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Leprosy Review

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Leprosy Review is published by the British Leprosy Relief Association (LEPRA) with the main objective of contributing towards the better understanding of leprosy and its control. Original papers on all aspects of leprosy, including research, are welcomed. In addition, *Leprosy Review* seeks to publish information of educational value which is of direct benefit to the control of leprosy under field conditions, and hence to the individual patient. The Journal aims to interpret what is being done in other disciplines, particularly for field workers.

From time to time the Editorial Board invites special articles or editorials from experts in various parts of the world, and gives consideration to the production of a supplement or special number devoted to a particular subject or theme of major importance.

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Editorial

RELAPSE FOLLOWING VARIOUS TYPES OF MULTIDRUG THERAPY IN MULTIBACILLARY LEPROSY

Introduction

One of commonest questions asked by leprosy patients on their completing multidrug therapy (MDT) is, 'Am I cured?'. There are, of course, several different facets to this question, including the risk of subsequent physical deterioration due to damage to anaesthetic hands or feet. But, from the point of view of chemotherapy, the questioner is in effect enquiring whether all the leprosy bacilli in his or her body have been successfully killed, or, if tiny numbers of dormant living bacilli ('persisters') still survive, what is the risk that these may subsequently resume multiplication and eventually cause clinical relapse? The fear that the disease might 'come back' and that the patient could infect family members, especially new children or grandchildren, is very deep seated. How honestly can we reply, and what is the scientific evidence to date?

Relapse rates provide the ultimate proof of successful treatment of infectious diseases, even though regimens need also to be assessed in terms of acceptability, the incidence of toxic side-effects, duration and ease of treatment, and cost. In tuberculosis, an accurate assessment of relapse rates can be achieved within 2 years of stopping chemotherapy. Admittedly, small numbers of apparently cured patients may relapse years later, sometimes as a result of stress or intercurrent disease of immunosuppression, but these late failures do not materially affect the overall assessment of a regimen. Similarly, in cancer treatment, a good overall assessment is obtained by a 5-year survival rate, even though small numbers of patients may die from a recurrence a decade or even several decades later.

The duration of follow-up required in leprosy after completing MDT (release from treatment, RFT) to give an adequate overall assessment of subsequent relapse rates is not yet known. The WHO Study Group on the *Chemotherapy of leprosy for control programmes* report was published in 1982,[1] and therefore by 1994 we have at best only 10 years' post RFT experience in multibacillary leprosy (MBL). Therefore it is important to consider those results obtained, not only with WHO MDT, but with dapsone monotherapy and with other MDT regimens.

Perhaps dapsone monotherapy is the least helpful. But it is worth recalling that

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Pearson and his colleagues in Malaysia found that the average incubation period for dapsone-resistant relapse, in LL and BL patients *still* receiving dapsone, was 15·8 (range 3–24) years after commencing sulphone therapy;[2] in Ethiopia it was 8·7 (range 2–20) years;[3] and rarely, it can take over 30 years.[4]

Relapse rates and the timing of relapse after completing dapsone monotherapy vary somewhat, depending partly on the dose of dapsone given, and the duration and regularity of treatment. Becx-Bleumink[5] studied 1123 MBL patients who had undergone RFT between December 1983 and July 1987. All had been skin-smear negative at RFT. On a 6–7 year follow-up, 148 patients were considered to have relapsed, giving an overall relapse rate of 13·2%, or 24·8 per 1000 patient years. Although the annual relapse rate was significantly lower during the 5th to 7th years after stopping treatment (10·0–13·5 per 1000 patient years after relapse) compared to the first 4 years (25·2–39·4 per 1000 patient years), it was still unacceptably high. Cartel *et al.* followed a small group of 131 MBL patients for up to 36 years after RFT; the average risk of relapse was 1·39 per 100 patient years, and the risk did not vary significantly with time.[6] Waters *et al.*[7] studied 362 LL and BL in-patients treated with supervised dapsone monotherapy in Malaysia for 18·5–22 years until July 1970. All had been smear negative for at least 5 years at RFT. Over the next 8–9 years, 25 patients (8·6%) relapsed, with an average relapse risk of 1·04 per 100 patient years of observation, and there was no significant difference in the relapse rate throughout the 9 years. Of 8 strains of *Mycobacterium leprae* isolated from patients in relapse, 5 were found to exhibit some level of dapsone resistance in mice, whereas 3 were dapsone sensitive. If it can be assumed that in the remaining 17 relapse patients, whose strains of *M. leprae* were not tested, a similar proportion were also dapsone resistant, then the risk of relapse due to persisters was surprisingly small (0·4 per 100 person years). But, from among all the lepromatous patients who had commenced similar sulphone therapy in the leprosarium between 1948 and 1951, 52 had relapsed between 1960 and 1972 with proven dapsone resistance. Therefore failure of treatment due to the development of dapsone resistance proved to be a much greater risk overall than that of relapse due to microbial persistence on stopping treatment after 20 years of outstandingly good dapsone monotherapy. MDT is designed to prevent the emergence of drug resistance, but what effect it has on persistence is uncertain. Admittedly, untreated lepromatous patients who received rifampicin 600 mg plus dapsone 100 mg daily had at 6 months statistically fewer viable persisting bacilli detectable in immunosuppressed mice than did similar patients treated with dapsone 100 mg daily.[8] But this difference might have been due to the much faster rate of kill with rifampicin, the drug's ability to kill intermittently (by analogy with *M. tuberculosis*) or slowly metabolizing *M. leprae*, and also its ability to kill dapsone-resistant bacilli. What is important is to measure relapse rates after MBL MDT, whether given for 2 years only, or until skin-smear negativity, placing these results in the context of relapse rates obtained with other (non-WHO) MDT regimens, some of which have been followed up for long periods.

Relapse rates with various (non-WHO) regimens

1 THE MALTA PROJECT

Fear of persisters as a cause of relapse in LL and BL leprosy was reduced considerably

by the Malta project.[9] The trial commenced in 1972, at a time when most leprologists would not have had enough courage to stop chemotherapy after a limited period; it was brilliant in concept, although somewhat disappointing in details. Combined chemotherapy with daily rifampicin, dapsone, prothionamide, and isoniazid (the last 3 as Isoprodian) was administered to a mixed group of 257 patients, of whom about 200 were LL and BL, at all stages of treatment. Many had received years of dapsone monotherapy, and some had also received thiambutosine. The tuberculoid patients in general received about 6 months' treatment, and the LL and BL patients 21–25 months' treatment, although there was considerable variation, and the duration was longer for new patients. In about half the MBL patients, daily rifampicin was given for only the first 5–10 months, treatment being subsequently continued with Isoprodian alone. The criteria for stopping all treatment were not well defined. In 1992, a 20-year assessment was made of 92 MBL patients by Jacobson, which was reported to the Würzburg Symposium, and the 14th International Leprosy Congress.[10] Only 2 of the 92 patients were found to have relapsed. The first was diagnosed in 1949, received dapsone irregularly until 1972, and was smear positive in 1974, when she commenced 21 months of multidrug therapy. She relapsed in 1989 at the age of 85, with lesions which were clinically and bacteriologically LL. The second patient was diagnosed in 1967, and was smear negative on commencing, in 1972, 5 months of daily rifampicin and Isoprodian, followed by 16 months of Isoprodian. He relapsed in 1991 with BL leprosy. These results are undoubtedly very satisfactory, although almost all the patients had received years of dapsone monotherapy.

2 PARAGUAY LEPROSY CONTROL

Following the success of the Malta project, a similar treatment in the dosage of rifampicin 450 mg, prothionamide 350 mg, dapsone 100 mg, and isoniazid 350 mg daily, was commenced in Paraguay in 1979. To date, 5504 patients (MB 78%, PB 22%) have been treated, of whom 2396 were released, usually after 2 years (MBL), or 6 months (PBL), of treatment. In a post-therapy follow-up period of 5–10 (average 8·8) years, relapse has been diagnosed in 11 (0·6%) of 1846 MBL, but none in PBL patients. No relapses were detected during the first 5 years of follow-up (Alvarenga, Leguizamon and von Ballestrem; report submitted to the Pre-Congress Workshop on Chemotherapy, 14th International Leprosy Congress, 1993). It would be very helpful to know the number of previously untreated patients, as compared to those who had received dapsone monotherapy prior to rifampicin–Isoprodian MDT; nevertheless, these results strongly support the concept of limited duration MDT in MB leprosy.

3 STUDIES AT THE INSTITUT MARCHOUX

One of the most helpful pieces of opportunistic leprosy research in the past decade has been performed by the Marchoux Chemotherapy Study Group.[11] Influenced by short course chemotherapy in tuberculosis, Professor S. R. Pattyn of Antwerp and colleagues at the Institut Marchoux commenced in 1977 a series of chemotherapy trials of rifampicin-containing regimens of limited duration in LL and BL leprosy, and the Institut also joined the first THELEP controlled clinical trial in 1978. In these studies, in 11 of the 12 regimens, all drug doses were given supervised, 'daily' drugs being given on 6

days a week, and only in the 12th, the WHO MBL MDT regimen, were daily drugs given unsupervised 7 days a week. The first 2 regimens, given to untreated LL and BL patients, consisted either of rifampicin 600 mg twice weekly plus dapsone 'daily' for 6 months, followed by dapsone monotherapy for 6 months, before stopping all treatment (regimen A), or similar chemotherapy plus prothionamide 500 mg 'daily' during the first 6 months (regimen B). From 1978 to 1983, previously untreated LL patients were admitted to the WHO THELEP trial,[12] BL patients only being included during the second half of the 5-year intake. In all the other 7 studies, both LL and BL patients were admitted; untreated patients only were admitted to the 6-week (S6) regimen, otherwise the intake consisted of a mixture of untreated and relapsed patients (after dapsone monotherapy, or dapsone plus, usually, a single dose of rifampicin) or patients who had received dapsone for at least 5 years without clinical relapse, but still had a BI of 2+ or greater. Clofazimine-containing regimens were only given to patients who had received more than 5 years of dapsone monotherapy, or who had proven dapsone resistance. The THELEP and the WHO MDT regimens were given for 2 years, 4 regimens were given for 1 year (including regimens A and B), 2 for 3 months, 1 for 6 weeks, and 1 for 4 weeks. Thereafter, no chemotherapy was given. Of the 532 patients admitted to trials, 437 completed the prescribed treatments, and 384 were followed up for at least 1 year after RFT.

The Marchoux Chemotherapy Study Group studied subsequent relapses in the 384 patients. Relapse was carefully defined. It was suspected whenever the BI at any site showed an increase of at least 2+ greater than the previous value, or when a new lesion was observed with a BI greater than any pre-existing lesion. In most cases, biopsies were taken, both for histopathology (for the presence of young histiocyte granulomas containing solidly stained bacilli), and for separation of *M. leprae* for inoculation into the footpads of normal mice. The careful definition of relapse, together with confirmation of the presence of viable bacilli by mouse footpad inoculation, makes this study especially valuable.

By May 1991, relapse had been observed in 68 (17·7%) of the patients, and confirmed by multiplication of their strains of *M. leprae* in mice in 54 of the first 61. The relapses tended to occur late, about 5 ± 2 years after RFT; in general, the shorter the duration of rifampicin treatment, the earlier the appearance of relapse. It is unfortunate that the most powerful regimen THELEP A_2 ('daily' rifampicin, prothionamide, and dapsone) resulted in the frequent development of hepatitis so that intake was abandoned. Only 5 patients were followed long term; 1 of them relapsed in the 7th year, but it is notable that he had received steroid therapy for erythema nodosum leprosum for 1 year after stopping chemotherapy. The WHO MDT regimen had the shortest, inadequate follow up of only 4 years (see below). In the 2 oldest regimens from 1977, no patient had relapsed after 5 years, but at the end of 9 years, 5 of 18 (27·8%) regimen A and at the end of 10 years, 4 of 16 (25%) regimen B patients had relapsed. In regimen S_4, the 1-month regimen, the first relapse was detected in the 2nd year, at the end of 5 years 17 of 88 (19·3%) and at the end of 7 years 24 of 88 (27·3%) patients had relapsed. There was evidence that the risk of relapse was greater in patients with a high BI at RFT, than in those with a BI of $\leqslant 4+$, and the risk was also less in those who at some stage became smear negative.

A recent analysis of January 1993,[13] reported from 435 patients a total of 100 relapses, of which 66 had already been confirmed in mice; all the isolated strains

remained susceptible to rifampicin. Relapses occurred in the 2nd to the 14th (current) year after RFT.

At 5 years after RFT, the risk of relapse, 1·7%, was best in patients from the 12-month regimens, although it had risen to 20% at 10 years; the rate per 100 person years being 3·9; 3-month regimens gave a risk of relapse at 5 years of 5·1%, and the 1-month regimen of 12·5%. The 2-year regimens gave a 5-year risk of relapse of 5·2% (and 10 years of 33%), but it should be remembered that the THELEP C regimen consisted of only a single 1500 mg dose of rifampicin plus 2 years of dapsone, and their E_2 regimen of 13 weekly 900 mg doses of rifampicin plus 'daily' prothionamide, suggesting that the 2nd year of dapsone monotherapy had little effect on overall relapse rates.

4 PATTYN'S STUDIES

Not only was Pattyn associated with the Bamako studies, but also with other centres, especially in Zaire. Some regimens were common to both countries. Pattyn analysed both sets of figures,[14] although as he tabulated cumulative relapse rates calculated by a life table method, there is no straight comparison between the Marchoux results[11] and Pattyn's results.[14] Nevertheless, Pattyn made a number of important observations. Relapse rates were higher at the Institut Marchoux than in Zaire, where relapses tended to occur later. But BIs, at the start of treatment in the patients in Bamako were significantly higher than in the patients in Zaire. The higher the initial BI, the greater the risk of relapse, and this risk increased with time. Relapses occurring during the first 3 years after RFT at a rate higher than 1% might indicate a later high incidence of relapse, although the absence of relapses during the first 3 years did not exclude later high relapse rates. The combination of rifampicin with a thioamide in very short-course chemotherapy was insufficient (this surely indicates the need for careful controlled trials of combinations of rifampicin with ofloxacin, minocycline, and clarithromycin). Finally, he suggested that results with 'daily' rifampicin given for 8 weeks or 26 weeks were better than with intermittent rifampicin. However, the regimens given the 'best' results were only tried in Zaire and not at Bamako. Therefore, perhaps a full 10 years of follow-up are essential for the Zaire trails, especially as Pattyn himself emphasized that in the evaluation of the treatment of MB leprosy, it is necessary to follow up the patients for 9–10 years after RFT. Until then, an alternative explanation can be maintained, namely that the total dosage of rifampicin and to a lesser extent the total duration of treatment, are the important factors influencing relapse rates.

Relapse rates with WHO MDT

In chemotherapy research, once a drug or drug regimen has been shown to be active in a pilot trial, a controlled clinical trial is set up, in which individual patients are studied in detail, and if successful, the regimen is applied generally, and evaluated for its public health impact as well as individual effectiveness under field conditions. Because of the dapsone resistance epidemic in 1982, WHO MDT could not be evaluated in this classical programmed way, because it was considered unethical to set up controlled trials using dapsone monotherapy as the control regimen. Nevertheless, the WHO THELEP steering committee did set up, in 1982, very careful field studies in South India.

1 THELEP KARIGIRI AND POLAMBAKKAM MDT FIELD STUDIES

The aim was to include all LL and BL patients in the 2 control areas, save for those with significant intercurrent disease. Therefore, the majority of patients had received long-term dapsone monotherapy, although newly diagnosed LL and BL patients were admitted over the next 2 years. Patients received chemotherapy for 2 years, or until they achieved smear negativity, whichever was the longer. In all, 2 regimens were compared, WHO MBL MDT and a THELEP regimen in which 600 mg rifampicin and 600 mg clofazimine were given supervised on 2 consecutive days a month, plus unsupervised dapsone 100 mg daily. No significant difference has been found in their results as judged by relapse rates.

At Karigiri, about 980 patients were admitted to the trial, of whom almost 80% were LL. After a 7–8 year follow-up, relapses did not exceed 3 in number. A report on the Polambakkam results was presented at the 14th International Leprosy Congress.[15] Of 1174 patients admitted to the trial (of whom only 146 (12·4%) were smear positive), 979 were followed up for 8–9 years until 1993—8 relapses (0·82%) were detected, of whom 3 were smear positive BL, whereas the other 5 were smear negative, classified as PBL, BT or TT. The presence of viable bacilli from the first BL relapse was confirmed in mice, and the strain was fully sensitive to rifampicin, clofazimine and dapsone; 2 of the relapse patients had received the THELEP regimen and 6 WHO MDT. To date, therefore, 2 years of MDT given to long-term dapsone treated, smear negative LL and BL patients, appears to have been outstandingly successful.

2 KARIGIRI SECOND FIELD TRIAL

When the intake of the first field trial was completed, all newly-diagnosed MBL patients were given WHO MDT, for 2 years only. To date, patients' BIs have continued to fall at the normal rate, and by August 1993, no relapses had occurred.[16] Whereas in the first trial, almost 80% of patients admitted were LL, in the second trial almost 80% were BL. It is likely that, in this excellent control area, many were diagnosed relatively early, before their bacterial loads were high. The next 5 years' follow-up will be crucial.

3 INSTITUT MARCHOUX—EXPERIENCE WITH LIMITED DURATION WHO MDT

At Bamako, between 1984 and 1986, patients were recruited for limited duration MDT. Only 44 completed the course of 24 doses of monthly rifampicin, and 35 were available for long-term follow-up. By May 1991, 1 patient only had relapsed (in the 3rd year),[11] giving a relapse rate of 0·8 per 100 patient years of observation. By 1992,[14] the relapse rate had risen to 10%. In 1994, Ji and his colleagues reanalysed the WHO MBL MDT regimen (Ji, personal communication). By now, 7 of the 35 patients had relapsed, including 4 in the 6–7th years of follow-up and 1 in the 8th year, giving an overall relapse rate of 20%. The mean time of relapse, to date, was $62{\cdot}7 \pm 18{\cdot}7$ months. All 7 relapses had occurred in patients whose pretreatment BIs were $\geqslant 4{\cdot}0$. Therefore the risk of relapse was significantly greater in patients with an initial high bacterial load, which includes moderately advanced LL and advanced LL and BL patients. This series also suggested that follow-up needs to be greater than 5 years, probably 8–10 years to give a good assessment of relapse rates.

4 WHO POST-MDT QUESTIONNAIRE SURVEY

WHO has recently carried out 2 surveys of post-MDT relapse rates.[17] The pilot survey covered 92 194 MB patients; 467 were reported to have relapsed, giving an overall relapse rate of 0·23 per 100 person years. A 2nd, extended survey, of cohorts of patients at 28 selected centres, included 20 141 MB patients, of whom 1414 were followed until their 9th year after completing MDT—67 patients were considered to have relapsed, with a cumulative risk of relapse of 0·77%. Less than half the patients were followed for 5 or more years, therefore although the mean and median times of relapse were 3·42 and 3·0 years, respectively, it is important to note that the relapse rates in the 6th and 7th years were similar to those of the 3rd and 4th years. The WHO survey results are outstandingly good, fully substantiating faith in WHO MB MDT. But it should also be noted that no separate analyses have been made for patients who had previously received long-term dapsone monotherapy, and were smear negative on commencing MDT, and for previously untreated patients, subgrouped into those treated for 2 years only, and those treated until smear negativity. Further time is needed to collect these data.

Conclusions

The results for MBL following the introduction of WHO MDT have been excellent. The epidemic of secondary dapsone resistance has been aborted, and treatment of limited duration (even if continued until smear negativity) has been successfully introduced. Because of the epidemic of dapsone resistance, most thinking leprologists by the early 1970s concluded that some form of MDT was essential, analogous to combined chemotherapy in tuberculosis. The introduction of rifampicin in 1970,[18,19] and the early results from the pilot Malta project[20] showed that limited duration MDT was a real possibility. WHO MDT has proved very robust under field conditions.

The number of relapses reported to date have been minute, although the majority of patients followed for 8–10 years since release from treatment probably belong to the smear-negative long-term dapsone monotherapy group; as such their bacterial load of *M. leprae* at the start of MDT would have been tiny. In the first 5 years after the introduction of MDT most newly-diagnosed LL and BL patients and most relapse patients (whether from dapsone resistance or from discontinuing treatment) are likely to have been kept on MDT until becoming smear negative; that is for 4–10 years.

Criteria for defining relapse may vary from centre to centre,[17] and an apparently high relapse rate may reflect too loose a definition (Smith, Jesudasan and Jakeman, personal communication). This editorial has concentrated on centres where relapse has been defined clinically, bacteriologically, and histologically, and where the presence of viable *M. leprae* (proof of bacteriological relapse) has usually been confirmed by multiplication in mouse footpads. These results suggest that:

1 Risk of relapse is very low in old smear negative LL and BL patients, some of whom may relapse with localized BT lesions.[21]
2 Risk of relapse is not yet fully known in previously untreated LL and BL patients given WHO MDT until smear negativity, although provisional data suggest that the risk is very small.
3 In previously untreated LL and BL patients treated with WHO MDT for 2 years only,

the risk of relapse is related to the pretreatment load of *M. leprae*; the more severe the infection, the greater the risk of relapse.

4 The timing of relapse is important. Very few well-authenticated relapses occur in the first 3 years after RFT, and the claim that the majority occur in the first 5 years[17] is based on absolute numbers reported from large cohorts at 1–4 years, rather than relapse rates. Both from experience with WHO MDT and from other regimens, a follow-up of 8–10 years appears essential.

5 There is a great desire among leprosy control experts to give WHO MBL MDT for a set duration of 2 years,[22,23] especially in 'rolling programmes' with outside funding. Such short-duration MDT is operationally essential in such circumstances. It has been claimed that by lowering the incidence of tuberculosis (and by inference, of leprosy) by 80%, the endemic of disease should decline.[14] This is almost certainly true. But it is in those areas which previously had the poorest leprosy control which are likely to have the most advanced patients with the highest bacterial loads. Therefore, significant numbers of relapses will almost certainly occur in such circumstances, and therefore an effective residual structure must remain in an area to detect relapses early and to treat them well, both as a duty to the patients and to allay any threat to the credibility of the programme. Furthermore, Jamet, Ji, and their colleagues are now proposing that the duration of MDT should be doubled to 4 years in patients with an initial average BI $\geqslant$ 4·0 before commencing MDT.[24] This would be a simple measure to implement in high endemic areas. In low endemic areas with a declining endemic approaching the WHO 'elimination' target, where leprosy may already be tending to persist in 'clusters', and where the general health services are likely to be of a high standard, it may well be worth keeping patients on MDT until they achieve smear negativity as originally advised by the WHO Study Group in 1982;[1] this should ensure that the endemic continues to die out at the maximum possible speed, because of minimal relapses. For although the results with WHO MDT are still incomplete, experience in Africa does suggest that the relapse rates after different forms of MDT are usually related both to the initial BI and to the total number of rifampicin doses and the total duration over which they are given.

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IgG subclass antibodies to mycobacterial sonicate and recombinant antigens in leprosy

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Summary In this study the IgG subclass antibodies to sonicated preparations of *Mycobacterium leprae* (leprosin A) and BCG (BCG-S) as well as to purified recombinant 65 kDa protein of *M. leprae* (rML65) were analysed in sera from leprosy patients and healthy household contacts (HFC) and noncontacts (HNC) in a leprosy endemic population. In LBI + (lepromatous bacterial index positive) patients, IgG3 was predominant in the responses to sonicated antigens of *M. leprae*. Following chemotherapy, IgG3 responses were reduced while IgG2 levels were increased. On the other hand, IgG response to rML65 was dominated by IgG1 in all the patient and control groups. Interestingly, the level of antileprosin A IgG antibody in erythema nodosum leprosum (ENL) was similar to that of lepromatous groups, while the level of anti-rML65 IgG antibody was significantly reduced in ENL. IgG4 antibodies to the antigens studied were only at low levels in all groups, including ENL. Significant differences were observed between HNC and HFC in the pattern of IgG subclass antibodies to sonicated antigens, even though their antigen specific IgG levels were similar. While HNC showed equivalent proportion of IgG1 and IgG2 in their responses to leprosin A and BCG-S, HFC showed a specific increase in IgG1 levels, suggesting that both groups are distinctly different. Further studies are required to elucidate the functional significance of IgG subclass pattern in pathogenesis and the mechanism of immunoregulation resulting in the high levels of IgG1 and IgG3 antibodies to *M. leprae* protein antigens in lepromatous leprosy.

Introduction

Leprosy, a chronic infectious disease caused by *Mycobacterium leprae*, presents a wide immunological spectrum. At one end, tuberculoid leprosy patients mount a good cell-

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mediated immune response and contain the disease.[1,2] At the other end, lepromatous patients exhibit a specific T-cell anergy[3] and have elevated serum IgG levels compared to normal individuals.[4–6] Antibodies to *M. leprae* antigens occur in increasing amounts from the tuberculoid to the lepromatous end of the spectrum.[7,8] However, the subclasses of these antibodies have not been analysed in detail except in the report of Dhandayuthapani *et al.*[9]

The 4 different IgG subclasses have different effector functions and appear to be differently regulated in humans.[10] Antibodies to protein antigens predominantly belong to IgG1, IgG3 or IgG4, while polysaccharide antigens preferentially elicit IgG2 antibodies.[11,12] IgG2 and IgG4 are inefficient in fixing complement.[13] Specific subclasses of IgG have been observed to become predominant after certain bacterial,[12] viral[14,15] and parasitic[16,17] infections. Expression of different Ig subclasses is regulated by cytokines. While this regulation is well documented in the murine system,[18] little is known about it in humans.[19] This study was aimed at analysing the subclasses of IgG antibodies to sonicate antigens of *M. leprae* and BCG as well as to recombinant *M. leprae* 65 kDa protein in leprosy patients and endemic controls.

Materials and methods

SERA

Blood samples were obtained from leprosy patients attending a leprosy hospital located at Sakthinagar in Periyar District, about 300 km from Madurai. The hospital, which is managed by Sakthi Sugars Limited, is a leprosy control unit under the Tamil Nadu Government Health Services. Leprosy patients were diagnosed clinically and the bacterial index (BI) was determined in all patients by the slit–skin smear test. The patients were classified according to clinical criteria into 5 groups: polar lepromatous (LL), borderline lepromatous (BL), borderline tuberculoid (BT) and polar tuberculoid (TT).[20] Both untreated cases and patients treated for varying lengths of time were included. LL and BL were grouped together and segregated into BI positive (LBI+) and BI negative (LBI−) lepromatous patients. Samples were collected from ENL patients at the time of reactions. Household contacts of leprosy patients (healthy family contacts, HFC) were thoroughly examined for leprosy lesions before taking blood samples. Leprosy patients and their family contacts were selected randomly without any bias towards sex. Their age varied from 15 to 60 years. Healthy noncontacts (HNC) were members of the staff and students of the School of Biological Sciences, Madurai Kamaraj University, who have not had any known habitual contact with leprosy patients even though they live in leprosy endemic area. Both sexes were included and their age varied from 21 to 40 years. All blood samples were collected with prior consent.

ANTIGENS

Leprosin A (batch CD141) was obtained from Dr R. J. W. Rees (WHO/UNDP/IMMLEP *M. leprae* bank). The sonicate antigen was prepared from armadillo derived *M. leprae* by ultrasonication. After centrifugation at 12,000 × **g** the supernatant was filtered through a 0·22 μM membrane filter and the protein concentration was adjusted to 1 mg/ml. Aliquots were stored at −70°C as recommended. *M. bovis* BCG sonicate

(BCG-S) was prepared from 1 g of bacilli following the procedure used for leprosin A preparation. Recombinant *M. leprae* 65 kDa (rML65) antigen was generously supplied by Dr Van Embden, Bilthoven, The Netherlands through the UNDP/World Bank/ WHO Special Programme for Research and Training in Tropical Diseases. The freeze-dried material was reconstituted with Dulbecco's phosphate-buffered saline at a concentration of 1 mg/ml.

ESTIMATION OF IgG SUBCLASS ANTIBODIES TO BCG-S, LEPROSIN A AND rML65

IgG subclass levels to BCG-S, leprosin A and eML65 were determined by ELISA using monoclonal antibody (MoAb) reagents from Boehringer Mannheim GmnH (Germany). Briefly, Nunc flat bottomed microtitre plates were coated with 50 μl/well of antigens at appropriate concentration for 1 h at 37°C and overnight at 4°C. After blocking with 1% gelatin, pooled lepromatous serum (LSP) or the serum samples were added at a dilution of 1:20. The plates were incubated for 1 h at 37°C. MoAb directed against the human IgG subclasses (IgG1 (clone NL16), IgG2 (clone GOM1), IgG3 (clone ZG4) and IgG4 (clone RJ4))[21,22] were added at a dilution of 1:500 (50 μl/well) and incubated at 37°C for 2 h followed by 50 μl of peroxidase conjugated rabbit antimouse IgG (1:2000) (Cappel; preadsorbed with human Ig) and incubated for 1 h at 37°C. In parallel, 1 set of plates were treated with goat antihuman IgG (1:2000) for 1 h. Subsequently the reaction was developed with OPD substrate.

Optimal dilution for MoAb against the human IgG subclasses was 1:500. Optimal coating concentration of the antigens and serum dilution was determined by checker board titration. BCG-S, Leprosin A and rML65 were coated at a concentration of 4 μg/ml and the serum samples were used at a dilution of 1:20. LSP was included every time ELISA was performed and the coefficient of variation for 6 separate assays was 5·2%. Individual IgG subclass response was expressed as a percentage of the total of absorbance values for the 4 subclasses.[23] Mean + 2SD of HNC was used as cut-off value to determine the percentage of positivity.

Results

The levels of IgG antibodies against BCG-S and leprosin A were significantly elevated in lepromatous patients, compared to HFC, HNC and TT/BT groups ($p < 0{\cdot}05$; Figure 1). However, they were similar among LBI+ and LBI− groups. Antileprosin A IgG response in ENL was comparable with lepromatous patients, while the level of anti-rML65 IgG antibody was significantly reduced in ENL. With regard to rML65 specific IgG antibodies, LBI + patients had the highest and ENL the lowest levels and the difference ($p < 0{\cdot}05$) was significant. Further, with the reduction in bacterial load, there was concomitant decrease in the level of IgG antibodies to rML65.

Several major differences were observed in the pattern of IgG subclass antibodies to leprosin A and BCG-S among different groups of patients and controls. The proportion of antileprosin A IgG3 antibody was significantly higher in the LBI + than in the LBI− group ($p < 0{\cdot}05$; Table 1). This increase was also reflected in the percentage of individuals with higher IgG3 response (mean + 2SD of HNC; Table 2). Following chemotherapy, IgG3 levels were decreased with a concomitant increase in antibodies of

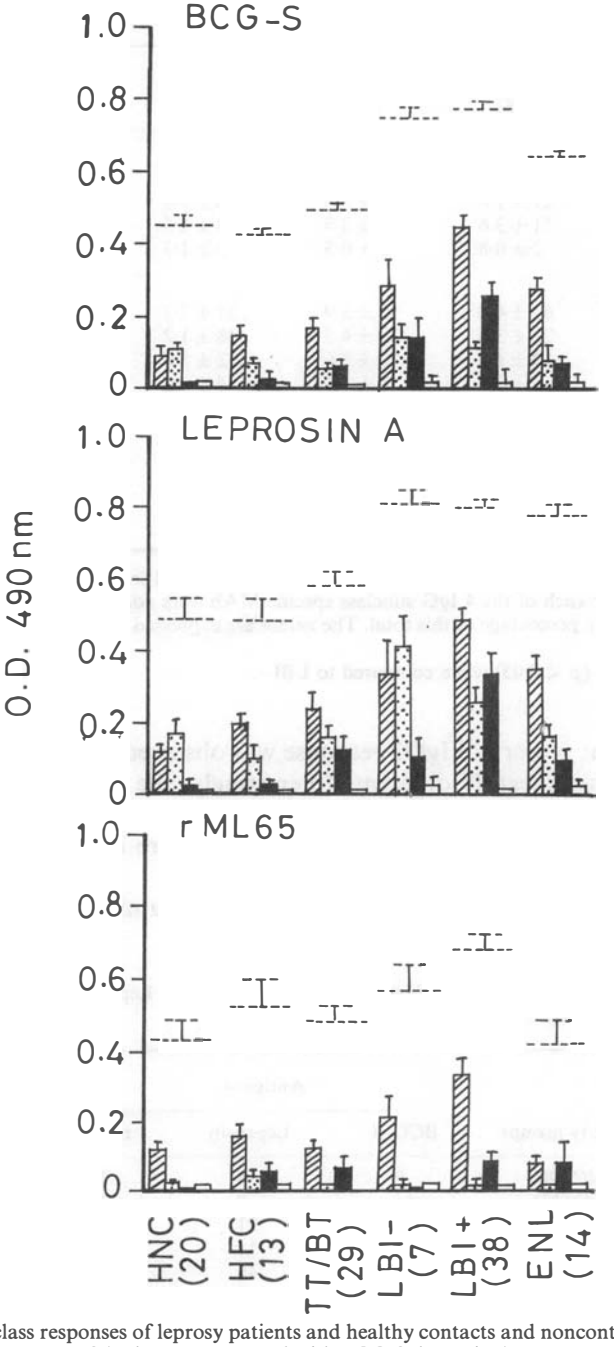

Figure 1. IgG subclass responses of leprosy patients and healthy contacts and noncontacts to BCG-S, leprosin A and rML65 antigens. ELISA plates were coated with BCG-S, leprosin A or rML65 (4 μg/ml) and incubated with 1 : 20 dilution of serum samples. Antihuman IgG subclass MAb (▨, IgG1; ▩, IgG2; ■, IgG3; □, IgG4) were added at a dilution of 1 : 500 followed by 1 : 2000 dilution of antimouse IgG-HRP. Parallely, 1 set of plates was treated with antihuman IgG HRP (1 : 1000) after incubation with the serum samples to measure the IgG levels (dashed lines). The number of serum samples tested for each group is given in parentheses.

Table 1. Relative proportions of IgG subclass responses to BCG-S, leprosin A and rML65

	HNC [20]	HFC [13]	TT/BT [29]	LBI− [7]	LBI+ [38]	ENL [14]
BCG-S						
IgG1	40 ± 4·2	60 ± 3·5	55 ± 3·5	49 ± 3·2	55 ± 2·8	60 ± 3·6
IgG2	52 ± 4·9	27 ± 1·8	24 ± 3·5	30 ± 7·9	18 ± 2.2	21 ± 3·6
IgG3	5 ± 1·4	11 ± 3·6	18 ± 3·5	18 ± 6·5	26 ± 2·8	15 ± 3·5
IgG4	3 ± 0·7	2 ± 0·8	3 ± 0·5	3 ± 1·7	2 ± 0·3	4 ± 0·5
leprosin A						
IgG1	39 ± 4·8	60 ± 4·4	50 ± 3·9	37 ± 7·2	49 ± 3·5	54 ± 6·5
IgG2	50 ± 5·5	29 ± 3·6	33 ± 4·5	48 ± 1·2	24 ± 3.1	25 ± 4·9
IgG3	8 ± 3·0	10 ± 1·9	18 ± 3·6	12 ± 5·8	25 ± 3·3*	16 ± 4·9
IgG4	3 ± 0·9	3 ± 1·1	4 ± 1·6	4 ± 1·6	2 ± 0·5	5 ± 0·8
rML65						
IgG1	75 ± 5·6	65 ± 6·1	72 ± 5·0	86 ± 5·8	73 ± 4·2	47 ± 7·5
IgG2	16 ± 3·7	17 ± 4·3	9 ± 1·8	5 ± 2·9	8 ± 2.3	22 ± 7·3
IgG3	4 ± 2·0	14 ± 5·6	19 ± 4·3	5 ± 1·8	16 ± 3·2*	15 ± 6·9
IgG4	6 ± 3·7	5 ± 2·6	1 ± 0·4	5 ± 3·4	4 ± 1·4	15 ± 6·2

The proportion of IgG subclass response was calculated as described in ref. 23. The absorbance values (presented in Figure 1) for each of the 4 IgG subclass specific MAb were added and individual IgG subclass response was expressed as a percentage of this total. The values are expressed as mean ± SE of the percentage response.

*Significantly different ($p < 0·05$) when compared to LBI−.

IgG2 subclass. Similar pattern of IgG3 response was observed for BCG-S, even though the levels were not significantly different. Interestingly, the subclass pattern of anti-leprosin A and antiBCG-S IgG antibodies in ENL patients was similar to that of TT/BT group (Figure 1, Table 1), even though ENL reactions are observed in lepromatous patients.

The IgG subclass distribution of antileprosin A and the antiBCG-S response in HFC

Table 2. Proportion of IgG3 responders to BCG-S, Leprosin A and rML65

	Antigens		
Study groups	BCG-S	Leprosin	rML65
HNC (20)	0	5	10
HFC (13)	15	0	15
TT/BT (29)	45	21	31
LBI− (7)	43	14	0
LBI + (38)	68	40	26
ENL (14)	36	14	21

The proportion of individuals among the various study groups with IgG3 antibody levels above the positive cut-off value (mean +2SD of HNC) is given.

The number of serum samples tested for each group is given in parentheses.

was different from HNC, even though their IgG levels to these antigens were comparable (Figure 1). In HFC, IgG1 response was predominant, while HNC showed equivalent proportion of IgG1 and IgG2 antibodies to leprosin A and BCG-S (Table 1).

Minor differences were also observed in the IgG subclass pattern in the anti-rML65 response. However, IgG1 was the predominant subclass in the rML65 response in all the groups of patients and controls. ENL patients had equivalent levels of IgG1 and IgG3 (Table 1). On the other hand, antibodies of IgG4 subclass against the 3 antigens studied were at low levels in all the groups (Figure 1).

Discussion

The 4 IgG subclasses have distinct effector functions. While IgG1 and IgG3 are efficient in fixing complement and mediating ADCC, IgG2 can mediate opsonization only at very high epitope densities.[10] On the basis of effector functions, the importance of IgG2 subclass in the clearance of capsulated microorganisms is well documented.[24] However, the subclass antibody responses in leprosy has not been addressed in detail. In the leprosy spectrum, despite the high levels of *M. leprae* specific IgG antibodies in the lepromatous patients, the bacterial load remains high.[1] In this study, an attempt was made to analyse the subclass distribution of the antimycobacterial antibodies in a leprosy endemic population. With the availability of purified recombinant proteins of *M. leprae* and defined MAb reagents to human IgG subclasses,[21,22] it is now possible to address the questions on the subclass responses.

In accordance with published reports, IgG antibodies to sonicated antigens of *M. leprae* and BCG were higher in lepromatous patients than in all other groups. However, in this study, the levels were the same in both BI positive and negative lepromatous patients (Figure 1). In a larger study,[25] serum IgG antibodies to *M. leprae* sonicate were at significantly higher levels in LBI+ than in LBI− patients. The observed difference may be either due to the variations in the antigenic composition of leprosin A used[26] or due to the fewer LBI− cases studied here. The salient observation of this study is the difference in the pattern of IgG subclass responses between different groups of patients and contacts. Assuming that proteins are the predominant antigens in the sonicate preparations of BCG and *M. leprae*, the IgG3 responses were elevated both in quantity and proportion, in the LBI+ group when compared to LBI− group. On the other hand, in the LBI− patients, IgG2 responses were dominant (Figure 1, Table 1). Since the antigens are sonicate preparations, the differences in the subclass responses suggest two possibilities. (A) There is a switch in the subclass responses to the same antigens during different phases of the disease; or (B) different antigens are immunodominant during the different phases of the disease.

The first possibility was tested by analysing the IgG subclass antibody levels to purified recombinant proteins of *M. leprae*. It has already been demonstrated that the IgG response to purified recombinant 65 kDa of *M. leprae* and *M. bovis* and 70 kDa of *M. tuberculosis* was significantly decreased in LBI− patients when compared to LBI+ patients (Figure 1 and ref. 27). In this study it was observed that IgG1 was the dominant subclass in response to rML65 in all the groups studied, suggesting that any given antigen preferentially elicits only one type of subclass antibodies in leprosy. Moreover, LAM has been shown to elicit predominantly IgG2 response.[9] Together, these observations indicate that the second suggestion may be more relevant. Analysis of

subclass antibodies to other purified proteins are underway to understand more about the pattern of subclass response and its relevance to the disease process.

The observed increases in the level of IgG3 antibodies to leprosin A in LBI+ patients when compared to LBI− and tuberculoid patients in this study are contrary to the findings of Dhandayuthapani *et al.*,[9] wherein IgG2 was the predominant subclass to leprosin A over and above the IgG1 levels. Based on this observation, the authors concluded that most of the IgG subclass responses to leprosin A could be accounted for by the reactivity with lipoarabinomannan, a carbohydrate antigen which elicited a predominantly IgG2 response. This discrepancy may well be due to their use of a monoclonal (SG-16) to IgG1 proteins of G1m(f) allotype which is represented at low frequency in the study population.[28] However, in the present study, based on the presence of IgG1 and IgG3 responses, it is clear that apart from LAM, other dominant protein antigens are also present in leprosin A.

In lepromatous patients undergoing ENL reactions, the pattern of IgG subclass response observed was similar to that of tuberculoid patients. Their anti-rML65 IgG, especially IgG1 levels, were decreased significantly. We do not know the basis for the observed differences. A longitudinal study of lepromatous patients undergoing reactions may help in correlating the lymphokine patterns[29,30] with IgG subclass responses. However, these patients do not have elevated levels of IgG4, even though the anti-IgG4 monoclonal antibody reagent used in this study has high affinity.[31]

Another salient finding in this study is the difference in the pattern of subclass response among healthy family contacts and endemic controls (HNC). In fact, IgG subclass pattern of HFC was more similar to tuberculoid patients than to HNC. The specific increase in the proportion of IgG1 and IgG3 in HFC (Table 1) suggests that their immune system is primed selectively to mycobacterial protein antigens. In accordance with our other studies in the same population,[27] the immune profile against mycobacterial antigens is specifically altered in HFC even though both groups of healthy controls are derived from the same endemic area.

The elevated IgG antibody response to rML65 in lepromatous patients was dominated by IgG1 and to a lesser extent by IgG3 (Figure 1), which are the predominant isotypes expected in response to protein antigens.[11,12] It is of interest to note that both IgG1 and IgG3 levels to rML65 were reduced in LBI− patients and IgG1 in ENL patients. At present the meaning of these alterations in subclass pattern is not clear and neither do we understand the functional significance of IgG subclass pattern in relation to pathogenesis. However, it is possible that certain regulatory mechanisms play a crucial role in lepromatous leprosy. Such a regulatory mechanism is even more relevant because of our recent finding of an inverse relationship between cell mediated and humoral immunity of rML65 in both leprosy patients and endemic controls.[32]

Recent studies have demonstrated distinct patterns of expression of cytokines in lepromatous and tuberculoid patients *in vitro*, thereby implicating a role for T-cell subsets in the immune spectrum of leprosy.[29] The regulatory role of cytokines in the class switching of immunoglobulin isotypes is well documented in the murine system but not in humans.[18] However, recent studies by Kitani & Strober[19] have shown that the various human IgG subclasses manifest distinct requirements of cytokines for the regulation of early steps in isotype differentiation. Further analysis of the pattern of IgG isotypes induced in response to purified mycobacterial antigens may help in understanding the dichotomy of the immune response observed in leprosy.

Yamamura *et al.*[29,30] have demonstrated the differences in the expression of T helper subsets in leprosy across the spectrum with the expression of Th2-like pattern of cytokines in the lesions of lepromatous leprosy patients. However, no overt increase in IgG4 was observed in these patients, unlike in parasitic infections[16,17] wherein elevated IL-4 has been shown to be associated with IgG4 and IgE. While the regulation of IgE and IgG4 by IL-4 is well documented in humans, the regulation of other subclasses is not clear. Studies on the expression of IgG subclass germline transcripts[19] have revealed that in the presence of a proliferative stimulus IgG3 in augmented by IL-4 and IgG1 by IL-2, while cytokines are sufficient for the induction of IgG1 and IgG4 (by IFN-γ and IL-4, respectively). Therefore, it is possible that induction of IgG3 in lepromatous leprosy patients may be accomplished specifically by Th2-like cells induced by certain antigens of *M. leprae*, and that it is downregulated following chemotherapy. It appears that such a modulation is brought about more specifically in response to *M. leprae*, since we did not observe any difference between patients and controls in the IgG subclass pattern to several autoantigens (S. Ilangumaran and R. Sheela, unpublished observations).

It is well documented that in the highly bacilliferous lepromatous leprosy patients effective cell-mediated immune response is not mounted, partially due to the absence of IL-2 and IFN-γ. However, antigen specific T cell help to B cell is present in order to account for the high levels of IgG, especially IgG1 and IgG3 subclasses to protein antigens. With more knowledge on the regulation of IgG subclasses in humans, it should be possible to understand the regulation of the antibody response by T-cell subsets in leprosy.

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Sustained T-cell reactivity to *Mycobacterium tuberculosis* specific antigens in 'split-anergic' leprosy

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Summary Split anergy represented by delayed-type hypersensitivity skin reaction to tuberculin, but not to leprosin, is known to occur in a distinct proportion of leprosy patients. The mechanism was originally attributed to *Mycobacterium leprae*-specific suppression of T cells toward common mycobacterial antigens. This study ascertained an alternative explanation, attributing the phenomenon to selective responsiveness to *M. tuberculosis*-specific epitopes. Indeed, the results of blood T-cell proliferative responses in 11 split-anergic patients showed normal responsiveness to the *M. tuberculosis*-specific 38 kDa lipoprotein and peptide 71–91 of the 16 kDa antigen but diminished responsiveness to 2 common mycobacterial antigens, represented by the 65 kDa heat shock protein and the fibronectin-binding Ag85 complex, as compared with leprosin responsive patients and healthy contacts. These findings support the hypothesis that split anergy is due to selective recognition of *M. tuberculosis*-specific epitopes and deletion of T cells reacting to shared mycobacterial antigens.

Introduction

The majority of patients with multibacillary leprosy manifest T-cell anergy represented by the lack of delayed-type hypersensitivity (DTH) skin reactions to soluble extracts from both *Mycobacterium leprae* (leprosin) and *M. tuberculosis* (tuberculin). This anergy was attributed originally to a deletion of T cells[1] but various suppressor mechanisms were also proposed.[2] A distinct proportion of lepromatous patients show 'split anergy', manifested by DTH response to tuberculin whilst being anergic to leprosin.[3] It has been

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postulated that the mechanism of split anergy is due to suppression of T-cell responses to common mycobacterial antigens present in both leprosin and tuberculin by T cells to *M. leprae*-specific, either phenolic glycolipid[4] or protein[5] antigens contained only in leprosin. An alternative explanation, which suggested the role of macrophage mediated suppression,[6] did not explain, however, the basis for the split specificity of anergy. A selective defect of IL-2 secretion was suggested following the restoration of T-cell proliferation by exogenous IL-2,[7] but this could be interpreted in support of either reversal of suppression or as amplification of responder T cells.[8] Analysis of the specificity of the T-cell repertoire of leprosy patients using fractions separated by polyacrylamide gel electrophoresis has been inconclusive.[9] Taken together, there is at present no satisfactory explanation of mechanisms underlying the 'split anergy' phenomenon in leprosy.

Our experimental approach hinged on the analysis of specificity of proliferative T-cell responses in leprosy. We considered as our working hypothesis, that 'split anergy' could be explained on the basis of a sustained T-cell responsiveness to *M. tuberculosis*-specific epitopes, coinciding with the deletion of T cells which respond to common antigens. This assumption has been supported by the results presented in this study in which the T-cell proliferative responses toward 2 *M. tuberculosis*-specific antigens or peptides (38 and 16 kDa) and 2 largely cross-reactive antigens (hsp65 and Ag85) have been compared.

Materials and methods

SUBJECTS

We examined 40 leprosy patients of both sexes, 15–50 years old (29 multibacillary; 11 paucibacillary) and 7 professional contacts, selected from the Shashemene Leprosy Control Unit, or the All Africa Leprosy Rehabilitation and Training Hospital (ALERT) or from the Armauer Hansen Research Institute (AHRI). Patients had received multidrug therapy, according to the WHO regimen, for between 1 and 48 months—4 of the paucibacillary and 8 of the multibacillary patients suffered from reversal reactions and were under steroid therapy, and 2 of these patients were in the 'split anergy' group. None of the patients had active tuberculosis. Blood samples were obtained with informed consent of patients and with ethical research committee approval.

SKIN TESTING

Patients were tested for reactivity to intradermal inoculation with 50 μl of leprosin ('Rees antigen', WHO Bank, National Institute for Medical Research, London, UK) and 10 units of tuberculin (Purified protein derivative, PPD, Evans Med. Ltd, Langhurst, UK). Skin indurations of more than 10 cm diameter at 48 hrs after administration were classified as positive reactions.

ANTIGENS

Tuberculin (PPD) was purchased from Evans Medical Ltd (Liverpool, UK). *M. leprae* soluble extract (MLSE), the recombinant 65 kDa heatshock protein (hsp65) from

M. bovis and the recombinant 38 kDa protein (P38kDa) from *M. tuberculosis*[10] were obtained from the WHO Banks in London or Bilthoven. The recombinant purified Ag85 complex from *M. leprae*[11] was a gift from Dr Jelle Thole (The Academic Hospital of Leiden, The Netherlands). The synthetic 20mer peptide 71–91 (RDGQLTIKAER-TEQKDFDGRS), derived from the sequence of the 16 kDa protein of *M. tuberculosis* (p71–91), was prepared as described earlier.[12]

LYMPHOCYTE PROLIFERATION

Peripheral blood mononuclear cells (PBMC) were isolated from fresh defibrinated whole blood by Ficoll gradient centrifugation (Ficoll-Isopack, Pharmacia, Uppsala, Sweden) and resuspended in culture medium RPMI 1640, containing 5% human serum and 1% penicillin & streptomycin and 1% glutamine). PBMCs at $1{\cdot}56 \times 10^6$ in a volume of 100 μl were cultured using routine conditions[9] in round bottomed 96-well microtitre plates in the presence of purified or complex antigens at 5 μg/ml or 40 μg/ml of peptide p71–91. Following incubation in a humidified 5% CO_2 incubator at 37°C for 6 days, 1 μci/well of [^{3}H]TdR (Amersham International, Amersham, UK) was added and radioactive counts were quantified after an overnight incubation using liquid scintillation fluid in a betaplate counter. The results have been expressed as cpm in the antigen stimulated culture following subtraction of background counts (Δcpm). Student's *t*-test was used for statistical evaluation of differences between group mean values.

Results

From the total of 29 patients with lepromatous leprosy, initially included in the study, 18 were found by skin testing to be anergic to both leprosin and tuberculin, and also failed to respond with significant *in vitro* proliferative response to both antigenic extracts. DTH skin responsiveness with split responsiveness to tuberculin, but not to leprosin, was observed in only 11 patients, of whom 7 were clinically classified as borderline lepromatous (BL) and 2 were borderline tuberculous (BT). Despite the lack of skin DTH, the state of T-cell anergy to leprosin was merely partial, since a diminished degree of proliferative responsiveness was sustained. The proliferative responses to individual antigens were analysed also in 7 borderline leprosy patients, including both lepromatous and tuberculoid cases, with positive DTH skin responses to both tuberculin and leprosin and 7 healthy hospital contact responders.

The results of *in vitro* stimulation with: (i) hsp65 and Ag85, representative of common mycobacterial antigens; (ii) the 38 kDa protein and the p71–91 synthetic peptide, representative of *M. tuberculosis* specific antigens; and (iii) tuberculin and leprosin as complex antigenic extracts are shown as individual Δcpm values from split anergic leprosy patients and from responder patients and healthy controls (Figure 1). Responsiveness of PBMCs from the majority of leprosin anergic patients (represented by empty squares) has been characterized by significantly diminished Δcpm values following incubation in the presence of either hsp65 (mean cpm 240, $p < 001$) or Ag85 (mean cpm 766; $p < 001$), when compared to skin-test responders, either on leprosy patients or on healthy contact controls. In contrast, responses to the 38 kDa protein and peptide p71–91 comparing split-anergic patients with DTH-responder

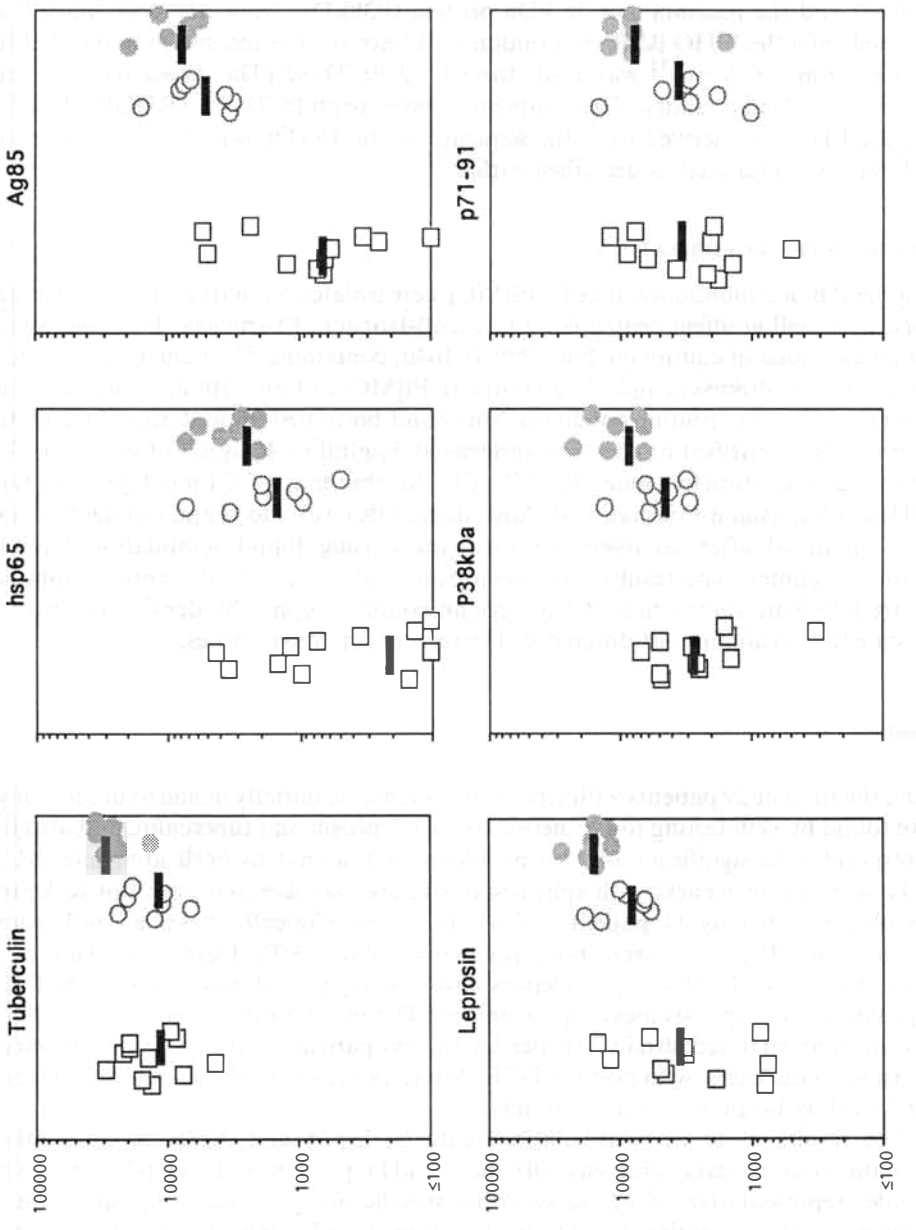

Figure 1. Proliferative responses to common mycobacterial and *M. tuberculosis* specific antigens. Individual Δcpm values obtained from 11 leprosy patients with DTH skin responses negative to leprosin but positive to tuberculin ('split anergy') (□), 7 leprosy patients with skin test responses to both reagents (○) and 7 healthy DTH responder healthy hospital contacts (◎). Vertical scale, thymidine incorporation, Δcpm; horizontal bars, geometric mean Δcpm values.

Table 1. Number of responder individuals based on proliferation to single antigens relative to individual responsiveness to PPD

Group tested	Skin test leprosin	Total tested	Number (%) of *in vitro* Responders*			
			P38kDa	p71–91	Ag85	hsp65
Leprosy	negative	11	10 (90)	9 (82)	4 (36)	2 (18)
Leprosy	positive	7	7 (100)	6 (86)	7 (100)	5 (71)
Contacts	positive	7	7 (100)	6 (86)	7 (100)	6 (86)

* > 75% of the relative response represented by: [Δcpm to Ag/Δ cpm to PPD] × 100, from individual values shown in Figure 1.

leprosy patients or healthy contacts were not significantly different. Despite the skin DTH anergy, significant proliferation in response to leprosin was probably due to the higher sensitivity of the latter assay. Therefore, it is appropriate to qualify the degree of T-cell anergy merely as partial.

In view of the observed pronounced variations in the magnitude of proliferative responses to PPD, it seemed desirable to express the Δcpm count following antigenic stimulation as a relative value in relation to the individual's response to PPD (100%). The relative values were calculated following the formula: [Δcpm with antigen/Δcpm with PPD] × 100 for each antigen. Using these relative values, tested individuals were classified as positive responders when their relative values exceeded the arbitrarily chosen 70% cut-off point. On the basis of such evaluation only 2 hsp65 responders and 4 Ag85 responders were found in the group of 11 split-anergic leprosy patients. However, responses to the 38 kDa protein and the p71–91 peptide were positive in the majority of split-anergic patients. Most patients and healthy contacts with positive skin DTH reactions to leprosin were found to be responders to all 4 antigens.

Discussion

The results obtained in 11 leprosy patients identified by DTH skin testing as 'split anergic', i.e. responders to tuberculin whilst anergic to leprosin, showed significantly diminished lymphocyte proliferation to the hsp65 and Ag85 antigens which are highly cross-reactive between *M. tuberculosis* and *M. leprae*.[13] In contrast, the same patients showed unimpaired responses to 38 kDa protein and the p71–91 peptide derived from the 16 kDa protein antigen which have previously been found specific for the *M. tuberculosis* complex.[12,13] This latter finding corroborates with the previous demonstration of elevated antibody levels to both the 16 kDa and 38 kDA protein antigens in patients with lepromatous leprosy.[14]

The argument in favour of suppressive mechanisms has previously been based on the assumption that partial T-cell responsiveness in leprosy is biased toward common mycobacterial epitopes.[4] However, this view is not supported by recent studies which demonstrated more profound impairment of responses to Ag85 than to whole *M. bovis* BCG.[15] The results of this study confirmed the impaired responsiveness to Ag85 and demonstrated a similar decline of response to hsp65, both antigens being representative

of cross-reactive common mycobacterial constituents. Furthermore, our finding of unimpaired responses in respect of the 2 tested *M. tuberculosis* specific antigens is alone sufficient to reconcile the occurrence of split specificity without a need to invoke *M. leprae*-specific suppression of responses to common epitopes.

The mechanisms of anergy in lepromatous leprosy are of fundamental interest, because they influence the rationale for immunotherapeutic intervention using the BCG vaccine or suitable adjuvants and cytokines.[7] Although the conclusions of this study are limited by the relatively small number of tested patients and by the lack of data on the T-cell cytokine profile, the obtained results clearly suggest a distinct, previously not identified, specificity pattern of the T repertoire in lepromatous leprosy.

Acknowledgments

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We thank Srs Genet Amare and Ethiopia Ghebreyes for technical assistance and Dr J. Thole (Leiden), Dr R. J. Rees (London), Dr J. van Embden (Bilthoven) for the supply of antigens produced with support from the UNDP/World Bank/WHO/TDR Special Programme.

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A study on performance of two serological assays for diagnosis of leprosy patients

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Summary We compared 2 serological tests for the diagnosis of leprosy to test their performances. The tests include the serum antibody competition test (SACT) for the detection of antibodies to *Mycobacterium leprae*-specific epitope on 35 KDa protein molecule, and *M. leprae* gelatin particle agglutination assay (MLPA), for the detection of antiphenolic glycolipid-1 (PGL-1) antibodies. In both the assays a higher serological positivity was seen amongst multibacillary (MB) patients than those in paucibacillary (PB) patients. Taking all leprosy patients together, the sensitivity of SACT (59·7%) was observed to be statistically comparable to that of MLPA (66·9%). However, SACT proved to be more specific (97·7%) than MLPA (75·0%). The agreement between these 2 assays was observed to be moderate.

Introduction

Leprosy is a major world health problem and in order to eliminate this disease two main strategies, accurate diagnosis and treatment, are playing a pivotal role. Recently a wide variety of serological tests have been developed for diagnosing leprosy patients by detecting anti *M. leprae* antibodies in their sera.[1] Of the assays described so far, the phenolic glycolipid-1 (PGL-1) utilizing enzyme-linked immunosorbent assay (PGL–ELISA), and the serum antibody competition test (SACT), have been studied widely and have been suggested to be highly promising for the serodiagnosis and monitoring of chemotherapy in lepromatous leprosy patients.[2–7] Recently, a microagglutination assay, called MLPA, that detects antiPGL-1 antibodies, has been introduced by Izumi *et al.*[8] It is a rapid and simple assay which can be evaluated by the naked eye. Further, its sensitivity and specificity have been shown to be comparable to that of PGL–ELISA. The present study was designed to evaluate the performances, in terms of sensitivities and specificities for diagnosis of leprosy patients, of SACT and MLPA using a set of sera.

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Materials and methods

SERUM SAMPLES

Sera were obtained from 68 multibacillary (MB) and 56 paucibacillary (PB) leprosy patients (who were active cases receiving antileprosy treatment) attending the hospital at the Central JALMA Institute for Leprosy, Agra, India. Sera from 28 pulmonary tuberculosis patients and 16 healthy volunteers, working at the Institute laboratories, were also included as controls. The sera were stored at −20°C until use.

DETECTION OF *M. LEPRAE* SPECIFIC ANTIBODIES USING SACT

In this approach, antibodies against a *M. leprae* specific epitope on 35 KDa antigen of *M. leprae* were detected by the competition test.[5,7] Briefly, the wells were coated with *M. leprae* antigen (supplied by IMMLEP, WHO, through Dr R. J. W. Rees). The wells were then incubated with peroxidase conjugated *M. leprae* specific monoclonal antibody (MLO-4) in the presence or absence of a 10-fold dilution of serum. Finally, colour was developed using o-phenylene-diamine dihydrochloride as substrate and optical density values were then read at 492 nm using an ELISA reader. Serum causing 50% or more inhibition of the binding of enzyme conjugated antibody to the specific epitope was regarded as positive.

DETECTION OF ANTIPGL-1 ANTIBODIES USING MLPA KIT

The microtitre agglutination test for screening of leprosy patients was performed using the Serodia leprae diagnostic kit (manufactured by Fusirebio Inc., Tokyo, Japan). Briefly, serum samples were diluted to 1 : 8 and 1 : 16 in wells of U-bottom microtitre plates giving a final volume of 25 μl/well. Then equal amounts of unsensitized and antigen-sensitized gelatin particles were mixed with these diluted sera giving 1 : 16 and 1 : 32 as the final dilutions. The contents in the wells were mixed thoroughly. Plates were covered and incubated at room temperature for 2 hr. Upon completion of the reaction the agglutination in the wells were read. Serum samples showing agglutination at 1 : 32 were taken as positive.

Results and discussion

Table 1 shows the positivities achieved by the 2 assays in various groups. In common with previously reported findings with SACT and MLPA[2,5,8] we have also found high sensitivity in the MB group of patients compared with the PB group of patients. When considering MB patients, the highest positivity (66 out of 68) was observed using SACT. MLPA showed more positivity in the case of the PB group of patients (21 out of 56). Taking all the patients together, the MLPA positivity was slightly more than SACT (83 *vs* 74). With MLPA, 4 out of 16 and 7 out of 28 sera were found to be positive in healthy individuals and tuberculosis patients, respectively. On the other hand, there was less false positivity with SACT, whereas all of the 16 sera from healthy subjects and 27 from 28 tuberculosis patients were negative. In all, only 1 out of 44 sera from nonleprosy

Table 1. Positivities for anti-*M. leprae* antibodies in sera from various groups using SACT and MLPA tests

Subjects	Number of sera tested	SACT positive	MLPA positive
Controls			
Healthy	16	0	4
Tuberculosis	28	1	7
Total	44	1	11
Leprosy patients			
Multibacillary	68	66	62
Paucibacillary	56	8	21
Total	124	74	83

controls (healthy persons and tuberculosis patients) was positive for SACT, whereas 11 of these 44 sera were positive using the MLPA test.

Based upon the above data, the percent sensitivities and specificities for the detection of leprosy patients of the 2 assays were determined. Table 2 shows these values. The results indicate that the sensitivity of SACT was 59·7%, whereas that of MLPA was 66·9%. The specificity of SACT was shown to be 97·7% and that of MLPA was 75·0%.

On analysis, though the sensitivity of MLPA was found to be slightly more than SACT, statistically there was no difference between the 2 tests ($\chi^2 = 1{\cdot}116$; $p < 0{\cdot}1$). As far as we know, the only report comparing MLPA and SACT which described MLPA as more sensitive than SACT is that of Dhandaya Chapani *et al.*[9] Our findings contradict their report. The reason for this discrepancy could be that in the previous study, analysis for sensitivity was carried out taking both leprosy patients as well as nonleprosy individuals into consideration, while in the present study we included leprosy patients only for this purpose.

Further, we have found (Table 2) that SACT is significantly more specific ($\chi^2 = 21{\cdot}859$; $p < 0{\cdot}001$) than MLPA. In order to distinguish between nonspecific agglutination reaction and agglutination due to antiPGL antibodies, the antiPGL-1-ELISA was run in parallel with MLPA. The results have shown that of the 11

Table 2. Sensitivities and specificities of SACT and MLPA for detection of anti-*M. leprae* antibodies in leprosy sera

Test	Sensitivity (%)	Specificity (%)
SACT	59·7	97·7
MLPA	66·9	75·0

Sensitivity of SACT and MLPA, for detection of leprosy patients, did not differ significantly ($p < 0{\cdot}1$).

SACT was more ($p < 0{\cdot}001$) specific than MLPA.

Table 3. Agreement between SACT and MLPA tests for the detection of anti-*M. leprae* antibodies using 124 leprosy sera

	MLPA results	
SACT results	Positive	Negative
Positive	64	10
Negative	19	31

Percent agreement, 76·7%; κ value, 0·5.

nonleprosy controls (which were positive by MLPA) only 2 were positive by PGL-1 ELISA (detailed data not given) indicating that the nonspecific positivities in MLPA were probably due to false agglutination caused by biophysical properties of sera from certain individuals, as has been proposed by Chanteau *et al.* than found in this work.[10] In contrast to our findings, Izumi *et al.*[8] demonstrated a lower frequency of MLPA positive sera among normal healthy persons and tuberculosis patients. These contradictory findings might be due to individuals belonging to different ethnic groups and locations that were used in these 2 studies.

As shown in Table 3 agreement (76·7%) between the 2 tests was found to be moderate (indicated by a κ value of 0·5). Some disagreement was expected, because the 2 assays measure different antibodies.

In conclusion, the sensitivity of SACT appears comparable to that of MLPA and SACT is more specific than MLPA. However, neither of these 2 assays seems to be very promising, even in diagnosing established leprosy. Therefore there is need of more specific and sensitive test(s) for the diagnosis of leprosy patients for use in screening the population.

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Immunoreactive antigens of a candidate leprosy vaccine: *Mycobacterium habana*

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Summary *Mycobacterium habana* (*M. simiae* serovar-1) is a candidate vaccine for mycobacterial infections on the basis of the protection shown by this strain. We prepared 3 fractions of *M. habana*, i.e. the cell wall (CW), the cell membrane (CM) and the cytosol (CS). Protein antigens of these fractions were resolved by SDS-PAGE and subsequently probed with the sera of leprosy and tuberculosis patients and also antiBCG antibodies.

We saw 3 major protein bands at ≃33 kD in the CW, ≃38 kD in the CM and ≃22 kD in the cytosol (CS) after coomassie blue staining of the gels. Pool leprosy patients' serum had identified proteins of ≃26 kD in CW, ≃35 and ≃18 kD in CM and ≃24 kD in the CS which have not been seen by the TB patient's serum pool. Pool serum of tuberculosis patients has identified 1 protein at ≃10 kD in the CW and a broad band between 20 and 24 kD and 1 at ≃4 kD in the CM which have not been visualized in the pool leprosy patient's serum lane. The proteins of *M. habana* which are recognized only by leprosy antisera or only by tuberculosis antisera could be exploited for developing diagnostic agents against these infections.

Introduction

Integral mycobacterial cells contain a pool of potentially pathogenic/redundant constituents which compromise the protective efficacy of such cells as vaccine. These vaccines may lead to the development of adverse immunological phenomenon on immune suppression[1,2] autoimmunity[3] and tissue necrosis.[4] It is imperative to identify the immunologically active molecules in such cells to further our understanding about the antigenic molecules in order to develop a subunit vaccine or diagnostic agents for mycobacterial infections.

Mycobacterium habana, TMC 5135 (now known as *M. simiae* serovar-1; the derivation is given elsewhere) is a cultivable[5] nonpathogenic mycobacterium which has shown promising protective efficacy against experimental leprosy[6] and tuberculosis[7] in a series of studies. It is an interesting organism, and its antigens deserve further exploration.

0305-7518/95/066031+08 $01.00

In this study we have identified the immunoreactive protein antigens in *M. habana* by probing them with the sera of leprosy and tuberculosis patients and also with antiBCG antibodies with a view to recognize immunoligically important antigens of this organism.

Materials and methods

GROWTH OF *MYCOBACTERIUM HABANA*

Mycobacterium habana was grown in liquid Sauton's medium containing glycerol and L-asparagine in a shaking incubator at 37°C. Defined mid-log phase (of 10 days) growth of the mycobacterium was harvested at 4000 × ***g*** at 4°C and washed 3 times with normal saline.

FRACTIONATION OF *M. HABANA*

The mycobacterial harvest was suspended in Tris-buffered saline (TBS; 0.01 M Tris, pH 7·4) containing $MgCl_2$ (10 mM) and protease inhibitors (1 mM) at a concentration of 200 mg ml^{-1}. The suspension was sonicated for 10 min at >20k cycles s^{-1} in phasic manner (50% s^{-1}) in Ultrasonic Processor XL Heat systems, sonicator. The sonicate was subjected to differential centrifugation to isolate the subcellular fractions of cell wall, cell membrane and the cytosol according to the procedure described by Brodie with slight modifications.[8] Briefly, the sonicate was first centrifuged at 15,000 × ***g*** for 30 min at 4°C to isolate the cell wall (pellet). The pellet was washed 3 times with TBS. The supernatant was again centrifuged at 150 000 × ***g*** for 60 min at 4°C, to obtain membrane (pellet) and cytosol (supernatant), fractions, respectively. Pure membrane was obtained by repeated centrifugation at 150 000 ×***g***.

ANTIBODIES

*Anti-*M. leprae *and anti-*M. tuberculosis *antibodies*

Sera from 12 lepromatous leprosy (LL) and 8 tuberculosis (TB) patients were collected separately from the Mission Leprosy Hospital, Barabanki and Tuberculosis Hospital, Rajendar Nagar, Lucknow. Separate pools of the sera of LL and TB patients were prepared. The serum pools, respectively, served as sources of polyclonal anti-*M. leprae* and anti-*M. tuberculosis* antibodies. Peroxidase conjugated antibodies against rabbit and human immunoglobulins (Igs) were procured from Sigma Chemicals (USA).

Anti-BCG Antibodies

Commercially available anti-BCG antibodies (rabbit raised; Dakoppats, Denmark) were used.

SDS-PAGE and Immunoblotting

Proteins of cell wall, cell membrane and cytosol fractions were separated on discontinuous 12·5% SDS-polyacrylamide gels (SDS-PAGE).[9] After electrophoresis, the resolved proteins were either stained or transferred to nitrocellulose paper (NCP, S&S,

0·45 μm).[10] After transfer the paper was cut into 4 mm wide strips and blocked with 3% skimmed milk powder (Anik-Spray, Lipton India Ltd) in TBS (20 mM Tris-HCl, pH 7·2, 500 mM NaCl) containing 0·05% Tween-20 (TTBS; dilution buffer) for 2 hr at room temperature (RT). Blocked strips were probed with heterologous antibodies diluted in 1% milk-TTBS (antiBCG antibodies 1 : 250 and anti-*M.leprae* and anti-*M. tuberculosis* antibodies 1 : 25) for 2 hr at room temperature. After washing (5 × 5 min each) with TTBS the strips were treated for 2 hr at room temperature (30°C) with peroxidase conjugated antirabbit and antihuman immunoglobulins (Igs) (1 : 500 in 1% milk TTBS). Strips were washed (5 × 5 min) with TTBS and finally once with TBS. Strips were developed with 4-chloronaphthol and reaction was stopped by extensive washing with distilled water.

Results

Analysis of the subcellular fractions of whole cell sonicate of *M. habana* revealed that the cell wall (CW) constituted 32%, the cell membrane (CM) only 2% and the cytosol (CS) about 46% of the cellular mass (in terms of proteins).

SDS-PAGE pattern of the proteins of CW, CM and CS after coomassie blue staining are shown in Figure 1. Cytosol fraction showed the greatest number of individual bands. It is apparent that the major protein bands are at ≃33 kD in CW, ≃38 kD in CM and ≃22 kD in CS. The consistency in protein migration pattern of fractions prepared in 2 different batches is also apparent in Figure 1.

Subcellular location of immunologically active protein antigens has been determined by probing the antigen loaded strips of the 3 fractions with heterologous polyclonal antibodies.

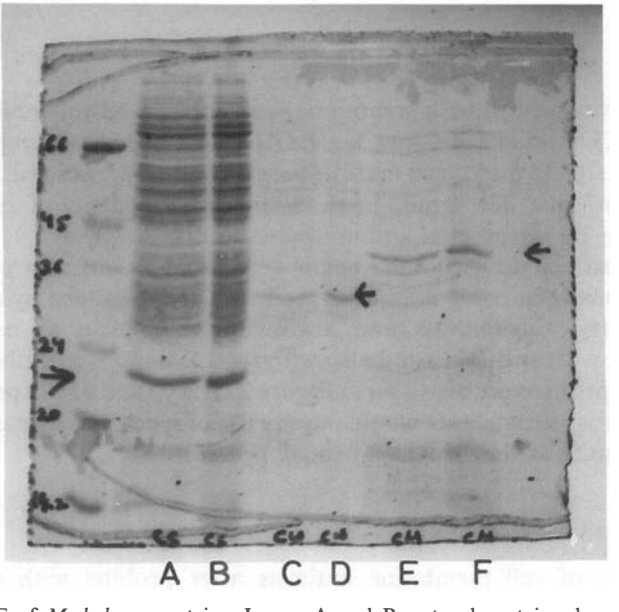

Figure 1. SDS-PAGE of *M. habana* proteins. Lanes: A and B, cytosol proteins; lanes C and D, cell wall proteins: E and F, cell membrane proteins. Molecular weight markers (MWM) are shown on the left.

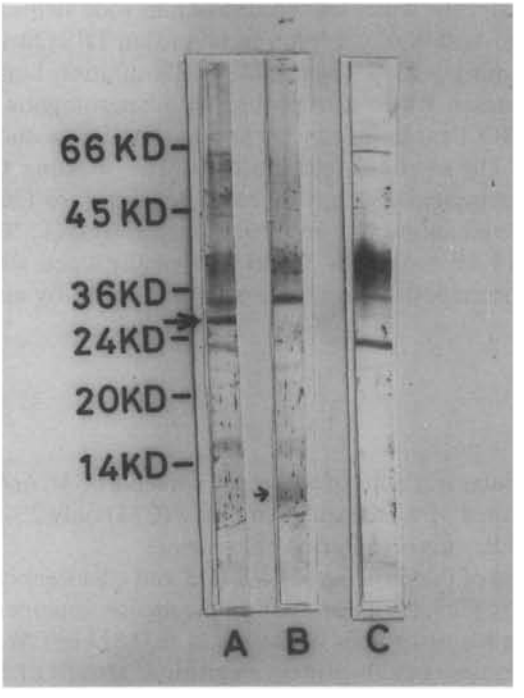

Figure 2. Probing of *M. habana* cell wall antigens with heterologous antibodies. Lane A: anti-*M. leprae* abs; lane B: anti-*M. tuberculosis* abs; and lane C: antiBCG abs. Molecular weight markers are shown on the left.

THE CELL WALL ANTIGENS

These antigens were probed with serum pools of leprosy and tuberculosis patients and also with antiBCG antibodies (Figure 2). The LL serum pool has recognized a dominant protein band at ≃26 kD molecular mass in the cell wall of *M. habana*, which is not seen after probing with the TB serum pool. Similarly, a protein of ≃10 kD has been recognized by the TB serum pool and not by LL sera.

A strong broad and diffused band below 40 kD and 1 dominant protein at ≃30 kD molecular mass has been recognized in the cell wall of *M. habana* by all the polyclonal antibodies (leprosy, tuberculosis and antiBCG). A protein at ≃23 kD has been prominantly seen with anti-BCG and also with the LL but not with the TB serum pool.

Several other proteins are also seen in Figure 2. The 60 and 65 kD proteins seen in the antiBCG and leprosy serum lanes would require use of specific monoclonal antibodies to confirm their identity as the Groel heat shock proteins.

THE CELL MEMBRANE ANTIGENS

The western blot of cell membrane antigens after probing with these 3 types of polyclonal antibodies (*vide supra*) is shown in Figure 3.

Proteins of ≃35 kD and ≃18 kD have been recognized only by the LL serum pool

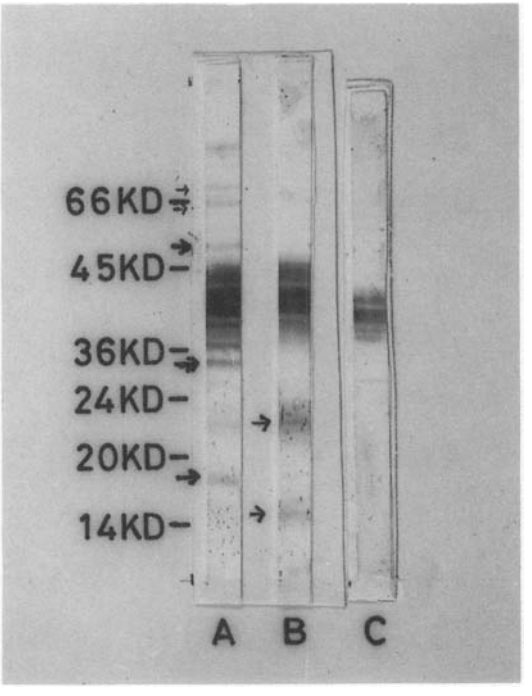

Figure 3. Probing of *M. habana* cell membrane antigens with heterologous antibodies (see Figure 2).

and not by the TB sera. Several other protein bands, i.e. at ≃70, ≃65, and ≃50 kD molecular mass are evident in the LL serum pool but is not so clearly seen in other lanes.

A diffuse broad band at 20–24 and 1 at ≃14 kD are seen in the TB serum lane which are not so well marked in the LL serum lane.

AntiBCG serum has recognized a broad and diffuse band of proteins at 36–40 kD which are also recognized by LL and TB serum.

CYTOSOLIC ANTIGENS

Cytosolic antigens of *M. habana* after probing with these 3 types of polyclonal antibodies have been shown in Figure 4.

A broad band at ≃24 kD and several other bands have been seen in the LL serum lane but not in TB serum lane.

AntiBCG serum has recognized several protein bands between 40–65 kD molecular mass which are also seen faintly/prominantly in the LL sera and feebly in the TB sera lane.

Discussion

Integral mycobacterial vaccines have generally provided less than desirable protection.[11] This may possibly be due to an adverse effect of certain mycobacterial constituents.

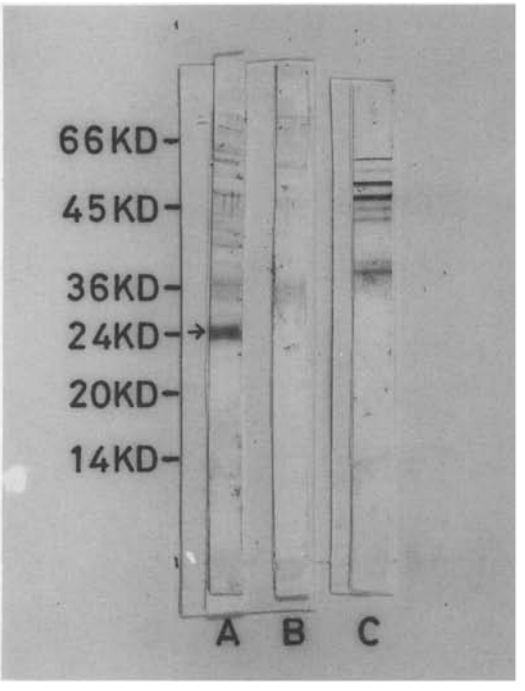

Figure 4. Probing of *M. habana* cytosol antigens with heterologous antibodies (see Figure 2).

Protection against intracellular mycobacterial infection is regulated through cell-mediated immune (CMI) response.[12] CMI response, in turn, is induced by the activation of relevant T cells (helper, $CD4^+$) by appropriate protein/peptides.[13] Mycobacterial proteins are known to bear both B and T cell epitopes and induce, through them, humoral and cell-mediated responses.[14–16] Research on mycobacterial proteins is still in a descriptive phase. Nevertheless, searching the antigens that might be involved in eliciting a protective CMI response by probing the epitopes that are recognized by B cell[17] is considered a justified approach. Therefore, immunological characterization of these proteins is done by studying their B cell reactivity through corresponding antibodies. Moreover, according to a prevalent view, protective, antigens of mycobacteria are those which are shared by other mycobacterial species rather than species specific.[6,18] These facts put together inspired us to undertake a systematic study to identify the myriad of protein antigens present in *Mycobacterium habana*, an atypical mycobacterium. The mycobacterium offers protection against experimental tuberculosis[7], leprosy[6], buruli ulcer[19] and shows antigenic cross-reactivity with *M. tuberculosis*, *M. leprae* and BCG.[20]

In this study we have identified some protein antigens in *M. habana* which are identified only by pooled LL serum and not by pooled TB serum or vice versa. These may be important for immunodiagnosis and/or immunoprophylaxis in these mycobacterial infections. In order to analyze the antigenic mosaic, mid-log cultures of *M. habana*

have been used to avoid the known contamination of autolytic cell products and irrelevant polysaccharides in old cultures.[21] Subcellular location of immunologically important proteins within the cell have been determined by preparing 3 broadly defined fractions, namely, the cell wall, the cell membrane and the cytosol. Protein profile of these subcellular fractions have been analysed by polyacrylamide gel electrophoresis under reducing conditions (SDS-PAGE) and 3 major proteins, ≃22 kD in cytosol, ≃38kD in membrane and 33 kD in wall fraction have been recognized by direct protein staining.

We identified 4 antigens of *M. habana*, i.e. ≃26 kD of CW, ≃35 kD and ≃18 kD of CM and ≃24 kD of CS, only by pooled LL serum. Similarly, 3 antigens ≃10 kD of CW, 20–24 kD and ≃14 kD of CM have been identified only by pooled TB serum. These findings indicate that these antigens may bear epitopes which are common between *M. habana*, and *M. leprae* only or *M. habana* and *M. tuberculosis* only. These may have diagnostic and/or protective potential in leprosy and tuberculosis.

A ≃40 kD antigen of cytosol fraction has been strongly recognized by anti-BCG antibodies. Similarly, ≃23 kD cell wall protein has been identified by anti-*M. leprae* and antiBCG antibodies. A ≃30 kD antigen of CW has been recognized by all the 3 antibodies. These observations suggest antigenic homology between *M. habana* and *M. leprae*, *M. tuberculosis* and BCG.

A 35 kD antigen of *M. leprae*[15,16] and *M. tuberculosis*[14] and a 14 kD antigen of *M. tuberculosis*[22] have already been reported to be of high protective and/or diagnostic value. Interestingly the proteins of corresponding molecular weights have been identified in *M. habana* also and more importantly by patient sera. Therefore, findings of this study will provide more appropriate information about the particular antigen of interest. The present effort to find subcellular location for some of the important predominant proteins may help in assigning a functional role of them.

Acknowledgment

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Lepr Rev (1995) **66,** 39–47

Inhibition of the multiplication of *Mycobacterium leprae* in nude mice by intermittent administration of a new rifamycin derivative, 3′-hydroxy-5′-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin (KRM-1648) combined with sparfloxacin

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Summary Inhibition of the multiplication of *Mycobacterium leprae* in the footpads of nude mice by the oral administration of sparfloxacin, a new quinolone, and 3′-hydroxy-5′-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin (KRM-1648), selected from a series of newly synthesized benzoxazinorifamycins, was studied. When the 2 drugs were administered alternately at intervals of 3 or 4 days, (i.e., each drug was administered once weekly), or simultaneously once weekly, between 3 and 5 months after inoculation of nude mice with *M. leprae*, 10 mg sparfloxacin and 0·6 mg KRM-1648 per kg bodyweight were sufficient to prevent multiplication of the organisms. Only partial inhibition of multiplication was achieved by alternate administration of 5 mg sparfloxacin and 0·3 mg KRM-1648 per kg, as was the case for 20 mg sparfloxacin per kg or 1 mg KRM-1648, each drug administered alone once weekly. The addition to these 2 drugs of dapsone, administered in the diet in a concentration of 0·001 g per 100 g, enhanced their effect.

The potential usefulness of multidrug regimens including these compounds is considered.

Introduction

We have previously reported that a new rifamycin derivative, 3′-hydroxy-5′-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin (KRM-1648) (see Figure 1 for structure) entirely prevented the multiplication of *M. leprae* in the footpads of congenitally athymic nu/nu (nude) mice, when the drug was administered twice weekly *per os* in dosages of 1 or 3 mg per kg bodyweight between 3 and 5 months after the animals had been inoculated.[1] We have also reported that administration of sparfloxacin (SPFX) in dosages of 10, 20 or 30 mg per kg by the same schedule partially inhibited the multiplication of the organisms.[2] In this paper, we report the results of several experiments

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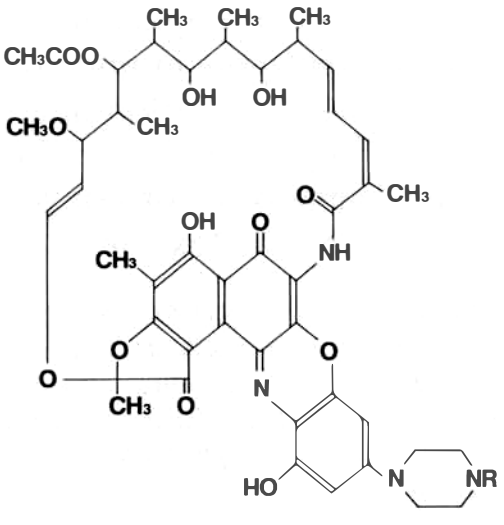

Figure 1. Chemical structure of KRM-1648.

in *M. leprae*-infected nude mice, in which these 2 drugs were administered alternately or simultaneously at weekly intervals.

Materials and methods

KRM-1648 was supplied by the Biochemical Research Laboratories, Kaneka Corporation, Ltd., and SPFX by the Bioscience Research Laboratories, Dainippon Pharmaceutical Co., Ltd. Emulsified suspensions of KRM-1648 and SPFX were prepared and preserved as described previously.[1,2] These 2 drugs were administered through mouse catheters alternately at intervals of 3 or 4 days (each drug was administered once weekly) in experiments 1 and 2, whereas they were administered simultaneously at intervals of 1 week in experiment 3. Dapsone, purchased from Wako Pure Chemicals Co., Ltd., Tokyo, and formulated into heat-stable pellets by Funabashi Nojyo Co., Ltd., Chiba, Japan, was administered incorporated in their diet in a concentration of 0·001 g per 100 g in experiment 4.

Female BALB/c *nu/nu* mice, aged 5 weeks, were purchased from Clea Japan Inc., Tokyo. The mice were bred and grouped as described earlier.[1,2]

M. leprae of the Thai 53 strain,[1,2,4] which had been maintained in nude mice through 10 or 11 passages, were employed. Inocula were prepared and the mice infected as described in our earlier reports,[1,2] except that 10^7 organism were inoculated into each hind footpad. Acid-fast bacilli (AFB) were harvested individually (2 hind footpads were pooled), and enumerated by the method of Shepard & McRae.[5] Each time 20 microscopic fields were counted for each of 2 circular smear spots of 1-cm diameter with fields of AFBs comparatively uniformly distributing, as a portion of supernatant from emulsified 2 hind footpads pooled from each animal, and the average count of AFB and the standard deviation (SD) were calculated for 2 or 3 animals.

Results

ALTERNATE ADMINISTRATION OF KRM-1648 AND SPFX

In experiment 1, KRM-1648 was administered *per os* in a dosage of 1 mg per kg bodyweight every Friday, and SPFX was administered *per os* in a dosage of 10 or 20 mg per kg every Monday, from the beginning of the 3rd month until the end of the 5th month after the nude mice had been inoculated with 10^7 *M. leprae* into each hind footpad. As shown in Figure 2, administration of KRM-1648 alone or of SPFX alone in

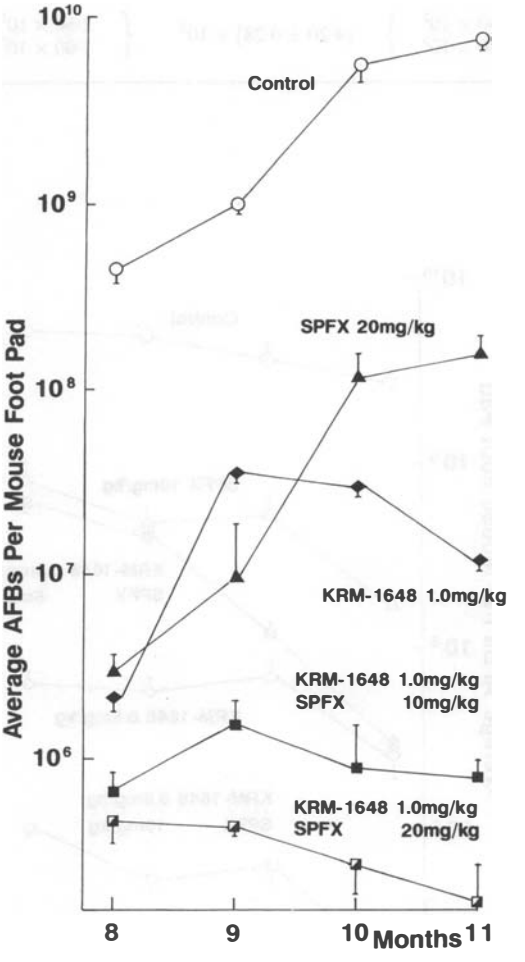

Figure 2. Inhibition of multiplication of *M. leprae* inoculated into nude mouse footpads by alternate treatment with KRM-1648 and sparfloxacin (SPFX) at intervals of 3 or 4 days. Groups of 10 nude mice were infected with *M. leprae*, strain Thai 53 by 1×10^7 bacilli into each of both hind footpads and then treated with oral administration of KRM-1648 and SPFX, alternately at intervals of 3 or 4 days (each drug was administered once weekly), between 3 and 5 months after inoculation. Otherwise, animals were orally treated with KRM-1648 or with SPFX, once weekly. We took 4 or 6 footpads of 2 or 3 mice at the indicated months after inoculation and AFBs in the footpads were counted up.

Table 1. Number of AFBs detected at 9 and 11 months after inoculation, in the 4 footpads of 2 mice, respectively, belonging to the 2 groups shown in Figure 2

	Number of acid-fast bacilli detected			
	9 months		11 months	
Group	AFB/FP	(Mean ± SD)	AFB/FP	(Mean ± SD)
Control	$1{\cdot}12 \times 10^9$ $0{\cdot}89 \times 10^9$	$(1{\cdot}01 \pm 0{\cdot}16) \times 10^9$	$7{\cdot}85 \times 10^9$ $7{\cdot}51 \times 10^9$	$(7{\cdot}68 \pm 0{\cdot}24) \times 10^9$
KRM 1 mg/kg+ SPFX 10 mg/kg	$1{\cdot}89 \times 10^6$ $1{\cdot}14 \times 10^6$	$(1{\cdot}52 \pm 0{\cdot}53) \times 10^6$	$8{\cdot}48 \times 10^5$ $7{\cdot}35 \times 10^5$	$(7{\cdot}88 \pm 0{\cdot}74) \times 10^5$
KRM 1 mg/kg+ SPFX 20 mg/kg	$4{\cdot}00 \times 10^5$ $4{\cdot}40 \times 10^5$	$(4{\cdot}20 \pm 0{\cdot}28) \times 10^5$	$2{\cdot}60 \times 10^5$ $1{\cdot}00 \times 10^5$	$(1{\cdot}80 \pm 1{\cdot}13) \times 10^5$

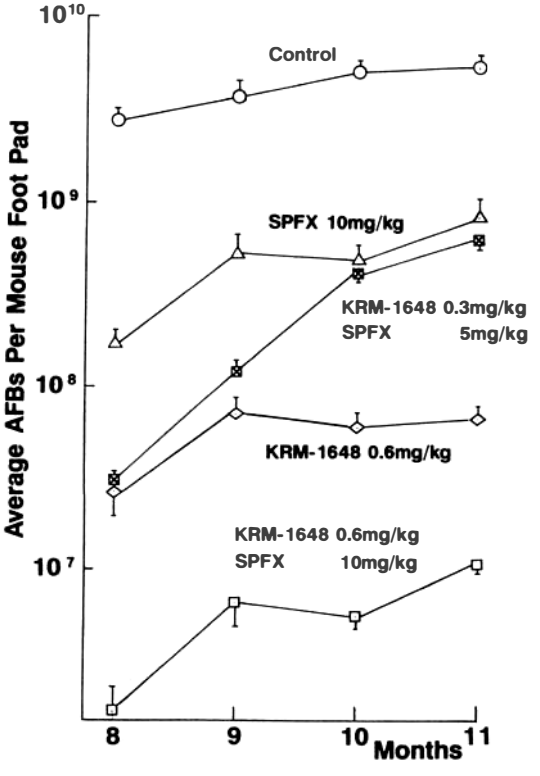

Figure 3. Inhibition of multiplication of *M. leprae* inoculated into nude mouse footpads by alternate treatment with low doses of KRM-1648 and SPFX at intervals of 3 or 4 days. Grouping of nude mice, inoculation of *M. leprae*, frequency of dose and counting of AFBs were performed according to the methods shown in the legend to Figure 2.

the higher dosage only partially inhibited multiplication of the *M. leprae*. On the other hand, alternate administration of these 2 drugs entirely prevented multiplication of the organisms, even when SPFX was administered in the lower dosage. As for 2 groups in Figure 2 in which detected AFBs were always below the number of *M. leprae* inoculated into each foot pad, the results are demonstrated by number of AFBs at 9 and 11 months after inoculation, as shown in Table 1.

In experiment 2, the efficacy was tested of the drugs administered in smaller dosages by the same schedule. As shown in Figure 3, weekly administration of SPFX in the dosage of 10 mg per kg only partially inhibited the multiplication of the *M. leprae*, as may also have been the case for KRM-1648 administered in a weekly dosage of 0·6 mg per kg. Similarly, the alternate administration of 5 mg SPFX and 0·3 mg KRM-1648 per kg was only partially inhibitory, whereas alternate administration of 10 mg SPFX and 0·6 mg KRM-1648 entirely prevented the multiplication of the organisms.

SIMULTANEOUS ADMINISTRATION OF KRM-1648 AND SPFX

In experiment 3, the 2 drugs were administered simultaneously on 12 occasions 1 week

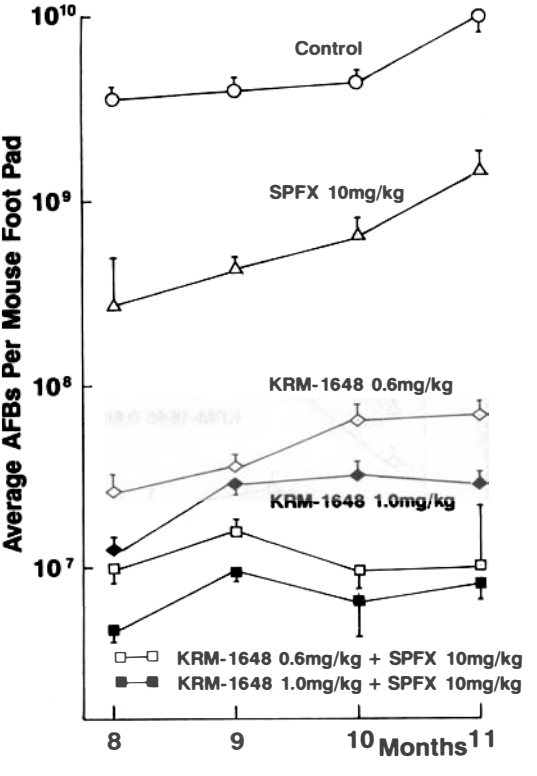

Figure 4. Inhibition of multiplication of *M. leprae* inoculated into nude mice footpads with simultaneous and once-weekly administration of KRM-1648 and SPFX. Grouping of nude mice, inoculation of *M. leprae* and counting of AFBs were performed according to the methods shown in the legend to Figure 2.

apart. As shown in Figure 4, the combination of 10 mg SPFX per kg with KRM-1648, the latter drug administered in a dosage of 0·6 or 1·0 mg per kg, entirely prevented multiplication of the organisms, as had been the case when the 2 drugs were administered alternately.

COMBINATION OF KRM-1648 AND SPFX WITH DAPSONE

In the final experiment, 2 groups of mice were administered dapsone in their diet at a concentration of 0·001 g per 100 g for 3 months, from the beginning of the 3rd month to the end of the 5th month after inoculation. In addition, 0·6 mg KRM-1648 and 10 mg SPFX were administered simultaneously once weekly to 1 of the groups from the start till the end of dapsone administration. Administration of these 3 drugs alone only partially inhibited the multiplication of the *M. leprae*, whereas the simultaneous administration of KRM-1648 and SPFX entirely prevented their multiplication. The addition of dapsone appears to have enhanced the efficacy of the 2-drug combination.

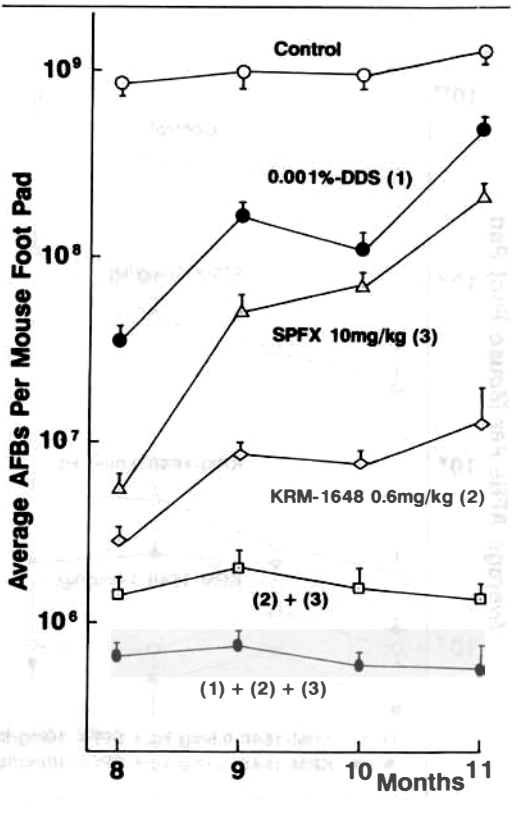

Figure 5. Inhibition of multiplication of *M. leprae* inoculated into nude mice footpads with simultaneous and once-weekly administration of KRM-1648 and SPFX, or with that further combined with dapsone. Grouping of nude mice, inoculation of *M. leprae* and counting of AFBs were performed according to the methods shown in the legend to Figure 2. Dapsone was administered between 3 and 5 months after inoculation through the diet prepared by containing 0·001 g dapsone per 100 g.

Table 2. Number of AFBs detected at 9 and 11 months after inoculation, in the 4 footpads of 2 mice, respectively, belonging to the 3 groups shown in Figure 5

	Number of acid-fast bacilli detected			
	9 months		11 months	
Group	AFB/FP	(Mean ± SD)	AFB/FP	(Mean ± SD)
Control	$9{\cdot}35 \times 10^8$ $10{\cdot}29 \times 10^8$	$(9{\cdot}82 \pm 0{\cdot}66) \times 10^8$	$1{\cdot}22 \times 10^9$ $1{\cdot}27 \times 10^9$	$(1{\cdot}25 \pm 0{\cdot}04) \times 10^9$
KRM-1648 0·6 mg/kg (2)	8.19×10^6 $8{\cdot}61 \times 10^6$	$(8{\cdot}40 \pm 0{\cdot}30) \times 10^6$	$1{\cdot}72 \times 10^7$ $0{\cdot}85 \times 10^7$	$(1{\cdot}29 \pm 0{\cdot}62) \times 10^7$
(2) +SPFX 10 mg/kg (3)	$2{\cdot}21 \times 10^6$ $1{\cdot}79 \times 10^6$	$(2{\cdot}00 \pm 0{\cdot}30) \times 10^6$	$1{\cdot}47 \times 10^6$ $1{\cdot}21 \times 10^6$	$(1{\cdot}34 \pm 0{\cdot}18) \times 10^6$
(2) + (3)+ 0·001%-DDS (1)	$6{\cdot}30 \times 10^5$ $8{\cdot}40 \times 10^5$	$(7{\cdot}35 \pm 1{\cdot}48) \times 10^5$	$6{\cdot}83 \times 10^5$ $4{\cdot}20 \times 10^5$	$(5{\cdot}52 \pm 1{\cdot}86) \times 10^5$

The results of 3 groups shown in Figure 5, in which multiplication of *M. leprae* was suppressed to a level comparable to or below the number of the organisms inoculated into each footpad, are demonstrated by the detected number of AFB in Table 2.

Discussion

The inhibitory effect on multiplication of *M. leprae* by sparfloxacin and KRM-1648 administered alternately at intervals of 3 or 4 days or simultaneously at intervals of 1 week were determined using an *M. leprae* infection model in nude mouse footpads. Findings demonstrated that 10 mg of sparfloxacin and 0·6 mg of KRM-1648 per kg bodyweight were sufficient to prevent entirely the multiplication of *M. leprae* in mouse footpads in both the dosing methods. Nevertheless, when the 2 agents were combined, administration of 5 mg of the former and 0·3 mg of the latter per kg of bodyweight at 1-week intervals proved to be insufficient. Review of the findings of our experiments suggests that the alternative administration of 20 mg of sparfloxacin and 1 mg of KRM-1648 per kg of bodyweight at intervals of 3 or 4 days (1st experiment) and the simultaneous administration of 10 mg of sparfloxacin with 0·6 mg of KRM-1648 per kg of bodyweight combined with dapsone (4th experiment) seem to be the 2 most potent regimens.

An adverse effect of rifamycin analogues is the toxicity to hepatic enzymes[7] and blood cells at high dosages.[8] Repeated use of high-dose rifabutin or rifampicin induces predominancy of extrahepatic metabolism, which appears to reduce AUC.[9] For this reason, lower dosage and frequency of administration are best for rifamycin analogues. KRM-1648 is useful not only because it can be administered in low doses but also because it may achieve economical inhibition of the multiplication of *M. leprae*.

Regarding the selection of a companion drug in multidrug therapy (MDT), Banerjee *et al.*[10] reported a 100-fold reduction in the viability of *M. leprae* in nude mouse footpads achieved with the combined administration of high-dose ofloxacin and rifabutin compared that with administration of either drug alone, suggesting the possibility of

synergism between this new quinolone, sparfloxacin and this new rifamycin derivative, KRM-1648. In fact, a co-operative effect of sparfloxacin in combination with KRM-1648 was detected in the 1st experiment.

Yoder *et al.*[11] reported the cross-resistance of rifabutin to rifampicin in a strain of rifampicin-resistant *M. leprae* in nude mice. Saito *et al.*[12] found strain-specific cross-resistance of KRM-1648 to rifampicin in various rifampicin-resistant mycobacterial strains. Partial cross-resistance of sparfloxacin and ofloxacin in the treatment of *M. tuberculosis in vitro* has also been reported.[13] These findings remind us of the importance of completely eradicating *M. leprae* as rapidly as possible with strong MDT.

In the 4th experiment, dapsone was tested in combination with KRM-1648 and sparfloxacin. Pronounced inhibition of multiplication as evidenced by reduction even in the number of nonsolid bacilli was observed. However, if the amount of dapsone contained in the 0·001%-DDS diet is converted to the amount in the diet of an adult weighing 60 kg, it corresponds to approximately 72–150 mg daily. Although the tissue concentrations of KRM-1648 are higher in the spleen than in the liver,[1] while those of sparfloxacin[14] and dapsone are the opposite, a devised administration of dapsone to decrease the metabolic load on the host due to this multidrug regimen can be expected for clinical use, particularly because both KRM-1648 and sparfloxacin are long-acting substances, but also because dapsone exhibits slow clearance.

Recently, Franzblau *et al.* (personal communication) examined the clinical effect of sparfloxacin on 9 lepromatous cases using assay of PGL-1 antigen, determination of killing of leprosy bacilli with the mouse footpad method, and a radiorespirometry. Daily treatment with 200 mg sparfloxacin for 12 weeks was found to be sufficiently effective. This dosage of sparfloxacin corresponds at most to only 20–40% of those used for nude mice in the present study.

Given these findings, studies on rapid and complete eradication of *M. leprae* with a minimal number of combined administration of KRM-1648 and sparfloxacin with or without dapsone will be continued using the *M. leprae* infection model in nude mice as described here.

Acknowledgment

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Lepr Rev (1995) **66,** 48–54

Vascular surgery of the posterior tibial compartment for plantar ulceration in leprosy

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Summary Traditional surgical decompression of the posterior tibial nerve yields equivocal results. The authors postulate that the posterior tibial artery is the most compromised structure in the neurovascular compartment and that the best surgical results in healing of plantar ulcers are achieved by the rechannelling of the blood flow in the posterior tibial artery during posterior tibial neurovascular compartment surgery.

This procedure has been of benefit to patients with plantar ulcers of greater than 7–10 years' duration in whom all other modes of healing had failed. It has been undertaken as an outpatient procedure under local anaesthesia, supported by postoperative vasodilator drugs. The use of tourniquet, antibiotics and surgical interference with the ulcer *per se* was eschewed. A report of 156 patients is presented with follow-up of up to 6 years for the earlier cases.

Introduction

Recurrent plantar ulceration of the anaesthetic foot is a severely disabling deformity in leprosy, the most severe deformity leading to loss of toes. This presents a difficult problem in the rehabilitation of these patients and may even necessitate amputation. According to Srinivasan[1] 10–15% of leprosy patients suffer from neuropathic plantar ulceration. Ulceration also occurs in other neuropathies like diabetes mellitus, syringomyelia, tabes dorsalis and alcoholic peripheral neuropathy.

The commonest form of treatment for plantar ulceration has been evaluation and rest. A Plaster of Paris (POP) cast for 6–8 weeks heals most superficial ulcers.[12] Curettage and debridement of the ulcer followed by a plaster cast is employed for deeper ulcers.

Radical metatarsectomy for deep ulcers over the 2nd, 3rd and 4th metatarsal heads[13,14] and also protective footwear for leprosy patients was described by Robertson[15] and Antia *et al.*[16]

The importance of damage to the posterior tibial nerve as a cause of anaesthesia has been well documented. Decompression of the nerve in the neurovascular compartment has given equivocal results even though this was regarded as the most important site for surgical intervention as far back as the mid 1970s.[2] However, in 1955 Dharmendra *et al.*[5]

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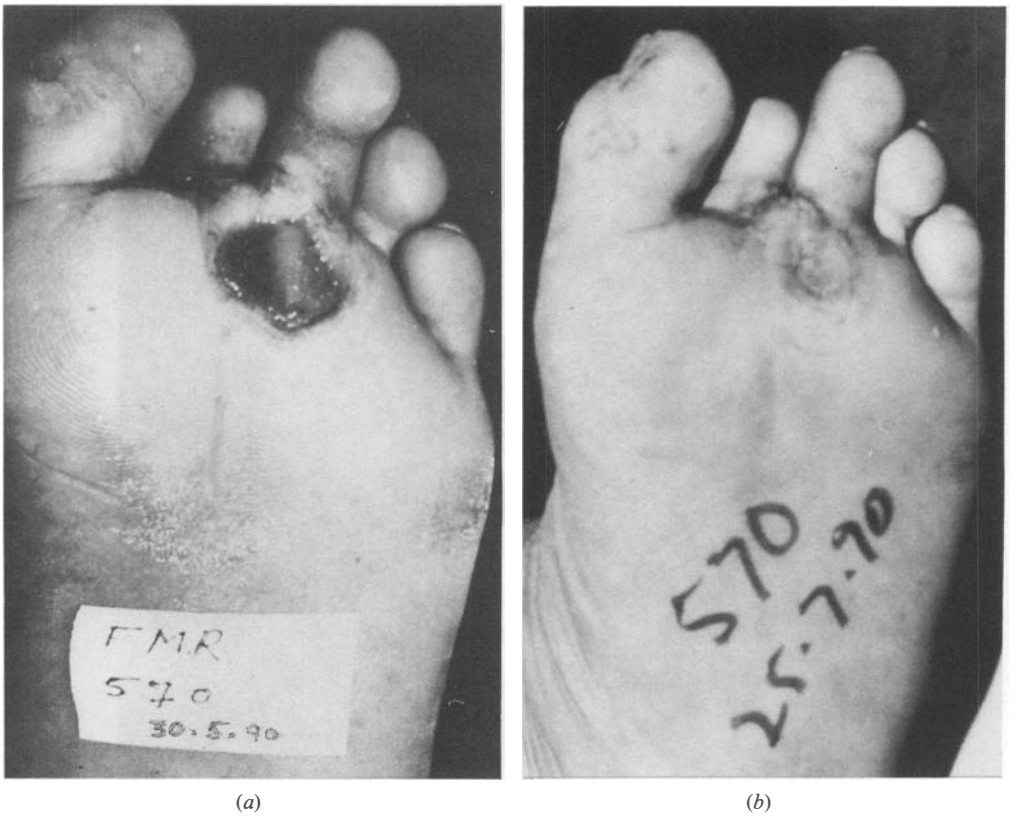
stated that a reduction in blood supply was an important factor in plantar ulceration, and Chatterjee[7] also suggested that neurological symptoms in leprosy were not so much due to uncomplicated Wallerian degeneration as to reduced neural circulation.

Vascular changes in leprosy have been documented in the literature. Terminal arteritis and vasculitis of vasa nervorum was described by Mitsuda.[6] Carayon's[3] studies of the posterior tarsal and calcaneal canals demonstrated venous hyperplasia and hypertrophy around the nerve. Cochrane & Davey[8] postulated that neuroparesis of the arterial wall in leprosy led to flaccidity, and the slowing of circulation with associated tortuosity due to external compression. Carayon[3] also demonstrated improvement of the 'spastic' state of the posterior tibial artery by unroofing the tarsal tunnel. However, he found that decompression of the vessel was ineffective in the presence of endovascular obstruction. Debi *et al.*[9] reported obliteration of the vascular lumen in 5 out of 20 lepromatous leprosy cases with plantar ulceration of over 2 year's duration. There was a correlation between the age of the patients, the duration of the ulcer and arteriographic findings, so they concluded that advanced vascular changes in distal digital vessels had contributed to ischemia and ulceration. Agarwal[10] performed percutaneous arteriography and muscle biopsy, finding tapering, occlusion and tortuosity of vessels with absence of collaterals. He considered that vascular thickening, perivascular granuloma and lymphocytic infiltration in the smooth muscle of the artery might be responsible for trophic ulceration.



(*a*) (*b*)

Figure 1. (a) Preoperative; (b) postoperative (9 weeks) view of plantar ulcer.

There is thus increasing evidence in the literature of a shift in emphasis from a neural to a vascular aetiology of plantar ulceration. Possibly both factors should be considered.

In September 1984 Arolkar devised an entirely different surgical approach, based on the posterior tibial artery rather than on the nerve, and the details follow.

Method

Arolkar's surgical approach was undertaken as an outpatient procedure under local anaesthesia and without the use of a tourniquet. The dissection can be undertaken without an operating microscope. The traditional postoperative use of a POP

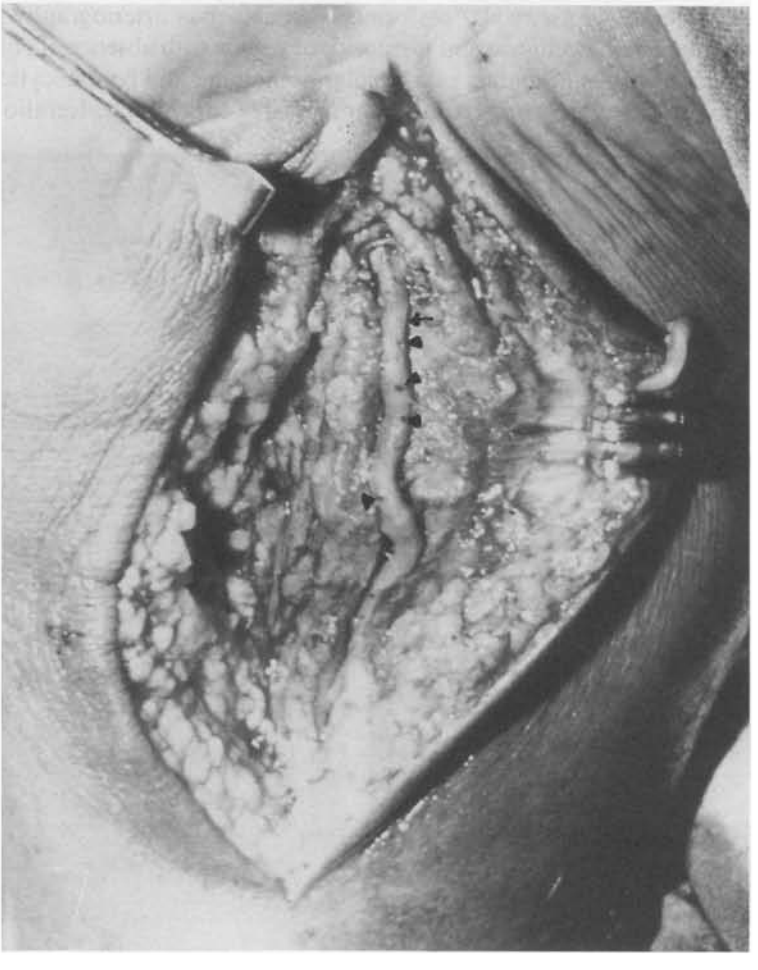


Figure 2. Operative field showing the freed posterior tibial artery (→) with some of its branches cauterized (►).

immobilization for 3–6 weeks has been reduced to 1 week, followed by the use of the patient's usual footwear postoperatively. Oral vasodilators like isoxprine were prescribed from 6 weeks to 6 months after surgery. No antibiotic is used even in the presence of active ulceration (Figure 1).

To date 156 ulcerated feet have been treated using this technique.

PREOPERATIVE PREPARATION

The foot and the ulcer are cleaned with soap and water only. No physiotherapy is prescribed.

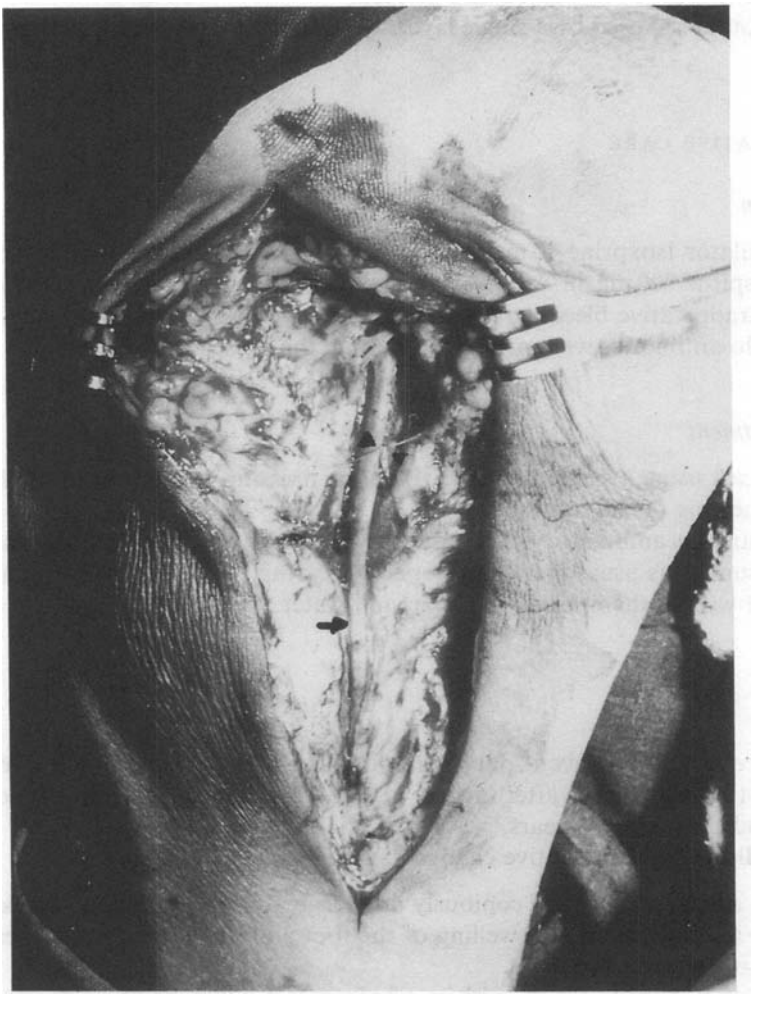


Figure 3. Operative field showing the vein (▶) straddling the posterior tibial artery (→) at its bifurcation above the mouth of the tarsal tunnel.

SURGICAL TECHNIQUE

Local anaesthesia, 1% xylocaine with adrenaline (1 : 2,000,000) was infiltrated midway between the medial malleolus and heel, from 4·2 cm below to 8·5 cm above the medial malleolus. The posterior tibial neurovascular compartment was exposed by incising the flexor retinaculum. A longitudinal strip 1-cm wide of the thickened sheath overlying the neurovascular compartment is dissected out and excised. Care is taken to avoid any interference with the nerve or its vascularization from the underlying bed. The posterior tibial artery is completely separated from the accompanying nerve, and from its bed. All the branches varying from 8 to 14 in number along the entire exposed length of this segment of the artery are cauterized in continuity up to the bifurcation of the vessel into the medial and lateral plantar divisions in the tarsal canal (Figure 2). A vein, which is consistently observed to straddle the bifurcation, and possibly obstruct the arterial blood flow (Figure 3), is ligated and divided.

The wound is closed by a single layer subcuticular suture which is removed 6 weeks later.

POSTOPERATIVE CARE

Medication

The vasodilator Isoxprine 40 mg is given orally in 4 divided doses daily, together with soluble Aspirin 700 mg in 4 divided doses. If used preoperatively these drugs cause intense intraoperative bleeding and hence were given only postoperatively, for up to 6 months. No antibiotics were prescribed despite the presence of ulcers.

Local treatment

A plaster cast using a single roll of POP on the dressing is maintained for 1 week. This helps the healing of the sutureline.

The patient is ambulatory 48 h postoperatively. After removal of the plaster cast the patient resumes his usual footwear, possibly wearing socks. No dressings are used and the patient washes the wound with soap and water.

Results

Table 1 provides the relevant data regarding the size (a), site (b) and duration (c) of ulcers and the healing time after surgery (d). The shortest postoperative follow-up was 6 months and the longest 6 years.

The following postoperative changes were observed:

There was drying of previous copiously discharging ulcers within 2–3 weeks.
There was a reduction in the swelling of the foot and therefore a subsequent ability to wear previously worn footwear.
The foot felt warmer to touch, with re-emergence of 'filled' veins on the dorsum of the foot.

Table 1

(a)			
Size of ulcer	<3cm diam— 51 cases >3cm diam—105 cases		
Total number of ulcers	—156		
(b)			
Site of ulcers			
Forefoot	—138 cases		
Lateral border of the foot	— 12 cases		
Heel	— 6 cases		
(c) Duration of ulceration (years)			
	No. of patients	Postoperative healing time (weeks)	
0–5	51	3–5	
5–10	73	4–6	
>10	32	>6 and more	
(d) Duration of postoperative follow-up			
	Patients		
6 months–2 years	24		
2 years–4 years	58		
4 years–6 years	74		

Chronic recurrent ulcers of up to 15-year's duration healed within 8–10 weeks without resort to split-skin grafts of flaps, utilizing the footwear used preoperatively. This was usually plastic footwear sold in the open market.

Because of an underlying necrotic bony tissue (which was confirmed by X-rays) 30 deep ulcers failed to heal postoperatively. (All ulcerated feet were not routinely X-rayed before surgery.) All but 2 of these ulcers healed by the 6th month with daily dry dressings.

The 2 cases with persistant ulceration and fungation after up to 6 months of conservative treatment had a below the knee amputation, squamous cell carcinoma being suspected and later confirmed histologically.

Conclusion

The experience of 156 cases to date suggests the probable importance of a combination of: 1, decompression of the neurovascular compartment by excision of the sheath; 2, sympathetic denervation of the posterior tibial vessels; and 3, selective diversion of the blood supply to the sole of the foot, by cauterizing branches of the posterior tibial artery, the last possibly playing the most significant role.

The operation has shown consistent results and its simplicity merits its use as a procedure of choice in the rehabilitation of those suffering from chronic plantar ulceration due to leprosy. Its use in other conditions, e.g. diabetes, has been beneficial especially to limit gangrene.

In order to provide a better understanding of the scientific basis of these results, in future we propose to correlate the clinical findings with studies of motor and sensory nerve conduction as well as measuring alterations in blood flow and vascularity of the foot. Raised skin temperature would also reflect increased vascularity and is measurable.

If increased vascularity is proven, the reason for this requires further physiological investigation since reducing the peripheral field of distribution of an artery by ligating branches may not automatically increase the flow in the parent stem vessel.

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Lepr Rev (1995) **66,** 55–62

Assessment of the methods available for testing sensation in leprosy patients in a rural setting

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Summary The aim of this study was to assess the efficacy, practicality and patient understanding of 5 methods used for testing sensation in leprosy patients, in a rural setting. The tests used were the WHO test, cottonwool, pin-prick, monofilaments and the biothesiometer. We concentrated on testing sensation in the hands, and the various tests were carried out on 75 patients and 32 controls, all taken from villagers living at Kindwitwi Leprosy Village, Tanzania. Our results showed that although the WHO test, cottonwool and pin-prick were all easy to use, cheap and well accepted they were not sensitive enough to be of any practical value. We found that the monofilaments, as well as being cheap and easy to use, had great potential value, as the 2-g monofilament could be used as a threshold value (indicative of leprosy, but not diagnostic) for protective sensation with a combined false-positive and false-negative value of only 4%. Finally, the biothesiometer was found to be a precise test that can accurately identify leprosy patients from controls and identify patients at risk of ulceration. It was, however, associated with its own problems, chiefly those of expense and its need of electricity, although we found this latter problem could be easily and relatively cheaply solved by the use of a solar powered recharger (Appendix).

Introduction

Leprosy is feared mainly because of the unsightly deformities and crippling disabilities that may follow. The real goal of leprosy programmes all over the world is to prevent deformities and disabilities caused by leprosy, by arresting the spread of the disease.[1] One of the most important factors in the prevention of disability is careful monitoring even after completion of treatment. As 'antileprosy' treatment does not necessarily prevent disability, it is essential to follow-up patients at high risk, and most in need of special attention, to prevent the development of disability.

The severe disability and disfigurement resulting from the destruction of tissue is exacerbated by the involvement of nerves.[2] Sensory testing has been shown to be much more reproducible, and therefore a more reliable method of testing for nerve damage than voluntary muscle testing alone,[3] although in an ideal situation both would be used. The testing of motor nerve conduction velocity requires capital investment and hospital

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conditions and is therefore not applicable everywhere.[4] Due to the problems associated with the other forms of testing, testing sensation is probably the best way of detecting nerve damage in rural clinics and the field. Therefore this study deals only with sensory testing. The aim is to assess the efficacy and practicality of the various methods available for testing sensation in the field.

The efficacy of the tests will be assessed by looking at their ability to identify a threshold value indicative of neural involvement and a level of protective sensation. This is a level of sensory loss beyond which patients are at a high risk of developing ulcers and thus becoming disabled. This is important as pressures on money and time make it essential for health workers to identify quickly those patients at the greatest risk of disability.

The various practical values and the problems associated with each test will also be assessed, looking particularly at their expense, requirements, e.g. electricity and special training, and their availability. Also the ease with which patients understood the tests and their acceptance or reluctance to participate in them will be investigated.

The tests selected for this study are the WHO sensory test (using a ball-point pen), cottonwool, pin-prick, monofilaments and the biothesiometer. The first 3 of these tests are all widely used in the Third World. Monofilaments, although widely used in the USA, have only been used in a few areas in the developing world. The biothesiometer has been used in the USA for at least 55 years;[5] however, its use in the UK has been limited to the last 10–12 years. It was designed to measure the threshold amplitude at which vibration becomes perceptible, and to measure any rise in the threshold during the disease, demonstrating a loss of vibratory sensation, e.g. as in diabetic peripheral neuropathy.[6]

Method

This study concentrates on testing the sensation in the hands rather than the foot, as there is less person-to-person variation in skin consistancy of the hands. This similarity between subjects is preferable when comparing different testing methods. In assessing the sensitivity of each test, sensation in the ulnar and median nerves in a group of 32 controls, 38 leprosy patients without hand ulcers and 37 leprosy patients with ulcers was recorded. These were then compared and questions were addressed as to whether threshold values, indicative of neural involvement, and a significant and critical loss of protective sensation could be found for each of the tests. The reproducibility of the test was assessed by testing each site 3 times and looking at the variation in values recorded.

SAMPLE OF PATIENTS AND CONTROLS

The patients and controls used in this study were all villagers at Kindwitwi Leprosy Village, Tanzania. The study was carried out in July and August, which was during the dry season. This is a village of around 600 people, about half of whom have leprosy. Within the village there is a wide range of disability, ranging from many patients with no visible signs of leprosy to others who have lost limbs due to chronic ulceration and infection. There were also 3 patients who did not live in Kindwitwi but came from the Rufiji delta and were identified as leprosy patients at outreach programmes.

The leprosy patients were divided into 2 groups, those who had never suffered from hand ulcers and those who were at present suffering from hand ulcers. Patients who had a past history of ulceration but without ulcers at the time of the study were excluded.

SUMMARY OF THE TESTS AND HOW THEY WERE USED

In order to try and minimize false results, testing with all the methods was carried out using the following guidelines and principles:

the tests were carried out in a private and quiet room and using an interpreter;
no testing session lasted more than 20 mins, as patients can lose interest;
a preliminary test was carried out, so the patient understood the test; and
after the preliminary test the patient was blindfolded and the selected areas were tested, each area being tested 3 times (a response to all 3 of the stimuli was regarded as 'having sensation'). To maintain concentration in a patient unable to feel at the test sites, areas where sensation was normal (not necessarily test sites) were stimulated after every 4 or 5 unfelt stimuli.

The tests were carried out with the arm and hand relaxed, supinated and fully supported. In order to test the ulnar and median nerve 2 sites were used; distal pulp of the little finger (ulnar nerve) and the distal pulp of the index finger (median nerve). The tests were always carried out in the same order.

WHO sensory test

This involves the application of firm pressure (sufficient to cause a 2-mm skin indentation) with a ball-point pen on to an area to be tested with the patient blindfolded (all the tests were carried out with the patients blindfolded to minimalize bias). The patient was asked to point to where he felt the pen, and if he was within 2 cm in both tested areas the test was recorded as positive. If he was unable to do so in 1 or both areas the test was concluded to be negative.

Cottonwool test

The patient was touched lightly (not stroked across the skin) with a piece of cottonwool rolled into a point. The patient was asked to identify the site touched. It was repeated 3 times at each site and the result for the hand was either positive or negative.

Pin-prick test

The pin used was sterile and not too sharp. The point was lightly touched on the skin; the test must not cause bleeding. If the individual felt the stimulus, he stated if it was sharp or blunt when compared with the sensation evoked in the normally sensitive skin on the arm.

Monofilaments

The monofilaments are a set of 4 graded nylon threads, that are calibrated to bend slightly when forces of 0·5 g, 2 g, 5 g and 10 g, respectively, are applied to them. The

threads are individually touched in ascending order, until they bend slightly, and then withdrawn on the skin at the test site. The patient is asked to point to where he felt the stimulus; for obvious reasons the sites should be varied, so nontest sites on the hand are also used. The value that a patient can reliably detect 3 times, at each site is then recorded. The lowest appreciated threshold was recorded as the hand threshold.

Biothesiometer

The biothesiometer has a handheld plastic probe which is held firmly on the skin. It vibrates at 120 Hz, and is powered by a rechargeable battery. The amplitude at which the probe vibrates can be altered within a range of 0–25 μm, by varying the voltage (the amplitude being proportional to the square of the voltage). As a preliminary each patient was familiarized with the sensation by turning the amplitude to a maximum. The patient was then blindfolded and tests were carried out by gradually increasing the amplitude from 0 until it was felt and the voltage was noted. The mean of the 3 values for each test site was then calculated and recorded.

Results

The monofilaments of the stated specifications were unable to demonstrate a clear threshold value, although the 2-g monofilament gave a level of protective sensation. If this level were to be used in a screening test it would be associated with a combined false-positive and false-negative level of only 4% (Table 1).

It can be seen that all the tests used could only differentiate between a positive and negative result, rather than having a spectrum of graded results, and were not sensitive enough to differentiate any sensory difference between the control group and the group with leprosy (Table 2). Only the cottonwool test produced results that suggested that it could be used as a field test to detect patients at risk of ulceration.

The biothesiometer results demonstrated clear differences between the 3 groups (Figure 1), allowing both threshold and protective sensation values to be identified. Using the value of 4 mV as a threshold value, the results show statistically significant differences between the control group and the leprosy patients (using the χ^2-test, $p < 0{\cdot}0005$). If this value was to be used as a screening level the results would suggest that it would be associated with a combined false-positive, false-negative rate of less than 8% (Table 3).

If the results are further broken down looking at leprosy patients with ulcers and those without it can be seen (Table 4) that if the 10 mV value is used there is again a

Table 1. The distribution of hand thresholds across the 3 groups

Group classification	0·5 g	2 g	5 g	10 g	Negative
Control ($n = 64$)	62	2	0	0	0
Leprosy +, ulcer −	50	22	4	0	0
Leprosy +, ulcer +	0	2	16	12	44

Table 2. Number of hands responding to the WHO, cottonwool and pin-prick tests

Group classification	WHO +ve	WHO −ve	Wool +ve	Wool −ve	Pin +ve	Pin −ve
Control ($n = 64$)	64	0	64	0	64	0
Lep+ ulcer− ($n = 76$)	76	0	72	4	54	22
Lep+, ulcer− ($n = 74$)	44	30	6	68	22	52

significant difference (using the χ^2-test, $p < 0{\cdot}0005$). Using this level as a screening value for protective sensation there would be an associated combined false-positive, false-negative level of approximately 8·5%.

Discussion

This study set out to assess the efficacy, practicality and the patients' understanding of 5 different methods of sensory testing. Our results agreed with previous studies,[4] in finding that although the WHO test is cheap, requires no specialized equipment and is easily understood, as it is not a graded test it is of little practical value. Similarly the cottonwool test requires little equipment and is easy to understand; however, it is again limited in not having a graded response. This limitation could explain why although it was able to identify a protective sensation level, it was unable to identify a threshold value. In addition this test is assessing fine touch and it is agreed that it is loss of firm touch that is of prime importance from the point of view of protection, and determines the likelihood of suffering injury. When testing the ability of patients to distinguish between a sharp

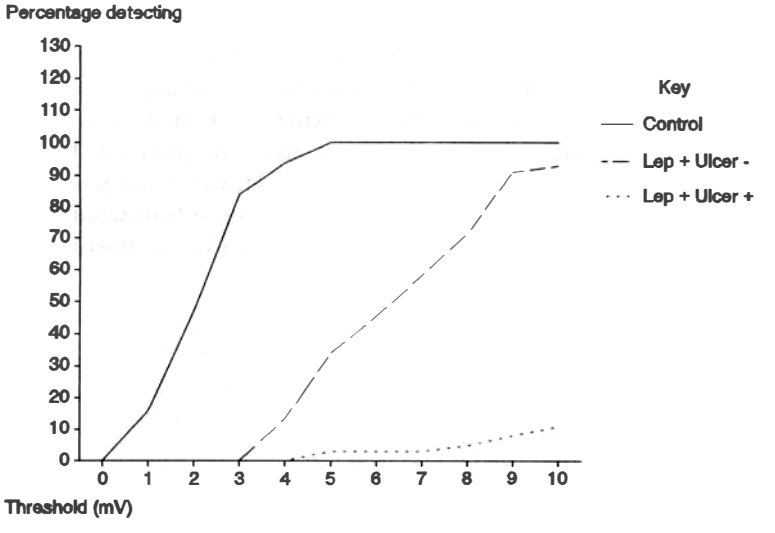

Figure 1. Distribution of vibration thresholds in the 3 groups.

Table 3. Threshold value detecting leprosy patients

Group	Felt 4	Could not feel
Control ($n = 64$)	56	8
Leprosy patients*	9	139

* Two patients did not understand.

and blunt stimulus, there were no difficulties in understanding. However, we feel this test is of limited value for 2 reasons. First it is essential that the needle used in the test is sterile,[7] unless a sliding pin is used which cannot puncture the skin.[2] Second, our results demonstrate that it is insufficiently specific to be of any value.

Monofilaments are a specialized piece of equipment. They are, however, inexpensive (£12 for a set of 3),[8] and it is also possible to make and calibrate them locally,[2,7] which is obviously much cheaper. The test can be carried out by any health worker after training for only 10 min and it is very easy for the patient to understand. We found it to be reproducible and rapid, taking less than 1 min to complete. Our results illustrated the practical importance of the monofilaments, as the 2-g monofilament can be used as a threshold value for protective sensation, with a low combined false-positive and false-negative level of 4%. We felt that the inability to identify a threshold level could be remedied by using more finely divided monofilaments, particularly in the lower range.

The biothesiometer has many practical difficulties. It requires an initial financial outlay of around £400 and also requires specialized knowledge in order for it to be repaired or maintained. A major problem associated with its use is due to the fact that it is electrical and it will only work for just over 1·5 hr before it needs recharging. In the area where this study was carried out there was no electricity and so in order to recharge the biothesiometer we designed and made a solar recharger. This itself was quite simple to make, requiring only 2 large solar cells and a few basic electrical components. (Details of this are to be found in the Appendix.) The equipment for this, however, would be hard to get hold of in the Third World and so would need to be imported in with the biothesiometer, again increasing expenses. We found the test was easy for health workers to carry out but took a lot of time and effort to explain to patients as in rural areas where there is no electricity, vibration is not a sensation easily comprehended, and occasionally that patients were unable to understand the concept. However, despite these problems it is important not to discard it as a method of testing, as our results demonstrated it to be a precise test that can accurately identify leprosy patients from controls and also which

Table 4. Protective sensation value detecting risk of ulcers

Group	Felt 10 or less	Could not feel
Lep+, ulcer−*	70	5
Lep+, ulcer+*	8	65

* Two patients did not understand.

patients are at risk of ulceration. Thus if a biothesiometer and methods of recharging it are available, that area is then in the position to carry out an effective screening programme. It is because of this, that we feel that the biothesiometer has and will have an important role in leprosy screening programmes, and should at the very least be available in reference centres.

Acknowledgments

We would like to thank Drs Mbega and Kombo and Father Robin Lamburn, MBE, who has now sadly passed away, at Kindwitwi Leprosarium for their help in carrying out this project. We would also like to thank Professor K. G. M. M. Alberti for providing us with equipment and ideas.

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Appendix

In order to recharge the biothesiometer we designed a simple solar powered piece of apparatus that could be used in the field (Figure 2). It consisted of 2 separate 12 V solar cells, which were placed in series and could therefore work independently of each other, should one of them break. These cells could be connected to the biothesiometer, and it could be completely recharged within 3 hr in bright sunlight. The cells were stored in a purpose-built wooden carrying case for protection. The total cost of the components for this unit was less than £25.

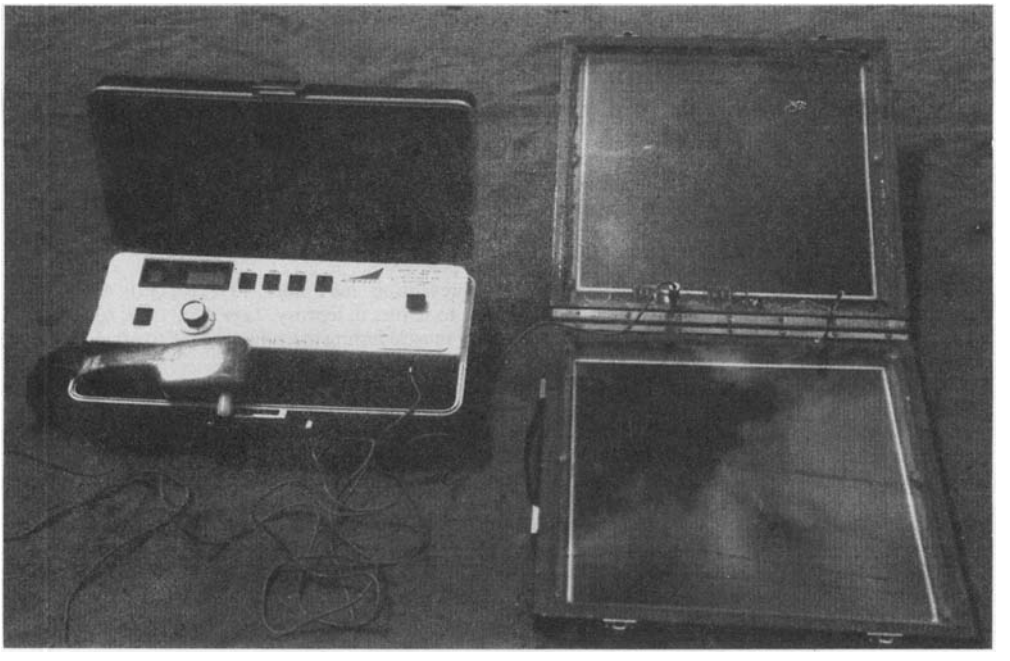

Figure 2. The biothesiometer and its solar recharger.

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Clofazimine induced cardiotoxicity—a case report

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Summary A 66-year-old Indian male who had been treated for recurrent erythema nodosum leprosum with 300 mg of clofazimine per day for 11 months presented to hospital with a 4 week history of severe gastrointestinal upset. Soon after admission he developed several short runs of ventricular tachycardia with a morphology suggestive of torsade de pointe. The patient had a slightly low magnesium level which was corrected within 2 days; however, his rhythm disturbance persisted for 5 days despite management with intravenous lidocaine. His gastrointestinal symptoms abated 2 weeks after clofazimine was discontinued.

Subsequent investigations showed that the patient had a keratopathy and myelin-type figures in his polymorphonuclear white cells similar to that seen with the cardiotoxic drugs chloroquine and amiodarone. It is postulated that clofazimine alone or in conjunction with electrolyte disturbance was responsible for the patient's cardiac arrythmia.

Introduction

Clofazimine has been used in the treatment of leprosy since 1962.[1] Chronic clofazimine use can cause accumulation of the drug in various tissues where it can persist for some time due to its long half-life. Tissue deposition of clofazimine also results in the well-described adverse effects of skin discoloration and gastrointestinal toxicity. Other side-effects include the production of anticholinergic symptoms and, in rare cases, phototoxicity.[2] We describe here the first known case of clofazimine-induced cardiotoxicity and speculate on the possible pathophysiological mechanisms.

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Case Report

The patient was a 66-year-old Indian male who emigrated to Canada in May 1986. Lepromatous leprosy was diagnosed 1 month before the patient's arrival in Canada and treatment with dapsone, rifampicin and clofazimine was begun. After 2 weeks of the commencement of therapy, the patient developed erythema nodosum leprosum (ENL) which was managed with chloroquine and subsequently with corticosteroids. Rifampicin was discontinued at this point because of abnormal liver function tests. Over the next 14 months he had recurrent flares of ENL whenever an attempt to taper the corticosteroid dose was made.

On 17 October 1987 the patient was admitted to hospital with a 4-week history of generalized abdominal pain, nausea and vomiting. He had been taking 100 mg of clofazimine tid since November 1986, but this was stopped 1 week before admission due to suspected gastrointestinal (GI) toxicity. Despite this, his GI symptoms continued to worsen with increasing abdominal pain and vomiting. Medications at the time of admission consisted of: dapsone 100 mg od; azathioprine 100 mg od; prednisone 27·5 mg od; sucralfate 1 g qid; warfarin 5 mg od; hydrochlorthiazide 50 mg od; buscopan 10 mg AC meals and at HS, fluoride 20 mg BID, 32 units of NPH and 10 units of regular humulin insulin in the morning, thalidomide 100 mg od and bid on alternate days, as well as vitamin D supplements and an antacid on a prn basis.

Past medical history revealed that the patient had a 10-year history of adult-onset diabetes which had been managed with diet alone. He required insulin when corticosteroid therapy was begun. The patient suffered from chest pain at rest during his initial hospital admission in May 1986 but a graded exercise stress test and a stress-Thalium test were negative. Ventriculography revealed some mild anteroapical hypokinesis with normal left ventricular ejection fraction while coronary angiography showed insignificant coronary artery disease. Other studies showed oesophageal dysmotility as well as gastroesophageal reflux. On the basis of these studies, the chest pain was felt to be of gastroesophageal origin. His course was also complicated by ENL induced femoral vein phlebitis complicated by recurrent pulmonary emboli which were diagnosed by a pulmonary arteriogram in October 1986. The patient was a nonsmoker and denied any ethanol use. There was no history of drug allergies. The family history was noncontributory.

On admission, the patient looked ill and complained of periumbilical and epigastric abdominal pain. The blood pressure was 110/56, pulse 72, respiratory rate 18, and the temperature 36·7°C. Pertinent physical findings included a soft, diffusely tender abdomen, marked tenderness in the epigastric area and mild rebound tenderness. There was no guarding. Bowel sounds were infrequent. On cardiovascular examination, there was no evidence of heart failure. A previously-documented grade II/VI systolic ejection murmur was present at the apex. Pitting oedema was present in both lower limbs up to the knees.

A chest radiograph obtained upon admission was normal. Flat plate examination of the abdomen was unremarkable apart from the presence of large amounts of stool in the descending colon. The leucocyte count was $8{\cdot}9 \times 10^9$/l with 89% granulocytes. Haemoglobin was 103 g/l and the platelet count was 147×10^9/l. Erythrocyte sedimentation rate was 57 mm. Serum electrolytes were normal on admission with the exception of the serum magnesium level which was 0·68 mmol/l (normal rate 0·8–1·20 mmol/l) and the

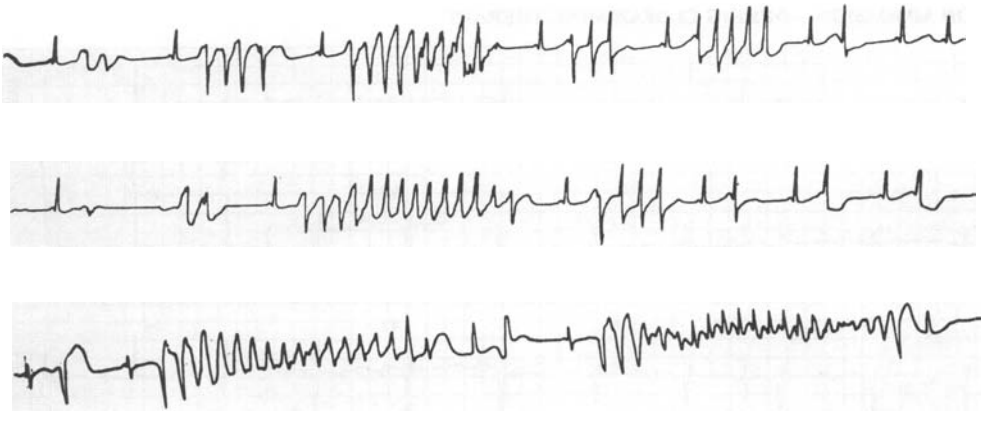

Figure 1. Continuous electrocardiographic recording obtained on admission to hospital. This reveals frequent self-terminating salvoes of polymorphic ventricular tachycardia in the setting of marked QT prolongation. The runs of tachycardia are consistent with the diagnosis of torsade de pointes.

serum phosphate which was 0·83 mmol/l (normal range 0·70–1·30 mmol/l). The urea, creatinine, and cardiac enzyme levels were normal.

Soon after admission, the patient developed several short runs of ventricular tachycardia with a morphology suggestive of torsade de pointe (Figure 1). These were managed with intravenous lidocaine and intravenous magnesium sulphate. An electrocardiogram obtained at the time of the torsade revealed a prolonged QT interval of 0·60 s and repolarization abnormalities (Figure 2).

The patient was transferred to the coronary care unit and intravenous lidocaine as well as intravenous magnesium supplementation were continued. Serial assays of cardiac enzymes were obtained and these remained within the normal range throughout his hospital stay. Intermittent runs of ventricular tachycardia continued over the next 5 days. QT prolongation and T-wave changes persisted despite normalization of the magnesium and phosphate levels 2 days after admission. No further arrhythmias were observed after 5 days and lidocaine was discontinued. The electrocardiogram reverted to normal 9 days after admission. At this time the QT interval was 0·44 s and the T-wave changes had resolved (Figure 3). A MUGA scan obtained during the patient's hospital stay showed normal left ventricular function with an ejection fraction of 50%.

Abdominal ultrasound examination was normal apart from the finding of dilated small bowel loops. A subsequent small bowel barium enema showed jejunal oedema, consistent with a picture of clofazimine toxicity. The gastrointestinal symptoms, managed conservatively with nasogastric suction, resolved 1 week after admission.

Several additional investigations were carried out in an attempt to elucidate the mechanisms behind the cardiac toxicity. An ophthalmologic examination revealed a keratopathy with corneal deposits similar to those seen with chronic chloroquine or amiodarone administration. On examination of a buffy coat preparation, the polymorphonucleur cells were found to contain increased amounts of glycogen and occasional myelin-type figures. A skin biopsy, when examined by electron microscopy, revealed irregular electron dense granules in the cytoplasm of epithelial cells.

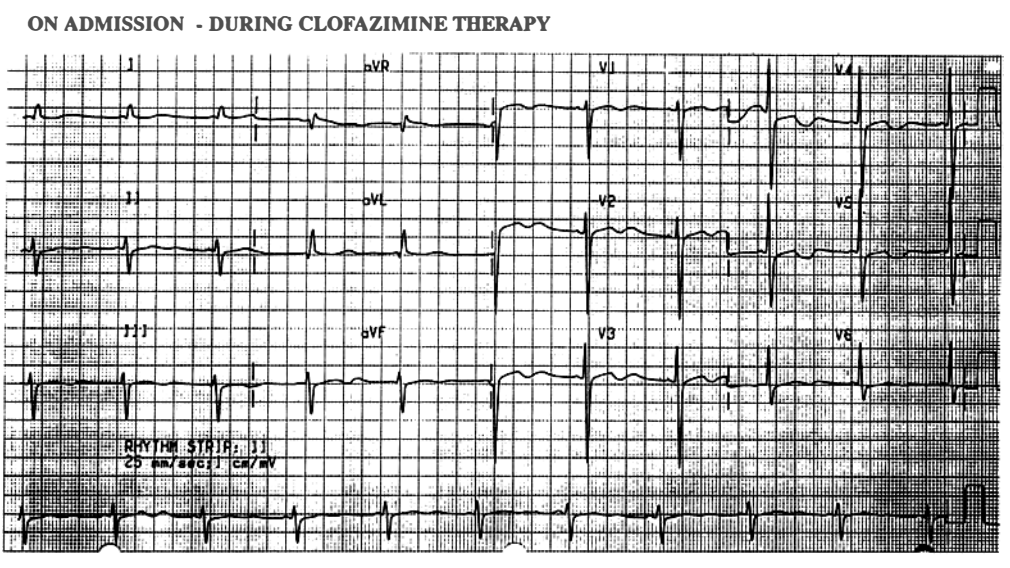

Figure 2. A 12-lead electrocardiogram obtained upon the patient's admission to hospital. Marked repolarization abnormalities with QT prolongation are evident on this electrocardiogram and most pronounced in the precordial leads.

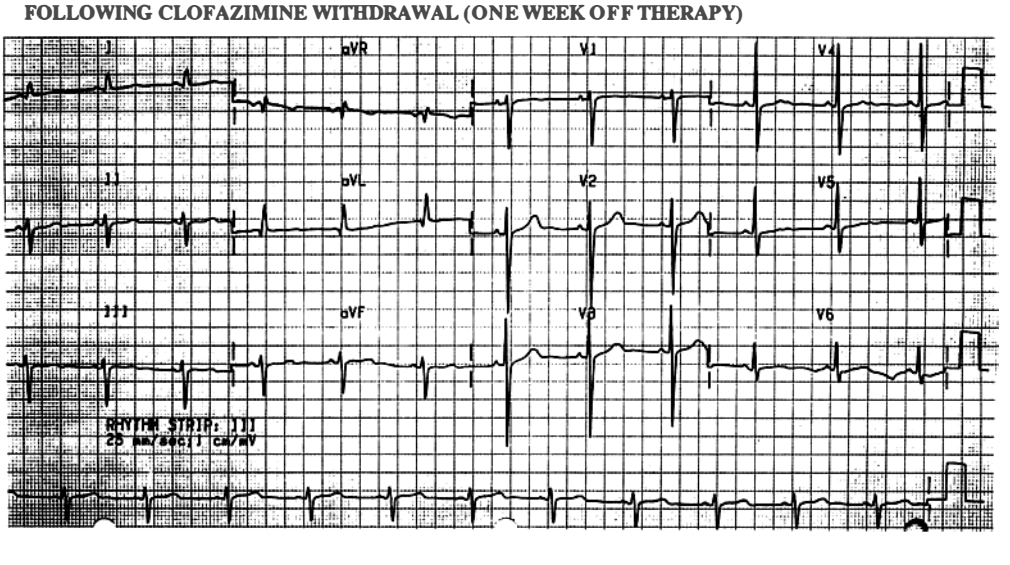

Figure 3. A 12-lead electrocardiogram from the same patient 1 week after drug withdrawal. Following drug withdrawal, the QT interval has returned to normal.

Discussion

Clofazimine, a substitute phenazine dye, is used extensively for the treatment of leprosy. Due to its highly lipophilic nature and prolonged half-life of 70 days, extensive tissue deposition of the drug occurs in patients who are on prolonged clofazimine therapy.[2] Autopsy series have shown that drug accumulation occurs in skin, subcutaneous fat, gall bladder, spleen, mesenteric lymph nodes, small intestine, kidneys, adrenals, heart, pancreas, muscle and bone.[4] Tissue deposition leads to the well-known adverse effects of altered skin pigmentation and small bowel toxicity. Clofazimine induced cardiotoxicity has, however, not been documented.[4]

Our patient had cardiotoxicity as manifested by nonspecific ST and T wave changes and ventricular arrhythmias (torsade de pointe) on electrocardiography. Although the patient was on multiple medications, none of these are known to cause cardiac toxicity, aside from the diuretics which may initiate or aggravate an arrhythmia by producing electrolyte disturbances. While an ischaemic event may produce similar changes, this was excluded by serial assays of the cardiac enzymes.

Torsade de pointe, first described by Stratmann & Kennedy,[7] is a ventricular arrhythmia in which the QRS complexes are seen to twist about the isoelectric line of the electrocardiogram. The QT interval is characteristically prolonged. A wide variety of drugs and toxins are known to cause torsade, including the anti-arrhythmic drug amiodarone, and antimalarials such as chloroquine. Torsade is also associated with various electrolyte abnormalities.[7] Amongst electrolyte abnormalities, hypomagnesemia is a known cause of 'torsade'[7] and magnesium is a recommended therapy for this arrhythmia.[8,3] Our patient's magnesium level was only moderately low and was quickly corrected into the normal range by intravenous magnesium supplementation. The electrocardiographic changes and recurrent episodes of arrythmia persisted even after this correction, suggesting that the cardiotoxicity was not due to electrolyte abnormalities alone. However, since tissue levels of magnesium may take longer to correct than serum levels, we cannot rule out the possibility that hypomagnesemia was a substantial contributing cause to our patient's arrhythmia.

Although cardiotoxicity has not been seen with clofazimine in the past,[4–6] we believe that it did play a role in producing the electrocardiographic abnormalities and arrhythmias seen in this patient. The cardiotoxicity was first noted after the patient presented with clofazimine-induced GI toxicity and resolved when the patient's GI symptoms resolved 2 weeks after the drug was stopped. Correction of the patient's electrolyte abnormalities did not alter the course of the arrhythmias or normalize the electrocardiographic abnormalities. This patient had a keratopathy similar to that seen with chloroquine or amiodarone. In particular, the myelin-type figures seen in the patient's polymorphonuclear cells and the electrondense granules in the cytoplasm of his epithelial cells are similar to those seen with amiodarone and chloroquine therapy. In both instances, they are felt to be layers of phospholipid which accumulate within lysosomes due to inhibition of phospholipase. Taken together, these findings suggest that clofazimine may have produced torsade de pointe in this patient via a mechanism similar to that seen with chloroquine or amiodarone.

The accumulated literature on clofazimine-induced adverse effects does not contain any reference to cardiotoxicity,[4–6] implying that this is a rare side-effect. While clofazimine may accumulate in the heart, other predisposing factors may be required for

the full syndrome of cardiotoxicity to develop. In this patient, hypomagnesemia secondary to the thiazide diuretics and clofazimine induced small bowel toxicity, may have served this role. Conceivably, subclinical cardiotoxicity was present for some time but only became significant when the patient developed electrolyte abnormalities. Interestingly, in the largest review of clofazimine-induced side-effects, the incidence of gastrointestinal toxicity was only 0·04% and none of these patients had electrolyte abnormalities.[4] This may explain why cardiotoxicity was not seen in this large case series.

In summary, we have described the case of a patient with no underlying heart disease who we believe presented with clofazimine-induced cardiotoxicity. This side-effect has not been described before in the literature, possibly because it may manifest itself only when additional provoking factors such as electrolyte disturbances are present. We would recommend that patients on clofazimine who develop electrolyte abnormalities be screened with an electrocardiogram to rule out the presence of co-existing cardiotoxicity. An electrocardiogram should also be obtained whenever other signs of clofazimine toxicity are present. With confirmation of our findings, clofazimine may soon join the growing list of drugs and toxins that are known to cause torsade de pointe. Additional studies are needed to further elucidate the pathophysiology of clofazimine-induced cardiotoxity and to determine what critical tissue load, if any, is required before cardiotoxicity becomes a problem.

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Trends in Leprosy in the Kingdom of Bhutan, 1982-1992

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Summary An evaluation programme was undertaken 11 years after the introduction of multidrug therapy (MDT) into Bhutan, by examining the case notes of 3239 leprosy patients who had been under treatment at any time during the period. The registered prevalence was found to have fallen markedly, as expected, and this had been accompanied by a clear fall in the case detection rate as well. The lepromatous rate among new patients rose considerably, giving epidemiological hope that the disease may be coming under control. However, no concomitant fall in the proportion of child cases was seen. The disability rate at detection rose slightly, although numbers were small. New cases were increasingly likely to have more highly positive skin smears, and to be self-reported. Programme planners should give thought to the implications of these findings.

Introduction

The small Himalayan Kingdom of Bhutan was among the first countries in the world to commit itself to offering MDT to all leprosy patients who needed it. The National Leprosy Control Programme had been established in 1981. This brought within a unified structure the existing leprosy control activities of two nongovernmental organizations, The Leprosy Mission and the Norwegian Santal Mission. It also established a single control area to be run by the Royal Government of Bhutan's Health Services.

MDT was begun in 1982, using the World Health Organisation (WHO) regime,[1] but continuing the existing domiciliary strategy that was required by the remote, scattered population in the country. MDT was introduced gradually by each leprosy centre, covering hyperendemic pockets first in order to gain experience, and moving later into the areas where patients were more difficult to reach. Loose drugs were originally used,

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individually counted as monthly calendar courses by the paramedical staff; more recently blister–calendar packs have been adopted, and some of the strict requirements for monthly face-to-face encounters have been relaxed to allow MDT coverage of patients living in very inaccessible areas.

In 1993, the requirement to evaluate the MDT programme arose, as planning was needed for the integration of leprosy services into primary health care. The likely epidemiological developments had to be predicted in order to estimate the future case load and staffing requirements.

Method

Central records were not found to be accurate for various reasons, and the registers of some programmes were not complete. In general, the case notes give a more complete record of patients' treatment, so 2 of the authors undertook a review of all available leprosy case notes in the country, covering the period 1982–92. In 6 centres, 3239 patients were identified. Data were extracted from the case notes manually, using a specially designed questionnaire, and were analysed using the Epi-Info Program.[2] It is estimated that this study recovered the case notes of at least 85% of the actually registered patients, with a higher proportion from the most recent years.

The official population figure for Bhutan (1·4 million in 1993) is based on the annual updating of an estimate made many years ago, and is widely believed to be inaccurate. All calculations of rates in this study have therefore been based on the currrent informal estimate of 600,000. Population growth in recent years has similarly been estimated to be 2% per annum, but has been at least partly balanced by large-scale emigrations of people who were originally counted as part of the population. Figures for child population are also not available. Prevalence figures were taken as cases registered at 31 December of each year of the study period. The programme is operational throughout the country, all detected cases are registered, and there is not thought to be a significant backlog of cases remaining to be detected.

Active case detection included mass survey at first, and all 18 districts have been surveyed at least once. Most districts were then resurveyed prior to the introduction of MDT, and some have had further surveys since. In recent years, fewer mass surveys of whole districts have been done, but they have been replaced by focal surveys of areas known to have been hyperendemic in the past. The frequency of these varies between 2 and 5 years. Other leprosy control activities (including other active case-finding methods—contact surveys and surveys of groups such as school children and servicemen) have remained unchanged throughout the study period. No alterations in case definition have occurred. Any patient who has ever had a positive skin smear has been regarded as multibacillary for the purposes of MDT. Multibacillary (MB) treatment is given for 24 doses, or thereafter until 3 consecutive negative skin smears are obtained, at least a month apart. Active post-MDT surveillance is maintained for 4 years after paucibacillary (PB) treatment, and for 10 years for MB cases. Disability is recorded by staff according to the previous WHO definition,[3] and only visible deformity or damage (grades II–III) is included in this study. Patients with a slit-skin smear result of greater than 2+ were defined as highly positive for the purposes of this study.

Results

The complete data were analysed for trends in registered prevalence, MDT coverage and case detection rate (Figures 1 and 2). Additionally, the epidemiological characteristics of the 796 new cases detected during the study period were examined to assess whether the trends in these would give any indications on the course of the disease within the population, or on the operation of the programme (Table 1).

REGISTERED PREVALENCE, MDT COVERAGE, AND CASE DETECTION RATE

Registered prevalence was found to have fallen steadily throughout the study period, from 2534 to 143 patients. This is a fall from 42·2 per 10,000 to 2·4 per 10,000. MDT coverage had risen to over 89% at the end of 1992. The maximum number of patients taking MDT in any year was 861 in 1986, after which the actual numbers declined, although the coverage increased.

The case detection rate had fallen from 1·9 per 10,000 to 0·65 per 10,000 during the study period, that is from 114 to 39 new patients annually.

NEW CASE CHARACTERISTICS

The lepromatous rate was found to be gradually rising, from around 50% to around 70% by the end of the study period. The child rate varied between 3·7% and 12·8%, with a mean of 7·1%, but the numbers are small and the trend is unclear. The M : F sex ratio varied between 1·4 : 1 and 3·8 : 1, but there was no clear trend away from the mean of 2·4 : 1 (male = 71%).

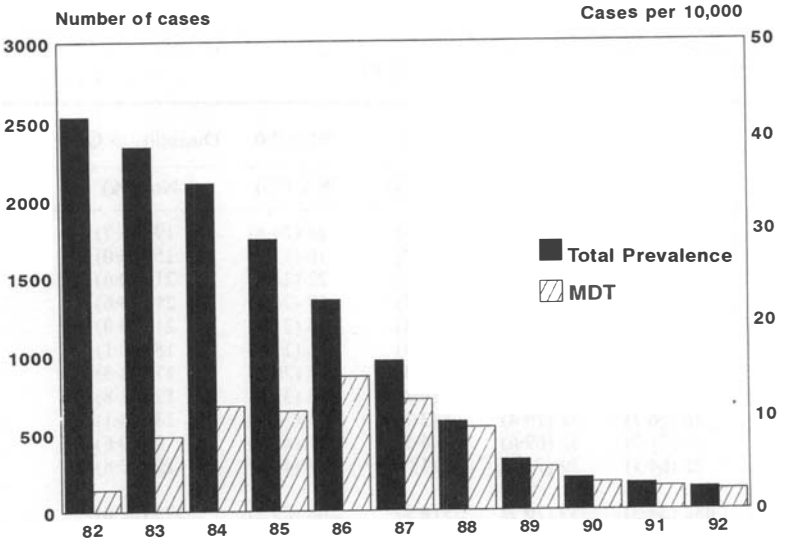

Figure 1. Prevalence of leprosy by treatment: MDT and total.

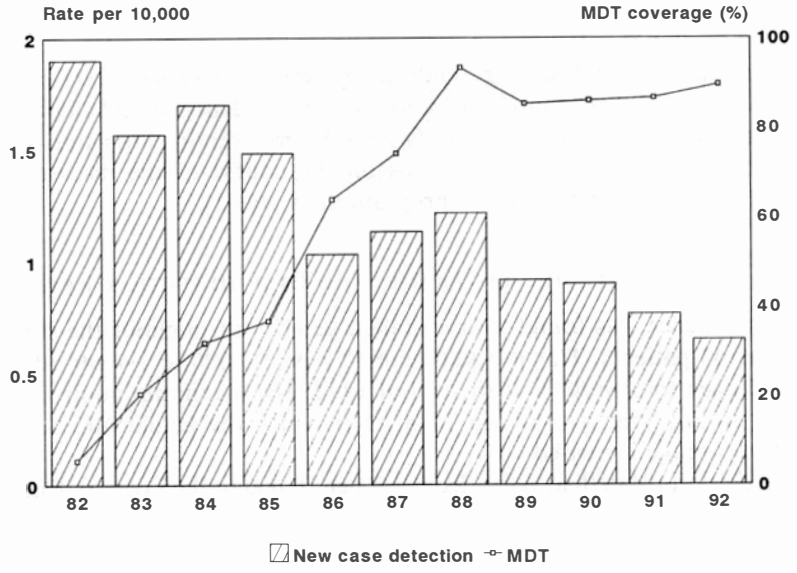

Figure 2. New case detection and MDT coverage.

OPERATIONAL INDICATORS

Certain indices give information that is useful in operational monitoring of the programme. The trend in disability rate shows a slight but definite increase, from under 20% to over 25%, though the actual numbers fell . The mode of detection was not fully reported until recently, and so in only 491 (62%) of the new cases in our study were these data available for analysis. However, a steadily increasing trend in the proportion

Table 1. Characteristics of new patients detected 1982–92

		MB	Male	Child	BI > 2·0	Disability > Gr 1	Self-reported
Year	Total	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)*
1982	114	58 (50·9)	67 (58·8)	10 (8·8)	28 (24·6)	19 (16·7)	10 (14·1)
1983	94	53 (56·4)	63 (67·0)	4 (4·3)	16 (17·2)	15 (16·0)	16 (37·2)
1984	102	41 (40·2)	79 (77·5)	8 (7·8)	22 (21·6)	21 (20·6)	12 (17·9)
1985	89	37 (41·6)	58 (65·2)	7 (7·9)	19 (21·8)	21 (23·6)	8 (12·1)
1986	62	39 (62·9)	49 (79·0)	3 (4·8)	13 (21·7)	21 (33·9)	11 (29·7)
1987	68	37 (54·4)	52 (76·5)	4 (5·9)	16 (23·9)	15 (22·1)	15 (34·9)
1988	73	35 (47·9)	52 (71·2)	4 (5·5)	14 (20·0)	17 (23·3)	6 (17·1)
1989	55	38 (69·1)	40 (72·7)	5 (9·1)	18 (33·3)	12 (21·8)	7 (21·3)
1990	54	36 (66·7)	38 (70·4)	2 (3·7)	21 (38·9)	13 (24·1)	14 (37·8)
1991	46	33 (71·7)	32 (69·6)	3 (6·5)	21 (45·7)	9 (19·6)	15 (48·4)
1992	39	25 (64·1)	29 (74·4)	5 (12·8)	15 (40·5)	12 (30·8)	10 (35·7)
Total	796	432 (54·3)	559 (70·2)	55 (6·9)	203 (25·5)	175 (22·0)	124 (25·2)

* Percentage of those whose mode of detection was recorded.

of self-reported cases is seen, from under 20% to over 35%. In 780 (98%) of the 796 new patients, a skin smear had been taken at detection and the result recorded. The percentage of new patients showing high smear positivity at detection increased from around 20% to around 40%.

Data on relapse was also collected during the study. Only 3 PB cases were given second courses of PB treatment after having been released from treatment, though others had initial courses extended, and others were reclassified as MB. There were 15 cases of MB leprosy who were restarted on treatment after release because of a diagnosis of relapse. However, no standardized definition of multibacillary relapse had been published, and MB relapses were based on slightly positive smear results found during active surveillance. A review of individual case notes detected no case of genuine post-MDT relapse among these cases.

Discussion

The decline in registered prevalence can be attributed both to the register-cleaning that preceded MDT introduction, and to the shorter duration of MDT treatment than of monotherapy. The initial rate of 4·2 per 1000 is already much lower than rates reported in 1969,[4] which gave district prevalences of 13·4 per 1000 in (low-endemic) Paro, and 24·8 per 1000 in (high-endemic) Lhuntsi, and this drop clearly occurred before MDT was introduced.

The MDT coverage did not increase consistently. There were some small groups of patients persistently not taking MDT, due to refusal or inaccessibility. As the overall numbers fell, they had a disproportionate effect on the coverage percentage.

The decline in case detection rate is less marked than the fall in registered prevalence, but is more significant in terms of the progress of leprosy control.[5] These rates cannot, of course, be regarded as exactly representing the actual incidence. However, the amalgamation of results from 6 different centres, with differing case finding activities, means that the trend can be assumed to be reliable.

The fall in case detection rate started from the beginning of the period of this study, long before the influence of MDT can have had an effect. This may reflect the fact that Bhutan had a well-established and fairly comprehensive vertical monotherapy programme in place for some years before MDT was introduced. Other considerations are the introduction of BCG immunization[6] in the 1960s, which had attained effectively 100% coverage by the time Universal Child Immunization was achieved in 1991. The rapid socioeconomic improvements seen in the last 20 years as development activities were established may also have made a contribution. Although the case detection rate appears to fall more consistently in the second half of the study period, there is no overall change in the rate of fall. This finding is in keeping with recent findings from Malawi.[7]

The trends in the characteristics of the new cases can give some information on the progress of a control programme.[5] There were no alterations of case definition during the study period, and the increase in the lepromatous rate can therefore be taken to represent a genuine change. This provides encouraging evidence that control of the disease is actually occurring. The child rate would be expected to fall as transmission decreases, and this has not been seen. Apart from the small numbers, which make trend analysis unreliable, a possible reason for this is the cautious over-diagnosis of

single-lesion disease, especially in school surveys. The lack of an accurate denominator of the child population further confounds the interpretation, though the consistently low numbers are themselves an encouragement.

The M : F sex ratio is higher than is usually reported. In Bhutan, health facilities are fully utilized by women, and although female paramedical staff are not employed within the leprosy programme, the examination of female patients by men is not usually a problem in the local culture. This suggests that there may be a real biological difference in the incidence between the sexes, and that neither the fall in prevalence nor the decrease in case detection rate brings this ratio towards unity.

The absence of MB relapse following MDT is likely to be irrelevant. The earliest cases treated with MDT had mostly been in the monotherapy treatment programme for many years. If they still harboured viable bacilli at the start of MDT, bactericidal treatment of almost any duration is likely to have eradicated these. The duration of this study is probably too short to expect to see genuine relapse from active cases treated with MDT. The matter of relapse among DDS-treated patients released from treatment without receiving MDT was not addressed in this study.

Any deterioration in disability rate is a worrying situation for programme managers, as it indicates a delay in case detection. In Bhutan, the delay is probably due to the decrease in the mass surveys that are being done, the overall low prevalence making such surveys non-cost-effective. The numbers of patients involved is small, but it is a matter of operational concern. The reduction in mass surveys contributes to the increasing proportion of self-reported patients, and this trend will need to be encouraged in order to promote early detection and prevention of disability, as well as to treat possible infectious cases.

As well as the epidemiological implications linked with the rising lepromatous rate, the increase in the proportion of new cases with high smear positivity is a trend that has operational implications as well. These patients who begin their MDT with a high bacterial load have a greater likelihood of reactions; and there are implications for the duration (and therefore cost) of treatment if the until 'smear negative treatment' regime continues to be used.

In interpreting all these results, however, the influence of active case finding has to be considered. There has been a reduction in mass surveys in recent years, largely due to the reduction in the team of vertical staff in response to the falling prevalence. This reduction by itself could explain away most of the encouraging findings of this study. However, the trends that are seen were established in the early part of the study period, when mass surveys were still an important strategy. These continued on a regular basis until 1988, and then were gradually replaced by more cost-effective focal surveys in areas of previously known high prevalence. Contact and group surveys have continued throughout.

Conclusion

Bhutan has seen a remarkable drop in the registered prevalence of leprosy over the past 11 years, and the WHO target of 1 per 10,000 might be achieved by the year 1995, about 5 years before the global goal. This situation was reached through a comprehensive monotherapy programme followed by intensive MDT, with coverage approaching 90%

in 1992. Many of the older cases treated with MDT may have actually been cured by years of dapsone monotherapy. Doubt has been expressed about the effect of MDT on the incidence of leprosy, and there is no change in the rate of fall of the case detection rate that can be attributed to MDT. The time elapsed since adequate MDT coverage has been achieved may still be too short for an effect to be seen. The lepromatous rate, rising to 70%, seems to confirm that the disease is coming under control, although the child rate has not fallen as would be expected. A small rise in the disability rate has been seen, but this may be reversed in time by encouraging early self-reporting by patients. The increasing number of patients who are now starting MDT with highly positive skin smears is important in planning the programme, both in ensuring staff awareness about lepra reactions, and in decision-making about programme policy and the economics of the MDT delivery options.

Acknowledgments

This study would have been impossible without the help and co-operation of numerous colleagues, both national and expatriate, and their contribution is warmly acknowledged, as is the permission of the Royal Government of Bhutan for publication. Some of the patients studied had received MDT in blister-calendar packs donated by Ciba-Geigy. Dr W. C. S. Smith made valuable suggestions on earlier versions of this paper, as did 2 anonymous referees. P.J. is employed by The Leprosy Mission International.

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Letters to the Editor

COMMENT: BLISTER–CALENDAR PACKS FOR MULTIPLE DRUG THERAPY IN LEPROSY

Sir,

I have read with interest the article 'Further observations on MDT blister–calendar packs in vertical leprosy eradication programmes—a multicentre study (Phase II)' by Revankar *et al. Lep Rev* (1993), **64,** 250–4, in which no significant difference was found in compliance rates for self-administered doses between the 2 groups studied (blister–calendar packs *versus* loose drugs). Their observations prompt me to submit some information from a trial of blister–calendar packs (BCPs) which was set up some years ago in Thailand. Although for various reasons (described below) the trial was terminated without analysis or publication, the attempt was of considerable interest, not only in the context of the historical development of BCPs for leprosy in recent years, but also as an illustration of some of the inherent difficulties which are likely to be encountered in trials of this kind.

In association with colleagues in the Ministry of Public Health in Bangkok, the Medical Department of Ciba-Geigy, Basle, Switzerland, set up a comparative trial in Thailand in 1987 to assess: 1, regularity of attendance for monthly medication; 2, compliance to the ingestion of daily unsupervised medication; 3, the logistics involved in the distribution of anti-leprosy drugs to patients; and 4, attitudes, motivation and performance of patients and staff, with particular regard to the dispensing of antileprosy drugs. Emphasis was given to the use of both vertical (specialized) and horizontal (integrated) programmes in the belief (at that time) that the most useful long-term application of BCPs might be in integrated programmes, using the primary health care approach, with supervision at district level, when prevalence rates had fallen to low levels. Multibacillary patients, either new or under treatment, were included, with a proposed total intake of 480, subdivided into 4 groups of 120 (BCP and loose drug groups in both vertical and horizontal programmes). The area chosen was situated in the north-eastern part of Thailand, extending towards the border with Laos. Each case was to be followed in the trial for a period of 6 months and apart from routine clinical and administrative details, records were to be kept of attendance rates and compliance, the latter based on dapsone–creatinine tests of the urine for the presence of (self-administered) dapsone. A questionnaire was included to record 'soft' data on motivation and attitude of patients and staff to treatment, notably with regard to the use of BCPs versus loose drugs. Preliminary descriptions of the techniques used in assessment[1] and the methodology of the trial[2] were given at the 13th International Leprosy Congress in the Hague in 1988.

Despite a reasonably good intake of patients, with satisfactory participation and completion by the majority, numerous problems arose with regard to the final collection, analysis and interpretation of data from the field, which eventually proved insurmountable. These were partly related to protracted difficulties with the analysis of the urine specimens for the dapsone : creatinine ratio, but also to problems of communication with the trial 'managers' in Oxford, Basle, Bangkok and the field areas. In 1990, it was reluctantly concluded that no further

progress could be made and the trial was therefore closed. In retrospect, it is in fact doubtful if the data collected would have been adequate to demonstrate a statistically significant difference between results in vertical (specialized) and horizontal (integrated) programmes, and it is also clear that the 'soft' data on attitudes and motivation would have been extremely difficult to analyse in objective terms. Furthermore, the overall value of the trial was considerably weakened, even before it got under way, by: 1, rapidly mounting evidence that many national and international agencies working in leprosy had already decided to use BCPs, in some cases on quite a large scale; and 2, numerous reports from WHO and other agencies of high levels of attendance and compliance to prescribed medication, using loose drugs for multiple drug therapy, in routine control programmes in different parts of the world.

In the years since the publication of the first designs and recommendations for the use of BCPs in leprosy by Winsley *et al.*[3] it has become increasingly clear that they are unlikely, in most circumstances, to improve attendance and/or compliance figures above those which are frequently achieved using loose drugs. As indicated by Revankar *et al.* in the article referred to in Ref. 3, and in a detailed editorial account of the development and potential of BCPs in leprosy,[4] other operational benefits may be of much greater importance. Their wider use in leprosy has, almost certainly, been impeded by the element of higher cost, compared with loose drugs. However, there have also been remarkable (and in some ways inexplicable) contrasts between agencies and control programmes which have accepted and used BCPs extensively (for example the Philippines and the DANIDA-supported districts in India), as having obvious advantages, without the need for formal trial or assessment, whilst others have been slow to see operational benefits or cost-effectiveness.

As far as India is concerned, the issue may now be resolved, for it has recently been reported that BCPs will be used for all the remaining districts in need of MDT in the National Leprosy Eradication Programme, with support from the World Bank. As this involves 143 districts with over 1·5 million cases in the next 5 years, inclusive of new cases likely to arise,[5] this clearly represents a very high level of interest and confidence in the use of BCPs for this purpose. In addition to the information which is already available from the use of BCPs over a period of many years by the DANIDA-Assisted National Leprosy Control Programme, the opportunities to further assess the value of BCPs in leprosy (and their potential for other diseases, notably tuberculosis) are likely to be immense.

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TASK-ORIENTED SHORT-TERM TRAINING TO CONTRACT LEPROSY WORKERS IN A NATIONAL LEPROSY ERADICATION PROGRAMME

Sir,

To hasten multidrug therapy (MDT) coverage in difficult endemic districts in Northern India, the National Leprosy Eradication Programme (NLEP) authorities decided to launch a novel scheme involving contract leprosy workers who were trained to deliver MDT.[1] A main obstacle in these northern states stopping rapid MDT coverage was inadequate infrastructural facilities, especially manpower at the field level able to operate vertical MDT programmes. To develop manpower to deliver MDS, leprosy workers are usually given 4 months conventional basic training in leprosy training centres. To achieve rapid MDT coverage, the NLEP recommended that 4–5 days training should be offered to these contract leprosy workers (who will work on a contractual basis).[2] The Bombay Leprosy Project was entrusted to design a suitable training module and offer training in 10 districts in Madhya Pradesh and Uttar Pradesh.

The main tasks of these contractual workers are to detect leprosy cases, prepare patient treatment records after leprosy is confirmed, deliver MDT under the supervision of supervisory staff, report suspect reactions, toxicity and identify deformities, etc. To develop adequate knowledge and skills, a task-oriented, simple, practical and unstructured training programme was designed. No theoretical lectures on anatomy, physiology and epidemiology were included. All the sessions were held with demonstrations, discussions and actual fieldwork. Patients, records, clinical photographs, slides and simple notes were used as training materials. All the sessions were arranged according to trainees' needs and feedback. During training, the stress was on multibacillary case detection (skin smear positive), and their importance in leprosy control, MDT drugs, regularity, defaulter retrieval, etc. The village visits were arranged to demonstrate population surveys and to study suspect leprosy cases. To determine the immediate impact of training, the trainees were asked to undertake population surveys, to investigate suspect leprosy cases and prepare patients' records including charting, clinical details, etc.

The study group comprised of 446 contract workers from 2 districts in Madhya Pradesh and 8 districts in Uttar Pradesh, who were given task-oriented training. During the training, they detected 138 leprosy cases during survey and clinic exercise; 34 were new cases, out of whom 4 were smear positive. The rest were old, treated cases. These findings indicate that with 4–5 days' training, adequate knowledge and skills could be developed to detect leprosy cases. Feedback obtained from 2 districts from Madhya Pradesh (Satna and Khandwa) revealed that 71 contract workers suspected 838 new cases, of whom 811 (97%) were confirmed as cases, 366 were MB type, and 120 (33%) out of 366 MB cases were smear positive. They also recorded 96 (12%) patients with deformities, and 2586 active cases also received MDT, in their respective areas.

These observations indicate that a short-term task-oriented training for 4–5 days is quite adequate for untrained contract leprosy workers to detect and treat leprosy cases with MDT in an MDT campaign.

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COMMENT: SENSORY TESTING WITH NYLON FILAMENTS AND PENS

Sir,

Regarding the comprehensive and thorough paper by Lienhardt & Fine (*Lepr Rev*, 1994; **65;** 9–33), entitled 'Type 1 Reaction, neuritis, and disability in leprosy. What is the current epidemiological situation?', I would like to submit a few points:

The nylon filaments (Semmes–Weinstein filaments) are *not* expensive, unless purchased in readymade kits from the USA. In fact, they have been disseminated to scores of clinicians and leprosy centres throughout India since I returned to India in 1991 as an ALM disability prevention consultant. They are available free to any interested party within India (such availability in East Asia, Africa, and Latin America is to follow soon). The filaments are easy to assemble (instructions and procedures are included in the packet).*

In the Appendix it is stated that 'each filament is applied 3 times in each tested area. If the patient points at least twice . . . the response is judged correct.' This is not correct. On the contrary, 1 response, not 2, is both sufficient and more accurate. By requiring 2 responses, the evaluator changes the detection threshold significantly; the false detection rate is much too high.[1]

Caution needs to be voiced concerning the use of the pen as a tool for sensory testing in many clinic and field contexts. Why? Although it does boast such assets as simplicity and low cost, in many parts of the world, including India where the majority of leprosy cases are believed to be found, the detection thresholds are considerably less than the pen can deliver. In Stratford & Owen[2] results showed that less than 2 g of pressure with the filament is necessary for detection. A few graded filaments of higher pressure values would ensure detection of change, serial change, of sensory status over time. As a pen's application of pressure averages several grams above 2 g[3] many patients with sensory involvement and underlying nerve impairment would be missed, i.e. those with WHO Grade 0 disability who, left untreated, would likely progress to Grade 1 disability, if the pen alone were to be used.

There are some regions where the plantar surface of the foot has an unusually high detection threshold to pressure application, such as Ethiopia.[4] In most countries, however, a much lower

* Interested parties can write to the distributor for India: Dr R. Premkumar, c/o S.L.R.T.C. Post, Karigiri, Via Katpadi, T.N. 632106, India.

detection threshold is exhibited, such as is exemplified in Stratford & Owen's paper. In this author's study of hundreds of normal hands and feet in India[5] the vast majority of subjects without any footwear were able to respond to the 2-g filament, with the exception of the heel; in that case the vast majority responded to the 4-g filament again, less than the pen can deliver. Details and tables of this study are to be published later this year in an Indian publication.

It is vital that your readership and diverse community of authors do not dismiss the value of the filaments which are available, reliable, and very practical in most settings, as well as being cheap. Certainly there are geographical settings where the pen may be optimal. But any prevention of disability (POD) programme that is earnest on addressing the large reservoir of patients (whether under treatment or RFT) with the Grade 0 disability, working to monitor them so their '0' status does not transform to Grade 1, would be well advised that with the stroke of the pen may likely miss such patients. Currently there are 6 project sites in India which are participating in a multicentre study, 'International project to measure peripheral nerve involvement underlying disability in leprosy'. The nylon filaments are an integral part of the compact screening format which, among other features, identifies nerve involvement at early stages, i.e. the Grade 0 level of disability according to the WHO grading system. Participating clinicians are excited to harness these features into their treatment and management regimes. A preliminary report on this study based in Carville is to follow.

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Teaching Materials and Services

Collection of leprosy literature 1913–91 on compact disk

Now, CD-ROM technology allows for rapid access to important leprosy literature. By searching the author, title, or source, every citation can be accessed and printed. The speed and completeness with which the computer finds the information is the power of having the data on computer.

The items have been selected from 2874 journals and publications and totals 41,168 citations.

Specifications: IBM/Compatible; DOS 3.2 (or higher); minimum RAM required, 512K; EMS usage; program helps languages—English, French and German. Price: US$20.00. For further details apply: Leprosy Research Foundation, 11588 Lawton Court, Loma Linda, CA 92354, USA.

Teaching and learning materials for leprosy in Portuguese

The librarian of the *Biblioteca do Instituto Lauro de Souza Lima (LSL)*, Caiza Postal 62, Bauru-SP, 17001-970, Brasil, has kindly sent a copy of the latest catalogue of teaching and learning materials for leprosy, in Portuguese. The main headings are: leprosy in general, epidemiology and control, prevention and rehabilitation, health education. Mainly because of the extremely high cost of postage within Brasil, it is not the policy of the above Institute to send out copies, unless advance payment is received, but the large number of training courses held at Bauru gives an opportunity for direct distribution to students and field workers, which has proved satisfactory. The items have either been produced in Brasil, or modified from English language texts. The list is available on writing to Bauru or to CERPHA (Palavra & Ação), Rua Guapeni 54/101, Tijuca, Rio de Janeiro-RJ, 20 520, Brasil.

Core list in ophthalmology for developing countries

Connie Dickinson, Director of the Medical Library of the King Khaled Eye Specialist Hospital, P.O. Box 7191, Riyadh 11462, Kingdom of Saudi Arabia, has supplied information and a core list on ophthalmology in developing countries. Apart from covering all sub-specialities (optics, refraction, contact lenses, neuro-ophthalmology, paediatric ophthalmology, external disease and cornea, intraocular inflammation and uveitis, glaucoma, lens, anterior segment trauma, retina and vitreous, fundamentals and principles of ophthalmology), there is also a valuable list of publications on leprosy (Medicine 1989–September 1993).

Library packages for district hospitals and district health workers

Teaching-aids at low cost (TALC) has recently announced a scheme which makes small libraries of vital medical books available. The first library is for district hospitals. It contains 17 books,

including 2 on primary surgery, an AIDS handbook and the latest book on tuberculosis. It costs £85, including postage and packing by surface mail worldwide. The second, aimed at district health workers, has 14 books, including *Where there is no doctor*, a revised book on nutrition and a book on obstetric emergencies. It costs £60.00, including postage and packing by surface mail worldwide.

Apply: TALC (Teaching Aids at Low Cost), PO Box 49, St Albans, Herts AL1 4AX, England.

New publications on ocular leprosy—books and videos

In the last few months several publications, slides and videos on ocular leprosy have been newly developed or reprinted, and here we give them a short overview.

TALMILEP produced 2 training booklets written by Paul Courtright and Susan Lewallen entitled:

Guide to Ocular Leprosy for Health Workers: A Training Manual for Eye Care in Leprosy. Singapore etc., World Scientific, 1993, 46 pages. ISBN 981-02-1328-X, free of charge. (Available from TLMI, address see below.)

Written in simple English with illustrations, this booklet provides a step by step approach to eye care for middle level health workers. Contents include a list of tasks such as vision screening, how to recognize and treat or refer ocular complications, and some useful questions and answers.

Training Health Workers to Recognise, Treat, Refer and Educate Patients about Ocular Leprosy. Singapore etc., World Scientific, 1993, 52 pages. ISBN 981-02-1329-8, £1.00. (Available from TLMI, address see below.)

Accompanying training manual to above-named booklet. This manual contains material for a 5-day course on ocular leprosy and a list of tasks for health workers, course teaching objectives, pre- and post-tests, teaching methods and a suggested course schedule.

TALMILEP also produced a third revised version of:

Care of the Eye in Hansen's Disease. By Margaret Brand. Brentford, The Leprosy Mission International, 1993, 40 pages. ISBN 0-902731-36-X, free of charge.

This 40-page booklet deals with the management of eye complications in leprosy, paying special attention to corneal sensation.

You can order these publications from: Teaching & Learning Materials, The Leprosy Mission International, 80 Windmill Road, Brentford, Middlesex TW8 0QH, United Kingdom.

NSL/INFOLEP has produced 2 slide series and a video film on ocular leprosy developed by Margreet Hogeweg:

Ocular Complications in Leprosy. Africa. Series of 24 slides, accompanied by a booklet of 18 pages. Amsterdam, NSL-INFOLEP, 1993, free of charge.

Ocular Complications in Leprosy. Asia. Series of 24 slides, accompanied by a booklet of 18 pages. Amsterdam, NSL-INFOLEP, 1993, free of charge.

Both series illustrate the common eye complications in leprosy. The booklet demonstrates emphasis on a systematic, step by step diagnosis and gives guidelines for treatment. The slides feature African and Asian patients, respectively. They are meant to help teachers in their lessons on ocular leprosy and for self-tuition for leprosy field staff, eye nurses, medical students and ophthalmologists in training.

Lagophthalmos and Simple Lid Surgery in Leprosy. Video film, 14 minutes, VHS/PAL, English. Amsterdam, NSL, 1993. Target group: leprosy field staff. US$20.00.

This video features several patients with lagophthalmos. It focusses on the commonest cause of lagophthalmos: reversal reaction in patients with facial patches. It then shows the dangers of lagophthalmos for the eye: exposure keratitis and corneal ulcer. It covers the treatment of recent lagophthalmos by systemic steroids and blinking exercises and the importance of sunglasses for protection in longstanding lagophthalmos. Finally it shows the simplest type of lid surgery in lagophthalmos: temporal tarsorraphy and the results of surgery after 3 months.

These slide series and video film are available from: The Netherlands Leprosy Relief Association (NSL), INFOLEP Leprosy Information Services, Wibautstr. 135, 1097 DN Amsterdam, The Netherlands.

The Study Aid Foundation Saint-Lazare (SLSL) reprinted its poster titled: *Dangers to the Eye in Leprosy*. Format A1, coloured, which is available free of charge from SLSL, c/o NSL, Wibautstr. 135, 1097 DN Amsterdam, The Netherlands.

WHO Catalogue on Hospitals in the District Health System, 1993 (updated)

This 35-page catalogue provides bibliographic and descriptive information for over 100 WHO publications offering practical and technical information that can help strengthen the medical care provided in small hospitals. Publications are grouped according to the role of hospitals in the district health system and the functions commonly performed at the first-referral level, as follows: hospitals and the health system, clinical functions, guides to the management of common diseases (includes AIDS, sexually transmitted diseases, malaria, cholera, tuberculosis, the leishmaniases, leprosy, schistosomiasis, Chagas disease, sleeping sickness, lymphatic filariasis, viral diseases, hookworm infection), clinical support, hospital planning and design. Because of the wide diversity of conditions that may be seen at the first-referral hospital, many of these books serve as authoritative how-to guides for the safe management of illnesses and emergencies—whether involving the treatment of children hospitalized because of respiratory infections or the emergency management of life-threatening complications of malaria.

Aware that small hospitals often struggle under the burden of inadequate resources and supplies, other books offer advice on how to improve services by making the best choices when selecting drugs, ordering laboratory tests, or purchasing equipment. Still others guide hospital administrators and clinicians in the steps needed to upgrade hospital services, build new clinical skills, and extend competence.

An order form listing all titles included in this catalogue appears on pages 33–34. WHO publications can be ordered from booksellers, subscription agencies, or directly from WHO. Orders addressed to WHO must be accompanied by payment in Swiss francs, US dollars, or UNESCO coupons. Payment by credit card is accepted.

For developing countries 30% can be deducted from the prices given in the catalogue. For further information: WHO Publications, Distribution & Sales, 1211 Geneva 27, Switzerland.

International Health Exchange, London, UK

International Health Exchange aims to facilitate the provision of health workers to developing countries; to promote appropriate training for those preparing to work overseas and to raise awareness among health workers of the health and human resource needs of developing countries.

International Health Exchange provides:

- an international register of health professionals for work in developing countries;
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The Training Programme provides a comprehensive approach to training in human resource development. Health workers are considered to be our best asset and the training programme aims to provide a forum for health workers to develop their knowledge, attitudes and practical skills in health development.

Apply: International Health Exchange, 38 King Street, London WC2E 8JT, England.

Clinical tuberculosis. John Crofton, Norman Horne & Fred Miller (1993)

This book is sponsored by the International Union against Tuberculosis and Lung Disease and by TALC. A low cost edition for developing countries has been financially supported by the World Health Organization and other bodies. It is written primarily as a practical guide for busy non-specialist doctors working in areas with few resources. The language is simple and there is an extensive glossary. The book can therefore be useful to Health (Medical) Assistants and senior nurses with a limited knowledge of English. It can also serve as a helpful reference for younger doctors in developed countries who now have less experience of tuberculosis.

The book covers diagnosis and treatment of all types of tuberculosis, pulmonary and non-pulmonary, both in adults and children. It deals fully with the effects of HIV infection on the disease and describes the essential elements of a National Tuberculosis Control Programme. There are many line drawings and flow charts as aids to training, learning and clinical practice. 'Stories' about individual patients highlight practical points.

The 3 authors have had many years experience of dealing with tuberculosis and of teaching both undergraduates and postgraduates. They have advised in many countries in Asia, Africa and South America. The final text incorporates constructive comments on an earlier draft by experienced consultants from the IUATLD, WHO and consultants working in several countries in Asia, Africa and the Pacific. The book therefore represents much collective wisdom.

Price: £3.00 per copy for developing countries; £10.99 for developed countries. Add 30% for postage and packing (minimum £2.00) by surface mail, or 60% (minimum £2.50) for airmail. Apply: TALC (Teaching Aids at Low Cost), PO Box 49, St Albans, Herts AL1 4AX, England.

African Medical and Research Foundation (AMREF)

AMREF is an independent non-profit organization which has been working to improve the health of the people in eastern Africa for over 30 years. It is one of the few international NGOs based in Africa. AMREF runs a wide variety of innovative projects with an emphasis on appropriate low-cost health care for rural areas. Current activities include:

—Training of community health workers.
—Training of rural health staff through continuing education, teacher training and correspondence courses.
—Development, printing and distribution of training manuals, journals and newsletters, and health education materials. This includes their *Afya*, *Defender* and *Cobasheca* newsletters.
—Health project development, planning and evaluation.
—Consultancies in these programme areas.

One of the units in AMREF is the Community Based Health Care Support Unit, which runs training for facilitators, trainers, Community Health Workers, Traditional Birth Attendants and Community Based Distributors of contraceptives. Supported by AMREF is the Health Education Network, with its own newsletter and Resource Centre.

AMREF has offices in a number of countries, in Africa and in the North. Its headquarters is at Wilson Airport, PO Box 30125, Nairobi, Kenya.

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News and Notes

Global strategy for elimination

The following item is reproduced from *LEP News*, December 1993:

BLUEPRINT FOR THE CONQUEST OF AN AGE-OLD DISEASE

The introduction of the WHO-recommended standard multidrug therapy (MDT) has dramatically changed the situation of leprosy in the world since the early 1980s. This treatment with a 'drug cocktail'—which in most multibacillary cases adds rifampicin and clofazimine to dapsone—has reduced almost to zero the possibility of *Mycobacterium leprae* becoming resistant to all 3 substances. By late 1993, some 4·3 million patients had been cured, and the global cumulative MDT coverage of registered patients had reached 85%. The number of registered cases has fallen from 5·4 million in 1985 to 1·9 million in 1993.

The striking progress had already encouraged the World Health Assembly in 1991 to set the goal of eliminating leprosy as a public health problem by the year 2000—specifically, reducing the prevalence to less than 1 case per 10,000 population in endemic areas. As a consequence, WHO has now elaborated a *Global Strategy for the Elimination of Leprosy as a Public Health Problem.* This blueprint for the conquest of an age-old disease calls for resource allocation and priority setting at global, regional and country levels, while underlining the fact that public awareness at the community level will be vital to ensure early detection of cases.

ESTIMATED COST

The elimination strategy envisages identifying and treating with MDT a total of about 6·5 million cases until the year 2000. The cost of dealing with these cases has been estimated at US$ 420 million, including US$ 140 million for the drugs. It will be possible to mobilize these resources over the next 5 to 7 years, provided that the need to eliminate leprosy as a public health problem is fully recognized, and provided all interested agencies actively work together in a spirit of partnership.

The elimination strategy aims to stratify the situation at different levels, identify priority areas for action, set intermediate targets and monitor them. The size and intensity of the problem and the accessibility of leprosy control services, including MDT, will determine the level of each stratum.

Political commitment as well as the mobilization and coordination of resources, including those from donor NGOs, will be essential prerequisites for the strategy. The core activities will continue to focus on implementing MDT, together with intensive case-detection. Programme monitoring and evaluation and epidemiological surveillance will also be important elements. The WHO Working Group on Leprosy Control will continue to monitor the progress towards elimination from the global point of view. For that goal to be attained, it is important for everyone to recognize and seize this opportunity to solve a major problem of international public health.

LEPROSY SITUATION 1993

No. of affected countries:	87		
Estimated cases:	5·5 million (1991) 3·1 million (1993)	Registered cases:	3·2 million (1991) 1·9 million (1993)
Cumulative total of patients cured through MDT over the last 8 years:			4·3 million
Global reduction in prevalence over the last 8 years:			64%
Cumulative MDT coverage:			85%

Leprosy on the way out, but still 'a formidable problem', *TDR News*

TDR News, published by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, No. 45, June 1994, carries the following section on leprosy:

'There are still about 2½ million people in the world with leprosy, according to estimates released by CTD's leprosy unit. This is, however, a nearly 70% fall from the 1985 estimate of 10–12 million cases. Actual registered cases have also plummeted over the past decade from 5·4 to 1·7 million, a 69% fall. The unit attributes the decline mostly to multidrug therapy (MDT), a treatment scheme introduced by WHO in 1981 that uses a combination of drugs.

Shaik K. Noordeen, chief of the unit, presented these figures in May to the World Health Assembly in Geneva. The trend, he said, is 'clearly promising and suggests that leprosy is not an endless problem and that it can be eliminated'. In its 1991 session, the Assembly made elimination of leprosy—which means cutting its prevalence rate down to less than 1 case per 10,000 population—a target for the year 2000.

'We're going in the right direction and the target seems to be within reach, but the task ahead,' Dr Noordeen cautioned, 'is formidable.' There are still 80 countries with prevalence rates greater than 1 per 10,000. Some, notably in South-East Asia, which has two-thirds of the world's estimated cases, have rates over 10 per 10,000. Africa's rate is 5·3 per 10,000. Moreover, of the estimated 2·5 million people with leprosy in the world, about 800,000 are not receiving any treatment.

With an additional 600,000 new cases being discovered every year, this means that to meet WHO's target, over 5 million patients will have to be put on MDT between now and the year 2000.'

The same issue includes the following under the heading:

'What has TDR been up to in the past years?'

Ofloxacin, a drug which holds the promise of 1-month or even 1-day treatments to halt leprosy, entered field trials in 15 centres in 8 endemic countries. Preliminary results should be available in 1997.

Two more drugs—minocycline and clarithromycin—were shown to have strong anti-leprosy action in experimental animals and short-term clinical trials.

Leprosy in women may be substantially under-reported and cause extreme social disgrace, hindering women's admission of the problem and access to care, according to a study from India.

By the end of 1994, more than 60% of the total genetic material of the organism *Mycobacterium leprae*, which causes leprosy, will have been mapped and sequenced, making it accessible to molecular genetics and the development of new vaccines, drugs and diagnostic tests.

Electronic Braille for anaesthetic fingers

The following item is reprinted from the latest issue of *Actionaid Disability News*, (1994) **5** (No. 2), page 95:

Until now, visually impaired people have had access to literacy through Braille which is a tactile script. However, what happens to those who are not only visually impaired but also have no sensation in their fingers to 'read' or 'write' tactile braille? Sensation in the fingers is lost due to leprosy which may incidentally cause blindness. Are they condemned to remain illiterate? An electronic substitute system devised by Mr K. G. K. Murthy, attempts to provide a universal cost-effective system that requires no computer backing and instead, standard morse code is modified and adopted in this device.

Fingers that cannot read tactile braille are figuratively termed as 'blind fingers'. This electronic substitute system is made to suit all visually impaired people in their reading and writing needs, particularly those who have no sensation in their fingers.

The system consists of an electronic device which is a simple A.F. oscillator that works on a 3 volt dry battery. It is an RC circuit whose frequency is inversely proportionate to varying resistance. The sensitized print material consists of paper which is a thin plastic sheet to ensure durability and dielectric properties. It has appropriate perforations—small and big. When the user's finger touches the small perforation, the oscillator produces low frequency a.f. and when the finger touches the big perforation, it produces high frequency a.f. The low frequency here is denoted by L and high frequency by H. The standard morse code is modified and adopted in this system. Morse code is made up of short sounds called dit (.) and a long sound called dah (–). There are a maximum of 6 notes in morse which is also there in the modified system. Between each alphabet there is a gap of note space. Thus the dits are equated to low frequency L and dahs are equated to high frequency H covering the alphabet. In practice, this combination is decoded as easily as in morse. Morse code can be learnt in about 2 months, and a speed of about 20 words per minute can be achieved after a few months of practice.

The present prototype works on 3 VDC. An oscillator circuit that is more compact and consumes less power is being researched. Also, rechargeable cells, photo-cells etc. are yet to be tried. For domestic use, a compact battery eliminator is useful thus economizing on battery cells. The perforating mechanical device needs development for ultimate use.

For those who have multiple disabilities like hearing and speech impairment, the output of this device can be made to drive another amplification circuit which gives safe but perceptible pulses on the skin comparable to the audible signals.

A diagram for assembly is supplied with the kit. Further developments towards a cost-effective oscillator with minimum maintenance and improved printing techniques are under consideration, subject to availability of funds. Since it is completely service oriented with no profit orientation, patent rights will be given to any agency/organization who will undertake its manufacture and promotion. Mr Murthy feels that this system deserves optimum development and welcomes any comments, suggestions or requests for more information.

For details write to: Mr K. G. K. Murthy, Health Aid Institute, P.B.2, Kothagundem 507 101, Andhra Pradesh, India.

Botswana combines leprosy with tuberculosis

Writing in the *East African Medical Journal*, Vol. 70, Number 10, 1993, page 635, Dr J. A. Kumaresan and colleagues describe a 'Case finding survey for leprosy in Botswana', carried out in July and August 1991 in the northern part of the country, where most cases have been recorded through the years. Out of a total of 799 contacts of 127 index cases and 8235 school children, 44 active cases of leprosy were registered and started on multiple drug therapy, but of this total only 32% were newly identified during the survey. In view of this modest outcome, surveillance and control of leprosy has now been integrated into the existing tuberculosis control programme (which has been well developed for many years). The majority of recorded cases are lepromatous and the age distribution indicates that 84% of the cases found were older than 25 years. The

authors consider that leprosy is possibly dying out in Botswana, but they draw attention to the considerable number of patients who still require medical attention for disability and deformity.

Tropical Health and Education Trust, London, UK

Fellows of the Society have always been actively involved in many tropical countries in establishing and developing medical schools and other training institutions. But some of these schools, particularly in poorer African countries, face severe hardships. Students have no books, there is no foreign exchange for journals, equipment lacks spares, research cannot be supported and external aid is directed towards primary health care.

The Tropical Health Education Trust has started to relieve, with support from many individuals, trusts and organizations, some of these disadvantages.

Basic books have been sent to all the rural hospitals in 2 African countries, sets of books have been given for students in a number of others. Links between medical schools overseas and home departments have been started with fellowships for students in training and research methods also.

The Tropical Health and Education Trust aims to extend support like this to more countries, hospitals, medical schools and students and needs funds to do it: Fellows of the Society who would like to take this opportunity to help our colleagues overcome some of their obstacles can do so through a single gift, a 4-year or a deposited covenant, or even through a legacy.

Trustees include: R. M. Anderson, K. P. W. J. McAdam, E. H. O. Parry (Chairman), D. A. Warrell.

For more information about THET please write or telephone: 21 Edenhurst Avenue, Fulham, London, SW6 3PD, UK. Tel: 071 927 2411, Fax: 071 637 4314.

Travelling abroad; personal protection against malaria, unclean water, non-sterile medical equipment and contaminated blood for transfusion

MASTA, Medical Services to Travellers Abroad, Keppel Street, London WC1E 7HT, England (071 631 4408), produce a wide range of products for personal protection against malaria, unclean water and non-sterile medical equipment, all of which have been approved and recommended by the London School of Hygiene and Tropical Medicine. These include various bed nets (adults and children), sprays and repellants for malaria, a medical equipment pack of syringes, needles, sutures and dressings and blood group label, water purifiers and an emergency dental pack to replace dental crowns, bridges and inlays. Their brochure also includes details of the 'Blood Care Foundation', a charity dedicated to the provision of fully screened and tested blood for travellers in countries where this is not readily available. The programme is based on a worldwide network of blood banks and regional supply points. In the event of an emergency abroad, a supply of grouped and tested clean blood will be made available to those registered in the programme by telephoning the BCF Alarm Centre in Switzerland. Further information on the BCF: (UK) 0274 531723, or through MASTA at the above address.

Erratum—Letter to the Editor

Comment: Distinguishing post-kala-azar dermal leishmaniasis from leprosy: experience in Sudan. This Letter to the Editor by V. V. Gurunatha Babu was published in *Lepr Rev* (1994) **65,** 150. The last two sentences of the Letter should read as follows:

'However a splenic biopsy of parasitic forms can confirm diagnosis. Under field conditions a course of sodium stibanate as a therapeutic test rapidly clears the lesions.

Instructions to Authors

Papers submitted for publication in *Leprosy Review* should be sent to the Editor, Professor J. L. Turk, LEPRA, Fairfax House, Causton Road, Colchester CO1 1PU, England. The name(s) of the author(s) and the place where the work was done should be clearly indicated below the title of the paper. Degrees and diplomas are not to be included.

It is understood that the paper is offered to *Leprosy Review* alone, that it will be subject to editorial revision, and that its copyright becomes the property of the British Leprosy Relief Association. Manuscripts should be typewritten, in double spacing, on one side of A4 (297 × 210 mm) paper, with wide margins (4 cm all round). Contributors must send three complete copies of the text, tables and figures. On a separate sheet give the title, short title, name and postal address of the author, together with the name of the institution where the work was done. Abbreviations of titles of journals should follow the list of journals indexed in *Index Medicus*. References to books should include the editor(s), publisher and place of publication. Once manuscripts have been accepted a copy on disk that matches the hard copies exactly would be very much appreciated.

Units and Abbreviations. The Journal recognizes the adoption of the Système International d'Unitès (SI Units) proposed in *Units, Symbols and Abbreviations* (1972) published by the Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE. Abbreviations should only be used for unwieldy names, and only when they occur frequently.

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