae* through a micro-ELISA test).

The vaccine we use contains 6×10^8 bacilli of *M. leprae* purified according to the method developed by Draper[8] from tissue of experimentally infected armadillos and killed in the autoclave at 121°C during 15 minutes, plus viable BCG in a variable amount (from 0·01 mg to 0·2 mg), according to the reaction of the patient to 2 units of PPD. The vaccine is applied intradermally in 3 sites, both deltoid regions and the upper part of the back.

Table 1 shows the patients studied, changes in the 48-reactivity towards *M. leprae* soluble antigen and clinical changes seen.

The group of 25 contacts, most of them adults, who had remained persistently

Table 1. Immunotherapy with a mixture of *M. leprae* plus BCG in leprosy groups studied and changes obtained

Classification period of vaccination	No.	Skin-test reactivity		Clinical and histopathological changes		
		SA pos. %	Mitsuda pos. %	Reduced infiltration %	Reversal reaction %	Total %
Active BL/LL						
6 mo.	18	0	—	0	8	6
18 mo.	74	20	—	27	20	47
> 19 mo.	259	38	—	43	27	71
Inactive BL/LL						
6 mo.	3	67	—			
18 mo.	39	46	—			
> 19 mo.	113	63	—			
Indeterminate						
6 mo.	2	100	—			
18 mo.	12	83	75			
> 19 mo.	32	97	88			
Contacts						
6 mo.	25	100	84			

Mitsuda-negative represents the most interesting group in relation to the potential use of this vaccine in the immunoprophylaxis of leprosy. All became positive towards *M. leprae* after 1, or rarely 2, vaccinations. The Mitsuda reaction has become positive in all who have been tested with this antigen.

We have seen important clinical, histopathological and immunological changes in the group of 46 patients with indeterminate leprosy. The clinical changes included the appearance of a papular eruption, with tuberculoid structure, which later regressed spontaneously; the lesions regressed and the hypopigmented areas became re-pigmented. A very high percentage became positive to soluble antigen and Mitsuda antigen. It was necessary to use 4 or more vaccinations to produce these changes, especially in the prelepromatous indeterminate patients who had bacilli at distant sites from their lesions (knees, ears, etc.).

As expected, it has been more difficult to produce changes in lepromatous or borderline lepromatous patients, even those who are bacteriologically negative after prolonged chemotherapy. Even in this group, 59% have responded to soluble antigen from *M. leprae* after 3 or more vaccinations.

The changes observed in active LL or BL patients who have been vaccinated 3 or more times are especially interesting. The clinical changes include reversal reactions, characterized by formation of nodules and plaques over their chronic

lesions, reactivation of lesions, better definition of borders, des-infiltration and progressive regression.

At the same time we have seen important histopathological changes, including infiltration by mononuclear cells, epithelioid differentiation in some cases and noticeable deterioration and reduction of the mycobacterial population. Of the 351 patients with active LL or BL, 62% showed some or all the changes described above; 32% became positive to soluble antigen, most of them after 5 or more vaccinations.

Most of the patients with active LL or BL presently receive chemotherapy with sulphones or rifampicin, but we have also seen the above changes in patients who are not receiving chemotherapy for various reasons (sulphone-resistance, very strong side-effects to chemotherapy or non-compliance with treatment). The immunological changes towards soluble *M. leprae* antigen have been seen very rarely in patients treated exclusively with chemotherapy.

Side-effects have been controlled with adequate treatment with thalidomide and triamcinolone and do not compare unfavourably to side-effects observed during conventional chemotherapy. In some cases with very large bacillary loads, we have observed fever and general malaise, which was controlled with small doses of corticosteroids. A very important observation has been the small number of adverse reactions in nerves. We have seen serious neuritis in only 4 cases and moderate reactions in 19 more. In 3 BL cases recently we have seen neuritic reactions of the external sciatico-popliteal nerve with Stepage (drop-foot). These side effects disappeared after 6 weeks' treatment with triamcinolone, 12 mg/day. The rest of the neuritic phenomena were managed easily with small doses of steroids, 4 mg/day, and left no permanent sequellae.

Another secondary phenomenon also seen recently is two cases of hepatitis with ictericia, also in BL cases with reversal phenomena; these cases were easily managed with 8 mg/day of triamcinolone.

The majority of secondary reactions have been observed in BL cases.

The results presented show the efficacy of the mixture of *M. leprae* and BCG to induce immunological changes in low-resistance leprosy and in Mitsuda-negative contacts. Therefore, the use of this same procedure would be justified as an immunoprophylaxis method for this disease.

Immunoprophylaxis

The bases for immunoprophylaxis as we understand it at present can be summarized as follows:

1 The population susceptible to leprosy, especially in its progressive forms, represents a very small proportion of the general population. This fact, plus the possibly high cost of producing a vaccine based on *Mycobacterium leprae* purified

from experimentally infected armadillos, justify a selective approach to vaccination, limited to high-risk populations, that is, contacts.

2 The high-risk population can be identified by two criteria, epidemiological and immunological. Through the first, we identify contacts around active leprosy cases. In Venezuela we estimate an average of 5 intradomiciliary and 45 extradomiciliary contacts per case, on the basis of previous epidemiological surveys. The interest of the group of extradomiciliary contacts lies on the fact that 75% of new cases of leprosy have been found among this group of persons. The immunological criterion used to identify high-risk contacts is a negative reaction towards soluble *M. leprae* antigen. Supposedly all contacts have been exposed to infection by *M. leprae* and those with normal immunological reactivity should have developed delayed hypersensitivity towards this organism.

3 From the evidence presented at the beginning of this presentation and the discouraging results of large-scale trials with BCG, the use of *M. leprae* or BCG alone do not offer much hope of an adequate vaccine. The favourable results obtained in the immunotherapy of leprosy with a mixture of *M. leprae* and BCG, plus the persistence of the immunological conversion seen in Mitsuda-negative contacts during an observation period of several years, indicate that this combination can represent a highly efficient vaccine.

4 The only absolute criterion to determine the efficacy of a preventive vaccine would be the incidence of new cases of leprosy; the evaluation would depend on a 5- or 10-year observation period. In any case, observations during the immunotherapy trial indicate that the induction of an immunological conversion of skin reactivity towards soluble *M. leprae* antigen can become a useful criterion to evaluate the response to the vaccine in terms of percentage of positive reactors and persistence of conversion.

Preliminary data obtained in an immunoprophylaxis trial carried out in two western states of Venezuela which are highly endemic for leprosy are extremely interesting. I will present only the most relevant results due to space limitations.

In the first stage of this trial we identified a total of 2,659 contacts in two work areas in Apure and Tachira States; 293 of these were household contacts (Figure 1 and Table 2). We examined them all clinically and neurologically, and skin tests with 2 units of PPD and 0·5 or 1·0 μg of soluble *M. leprae* antigen were applied; circulating antibodies were also studied in a micro-ELISA test.

Skin reactivity towards these soluble antigens is shown in Tables 3 and 4, according to age and sex. The reactivity to SA increases with age from the 12- to 14-year group, with an average reaction diameter less than 12 mm, up to a maximum in the 25–29-year group (20·7 and 16·9 mm in Apure and Tachira, respectively). Until this age group the correlation between age and reactivity is positive. From 40 years on, the phenomenon is inverted, reactivity is gradually reduced and the correlation between age and reactivity becomes negative.

In relation to reactivity to SA in age groups under 12 years, we did a

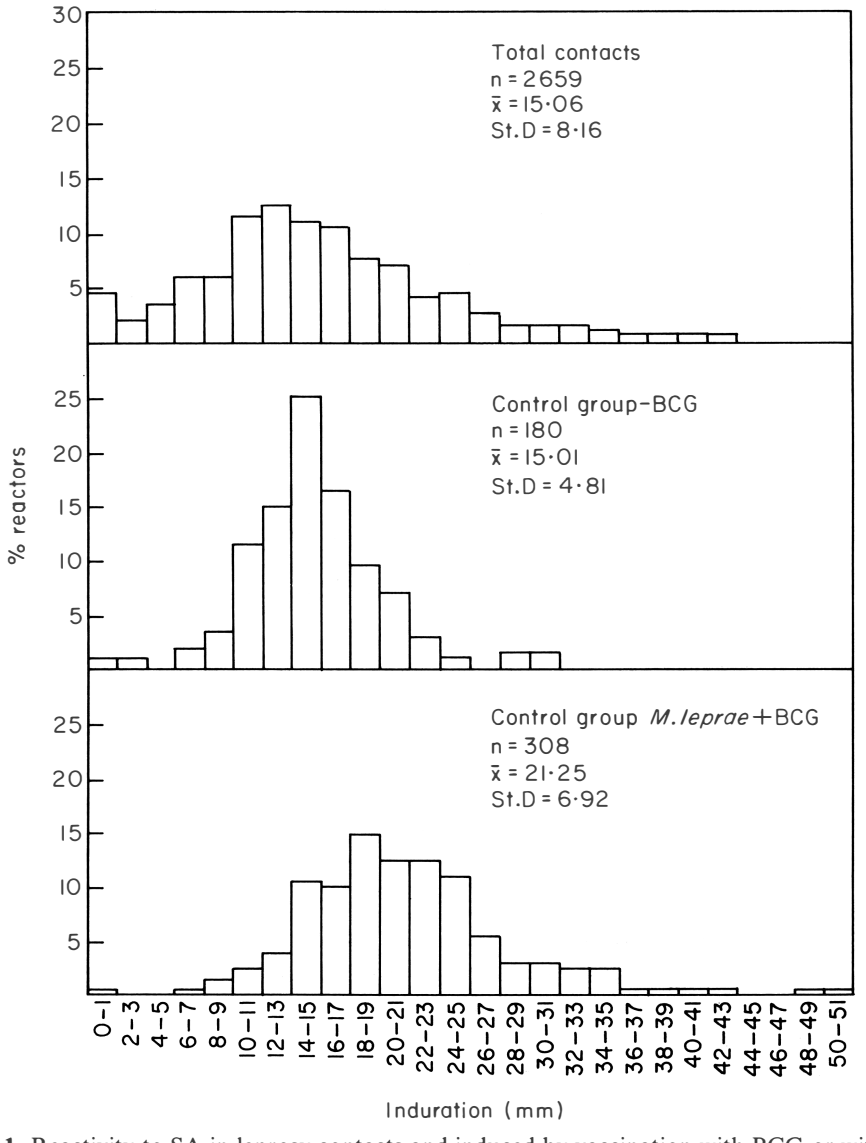

Figure 1. Reactivity to SA in leprosy contacts and induced by vaccination with BCG or with the mixture *M. leprae*–BCG in contacts initially negative. Sixty days after vaccination control, Apure–Tachira, 1981 (together).

preliminary trial with this antigen in 84 children in Apure and we saw that the 0–5 year group (30) had an average induration of 4·07 mm, with 90% under 10 mm. In children 6–11 years old the average was 10·6 mm and the proportion of 'negatives' (0–9 mm) was 40·7%.

The distribution by age and relationship with a leprosy case (Table 5), shows that in household contacts, reactivity increases earlier and apparently remains

Table 2. Immunoprophylaxis trial. Distribution of participants according to age groups. Apure and Tachira States, 1981

Age groups	Apure				Tachira			
	n	%	*N*-household	Household	*n*	%	*N*-household	Household
12–14	236	15·6	195	41	115	10·2	101	14
15–19	363	24·0	317	46	213	19·0	185	28
20–24	202	13·4	183	19	142	12·7	132	10
25–29	155	10·3	127	28	99	8·8	92	7
30–34	107	7·1	93	14	85	7·6	83	2
35–39	95	6·3	83	12	84	7·5	82	2
40–44	99	6·5	83	16	58	5·2	53	5
45–49	85	5·6	78	7	78	7·0	74	4
50–54	58	3·8	52	6	73	6·5	66	7
55–59	40	2·6	37	3	50	4·5	47	3
60–64	27	1·8	23	4	53	4·7	50	3
65–69	24	1·6	18	6	34	3·0	34	—
70 y+	21	1·4	16	5	38	3·4	37	1
Totals*	1,512	100·0	1,305	207	1,122	100·0	1,036	86

* The exact age could not be determined in 25 persons included in the study.

higher throughout. The percentage of negatives is less than that of non-household contacts in all age groups. According to this analysis, one of the groups at highest risk would be that of non-household contacts 40 years old or over, a fact which has shown an epidemiological corroboration during the preliminary stages of our trial.

We found 21·8% of 'non-reactors' in the population 12 years old or over when using criterion of induration of 9 mm or less at 48 hours (Table 6). The non-reactors were divided in two groups; one to be vaccinated with the same mixture of autoclaved *M. leprae* and BCG used for immunotherapy and the other, the control group, only with BCG.

Two months after vaccination both groups were again tested with *M. leprae* soluble antigen. As we can see in Table 6 and Figure 1, a high percentage of both groups became positive. In the group of 308 persons vacinated with the mixture *M. leprae*–BCG, we saw an average induration of 21·25 mm at 48 hours with soluble antigen; 56% of this group have strong reactions, 20 mm of induration or more and only 1·9% persisted as 'non-reactors' with reactions 9 mm or less of induration. On the other hand, the average induration in the group of 180 persons vaccinated with BCG alone was 15·0 mm; only 14% gave reactions 20 mm or more and almost 8% persisted as 'non-reactors'. This initial evaluation indicates that the *M. leprae*–BCG mixture induces an immunological conversion towards soluble antigen clearly superior to that induced by BCG alone.

Table 3. Reactivity to SA, according to age groups, in contacts. Apure and Tachira States, 1981

Age groups	Apure			Tachira		
	n	Avg Ind (mm)	% < 10 mm	*n*	Avg Ind (mm)	% < 10 mm
12–14	236	11·8	37·3	115	11·6	38·3
15–19	363	14·3	24·5	213	13·5	23·5
20–24	202	17·4	14·8	142	16·1	18·3
25–29	155	20·7	5·8	99	16·9	13·1
30–34	107	18·4	13·0	85	15·5	22·4
35–39	95	18·6	14·7	84	15·9	10·7
40–44	99	16·4	20·2	58	13·9	27·6
45–49	85	17·0	14·1	78	12·6	33·3
50–54	58	15·4	13·7	73	13·3	21·9
55–59	40	11·4	37·5	50	14·1	28·0
60–64	27	12·2	22·2	53	12·3	30·2
65–69	24	14·8	12·5	34	12·7	20·6
70 y+	21	14·4	9·5	38	12·0	26·3
Totals	1,512	15·75	20·3	1,122	14·1	23·7

Avg Ind = Average Induration

Apure:	*Age*	n	*(mm)*	*% < 10 mm*
	0–5	30	4·07	90·5
	6–11	54	10·6	40·7

Table 4. Reactivity to SA, distribution by age and sex. Apure and Tachira States, 1981

Age groups and sex		Apure			Tachira		
		n	Avg Ind (mm)	% < 10 mm	*n*	Avg Ind (mm)	% < 10 mm
12–19	Males	250	12·6	28·8	160	12·3	27·5
	Females	349	14·0	29·2	170	13·4	29·4
20–39	Males	173	18·1	12·1	134	13·8	15·7
	Females	386	18·9	11·9	276	17·2	17·0
40–59	Males	102	14·3	18·6	97	12·7	25·8
	Females	180	16·5	20·0	161	13·9	29·2
60 y+	Males	35	12·9	22·9	54	12·1	29·6
	Females	37	14·4	8·1	70	12·5	28·6
Total	Males	560	14·6	21·4	445	12·81	23·8
	Females	952	16·5	19·6	677	14·98	24·2

Table 5. Reactivity to SA antigen according to whether the contact lives in the same house with a leprosy patient or is a non-household contact. Apure and Tachira States, 1981 (summary)

Age groups	Household contacts			Non-household contacts		
	n	Avg Ind (mm)	% < 10 mm	*n*	Avg Ind (mm)	% < 10 mm
12–19	129	16·0	21·7	798	12·7	30·2
20–39	94	20·7	8·5	875	17·3	14·5
40 y+	70	17·5	12·9	668	13·8	24·1
Total	293	17·9	15·4	2,341	14·7	22·7

Table 6. 'Natural' reactivity to SA and sensitivity induced through BCG and the vaccine (*M.l.*) + BCG in contacts living in the areas selected for the trial of an antileprosy vaccine. Apure and Tachira States, 1981

Millimetres of induration	Initial population		Neg vac BCG		Neg vac *M.l.* + BCG	
	No.	%	No.	%	No.	%
0–9	579	21·8	14	7·8	6	1·9
10–14	792	29·8	73	40·6	33	10·7
15–19	622	23·4	67	37·2	97	31·5
20–24	366	13·7	20	11·1	90	29·3
25 y+	300	11·3	6	3·3	82	26·6
Total	2,659	100·0	180	100·0	308	100·0

1 'Negatives': reactions between 0 and 9 mm.
2 Second dose of SA 60 days after vaccination.

The difference in reactivity towards soluble antigen in both groups becomes much more evident at 8 months after vaccination (Figure 2 and Table 7). In the control group vaccinated with BCG, nearly 50% presented reactions 9 mm or less of induration (average of the whole group 8·99 mm); this fact shows that BCG-induced is not only weak, but also short-lived reactivity. The average of induration in the group vaccinated with the mixture was 16·32 mm at 8 months and 14% had reactions 9 mm or less. These results at 8 months support the initial conclusion obtained with the results at 2 months. It should be emphasized that the initial reactivity in the two negative groups (0–9 mm), subsequently vaccinated

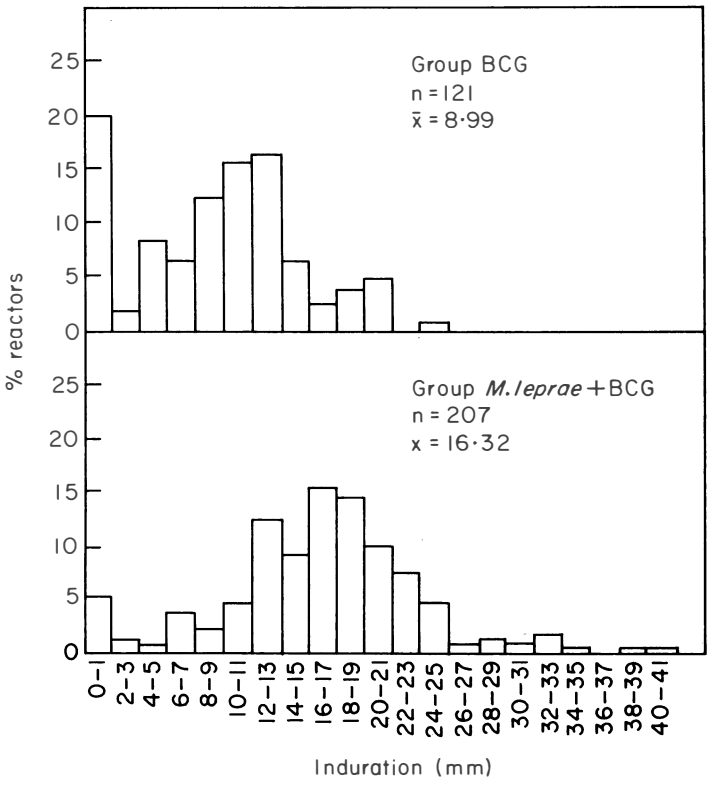

Figure 2. Reactivity to SA in leprosy contacts and induced by vaccination with BCG or with the mixture *M. leprae*–BCG in contacts initially negative. Eight months after vaccination control, Apure–Tachira, 1982 (together).

Table 7. Comparative sensitivity to SA antigen induced by BCG or *M. leprae* + BCG, at 60 days and 8 months after vaccination. Tachira and Apure States, June 1982

Millimetres of induration	Vaccination with BCG				Vaccination *M.l.* + BCG			
	60 days		8 months		60 days		8 months	
	n	%	*n*	%	*n*	%	*n*	%
0–9	14	7·8	59	48·8	6	1·9	29	14·0
10–14	73	40·6	43	35·5	33	10·7	45	21·7
15–19	67	37·2	12	9·9	97	31·5	72	34·8
20–24	20	11·1	7	5·8	90	29·3	41	19·8
25 y+	6	3·3	—	0·0	82	26·6	20	9·7
Total	180	100·0	121	100·0	308	100·0	207	100·0

Table 8. Initial reactivity of the negative contacts to SA and modifications induced by vaccination with BCG or *M. leprae* plus BCG, 2 and 8 months after vaccination

	Control group (BCG)			Test group (*M. leprae* + BCG)		
Evolution	*n*	$\bar{X}$ (mm)	SD	*n*	$\bar{X}$ (mm)	SD
Initial reaction	180	5·01	3·07	308	4·93	3·15
Skin test, 60 days	180	15·01	4·81	308	21·25	6·92
Skin test, 8 months	121	8·99	6·06	207	16·32	7·28

with BCG or with *M. leprae* and BCG, was of 5·01 and 4·93 mm average diameter, respectively, with very similar standard deviations (3·07 and 3·15 mm) (Table 8).

The future stages of this trial contemplate the repetition of the clinico-dermatological examination and skin tests with soluble *M. leprae* antigen at yearly intervals during 5 years and less frequent examinations in the next 5 years. The preliminary results have already become the basis for the protocol of a much larger trial in Venezuela, which will include the study of 61,000 contacts.

The results of the immunotherapy and immunoprophylaxis programmes developed in Venezuela offer the hope of new methods for antileprosy campaigns, at a moment when the alarming increase of sulphone-resistance makes it necessary to intensify the search for a new approach to solve this problem.

Before closing I would like to make some comments on the group of persons who, after being vaccinated with the mixture, appear as negatives (0–9 mm) or weakly positive (10–14 mm) after an intradermal test with soluble antigen.

A not yet determined percentage of these negative persons have high titres of circulating antibodies with the micro-ELISA test. This situation is similar to that seen in patients with low-resistant forms of leprosy (LL, BL) and Mitsuda-negative contacts, and we have considered the possibility that this group may require more than one vaccination to obtain favourable immunological changes.

According to these last considerations, the above mentioned group of non-reactors would be the root of the leprosy endemia. Therefore, the schedule of antileprosy campaigns could be the following: contact population divided in reactors and non-reactors to soluble antigen, vaccination with the *M. leprae*–BCG mixture and later re-vaccination of the persistently non-reacting group. With this approach the antileprosy campaign would be secondary to the immunotherapy of 'non-reactors' after the first dose of vaccine, since by producing immunological changes in this population group we would prevent the creation of new infected foci and, therefore, the maintenance of the endemia.

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Reactions in leprosy

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Introduction

Without leprosy reactions, this feared disease would have constituted little more than a cosmetic problem. *Mycobacterium leprae* is close to being 'the perfect parasite'. Lepromatous patients can harbour up to 10^{13} bacilli in their body without overt symptoms or discomfort. On the other side, a tuberculoid leprosy patient can be seriously crippled containing a number of bacilli which is below the present detection level. The reason for this great discrepancy between degree of infection and degree of disease is the variability of the immune response to the invader, justifying leprosy being named an 'immunological disease'. During the course of the disease, roughly 25% of all leprosy patients develop such serious immune reactions that they become chronically disabled. This, the public (including patients), know. They also know that the present antileprosy treatment offers little protection to leprosy reactions, and many thus have no urge to register for therapy. A better understanding and handling of reactions would probably result in a major reduction in the world's non-registered leprosy patients, presently estimated to be more than 50% of all cases—and thus to better leprosy control. Not to mention the tremendous amount of human suffering caused by these reactions. This is perhaps best illustrated by the observation that suicide was the commonest cause of death in some leprosaria prior to the introduction of efficient pain control by erythema nodosum leprosum.

History

Fairly precise clinical descriptions of leprosy reactions were recorded from 1895,[1] and reached some degree of classification in Cochrane's classical textbook, 1959.[2] A more pathogenic, and still valid classification, was reached by fundamental histopathological studies, 1969.[3] Reactions in borderline leprosy, type I leprosy reactions or reversal reaction, were characterized by a heavy mononuclear

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infiltration and destruction of dermal nerves. Reactions in lepromatous leprosy, type II leprosy reactions or erythema nodosum leprosum (ENL), were dominated by an acute inflammatory exudate with mainly polymorphonucleated granulocytes centred around heavily infected macrophages. In this classification 'Lucio reaction' with an intensive necrotizing vasculitis on an ENL-suggestive background, and 'downgrading reactions' causing a shift in the clinical picture towards the lepromatous pole, were not given a definitive place. Lucio reactions have later on defended a specific position as a variant of ENL, while downgrading reactions at present have only a historic interest, as 'downgrading' will not occur in patients on efficient antileprosy treatment.

Type I leprosy reactions

The pioneer work in elucidating the mechanism of type I leprosy reactions was done by Rees & Weddell.[4] In *Mycobacterium leprae* infected mice deprived of cell-mediated immune responses by thymectomy and whole body irradiation, and then bone-marrow reconstituted, they could provoke a type I-like reaction by injection of T lymphocytes. The reaction occurred about 10 days after T-cell reconstitution, and the inflammatory infiltrate was predominated by small lymphocytes and activated macrophages. This timing fits well with the peak of the 'tuberculin-type' of delayed-type hypersensitivity (DTH) in intact mice infected with mycobacteria.[5] Godal *et al.*[6] found higher *in vitro* lymphocyte responses to *M. leprae* antigens in reactions compared to non-reactional borderline leprosy patients. Longitudinal studies,[7,8] showed that there was a temporary increase in these responses during type I reactions and that the *in vitro* lymphocyte responses to *M. leprae* antigens correlated well with clinical and histological evidence of DTH in the lesions. The *in vitro* lymphocyte stimulation test gave on average higher responses in reactional BL patients than in non-reactional BT patients, and was virtually negative in BT patients with an indeterminate histology. The latter patients, however, control bacillary growth fairly well, and the picture thus became similar to tuberculosis where immune protection and DTH expressed as tuberculin reactivity can be clearly separated experimentally.[9] This does not mean that DTH to *M. leprae* only causes immune complications in leprosy. Probably the positive function of this immune mechanism in leprosy is containment and prevention of spread of the infection—similar to Koch's phenomenon in tuberculosis—rather than a sterilizing effect which is neccessary for immune resistance.

For a long time it was speculated that antileprosy treatment with dapsone caused a sudden release of mycobacterial antigens which could trigger reactions. It was thus recommended that dapsone treatment should start with a small dose and build up slowly. The likelihood for a bacteriostatic drug causing a sudden release of bacillary antigen is theoretically small, and the bactericidal drug,

rifampicin, has not caused the expected increase in reactions. Barnetson[10] compared BT patients started on 5 mg and 50 mg of dapsone daily, and found no evidence of increased reactions in the latter group. Neither were concomitant infections or immunizations found to precipitate reactions.

On the other hand, nerve reactions have a tendency to start in segments of the nerves that are easily traumatized. In this situation a shift of bacilli to cells that better expose their antigens to immunocompetent lymphocytes is possible. Ordinarily leprosy bacilli have two host cells in the human body, skin macrophages and Schwann cells of myelinated nerves. For lymphocytes to respond with a DTH reaction, the antigen has to be exposed on a cell surface together with histocompatibility antigens of the HLA-D series. These histocompatibility antigens are known to be present on skin macrophages (and some other cells which can be infected, like Langhans' cells in epidermis), but are possibly lacking from the surface of Schwann cells. This might explain why BT patients can have large numbers of bacilli in some nerves without any neuritis and at the same time the skin can be severely inflamed with only a few bacilli detectable. A traumatic release of bacilli from Schwann cells will bring them into infiltrating macrophages and stimulate a DTH neuritis. Why this happens in only a quarter of the leprosy patients seems to have a genetic basis. DeVries[11] showed that patients in the tuberculoid part of the leprosy spectrum had an HLA-linked susceptibility to develop this specific type of leprosy. They later showed that this tendency was associated to the HLA-DR-3 antigen, an antigen which is associated to vigorous DTH reactions to other antigens as well.

Type I reactions seem to occur earlier in the treatment phase of BT than of BL patients who have more immunosuppressive factors in their plasma.[12, 13] They are rare during pregnancy when cell-mediated immunity is suppressed, but increase drastically in the puerperium when this suppression wanes.[14] The reactions are also efficiently controlled by immunosuppressive drugs such as corticosteroids.[15] These observations indicate that type I reactions are susceptible to a dynamic immune regulation in the patients. During reactions, BT patients' plasma change from suppression of global T-lymphocyte responses to an augmentation of these responses compatible to a loss of 'immunological brakes'. This augmentation by reactional plasma came, however, later than clinical signs of reaction in BL patients. Either the augmenting factor(s) were absorbed out at the receptor site early in BL reactions, or the phenomenon is secondary rather than primary to the reactions.[13] Stress and inflammation-induced humoral factors like corticosteroids and prostaglandins would expectantly have the opposite effect on the immune response. Specific antibody levels were not raised during reactions although general immunoglobulin levels increased.[16] The nature of the plasma factor(s) 'normally' controlling type I reactions is still unknown, but should be studied as they might offer interesting therapeutic possibilities.

The immune response is also under strict control of immunoregulatory cells. In murine leprosy,[17, 18] in fungal infections[19] and in schistosomiasis,[20] suppressor T

cells are involved. Suppressor cells in leprosy were first suggested as a pathogenic factor in lepromatous leprosy[21] and evidence for such suppression has been published,[22,23] but Nath *et al.*[24] using a different suppressor cell assay, found evidence for a decreased suppressor activity in lepromatous leprosy. As T-cell suppression can be both general and antigen specific, and different assay systems will reflect this differently, these results might not be contradictory. T lymphocytes can suppress specific DTH in mice,[25] and a similar suppression of *M. leprae*-induced DTH might be an important mechanism in borderline leprosy, failing its purpose in those who develop inflamed lesions and reactions. Interestingly, impaired suppressor function in myastenia gravis is linked to HLA-DR3 and cold-reactive lymphotoxic antibodies;[26] both recorded increases in tuberculoid leprosy patients.[27,28] HLA-D typing and testing for cold-reactive lymphotoxic antibodies might thus be a method to identify borderline leprosy at risk to develop reactions, but data to substantiate this hypothesis are lacking.

The nature of the antigen(s) involved in DTH to *M. leprae* is also incompletely known. Closs *et al.*[29] has purified a cell wall component (*M. leprae* cell wall ag. 1) which is a powerful antigenic stimulus for sensitized lymphocytes *in vitro*. A cross-reacting cell wall component from *M. tuberculosis* is the principal stimulating antigen in tuberculin. This antigen is present both on whole bacilli and in sonicates of *M. leprae*, and will probably lodge in the tissues long after bacilli are dead. Other antigens might be involved,[7] as suggested by a predominance of the response to whole bacilli in skin reactions and to sonicated bacilli in nerve reactions. This does not, however, exclude that the same antigenic determinant could be involved in both, only differently exposed in skin (mainly cell wall remnants of dead bacilli) and nerves (mainly live bacilli). The re-establishment of a positive lepromin reaction by injection of dead *M. leprae* and BCG,[30] followed by skin inflammation but no neuritis, could similarly be explained by a difference in skin and nerve presentation of antigen. This creates hope that cell-mediated immunity could be re-established in lepromatous leprosy without necessarily provoking neuritis.

Erythema nodosum leprosum

In erythema nodosum leprosum (ENL), granular deposits of immunoglobulin and complement were found to correspond to areas of clinical lesions with polymorphonuclear infiltration.[31] This suggested an immune complex genesis of the lesions which could be caused either by local release of antigen (Arthus phenomenon) or systemic release with circulating immune complexes ('serum sickness'). Other symptoms compatible with serum sickness—fever, albuminuria, arthritis, etc.—can occur in ENL, but are relatively rare complications. This, together with the finding of raised levels of C3d only in ENL patients[32] and the presence of circulating immune complexes also in non-ENL lepromatous

patients, suggested the importance of locally, extravascularly formed immune complexes. Activation of C3 by antibodies could explain all the observed pathology expressed in ENL. In cases of inadequate antibody responses immune complexes would be formed in antigen excess. Such complexes would circulate for a prolonged time and cause chronic kidney disease,[33] but this complication does not correlate with the occurrence of ENL.[34] ENL is often precipitated by intercurrent diseases like tuberculosis (or BCG vaccination), which could cause antigen release by immune reactions to cross-reacting antigens, and viral diseases (or live virus vaccines), which could cause release of mycobacterial antigens from cells concomitantly infected with virus.

Chronic and recurrent ENL is a very distressing complication in leprosy, and the only type of ENL which can cause chronic disability. This should logically be treated with plasma exchange in combination with a B-cell cytotoxic drug like metotrexate. As yet no study has been published which could show the efficiency of such an approach.

Lucio's phenomenon

The study of Rea & Ridley[35] has given strong support to the view that Lucio's phenomenon is a variant of ENL. The reason for its special clinical picture seems to be the presence of *Mycobacterium leprae* in endothelial cells. Antigen release from this location can cause an intensive vasculitis with thrombotic occlusion of vessels due to activation of coagulation by C6. The ethnic peculiarity of Mexican patients versus leprosy patients in other parts of the world is thrilling. They show more diffuse lepromatous leprosy with less neurotropism and more endothelial tropism of the infection, as well as a high incidence of Lucio reactions. This might indicate the existence of specific cell receptors for the penetrance of *M. leprae* determinant for the specific localization of the infection to certain host cells and possibly to a certain fraction of the population.

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Evolution of the modern chemotherapy of leprosy

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Certainly, the modern chemotherapy of leprosy has depended upon the availability of potent drugs. It could be argued that new, more potent, drugs would have been introduced into the chemotherapy of leprosy without the prior development of laboratory means by which to assess their antimicrobial potency and their efficacy in the chemotherapy of leprosy. However, the history of the evolution of modern chemotherapy is, in large part, the history of the development of more sensitive and more precise means of measuring the potency of drugs against *Mycobacterium leprae*, and of measuring the response of patients with multibacillary (i.e. lepromatous and borderline-lepromatous) leprosy to chemotherapy. In other words, the availability of potent drugs was a necessary but not a sufficient condition.

Because assessment of the efficacy of antimicrobial chemotherapy requires measurements made on the microorganisms themselves, the chemotherapy of paucibacillary (i.e. non-lepromatous) leprosy will not be considered. As the term implies, patients with paucibacillary leprosy ordinarily have such small populations of *M. leprae* that the effects of antimicrobial drugs on the organisms cannot be studied directly.

Three phases of the evolution of the modern chemotherapy of leprosy may be identified. The first phase, comprising the 20-year period 1940–60, was one of attempting to screen drugs for antimicrobial potency and to assess the efficacy of drugs in therapy, with the aid of imprecise techniques of measurement. The second phase was ushered in by Shepard's descriptions of his technique for cultivating *M. leprae* in the mouse foot pad,[46,47] and was concluded in 1974 by the demonstration of Rees and his colleagues[16,41,64] that *M. leprae* possess the

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capability of persisting. This 15-year period also saw the detection of drug-resistant *M. leprae*. Although at this moment the third phase of the evolution of modern chemotherapy has endured for only 9 years, it is already possible to characterize it as a period of increasing concern over the apparently increasing incidence of relapse caused by the emergence of *M. leprae* resistant to dapsone, the recognition that persisting *M. leprae* do not pose the great danger to multibacillary patients that had been anticipated, and a change in emphasis from determination of the antimicrobial potency of individual drugs to investigation of the chemotherapeutic efficacy of combined drug regimens.

It appears useful to consider the evolution of modern antileprosy chemotherapy under the two main headings of 'drug-screening' and 'evaluation of chemotherapeutic efficacy'.

Drug-screening

Usually, drugs are screened for antimicrobial potency by exposing the target organisms to the drugs in cell-free culture. It has been necessary to screen drugs for activity against *Mycobacterium leprae* by other means. This was first accomplished in a necessarily imprecise and sometimes hazardous way, by employing the drugs in the treatment of leprosy patients. Because there existed no more sensitive means of assessing the effects of treatment on the patients' disease, potency of drugs was measured in terms of clinical response and the decrease of the bacterial index (BI). The series of multicentre trials conducted by the Leonard Wood Memorial during the 1950's[7–13, 59] exemplifies this method of testing the potency of drugs. These trials necessitated the exposure of large numbers of patients to the hazards of treatment by untried drugs, or, because it was at first necessary to employ placebo-treated controls, to the hazards of remaining untreated. In addition, these methods were insensitive; when a valid measurement was obtained, it was all-or-none in character.

Following Shepard's reports[46, 47] of the multiplication of *M. leprae* in the foot pad of the immunologically normal mouse, and his first demonstrations in this system of the potency or lack of potency against *M. leprae* of the drugs then commonly used in the treatment of tuberculosis and leprosy,[50, 51] this has become the accepted means of measuring the potency of antileprosy drugs. In this system and its variants,[4, 48, 49] the results of testing drugs of established potency are sufficiently reproducible, that it is no longer necessary to screen drugs in patients. Moreover, it has been possible to measure the minimal inhibitory concentrations of many drugs, and to distinguish between primarily bactericidal and primarily bacteriostatic effects.

Although application of *M. leprae* infection of the foot pad of the normal mouse has represented a tremendous advance in screening drugs, the technique is not without its limitations. Large quantities of drug and many mice are required.

Moreover, because the mouse is interposed between the drug and the organism, there is always the risk that active compounds will be found inactive and inactive compounds active, because of metabolic alterations of the compounds by the mouse.

In the absence of techniques for cultivating *M. leprae in vitro*, workers have been forced to consider the use of cultivable mycobacterial species as surrogates of *M. leprae*. At first, this was done uncritically, and potency of a drug against, for example, *M. tuberculosis* or *M. marinum* was accepted as presumptive evidence of potency against *M. leprae*. Modern workers have proceeded far more thoughtfully. Arguing that, among the numerous cultivable mycobacterial species, some must resemble *M. leprae* more closely than do others in terms of a specific target enzyme or enzyme system, they have begun by screening species for sensitivity to a drug equivalent to that of *M. leprae*, or for a pattern of sensitivity to drugs of a specific class like that of *M. leprae*.

Thus, Seydel has employed as a surrogate of *M. leprae* the cultivable *'M. lufu'*,[36] a species that is highly susceptible to the antimicrobial action of dapsone.[45] And Pattyn has employed a battery of strains of rapidly growing mycobacterial species, principally strains of *M. phlei*, that appeared to exhibit the same pattern of sensitivity to rifampicin and several of its analogues as did *M. leprae*.[27,28]

Another approach to drug-screening without interposing a mammalian host between drug and *M. leprae* has involved inhibition of the uptake of radioactively labelled substrates by *M. leprae* in short-term culture. Khanolkar[1,18,19] has worked with *M. leprae* incubated in a modification of Murohashi's medium, whereas Nath and her coworkers[26,37,44] have employed *M. leprae*-infected macrophage monolayers.

Chemotherapeutic efficacy

Having available a number of potent drugs, it is necessary to assess their efficacy in the chemotherapy of leprosy, in order to learn best how to employ them. These two measurements—of potency and of efficacy—are quite different. Typically, potency is measured against a small number of *Mycobacterium leprae*, in terms of killing or of preventing the multiplication of a small number of organisms, all of which are genotypically and phenotypically identical. On the other hand, chemotherapeutic efficacy, at least in the treatment of multibacillary leprosy, is measured against much larger numbers of organisms, larger by 6–8 orders of magnitude, when disease has been established, and there has developed genetic and physiologic diversity among the members of the bacterial population.

In parallel with the developments in drug-screening, our ability to measure chemotherapeutic efficacy has developed greatly in the 22 years since the introduction of the mouse foot-pad technique. In the absence of an experimental model of the lepromatous patient, however, chemotherapeutic efficacy must be measured in patients.

PHASE ONE

Thus, Faget and his coworkers at the National Hansen's Disease Center in Carville, studied the efficacy of a series of sulphones, beginning with glucosulphone (Promin®).[15] These investigators and others working at that time had available to them three imprecise means by which to measure the efficacy of leprosy chemotherapy: 1, the response of the patients as assessed by repeated clinical observation; 2, the decrease of the BI; and 3, the rate of relapse after withdrawal of chemotherapy.

As was the case when antimicrobial potency was measured by administering drugs to patients (in fact, no distinction was made at that time between measurements of potency and those of efficacy), the measurements required exposure of large numbers of patients to unknown risks for long periods of time. In addition, the measurements were insensitive. They were applied to events far removed from the critical event—the encounter between organism and drug. Measurement of the BI is the only method of the three that directly involves *M. leprae*. However, the decrease of the BI depends only secondarily on the rate at which *M. leprae* are killed during effective chemotherapy, and reflects primarily the degradation of already killed *M. leprae*, a phenomenon which is not an immediate consequence of bacterial killing.[63] Moreover, so long as *M. leprae* are being killed in the course of effective chemotherapy, the rate of decrease of the BI bears no relationship to the rate of killing. On the contrary, the rate of decrease of the BI appears to be constant, approximating 1 $\log_{10}$ unit (90%) per year, at least during the first years of therapy of LL leprosy.[42, 52] Not only is the measurement of the BI an insensitive means of assessing chemotherapeutic efficacy. It is also imprecise, because the error of the measurement in the individual patient may be as large as the change expected during effective treatment.

Although the yield of the first 20 years of studies of leprosy chemotherapy was disappointingly small, this first phase saw the establishment of the efficacy of dapsone as monotherapy.

PHASE TWO

The morphological index

The experimental basis for modern leprosy chemotherapy depended upon the development of two laboratory techniques that rendered possible the direct study of the effects of antimicrobial drugs on *M. leprae*. The first of these techniques, measurement of the morphological index (MI), was based on the morphological changes of the organism that accompany its death. After earlier workers had called attention to the morphological changes undergone by *M. leprae* during treatment of leprosy patients,[6, 22, 25] Rees and his coworkers[39, 40] found evidence that morphologically imperfect *M. leprae* were, in fact, dead, and employed the

decrease of the MI during treatment as a means of measuring therapeutic efficacy in a series of trials of chemotherapy of multibacillary leprosy carried out in the Sungei Buloh Leprosarium in Malaysia.

The trials at Sungei Buloh established that, during treatment of previously untreated patients with lepromatous leprosy with dapsone alone in dosages totalling from 300 to 600 mg weekly, the MI decreased to baseline values within 3–6 months.[62, 63] The addition of macrocylon[60, 63] or ditophal[62] to the dapsone did not result in a more rapid decrease of the MI. Clofazimine, 300 mg daily, 6 days per week,[35] and 100 mg twice weekly,[61] and dapsone, 50 mg twice weekly,[31, 34] appeared as effective by this criterion as the larger, conventional dose of dapsone. This change of the MI in the course of effective chemotherapy was consistent with death of *M. leprae*, and thus provided the first more-or-less direct measure of the effect of antimicrobial drug on the organism. Thus, the MI permitted one to recognize effective antileprosy drugs during clinical trials of only a few months' duration involving only small numbers of patients, a goal previously attainable only by clinical trials enduring at least 1 year and involving several tens of patients as a minimum.

However, the change of the MI appeared rather insensitive as a measure of response to antimicrobial therapy. The rate of decrease of the MI appeared no less rapid during treatment with much smaller dosages of dapsone and clofazimine as during treatment with these drugs administered in full dosage. One explanation for this insensitivity is suggested in the report of the Committee on Experimental Chemotherapy.[5] Using, instead of the MI, the more rigorously defined 'solid ratio' of Shepard,[23] it is apparent that the average patient with lepromatous leprosy presents before any treatment with a solid ratio no greater than 10 per 100, i.e. no more than 10% of his organisms are solidly staining and, therefore, presumed infective for mice (viable). The patient may harbour as many as 10^{11} (100,000,000,000) *M. leprae*. Because it is not feasible to examine many more than 100 individual organisms in the course of the measurement, the solid ratio will have fallen to <1 per 100 organisms after only 90% of the viable *M. leprae* have been killed. At this time the patient still harbours as many as 10^9 viable organisms. Thus, the rate of loss of viable *M. leprae*, in terms of the decrease of the solid ratio, is measured only during a very small segment of the patient's response to chemotherapy. A second explanation of the insensitivity of the solid ratio is that the death of *M. leprae* is probably not rate-limiting; rather, the rate-limiting step appears to be a secondary change of morphology of dead bacilli that occurs at a more-or-less constant rate, which may be slower than the rate at which the bacilli are killed. Thus, although *M. leprae* were killed much more rapidly during therapy with rifampicin than during dapsone monotherapy, as measured by mouse inoculation, the solid ratio decreased at the same rate in both groups of patients.[54]

The mouse foot-pad technique

The application of Shepard's mouse foot-pad technique, the second of the two new laboratory techniques, to the measurements of the rate at which *M. leprae* are killed during effective antimicrobial treatment of the patient with lepromatous leprosy, first reported in 1968,[52] provided a more quantitative and sensitive means of evaluating the efficacy of chemotherapy by individual drugs. In this application, mouse inoculation is employed in much the same way that sputum culture is employed in assessing the efficacy of an antituberculosis drug. A biopsy is performed before the start of treatment, a 50–100 mg specimen providing 10^6–10^8 organisms if the patient is lepromatous and previously untreated. The specimen is minced and homogenized, and an aliquot is spread over the measured area of a microscope slide for acid-fast staining and direct enumeration of the *M. leprae*. The bacterial suspension is diluted to provide an inoculum of from 5,000 to 10,000 *M. leprae* per foot pad, and the hind foot pads of from 10 to 20 normal mice are inoculated. According to Shepard's technique, one mouse is sacrificed each month, beginning about 3 months after inoculation. The inoculated hind foot pad is processed for histopathological examination, and paraffin sections are stained by an acid-fast stain. The presence in a section of a lesion filling at least one-fourth of a × 540 microscope field with brightly staining organisms indicates the end of the 'incubation period', and is the signal for performing a harvest. The tissues, usually of four inoculated foot pads, are pooled, minced, and homogenized, and aliquots of the resulting bacterial suspension are spread uniformly over measured areas on slides, fixed and stained, and the *M. leprae* are enumerated. The generation time is then calculated as if multiplication of *M. leprae* had occurred at a constant rate from the day of inoculation until the day of harvest. The killing of *M. leprae* during therapy is indicated by progressive increases of the values for the incubation period and the generation time.

Employing this technique, a series of clinical trials was carried out in San Francisco and in Cebu, the Philippines. These trials established that, on the average, *M. leprae* recovered from patients lost their infectivity for mice after: 100 days of treatment with dapsone, 50–100 mg daily;[2, 20, 52–4] 150 days of treatment with clofazimine, 100–200 mg daily or 100 mg three times weekly;[3, 20] longer than 150 days with acedapsone (4,4′-diacetamidodiphenylsulphone, DADDS), 225 mg intramuscularly every 77 days,[53] or clofazimine, 600 mg every 2 weeks or 1,200 mg every 4 weeks;[3] and within a few days of single 1,200 or 1,500 mg doses or daily 600 mg doses of rifampicin.[2, 21, 54, 55] In a much less extensive study, the anti-*M. leprae* activity of ethionamide was established (Levy L, Shepard CC, Fasal P, unpublished results).

Thus, the second phase of the evolution of leprosy chemotherapy was marked by establishment of the efficacy in both absolute and relative terms of chemotherapy of multibacillary leprosy by dapsone, acedapsone, clofazimine, rifampicin and ethionamide, administered as monotherapy. These results also

pointed up the limitations of the mouse foot-pad technique as a measure of response to antimicrobial therapy. One limitation of the sensitivity of the technique is imposed by the maximal number of *M. leprae* that may be inoculated and be seen to have multiplied, if multiplication has indeed occurred. In immunologically normal mice, multiplication ceases when the number of *M. leprae* approaches 1–2 million per foot pad; the mice then appear to have mounted an effective immune response. Inocula larger than 100,000 per foot pad fail to give rise to multiplication, and inocula must be no larger than 10,000 per foot pad, if multiplication is to be recognized reliably. The failure of multiplication in mice, each inoculated with 10,000 *M. leprae*, implies only that the inoculum included fewer than 1:10,000 viable organisms, and tells us nothing about the remaining *M. leprae*, as many as 10^7 of which may be viable. A second limitation is imposed by the great rapidity of the bactericidal action of rifampicin. By inoculating mice, one cannot measure a rate of killing of *M. leprae* faster than that produced by rifampicin alone, as might be the case if a second active drug were used in combination with rifampicin.

PHASE THREE

Dapsone resistance

The third and current phase of the evolution of the modern chemotherapy of leprosy had its beginnings in the first reports by Pettit and Rees[32, 33] of relapse of lepromatous leprosy associated with the emergence of dapsone-resistant *M. leprae*, and in the first reported demonstration, also by Waters, Rees and their colleagues,[64] of persisting (surviving, fully drug-susceptible despite apparently adequate chemotherapy) *M. leprae*. The recognition of these two phenomena—drug resistance and microbial persistence—emphasized the shortcomings of the chemotherapy (almost always monotherapy) in use at the time.

Pettit & Rees[32] demonstrated at Sungei Buloh 3 patients with lepromatous leprosy whose disease had relapsed after 13–15 years of apparently adequate dapsone therapy; as measured by clinical observation and determination of the MI, these patients failed to respond to dapsone administered by injection in a dosage of 300 mg twice weekly, despite the achievement of adequate blood dapsone concentrations. The *M. leprae* isolated from these 3 patients by mouse inoculation were not inhibited from multiplying by administration to the mice of diet containing dapsone in a concentration of 0·1 g per 100 g, 1000-fold the minimal effective dosage of dapsone for *M. leprae* in the mouse.

Although dapsone resistance had occasionally been suspected before this study was carried out, these were the first proven cases. In their discussion, the authors stated that 'there are . . . very few cases which relapse under treatment'. The current situation, which represents a dramatic change during the 18 years since this report appeared, has recently been exhaustively reviewed.[24,29] Evidence

of the magnitude of the threat to leprosy control efforts posed by the apparently increasing prevalence of secondary resistance to dapsone was furnished by reports of high prevalence of primary resistance to dapsone. Estimates of the prevalence of primary dapsone resistance have varied from 3·3 per 100 in Cebu, Philippines,[17] and 35 per 100 in Chingleput, South India and Bamako, Mali,[58] to 55 per 100 in Addis Abada.[30]

Microbial persistence

The demonstration in 1966 by Rees [38] that T-cell depleted (adult-thymectomized, whole-body-irradiated, and bone-marrow-reconstituted; T900R) mice permitted multiplication of *M. leprae* to a higher limit than did normal mice provided the basis for an important advance in the assessment of chemotherapeutic efficacy. T900R mice regularly permit multiplication after inoculation of as many as 100,000 *M. leprae* per foot pad. Inoculation of these immune-deficient rodents with 100,000 *M. leprae* recovered from skin biopsy specimens obtained at intervals from multibacillary patients during treatment, although theoretically capable of greater sensitivity than the technique employing smaller inocula in normal mice, has not permitted us to measure chemotherapeutic efficacy with greater sensitivity. But the problem is no longer a limitation of the technique, but rather an important feature of multibacillary leprosy—the ubiquity of persisting *M. leprae*.

The first evidence that *M. leprae* are capable of persisting was reported by Waters, Rees and their colleagues.[64] Seven of 12 lepromatous patients who had completed at least 10 years of continuous dapsone therapy were found to harbour small numbers of *M. leprae* capable of infecting mice; three strains were passaged and found to be fully susceptible to dapsone. The detection of persisting *M. leprae* was reported subsequently in patients with lepromatous leprosy after treatment with acedapsone for 3 or 4 years,[43] after treatment with rifampicin as monotherapy in a daily dose of 600 mg for from 2 to 5 years,[41] and after treatment with the combination rifampicin plus dapsone, each drug administered in a full daily dose, for 6 months.[16]

The identification of surviving *M. leprae* as persisters appears best established by the results of acedapsone therapy reported by Russell and his coworkers.[43] In this study, the clue to the continued presence of significant proportions of viable organisms was the finding of solidly-staining organisms in the smears of one patient after 3 years of treatment, and in the smears of additional patients after treatment for 4 years. After the solid organisms had been seen, skin lesions were biopsied and mice were inoculated, with the result that normal mice were infected with *M. leprae* that proved to be susceptible to dapsone. Although mice had not been inoculated earlier in this study, it had been reported[53] that, as the result of another trial of acedapsone, *M. leprae* became non-infective for mice after about 6 months of acedapsone treatment. Moreover, solidly staining *M. leprae* had

disappeared by the end of the first year of treatment. The absolute number of viable organisms appears to have reached a minimum after 2 years of treatment; thereafter, the total number of *M. leprae* decreased still further, unmasking the persisting organisms, the proportion of which actually increased.

The identification of drug-susceptible *M. leprae* that survive some period of treatment as persisters may appear somewhat arbitrary. However, there arose the need to explain the apparent paradox that, for example, *M. leprae* are rendered non-infective for normal mice after treatment for only a few days with daiiy rifampicin,[21, 54, 55] whereas viable (i.e. mouse-infective) organisms may again be detected after 2 years of this same therapy.[41] The explanation lies partially in the insensitivity of the normal mouse; T900R mice are capable of detecting smaller proportions of viable *M. leprae* than may be detected in normal mice. These data suggest that, despite continued chemotherapy, *M. leprae* are not killed at the same rate as that measured initially. It is the remaining viable organisms that are identified as persisters.

The demonstrations that current leprosy chemotherapy was incapable of eradicating the *M. leprae* infection in patients with lepromatous leprosy, who are known to be immunologically deficient, appeared to confirm the widely-held belief, largely based on anecdotal evidence, that patients with lepromatous leprosy whose chemotherapy was stopped were very likely to suffer relapse, in the course of which they would become infectious to their contacts. Based on this belief, very long-term and even life-time chemotherapy of lepromatous leprosy was usually recommended (see, for example,[65]). As a result, leprosy control programmes have become burdened with the supervision of patients for periods of many years' duration, an impossible burden for all but the best-funded of programmes. The addition to such a programme of a second drug, almost by definition more expensive and more toxic than dapsone, has generally been impossible.

The current phase of the development of an effective chemotherapy of leprosy may be considered to have begun, therefore, from the recognition of the need to prevent the emergence of drug-resistant *M. leprae*, and to minimize persisting populations of *M. leprae*. More rapidly effective combined drug regimens are under study among patients with previously untreated lepromatous leprosy in formal clinical trials, in which persisting *M. leprae* are systematically sought. Sponsored by the Chemotherapy of Leprosy (THELEP) Scientific Working Group of the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases,[57] controlled clinical trials of combined drug regimens have been undertaken in Bamako, Mali, and in Chingleput, South India. In each centre, a 'maximal' regimen (in Bamako, daily rifampicin, prothionamide and dapsone, each in full dosage, for 2 years; in Chingleput, daily rifampicin, clofazimine and dapsone for 2 years) is compared with a 'minimal' regimen (daily dapsone for 2 years, together with a single, initial 1500 mg dose of rifampicin in both centres) and a regimen intermediate in terms of the duration of

combined chemotherapy (in Bamako, a 3-month course of prothionamide and dapsone administered daily and rifampicin weekly in a 900 mg dose, followed by daily dapsone alone for 21 months; in Chingleput, a single initial dose of rifampicin daily clofazimine and dapsone for 3 months, followed by dapsone alone for 21 months). These regimens were selected, not because they were considered optimal, but rather to learn if the 'stronger' regimens would effect a greater reduction of the population of persisting *M. leprae*. In fact, this does not appear to have occurred. Persisting *M. leprae* have thus far been detected in very few of the patients, and at least as frequently among the patients treated by the maximal regimens as among those treated by the other regimens (Subcommittee on clinical trials, unpublished results).

Risk of relapse

Prior to 1979, a trial of chemotherapy of multibacillary leprosy that involved deliberate cessation of therapy appeared unethical. In that year, however, the results became available of an 8–9 year follow-up in Sungei Buloh of 362 patients with multibacillary leprosy who were 'released from control'—that is, their treatment was stopped—after having been treated with dapsone as monotherapy for 20 years (Waters MFR, Rees RJW *et al.*, unpublished data); the relapse rate averaged only 1% per year. At the same time, the results were reported (Leiker DL, unpublished data) from Malta of 85 multibacillary patients who, after varying periods of dapsone monotherapy, had been treated with a 2-year course (on average) of daily dapsone and prothionamide, together with daily rifampicin for the first 6 months, and whose treatment had then been stopped. No relapses had been noted in the course of the succeeding 4–5 years. The results of these two studies suggested that relapse of long—or intensively—treated multibacillary leprosy might be far less likely than had been predicted, given the universal presence of persisting *M. leprae*, and the specific immune defect characteristic of these patients.

As soon as it became clear that chemotherapy might safely be stopped after prolonged treatment of multibacillary leprosy with high-quality dapsone monotherapy, or after intensive treatment of much shorter duration, THELEP decided to undertake field trials among large numbers of multibacillary patients of a practical multi-drug regimen, in which efficacy was to be measured in terms of the relapse rate. The THELEP field-trial regimen consists of rifampicin, clofazimine and acedapsone, all administered intermittently, and dapsone administered daily. This regimen is based on the demonstration that single doses of 600 mg rifampicin are equivalent in bactericidal activity to a number of daily doses,[21] and that rifampicin administered on two consecutive days once monthly is no less effective than rifampicin, 600 mg daily (Rees RJW, *et al.*, unpublished data). Clofazimine has also been shown to be active when administered intermittently.[3] Acedapsone and the first of two monthly doses of rifampicin and

clofazimine are administered under full supervision, at the time of a monthly clinic visit, at which time the patient is given the next day's doses of rifampicin and clofazimine and a month's supply of dapsone, all for self-administration. This regimen is to be administered for 2 years to multibacillary patients who are already smear-negative, and until smear-negativity but for a minimum of 2 years to smear-positive patients. After completion of the course of chemotherapy, the patient is to continue under observation, and be given placebo tablets, and randomly-collected urine specimens are to be analysed for the presence of dapsone. Follow-up is to continue for 8 or so years. When relapse is suspected, it is to be verified by biopsy, inoculation of normal mice, and testing the susceptibility of the patient's *M. leprae* to dapsone, clofazimine and rifampicin. Sample-size calculations have shown that, at the end of the trial, information on relapse must be available from at least 200 patients per regimen to permit distinction between an annual relapse rate smaller than 1 per 100 and a measured rate greater than 2 per 100 patients at risk.

The WHO Study Group on the Chemotherapy of Leprosy for Control Programmes has recommended[56] a similar regimen for the treatment of multibacillary leprosy. In this regimen, rifampicin is administered monthly, clofazimine monthly in one dosage and daily in another, and dapsone daily. The monthly doses are administered under supervision, and the daily doses are unsupervised. This regimen is also undergoing trial in the two THELEP field trials.

Because they include two bactericidal drugs in addition to dapsone, the THELEP field trial regimen and the WHO Study Group regimen should be fully effective in patients whose organisms are already dapsone-resistant, as well as those who have responded well to dapsone monotherapy, but who may well harbour a larger-than-normal subpopulation of dapsone-resistant *M. leprae*. Thus, no relapses are expected to be caused by the emergence of drug-resistant organisms, and those relapses that do occur should respond readily to retreatment by the same regimen. To prevent the emergence of drug-resistant organisms, combined chemotherapy with bactericidal drugs should be administered for so long as the patient's bacterial population is large enough to contain drug-resistant mutants. If the supplies of rifampicin and clofazimine are limited, they will be used most effectively by intermittent administration, thus prolonging the period during which they may be administered. This is, at least theoretically, much more sound than administering these drugs daily in a much shorter course of combined treatment. Finally, there is an obvious advantage to the supervised administration of drugs in the treatment of leprosy, in which poor compliance of patients with the prescribed treatment regimens so often results in failure of the treatment.[14]

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Dapsone-resistant leprosy

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Summary It is likely that in 1983 over 10% of patients with lepromatous leprosy have developed dapsone resistance; the primary resistance rate of new cases is probably about 25%. Patients with active dapsone-resistant leprosy who are still receiving only dapsone monotherapy may well form a larger source of infection than all other infectious cases. Supervisable and cost-effective drug regimens designed to prevent the emergence of dapsone resistance and to control the infectivity of dapsone-resistant cases deserve urgent consideration.

Dapsone is a very good drug. In orthodox dosage (1–2 mg per kg per day, that is, 50–100 mg daily for adults) it is unusual to get any side effects; and when used in low dosage (as occurs with acedapsone (DADDS), which releases 2–3 mg of dapsone daily) it is still active against leprosy caused by strains of *Mycobacterium leprae* that are fully sensitive to dapsone.[1] Moreover, it has a half-life of about a day[2] which ensures that blood levels can remain in the therapeutic range even if patients' compliance with prescribed treatment is 50% or even less.

The very high therapeutic ratio (blood dapsone concentration/minimal inhibitory concentration) together with the long multiplication time (about 2 weeks) of *M. leprae* were responsible for the 10- or 12-year period that elapsed before cases of dapsone-resistant leprosy began to be seen. The earliest probable cases were observed in Sungei Buloh Leprosarium, Malaysia in about 1960 (unpublished data); these patients were among the first in the world to receive dapsone, which began to be used in Sungei Buloh in 1948. However, at that time it was only possible to suspect the diagnosis, as *M. leprae* had not yet been cultivated in the experimental animal.

One of the fortunate coincidences in leprosy was that, just when patients with dapsone-resistant leprosy began to appear, the mouse foot-pad technique for obtaining limited multiplication of *M. leprae* was discovered by Shepard.[3] Thus, exactly when it was needed, it became possible to test strains of *M. leprae* for dapsone resistance. For this purpose the mouse foot pad may be regarded as a free-range test-tube; if *M. leprae* multiply even when the mouse is fed dapsone incorporated into the diet, the bacilli must be resistant to dapsone.

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Rees was the first to use mouse foot-pad tests to study dapsone-resistant leprosy; and, with his clinical colleagues, was the dominant worker in the field for several years. The first cases of dapsone-resistant leprosy with mouse foot-pad proof were published in 1964,[4] and in 1968 the first case of 'partial' resistance was recorded[5] thus initiating study of the important question of different degrees of resistance. By 1975 a series of 100 cases had been reported from Sungei Buloh.[6]

By this time the fact that patients could develop dapsone-resistant leprosy was well recognized, but there was much doubt about how commonly it happened. The figures from Malaysia were from a selected population of patients, most (but not all) of whom were permanent hospital inmates. It was hard to determine the denominator of the figure (i.e. the number of patients at risk from whom these cases were derived); but on an estimate of 2,000, which is probably on the low side, the prevalence in 1975 was still only about 5%. More information was needed from other parts of the world, and preferably obtained by some sort of formal survey/review procedure.

At about this time the results of two such surveys became available, in Costa Rica[7] and Israel.[8] These gave prevalences of 6·8 and 3·7 respectively, that is, in the same order as that seen in Malaysia. These results, however, were rather quickly overshadowed by figures from Ethiopia. In 1973 Dr Rees and his co-workers initiated the Medical Research Council (MRC) Leprosy Project in the Addis Ababa Leprosy Hospital. This hospital, with its out-patient clinics, had an effective monopoly of anti-leprosy treatment in the Addis Ababa area; and all cases with clinical suspicion of dapsone-resistant leprosy were referred to the MRC Project for assessment. Thus, though not the result of formal surveys, it was possible to obtain a fairly accurate indication both of the prevalence and of the year by year incidence of dapsone-resistant leprosy in the Addis Ababa area.

The first results of these studies appeared in 1976[9] and showed a prevalence of about 10–20% (depending on whether cases with very strong suspicion, but not at that time clinically or mouse foot-pad proven, were or were not included). More alarming, however, was the incidence figure of some 50 new cases per year derived from an 'at risk' population of about 1,500 patients—that is, an incidence of 3% per year. This figure showed no change during the 5-year life-span of the MRC Project. If continued unchecked, it would ensure that about half the patients with lepromatous leprosy in the Addis Ababa area would develop dapsone resistance by the mid-1980's.

Since 1975 a number of surveys have been carried out in different parts of the world, many of them supported by THELEP and using a standard protocol developed for the purpose. These studies[10–15] indicate a rather uniform pattern of 5–10% prevalence of dapsone-resistant leprosy. These figures, while reassuring in comparison with the Ethiopian results, should not lead to complacency. First of all, new cases will continue to occur (unless preventive measures are undertaken); and an annual incidence of only 1% per year will raise the prevalence to almost

20% in a decade or so. Secondly, without early diagnosis and effective treatment these patients will initiate an epidemic of primary dapsone-resistant leprosy.

The first results of a series of patients tested for primary dapsone-resistant leprosy were reported by Rees and his group from the MRC Project in Addis Ababa in 1977;[16] they showed that 5 out of 8 patients tested were resistant to dapsone, though the resistance was in most cases low grade. Results of the full series[17] confirmed the initial findings; 5 out of 14 patients living in the Addis Ababa area were resistant, as were 11 out of 15 who lived away from the city.

These results were unexpected but in retrospect logical. By the early 1970's about 50 new cases of leprosy, previously untreated, were registered for treatment each year in the Addis Ababa area; and there were about the same number of patients per year who relapsed due to the emergence of dapsone-resistant leprosy. Thus there were about equal numbers of probably sensitive and probably resistant infectious cases; the finding that, a few years later, about half the new cases had been infected with dapsone-resistant *M. leprae* was by no means unreasonable.

The primary dapsone-resistant rate is a measure of the epidemiological importance of the cases of acquired (secondary) resistance. A number of surveys to determine the primary resistance rate have been initiated, but few results are currently available;[18–20] rates from 4 to 60% have been observed. The largest series has been obtained as part of the THELEP multidrug trials in India and West Africa.[21] Tests for primary dapsone resistance were initiated in all these patients before treatment was commenced; both centres show rates of about 30%.

One may optimistically summarize the probable general situation in regard to dapsone-resistant leprosy as follows:

1 In most parts of the world about 10% of patients with lepromatous leprosy have already relapsed with dapsone-resistant leprosy. A further 1% or more will continue to relapse each year. If they are not treated with non-sulphone drugs they will be transmitting dapsone-resistant leprosy to their contacts.
2 In most parts of the world a significant proportion of new cases are already infected with dapsone-resistant strains of *M. leprae*. These patients will, however, improve for a while on dapsone monotherapy in full dosage unless the bacilli show very high grade resistance.

Three large-scale objectives for leprosy chemotherapy can now be defined. The first, and the one to which most attention has been directed, is to apply 'maximal' treatment regimens which offer the prospect of cure to most patients, even those with lepromatous leprosy, and even if they are infected with dapsone-resistant strains of *M. leprae*. Such a regimen is currently advocated by WHO.[22] It is demanding both financially and operationally, particularly as regards supervision (often a weak link in leprosy control programmes). Even if government and voluntary agencies give the greatest priority to the supply of

drugs and training of personnel, it will be extremely difficult to apply this regimen universally in the next decade.

The second and third objectives are aimed towards controlling the spread of leprosy. The second, and easier to attain, is to apply supplementary chemotherapy to prevent the emergence of dapsone resistance in patients apparently responding well to dapsone monotherapy. Patients with secondary dapsone-resistant leprosy now make up a significant proportion of all infectious cases, and simple ways of cutting short the present epidemic of dapsone-resistant leprosy deserve urgent investigation.

The third objective is to apply cost effective and supervisable drug regimens which are as effective against dapsone-resistant leprosy as dapsone (taken regularly and in full dosage) is against fully sensitive strains of *M. leprae*. The spread of primary dapsone-resistant leprosy can only be controlled by the early diagnosis and effective treatment of patients with secondary dapsone resistance. Diagnosis can readily be achieved under field conditions by straightforward surveys; but more needs to be known about cost-effective treatment to control the disease, render patients non-infectious, maintain non-infectivity for many years, and avoid multiple drug resistance.

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I am grateful to many colleagues who have helped me to see the problem of dapsone-resistant leprosy in the context of leprosy control, and particularly Dr U Kyaw Lwin, Dr U Maung Maung Gyi, and Dr J C Cap. Dhoolpet Leprosy Research Centre is the 'City Centre Clinic' of Victoria Hospital, Dichpalli.

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A Note from the Editor to Contributors of *Leprosy Review*

During the past year we have occasionally had problems with the receipt, acknowledgement, posting and preparation of manuscripts, and we take this opportunity to draw attention to a few points which may ease the editorial 'process'.

1 Envelopes and packaging. Quite a number of manuscripts have been received with the envelope frayed, or even open along the edges. A strong envelope is essential and the use of plastic 'grips' or spines to hold pages together should be avoided, since they cut through the paper.

2 Originals and copies. We need a clear (black) original and an equally clear copy. We usually have to make at least two additional copies and this is impossible from a faint or poor quality original. Good quality photostat copies are preferable to carbon copies. Artwork, especially lettering, should be sufficiently clear to stand a reduction of about 60%.

3 Return of manuscripts to authors. The current costs of correspondence and air mail postage incurred by this Journal are already considerable. We regret that it is not possible, except under exceptional circumstances, to return manuscripts, photographs or artwork to authors. If a paper has not been found suitable for publication, we retain it here for reference for a period of 1 year, after which it is discarded.

4 Addressing. All matters to do with *manuscripts*, *publishing*, *printing* and the *editorial 'process'* should be addressed to the Editor or Editorial Assistant at the Slade Hospital, Headington, Oxford OX3 7JH, England. All matters to do with *subscriptions to the Journal*, *postage* and *distribution*, should be referred to LEPRA, Fairfax House, Causton Road, Colchester CO1 1PU, England. On several occasions, authors have changed address without letting us know. Please indicate your address for reply or any likely change of address in the near future.

5 Titles. Both for the purposes of our own indexing of this Journal, but even more importantly for general indexing and abstracting systems, it is important in most instances to get 'leprosy' (or some related word) into the title as a general guide to the subject matter.

6 Summaries. Authors, especially those working in pure or basic science, are asked to keep in mind that this Journal has a wide-ranging readership. Many subscribers do not appreciate the significance of scientific data *per se* and it would be of great help if authors could include in their summaries a brief note explaining *why* the study was undertaken and *what the results mean*, in terms which are likely to be comprehensible to the reader whose background is not scientific.

Thank you,
EDITOR

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